Does urban chemical exposure influence the skewing of the immune system in Thai infants?

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RESPONSIBILITIES

I was responsible as the principal investigator for completing all the activities listed below.

1. Reviewing the relevant literature and publishing the literature review as first and corresponding author
2. Creating the online questionnaires and biobank recoding system for data collection in the pilot project
3. Writing the research protocol and obtaining ethical approval from OxTREC and the local EC
4. Securing research funding
5. Setting up and running meetings to kick off the collaborative project between 3 hospitals in Thailand, monitor the project progression, and rectify the relevant problems in the project every 2-3 months, or as needed
6. Collecting participant informed consent at Ramathibodi hospital on enrollment.
7. Teaching and coaching each research assistant from 3 hospitals to complete the questionnaire and follow up with the participants as per the protocol
8. Monitoring data collection and proofing the data collected by the research assistants.
9. Liaising with the shipping company and co-researchers to ship the frozen specimens from Thailand to the UK (PHE, Oxford)
10. Laboratory analysing the targeted chemicals and biomarkers, including blood heavy metals, urine cotinine, microRNA, and plasma IgE
11. Contacting the Pollution Control Department of Thailand and obtaining the air monitoring data.
12. Conducting all of the statistical analysis

Note: Public Health England (PHE) transitioned to the United Kingdom Health Security Agency (UKHSA) in 2021. The Department in which this work was undertaken was transitioned intact from PHE to UKHSA and so both names are used in this work with the use of PHE or UKHSA reflecting the name at the time the results reported were generated.

ABSTRACT

Does urban chemical exposure influence the skewing of the immune system in Thai infants?

Ratchaneewan Sinitkul, Hertford, Clinical Medicine DPhil

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Introduction

Urbanisation and industrialisation have led to increased chemical pollution, potentially influencing infants' immune systems and allergies. This prospective cohort study aimed to investigate the impact of urban chemical exposure on the skewing of the immune system and allergy in Thai infants.

Methods

A pilot cohort of first-trimester pregnant women and infants born to them, attending Ramathibodi, Samut Prakan, and Rayong Hospitals in Thailand, was recruited. Their exposure to urban chemical pollutants was assessed via a self-assessment questionnaire (SAQ), estimated ambient $PM_{2.5}$ exposure quantified by spatial interpolation of reported concentrations, and biomarkers (whole blood heavy metals; As, Cd, Cr, Hg, Mn, and Pb) by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in the first trimester, urine cotinine/creatinine ratio (CCR) across pregnancy and infancy (early-life). The outcomes included allergy (allergic diseases, sensitisation, and total IgE) and immunological profiles of infants (cytokines). Sensitisation was indicated by measuring specific IgE (sIgE) to common allergen. IgE and sIgE were analysed multiple times during pregnancy and the first year of life. Statistical analyses of correlations and univariate logistic regression and multivariable logistic

regression with Bonferroni correction were conducted to determine associations between chemical exposure to immune system changes and allergic outcomes.

Results

Of 203 pregnant women, 86 maternal-child pairs were included in the analysis (sub-cohort study for this DPhil). Allergic diseases (allergic dermatitis; AD and food allergy; FA) were diagnosed in 24 infants (27.9%). There were no differences in sIgE and total IgE between individuals with and without allergies. Spearman's correlation found both significant positive and negative associations of allergy and concentrations of infant cytokines (Interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IL-22, IL-31, IL-33, interferon (IFN)-γ, tumour necrosis factor (TNF)-α, and thymic stromal lymphopoietin (TSLP)) with all of heavy metals except Cd and Pb, urine CCR, and $PM_{2.5}$. Univariate logistic regression analysis found that prepregnancy body mass index (BMI), maternal occupation, maternal education, maternal sensitisation, sibling allergy, gravida, birth weight, child gender, exclusive breastfeeding, type of residence, home ventilation system (open door/window, fan), air conditioner (AC) use, air purifier use, recently furnished, mould visibility at home, pet exposure, environmental tobacco smoke (ETS) exposure, season of data collection, birth season, Pb exposure, $PM_{2.5}$ exposure, and IL-12/IL-23 p40 concentration were significantly associated with allergic outcomes. Multivariable logistic regression analysis with Bonferroni correction and hospital (site of data collection) adjustment found that home ventilation (open door/window), using air purifier, visible mould, household ETS, and maternal sensitisation were significant associated with allergic outcome in infant children.

Conclusions

This study found statistically significant association between early-life exposure to urban environments and an increased risk of allergies (AD and FA) and immune skewing in Thai infants. These factors included residence type, ventilation practices, heavy metal exposure, environmental tobacco smoke, and household ETS. Exposure to urban pollution, including heavy metals, ETS, and $PM_{2.5}$ also significantly correlated with the production of critical cytokines related to allergies and inflammation, depending on the timing of exposure from pregnancy to infancy. These findings will help to inform public health strategies and policies for reducing environmental health risks in these and similar urban environments.

PUBLICATIONS ARISING FROM THIS THESIS

Publication of material included in this thesis

1. **Sinitkul R**, Wongrathanandha C, Sirirattanapruk S, Plitponkarnpim A, Maude RJ, Marczylo EL.Children's Environmental Health in Thailand: Past, Present, and Future. Ann Glob Health. 2018 Aug 31;84(3):306-329. doi: 10.29024/aogh.2301.

Publication of material arising from work-related to, but not included, in this thesis

1. **Sinitkul R,** Maude RJ, Nithirochananont U. Design of an Integrated Clinical Research Informatics System for a Multi-Centre and Multi-Visit Prospective Birth Cohort Study. Stud Health Technol Inform. 2022 Jun 6;290:125-129. doi: 10.3233/SHTI220045. PMID: 35672984; PMCID: PMC7614911.
2. Chankaew K, **Sinitkul R,** Manuyakorn W, Roekworachai K, Kamalaporn H. Spatial Estimation of PM2.5 Exposure and its Association with Asthma Exacerbation: A Prospective Study in Thai Children. Ann Glob Health. 2022 Mar 8;88(1):15. doi: 10.5334/aogh.3513. PMID: 35433288; PMCID: PMC8916056.

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ABBREVIATIONS

1-HOP	1-hydroxypyrene
8-OHdG	8-oxo-7, 8-dihydro-2'-deoxyguanosine
95%CI	95% confidence interval
AC	air conditioner
AD	Atopic dermatitis or allergic dermatitis
ADHD	Attention deficit hyperactivity disorder
ADME	Absorption, distribution, metabolisms and excretion
AML	Acute myelogenous leukaemia
ANC	Antenatal care clinic
AOP	Adverse Outcome Pathways
APO	adverse pregnancy outcomes
AQG	Air Quality Guidelines
AR	allergic rhinitis
ARR	Adjusted relative risks
As	Arsenic
AS	ankylosing spondylitis
ATSDR	Agency for Toxic Substances and Disease Registry
BCL-6	B cell lymphoma 6 protein
BLL	blood lead level
BMA	the Bangkok Metropolitan Administration
BMI	body mass index
BPA	Bisphenol A
CBA	Cytometric Bead Array

CBC	complete blood count
cBLL	capillary blood lead level
CBMC	Cord blood mononuclear cells
CCCEH	The Columbia Center for Children's Environmental Health
CCR	Cotinine-creatinine ratio (CCR) = urine cotinine/cretinine
CCR	C-C chemokine receptor
Cd	Cadmium
CD	cluster of differentiation
CDC	Centers for Disease Control and Prevention
CK	Collaborative Kriging
CMIR	cell-mediated immune response
CO	carbon monoxide
CoK	Co-Kriging
COPD	chronic obstructive pulmonary disease
COVID	Corona Virus Disease
Cr	Chromium
cr	creatinine
CRCE	the Centre for Radiation, Chemical and Environmental Hazards
CRF	Case Report Form
CRS	chronic rhinosinusitis
CRS	chronic rhinosinusitis
CSIP	Child Safety Promotion and Injury Prevention Research Center
CSV	Comma-separated values
CTSG	Clinical Trials Support Group
CVD	cardiovascular disease

CXCR	Chemokine receptor
CYP450	cytochrome P450
DALY	Disability-adjusted life years
DAP	dialkyl phosphate
DC	dendritic cells
DCCA	cis/ trans-2,2-(dichloro)-2-dimethylvinylcyclopropane carboxylic acid
DDE	1,1-dichloro-2,2-di(4-chlorophenyl)ethylene
DDT	dichlrodiphenyltrichloroethane
DEDTP	diethyl dithiophosphate
DEP	3-phenoxybenzoic acid
DEP	diesel exhaust particle
DES	Diethylstilbestrol
DETP	diethyl thiophosphate
DI	Deionised water
DM	diabetes mellitus
DMA	dimethylarsinic acid
DMAP	dimethylalkylphosphate
DMDTP	dimethyldithiophosphate
DMP	dimethylphosphate
DMTP	dimethylthiophosphate
DNA	Deoxyribonucleic Acid
DOHaD	Developmental origins of health and disease
EBLL	elevated blood lead level
EC	elemental carbon
EDC	endocrine disruptors

EDCM	Electronic Data Collection and Management
EDTA	Ethylene Diamine Tetra Acetic Acid
ELISA	Enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency
ESRI	Environmental Systems Research Institute
ETS	Environmental tobacco smoke
EU	European Union
FA	Food allergy
FANC	four-visit focused ANC
FDA	Food and Drug Administration
FEV	forced expiratory volume
FFQ	Food frequency questionnaires
FRM	federal reference method
FVC	forced vital capacity
GA	Gestational age
GC-MS	gas chromatography-mass spectrometry
gDNA	Genomic DNA
GIS	Geographic Information Systems
GNI	gross national income
GPS	Global Positioning System
GST	glutathione-S-transferases
Hb	haemoglobin
hCG	human chorionic gonadotropin
Hg	Mercury
HIV	human immunodeficiency viruses

HMIR	humeral immune response
HQ	hazard quotient
HTML5	Hypertext Markup Language version 5
IARC	International Agency for Research on Cancer
iAs	Inorganic arsenic
IBS	inflammatory bowel disease
ICP-MS	Inductively coupled plasma mass spectrometry
IDMS	Isotope-dilution mass spectrometry
IDW	Inverse Distance Weighting
IFN	interferon
IFN-γ	interferon gamma
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IL	interleukin
ILC	innate lymphoid cells
IQ	intelligence quotien
IQR	Interquartile ranges
IRF 4	interferon regulatory factor 4
ISFET	Ion Selective Field-Effect Transister
ISTD	internal standard
IT	interim target
KBE	Key Biological events
KE	Key events
KED	kinetic energy discrimination
LBW	Low birth weight (birth weight < 2,500 gram)

LC/MS	Liquid chromatography-mass spectrometry
LLOQ	Lower limit of quantification
LMP	Last Menstrual Period
LOD	Limit of Detection
LOQ	Limit of Quantitation
LPS	lipopolysaccharide
LUR	Land use regression
MA	muconic acid
MBRN	Medical Birth Registry of Norway
MDD	major depressive disorder
MG	macroglobulin
MHCII	major histocompatibility complex class II
MIE	Molecular Initiating Events
MIP	Molecularly imprinted polymer
ml	millilitre
MMA	monomethylarsonic acid
Mn	Manganese
MOA	Mode of Action
MOE	Ministry of Environment
MOPH	Ministry of Public Health
MORU	Mahidol-Oxford Research Unit
mRNA	messenger ribonucleic acid
MS	multiple sclerosis
MTA	Material Transfer Agreement
NAAQS	national ambient air quality standards

NCCHD	National Center for Child Health and Development
NCD	Non-communicable diseases
NEHAP	National Environmental Health Action Plan
NGS	next-generation sequencing
NIER	National Institute of Environmental Research
NIES	National Institute for Environmental Studies
NIH	National Institutes of Health
NIPH	Norwegian Institute of Public Health
NK	natural killer
NO_2	nitrogen dioxide
O_3	ozone
OD	optical density
ODK	Open Data Kit
OK	Ordinary kriging
OP	Organophosphates
OPD	Outpatient department
OR	odds ratios
OSMR	Oncostatin M Receptor
OTU	Operational Taxonomic Units
OxTRECs	Oxford Tropical Research Ethics Committees
PAF	population-attributable fractions
PAH	Polyaromatic hydrocarbons
PAU/L	Pharmacia Arbitrary Units per litre
Pb	Lead
PBA	3-phenoxybenzoic acid

PBMC	peripheral mononuclear white blood cells
PCB	polychlorinated bisphenols
PCD	the Pollution Controlled Department
PCDD	polychlorinated dibenzo-*p*-dioxins
PCDF	polychlorinated dibenzofurans
PEFR	peak expiratory flow rate
PFT	pulmonary function test
PHE	Public Health England
PHP	Hypertext Preprocessor
PI	Principal investigator
PICO	Problem/Patient/Population, Intervention/Indicator, Comparison, Outcome, and (optional)
PM	Particulate matter
PNP	paranitrophenol
POP	persistent organic pollutants
PYR	pyrethroid
QIIME	Quantitative Insights Into Microbial Ecology
RA	rheumatoid arthritis
REDCap	Research Data Electronic Capture
RF	Radio Frequency
RK	Regression Kriging
RMSE	Root mean square error
ROR	retinoid-acid receptor-related orphan receptors
RR	Relative risk
RSD	relative standard deviation

RSV	respiratory syncytial virus
RT-PCR	Reverse transcription polymerase chain reaction
SAQ	Self-assessment Questionnaire
SCFA	short-chain fatty acids
SD	Standard deviation
SES	Socioeconomic Status
SGA	Small for gestational age
sIgE	Specific immunoglobulin E
SIM	Sample information management system
SLE	Systemic Lupus Erythematosus
SO_2	Sulphur dioxide
SOE	Sarcosine oxidase
SPSS	the Statistical Products and Service Solutions
SQL	Structured Query Language
SQL	Structured Query Language
STAT	signal transducer and activator of transcription
TCA	trichloroacetic acid
TCE	Trichloroethylene
TCPy	3,5,6-trichloropyridinol
TEOM	tapered element oscillating microbalance
TGF	transforming growth factor
TH	T helper cell
THB	Thai Baht
TLR	Toll-like receptors
t-MA	t-muconic acid

TNF	tumour necrosis factor
TRAP	traffic-related air pollution
Tregs	Regulatory T cells
TS	Trend surface
TSLP	Thymic stromal lymphopoietin
TSNA	tobacco-specific nitrosamines
TSP	total suspended particulate
Ucot	Urone cotinine
UK	United Kingdom
UKHSA	The UK Health Security Agency
ULOQ	Upper limit of quantitation
US	United States
USA	United States of America
UV	Ultraviolet
VOC	volatile organic compounds
WAO	World Allergy Organization
WB	whole blood
WHO	World Health Organisation

OUTLINE

This thesis comprises 5 main components:

1. Background of this study in **Chapter 1: Introduction** and **Chapter 2: Literature review**

2. Study methods comprising birth cohort study design, data collection, bio-specimen collection, handling, storage, shipping, laboratory analysis and statistical analysis **(Chapter 3: Study design)**. The protocol which I developed for laboratory analysis is in **Appendix 5: Optimisation of laboratory and GIS methods**.

3. Results for the primary outcome, including epidemiological information on early life and allergic disease occurrences in infant children **(Chapter 4: Overview results of the birth cohort study)**, environmental chemical exposure **(Chapter 5: Exposure assessment result)** and immunological and atopic outcomes in infants **(Chapter 6: Outcome assessment result)**

4. Results for the secondary outcome to explore the influence of environmental exposure and atopic diseases in infants are presented in **Chapter 7: The association of in utero chemical exposures and infant allergies**. As well as the focused discussions were done at the end of each chapter.

5. The final part of the thesis summarises and discusses the study findings in a broader context linking toxicology, human health, and policy implication (**Chapter 8: Discussion**) and provides a conclusion, implications and plans for future work (**Chapter 9: Conclusions**).

In addition, the relevant documents from this study are attached in the **Appendix,** including details of laboratory measurements, calibration curves and standard curves, optimisation of laboratory and GIS methods, and subgroup analysis results of each infant allergic disease outcome.

Chapter 1: Introduction

This chapter is the background of my study which includes the chemical exposures situation in Thailand, the influence of the *in utero* environmental exposure on allergic outcomes in children, epigenetic alteration, challenges in environmental studies, conducting birth cohort in environmental health studies, Geographic Information Systems (GIS) in environmental health research, hypothesis, aims, and objectives of the study.

Chapter 2: Literature review

This chapter is based on the publication of a narrative review on Children's environmental health in Thailand: past, present, and future and a systematic review on "early life heavy metal exposure and children allergy". It also highlights the evidence gaps and their relevance to the cohort recruited for this study.

Chapter 3: Study design

This chapter explains all clinical study protocol and procedures and laboratory methods used including conducting the pilot birth cohort study in Thailand, outdoor air pollution assessment by GIS, metals analysis by ICP-MS, measurement of total IgE and IgA by ELISA, measurement of sIgE for common allergen by PhadiaTop®, measurement of metabolites of nicotine by LC-MS/MS, and measurement of cytokines by BD™ Cytometric Bead Array (CBA) and ELISA.

Chapter 4: Overview result of the birth cohort study

This chapter presents the questionnaire assessment results of the study participants and other relevant data collection such as geography of participant's residences, air monitoring station, and hospital (site of data collection).

Chapter 5: Exposure assessment result

This chapter has four parts. Starting with the home environment during pregnancy, followed by presenting the exposure data for the environmental tobacco smoke (ETS), heavy metals, and estimated $PM_{2.5}$ exposure by GIS interpolation.

Chapter 6: Outcome assessment result

The immunological outcomes (plasma cytokines, total IgE, and specific IgE) and allergic diseases in infants are presented in this chapter.

Chapter 7. The association of *in utero* chemical exposures and infant allergies

This chapter presents the associations and statistical analysis of all the data in chapters 5, 6 and 7. Statistically meaningful associations are highlighted in the discussion.

Chapter 8. Discussion

This chapter discusses the clinical and statistically significant associations of the study comparing to another publication and provides the strengths and limitations of this DPhil study. The discussion in a broader context to link between toxicology, human health, and

policy aspects has been added. Lesson learnt of conducting birth cohort study in limited resources setting also mentioned.

Chapter 9. Conclusions

The main findings from this study, including exposure assessment, immunological and allergic disease outcomes in children, and the association of early life environmental exposure and allergy in infant children, are summarised in this chapter. Implications for policy and plans are proposed.

Appendix

Relevant documents, e.g. standard curves of laboratory measurement and the information of ICP-MS comparing UKHSA and Ramathibodi Hospital are included in this chapter (**Appendix 1-4**). Additionally, optimisation of laboratory and GIS interpolation methods in **Appendix 5** describes all laboratory protocol development in this study. It includes ICP-MS for metal analysis in whole blood, ELISA for IgA and total IgE measurement in plasma/serum, and enzymatic creatinine measurement in urine. This appendix follows the common introduction format, methods, results, discussion, and conclusion to explain how I optimised these laboratory measures for this thesis. Comparing the suitability of two potential methods of GIS interpolation [inverse distance weighting (IDW) vs. Ordinary Kriging (OK)] is also explored in this chapter. The result of subgroup analysis of univariate and multivariable logistic regression analysis of independent variables and each allergic disease (AD, FA, combined AD and FA) is also provided.

Chapter 1: Introduction

The opening chapter of this DPhil thesis starts with the background to help understand Thailand from a health and environmental perspective, allergy in many aspects, from prevalence, pathophysiology, the potential link between gene and environmental concepts to the cause of allergy development, and developmental origins of health and diseases (DOHaD). The inquiry extends to exploring the potential connections between genetic factors and environmental exposures in the genesis of allergies, a pivotal theme underpinning this research. This chapter identifies gaps in current knowledge that motivate the subsequent research presented in this thesis.

The foundation for examining environmental exposures, including the Adverse Outcome Pathways (AOP) framework to elucidate possible links between environmental factors and the manifestation of allergic responses, and geographic information system (GIS) are also mentioned. Additionally, epigenetic modifications and alterations in the microbiome are defined as potential elements bridging the gap between environmental conditions and the development of allergies. This introductory chapter provides a comprehensive orientation to the multifaceted nature of allergies and their intricate interplay with the environment, offering the essential context and rationale for the ensuing research.

1.1 Background

The immune system protects the human body against pathogens and maintains overall health. Proper immune system development during early life is crucial for establishing a robust defence mechanism and reducing the risk of immune-related disorders later in life. However,

emerging evidence suggests that environmental and lifestyle factors, particularly urban chemical exposures, may influence the maturation and functioning of the immune system, potentially leading to immune dysregulation and increased susceptibility to diseases [1-3].

In recent decades, rapid urbanisation and industrialisation have transformed many world regions, including Thailand. The urban environment is characterised by a complex mixture of chemical pollutants arising from industrial activities, vehicular emissions, household products, and other sources. These chemicals, such as air pollutants, heavy metals, pesticides, and endocrine-disrupting chemicals (EDCs), can interact with the immune system and disrupt its delicate balance [4-7].

While the impact of urban chemical exposures on respiratory and cardiovascular health is well-documented, their effects on the immune system, particularly in early life, remain relatively understudied [8, 9]. Infancy is a critical period of immune system development and vulnerability, as the immune system is still maturing and adapting to the external environment. Therefore, investigating the potential influence of urban chemical exposures on immune system skewing[1] in Thai infants is very important for public health and environmental research.

The relationships between urban chemical exposures and the skewing of the immune system in Thai infants, after exposures during pregnancy (*in utero*) were explored. Specifically, it

[1] Immune system skewing refers to the alteration or shift in the balance of immune responses, leading to a dominance of one type of immune response over another. It involves a change in the proportions and activities of different immune cells, molecules, and pathways, often resulting in an overall change in the immune system characteristics and functions. It can occur in various context such as allergic reaction, autoimmune diseases, chronic inflammation, immune suppression, and immunosenescent.

seeks to examine whether exposure to urban chemical pollutants during early life is associated with alterations in cytokine profiles and regulatory mechanisms in infants residing in urban areas of Thailand. By understanding the potential impact of urban chemical exposures on immune system development, this study can contribute to identifying risk factors and developing preventive strategies to mitigate the health risks of allergy in Thai infants.

This thesis employed epidemiological and immunological approaches to achieve these objectives. It involved recruiting a cohort of Thai pregnant women from urban areas and assessing their exposure to various chemical pollutants through environmental monitoring and biomarker analysis. Following birth, the children of these mothers were assessed for immunological assessment of cytokine, total IgE and specific IgE to common food allergens were conducted to evaluate immune system status and potential alterations associated with urban chemical exposures.

The study was limited to a small cohort due to limited resources and high ambition. However, it was conceived with the anticipation that it would provide the basis for further work in Thailand. It was anticipated to provide valuable insights into the impact of urban chemical exposures on immune skewing in Thai infants and their association with allergic diseases. Identifying specific chemical pollutants and their immunomodulatory effects may affect public health interventions, policy development, and urban planning to minimise chemical exposures and promote healthy immune system development in early life.

In conclusion, understanding the potential influence of urban chemical exposures on the skewing of the immune system in Thai infants is crucial for addressing the emerging challenges

posed by environmental factors on immune health and allergy. This thesis aims to provide further evidence to bridge the knowledge gap by investigating the association between urban chemical exposures and immune system alterations in Thai infants, ultimately contributing to policy developments for protecting and promoting infant health in urban environments.

1.1.1 Thailand: geography and national health care

Thailand is in Southeast Asia and comprises six regions: Northern, North-eastern, Eastern, Western, Central, and Southern. The capital city is Bangkok. The climate of Thailand is tropical, with warm temperatures throughout the year. There are three seasons comprising two monsoon seasons [one rainy season due to the southwest monsoon (May to October) and a winter rainy season due to the northeast monsoon (November to February) and summer (March to May)]. The population in 2020 was 69,799,978, with 5.2% of children under five years old [10]. Before the 21st century, Thailand was considered a lower-middle-income country (LMIC). Recently, the World Bank re-categorised Thailand as an upper-middle-income economy (UMIC) based on gross national income (GNI) per capita, with the country's primary income source changing from agriculture to industrial [11].

The national health authority of Thailand is the Ministry of Public Health (MoPH), which is responsible for formulating, implementing, monitoring, and evaluating health policy [12]. Universal health coverage was successfully implemented in Thailand in 2002 [13]. Non-communicable diseases (NCDs) have greatly burdened the Thai health system. In the past decade, NCDs, including ischemic heart disease and stroke, were the most typical causes of death among the Thai population [14]. In the World Heald Organization (WHO) Report of NCDs Country Profiles 2018, NCDs were estimated to account for 74% of all deaths in

Thailand. Of these, cardiovascular diseases, cancers, and chronic respiratory diseases were the top three causes of mortality, contributing 23%, 18%, and 6%, respectively [15]. Allergy is an increasingly important cause of morbidity in Thailand, including asthma, which can be life-threatening in severe cases. Allergic diseases can cause a significant socioeconomic burden and impact the quality of life of patients and their families [16]. According to systematic sampling in a door-to-door survey in 2004, the prevalence of asthma in Thai adults was 4%, with the average childhood onset age at 15.8 years [17].

1.1.2 Allergy, as an early onset non-communicable disease (NCDs)

According to the WHO, NCDs are chronic diseases not passed from person to person [18]. They occur due to genetics, environment, and behaviour. NCDs contribute significantly to the disease burden in many parts of the world and disproportionately impact individuals residing in LMICs, where over 75% of the total global NCDs-related deaths (equivalent to 31.4 million) occur. NCDs are responsible for a substantial portion of illness, disability, and premature death in developed and LMICs. The main NCDs in adults are cardiovascular diseases, cancers, chronic respiratory diseases, and diabetes. Allergies are also categorised as NCDs that share common risk factors and similar pathology of chronic low-grade inflammation with other common NCDs [19]. While environmental factors like tobacco smoke, pollutants, physical inactivity, unhealthy diets, and alterations in the microbiome contribute to the risk of these conditions, genetic predisposition also plays a substantial role.

Allergies like allergic rhinitis (AR), asthma, and atopic/allergic dermatitis (AD) have a vital genetic component [20, 21]. Family history is a significant risk factor; if parents or close relatives have allergies, an individual is more likely to develop them [22]. Genetic variations

can influence immune responses, the production of IgE antibodies (common in allergies), and the body's ability to tolerate or react to specific allergens [23]. Genetic factors also contribute to NCDs, which include conditions like diabetes, cardiovascular diseases, obesity, and certain immune disorders [24]. Genetic variations can also influence metabolism, insulin sensitivity, lipid profiles, blood pressure regulation, and other physiological processes related to these diseases. These genetic predispositions interact with environmental factors to influence disease risk. Interestingly, genetic components can be shared between allergies and some NCDs, such as major depressive disorder (MDD) and autoimmune diseases [25, 26]. Evidence suggests that specific genetic variants associated with immune dysregulation and inflammation could increase the risk of allergies and NCDs [19, 27, 28].

The risk factors for both allergies and the broader group of common NCDs include tobacco and other pollutant exposures, physical inactivity, unhealthy diets and microbiome alteration, all of which influence systemic inflammation with downstream effects on the risk of insulin resistance, obesity, cardiovascular risk, immune diseases, and even mood and behaviour [29]. Some studies have also shown an association between allergic diseases and diabetes [30] and cardiovascular diseases [31] in adults. It has been proposed that gene-environmental interactions play a crucial role in NCDs development, particularly the specific vulnerability of the developing immune system to modern environmental change. Indeed, many environmental risk factors implicated in the rise of other NCDs have been shown to mediate their effects through immune pathways. Therefore, studies focusing on the causes of allergic diseases in children, the common and earliest-onset NCD, can inform allergy prevention and potentially additional opportunities to prevent other NCDs in adults.

This DPhil study, which will focus on the environmental contribution to early onset allergy, will provide evidence that may be used in later interventions or policies to prevent allergic disease in children and the economic and social burden of allergy.

1.1.3 Epithelial barrier hypothesis and environment

Recently, the epithelial barrier hypothesis has been proposed to explain the link between environmental factors related to technological development, particularly in urban environments and the increasing prevalence of NCDs, including allergy [32]. The evidence from animal studies suggests that potential hazards that injure epithelial cells and/or tight junctions can be found at low concentrations in household products and personal care items such as detergents, surfactants, or even food emulsifiers [32]. Epithelial injury can release cytokines, including members of the alarmin family such as IL-25, IL-33, and thymic stromal lymphopoietin (TSLP), which play a significant role in the development or aggravation of allergic diseases (**Figure 1.1-1**). In addition, epithelial barrier dysfunction can allow foreign substances and translocated bacteria to enter the body, activating an immune response and an inflammatory cascade. [33] The local epithelial dysfunction and immune response can cause tissue injury and affect systemic organ function through responses to cytokine release and migration of inflammatory cells from the leaky epithelial lesion to other sites [32]. Systemic low-grade immune systems are activated, and chronic inflammatory diseases can be exacerbated [19, 32].

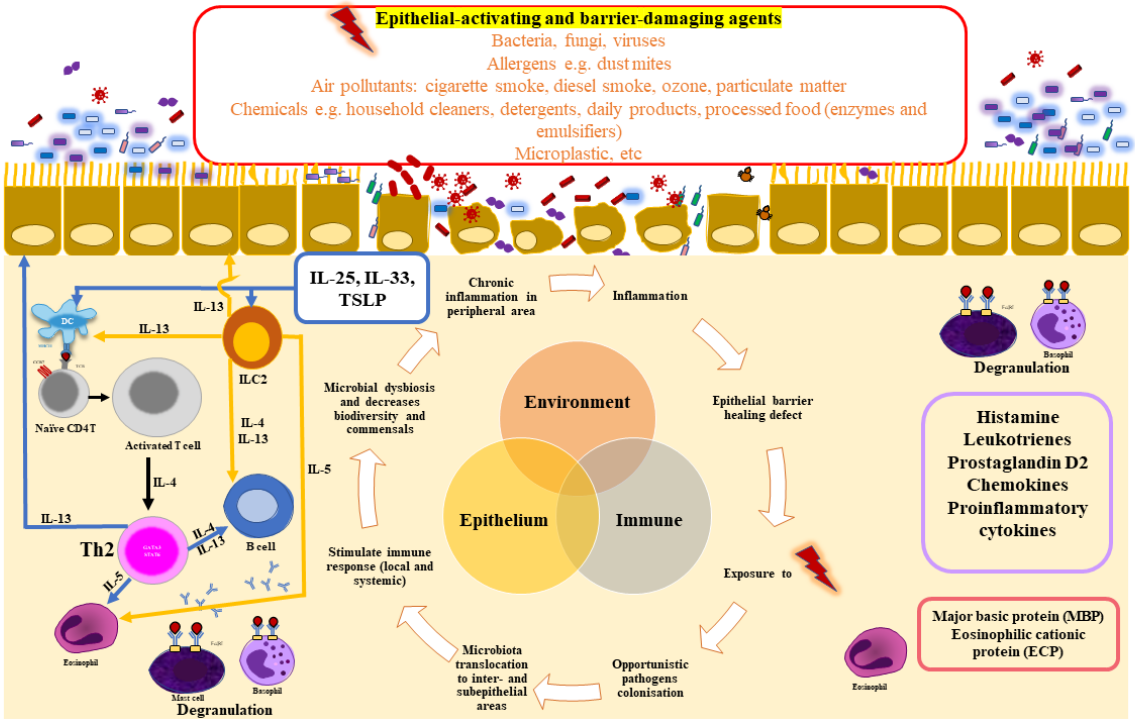


Figure 1.1-1: Environmental exposure, epithelial dysfunction and potential mechanisms to chronic inflammation leading to allergy development and exacerbation [32].

1.1.4 Prevalence of allergy in Thailand in a worldwide context

Allergic diseases are a significant public health issue worldwide. According to the World Allergy Organization (WAO) updated white book in 2013, allergic diseases have increased, particularly in industrialised and westernised countries [34]. AD or eczema, asthma, food allergy (FA), and AR or hay fever are the common allergies in children, which affect approximately 39%, 35%, 10%, and 9% of those under 18 years old [35-38]. Worldwide, sensitisation [i.e. development of specific IgE (sIgE) to the allergen] rates among school children were reported to be up to 40 - 50% [34]. In Thailand, a report from the International Study of Asthma and Allergies in Childhood (ISAAC) in 2003 found the prevalence of eczema, asthma symptoms, and allergic rhinoconjunctivitis symptoms in children were 16.7%, 11.9%, and 10.4%, respectively [36]. However, the prevalence of parent-reported food allergy in a

cross-sectional survey in the Northern region of Thailand in 2019 was 5.5%, with a decreasing trend compared to a cross-sectional survey in 2010 using the same method with 9.3% [39, 40].

While genetics contributes to allergies, the rise in prevalence may not be solely attributed to genetics. Rapid environmental changes due to technology are likely playing a role, raising concerns about the environmental connection. For example, epidemiologic studies suggested outdoor and indoor air pollution associated with asthma, asthma exacerbations, rhinitis, and rhinoconjunctivitis [34]. Additionally, as environmental factors can be modified, addressing them offers a chance to prevent allergies. This approach holds promise for reducing allergy prevalence and enhancing public health.

1.1.5 Early life, environmental exposures, and allergy development

Allergy is the immune response imbalance toward Th2 responses, including IgE-mediated mast cell stimulation, leading to allergic reactions to specific allergens. It tends to be more common in children than adults [41, 42]. Several allergic diseases occur in infants, and some may experience a set of allergic (atopic) marches, which are the natural history of allergic diseases that usually begin with a food allergy or AD and early wheezing in the infant period [43, 44]. In later childhood, allergic march is followed by asthma or airway hyperresponsiveness with/or without allergic rhinoconjunctivitis [44-46]. Epidemiological and animal studies indicate that children/animals with AD are highly likely to develop another allergic disease, such as IgE–mediated food allergy, and respiratory allergies due to the epithelial barrier impairment [47]. The disruption of immunological development may lead to an imbalance of immune responses, which is the key to hypersensitivity or allergy [19].

Environmental health hazards, they can be subdivided into interrelated categories, including biological (e.g., pathogen, allergen), chemical (e.g., air pollutants, heavy metals, pesticides, food contaminants, persistent organic pollutants (POPs), endocrine disruptors, physical (natural disaster, accidents, radiation), and psychosocial (stress, socioeconomic inequity) hazards [19, 48]. These hazards, particularly chemical hazards, can negatively influence the immune system or allergic outcomes, leading to the undesirable effect of allergic disease exacerbation or in some cases be causative [49-51]. Most studies can demonstrate an association between environmental factors and allergic outcomes, though the mechanism is poorly understood. One potential mechanism is an interaction of microbiota and epigenetics [48, 52]. This hypothesis arises because several chemical exposures, particularly *in utero*, possibly cause the direct effect of epigenetic change or indirectly by creating oxidative stress, altering commensal microbiota, or interfering with the stress hormone [45, 53-56]. Increased hygiene leading to decreased early life exposure and tolerance to allergens is also considered the increasing prevalence of allergic disease worldwide [57].

Understanding how environmental factors impact allergic diseases is challenging due to the complexities of the relationship between environment and disease outcome. One way forward is careful epidemiological analysis between a robust exposure assessment and analysis of disease outcome preferably on several occasions (longitudinal).

1.1.6 Exposure assessment

Exposure assessment is one of four components of the risk assessment (the others being hazard identification, dose-response assessment, and risk characterisation) and is a crucial risk analysis tool (**Figure 1.1-2**) [58]. Exposure assessment can be done by either direct methods, indirect

methods, or both. Indirect methods are usually done by environmental monitoring, models, questionnaires, and diary recording. Personal monitoring and indirect exposure assessment information can be constructed from the exposure models leading to exposure estimation. The typical direct methods are personal monitoring and biological monitoring. Personal monitoring is performed by wearing the measurement tools close to the exposure route, e.g. air monitoring at the breathing zone. Biological monitoring is a direct method to measure biomarkers predictably with the exposure of interest. It also can be modelled for pharmacokinetics and pharmacodynamics.

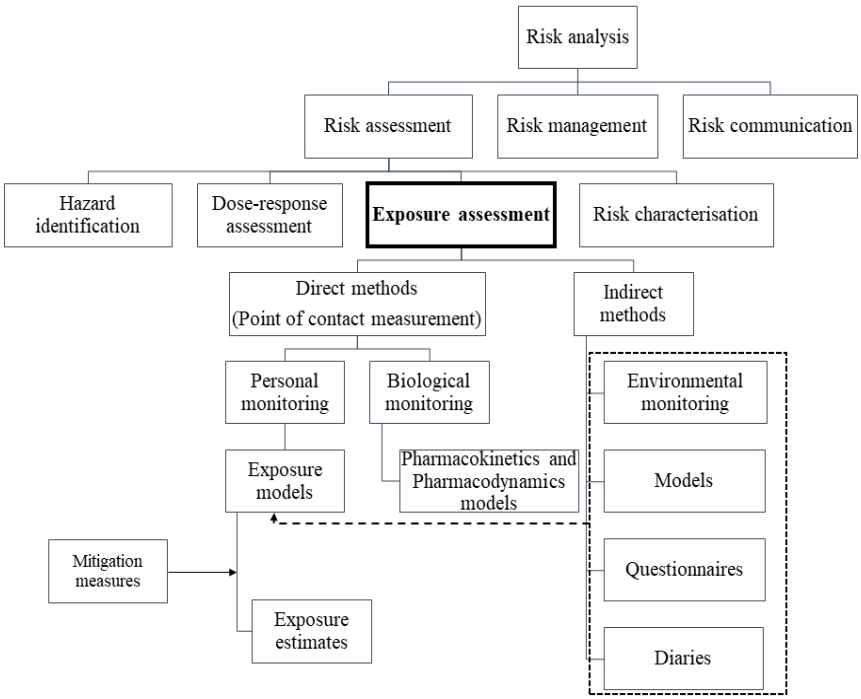


Figure 1.1-2: Diagram of the risk analysis

While exposure assessment is a systematic process used by researchers to quantify or measure human exposure to various environmental agents, such as pollutants, chemicals, allergens, and other factors that may affect health, it plays a critical role in epidemiological and environmental health research by providing data on the intensity, duration, frequency, and routes of exposure to specific environmental agents. The relatively new and comprehensive concept of exposome that aims to capture the totality of environmental exposures a person encounters throughout their lifetime, from conception to old age, is increasingly utilised [59-64].

1.1.7 Exposome

The exposome concept, introduced in 2005 by Dr. Christopher Wild, who served as the Director of the International Agency for Research on Cancer (IARC), represents a paradigm shift in how we approach the study of human health and disease [65]. Dr. Wild proposed the exposome as a complementary concept to the genome, emphasising the necessity of examining and measuring the environmental exposures individuals encounter throughout their lifetimes. This visionary concept emerged in response to the limitations of solely focusing on genetic factors when investigating the origins of diseases, recognising the pivotal role of environmental factors in shaping health outcomes.

The exposome concept has recently evolved to enhance our understanding of complex disease development by integrating biological processes across a person's lifespan. This refined definition emphasises two key components: a "cumulative measure" of the exposome, which quantifies environmental influences, and the "differential response" in biological processes [66]. This evolution allows a more precise and encompassing exploration of how environmental factors influence health outcomes over time.

The exposome can be broadly categorised into internal, general external, and specific external exposure (**Table 1.1-1**) [66].

Table 1.1-1: The domains of the exposome concept [66]

Domains	Explanation
Internal Exposome:	This domain encompasses internal biological processes, including oxidative stress, inflammation, epigenetic changes, metabolism, and the internal microbiota. These processes reflect how the body responds to various environmental exposures.
General External Exposome	Factors within this domain encompass socioeconomic determinants, the urban environment, and climate-related factors. They capture the broader external context in which individuals live and interact.
Specific External Exposome	This domain relates to an individual's immediate local environment and includes chemicals, diet, physical activity, tobacco exposure, and infection.

Critical aspects of the exposome concept include recognising cumulative effects, considering timing and life course stages, and acknowledging the intricate interplay between different environmental factors. By adopting a holistic approach that integrates genetic, environmental, and lifestyle factors, the exposome concept offers a comprehensive understanding of the multifaceted influences on human health and disease. It represents a significant advancement in environmental health and epidemiology, enabling researchers to unravel the complex web of factors contributing to various diseases and health outcomes.

1.1.8 Developmental origins of health and disease (DOHaD)

The window period of exposure also plays a critical role in determining health problems. Growing evidence suggests that early life exposures, including during pregnancy, may have the most significant impact on promoting disease development [67-69]. For example, maternal exposure to certain air pollutants such as NO_2, particulate matter 10 (PM_{10}), and particulate matter 2.5 ($PM_{2.5}$) during pregnancy, especially in the second trimester, is associated with childhood allergic diseases [69]. This observation is supported by the DOHaD concept. The DOHaD concept was recognised in 1992 when Barker and Hales developed the 'thrifty

phenotype' theory to explain the increased risk of ischemic heart disease after the UK famine period by the "trade-offs" mechanism. The foetuses developed insulin resistance *in utero* to adapt to the nutritionally deficient environment and survive till delivery. However, later in life, exposure to enriched environments may raise the incidence of non-insulin-dependent (Type 2) Diabetes Mellitus (DM) [70]. However, this hypothesis was questioned due to its limitations in explaining the development of DM type 2 in the average birth weight population. Later research revealed that insulin resistance in this population occurs in adults, not foetuses.

DOHaD is currently a manifestation of normal developmental plasticity processes, the ability of an organism to change its structure and function to changing conditions during development that enable the ability to respond to environmental change. It can occur in a regular or irregular pattern and on a time scale ranging from one to several generations [71]. Human plasticity happens during the early stages of life, including the time between pregnancies and early infancy. As a result, any stressor can easily disrupt the process within this sensitivity window. This concept is also known as a "predictive adaptive responses" mechanism, as demonstrated in **Figure 1.1-3**. Epigenetic processes are changes in gene expression and can be altered by environmental factors such as nutrition, stress, and toxins. They can also last a lifetime or even generations.

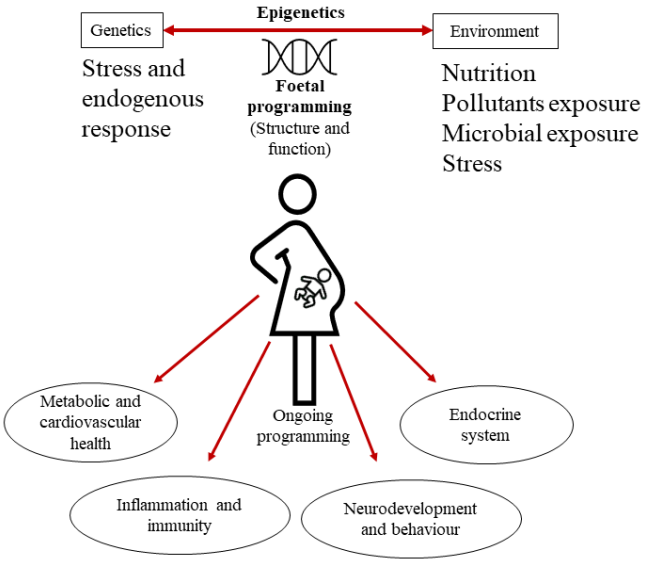


Figure 1.1-3: Diagram of Developmental Origins of Health and Diseases (DOHaD) concept

1.2 The potential environmental exposures in Thailand that may associated with allergy

Environmental problems such as air pollution, especially $PM_{2.5,}$ in urban areas and industrialised provinces, extensive use of pesticides in agricultural activity, and heavy metal contamination from non-regulated sectors remain Thailand's top three concerning hazards [19, 72-79]. However, the burden of diseases related to environmental exposure is still under evaluation, particularly in LMICs, including Thailand, due to a lack of paediatric environmental health research and prevention policy [73]. Environmental exposures occur from three main matrices: Air, Land and Water (**Figure 1.2-1**). Air and land are the most pertinent for the work here and are considered in separate sections below. Furthermore, exposures can be measured as external or internal at the site of action, meaning the pollutant has to enter the body, which is subject to absorption, distribution, metabolisms and excretion

(ADME), all physiological processes that will affect the amount reaches the internal site of action.

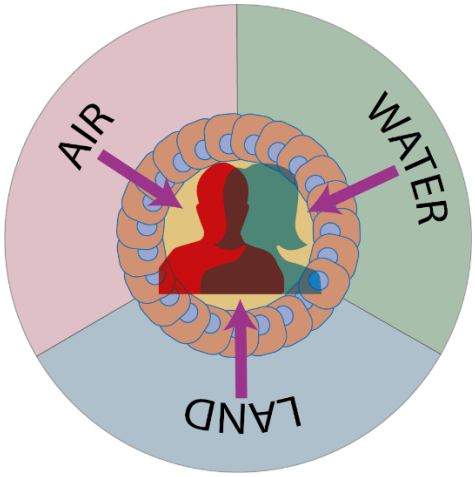


Figure 1.2-1: The schematic represents external exposures to the receptor and the transfer of these exposures across cell membranes to become internal exposures to organs.

1.2.1 Air pollution

Air pollution is the contamination of the atmosphere with any chemical, physical, or biological pollutants in an indoor or outdoor environment, which modifies the natural characteristics of the atmosphere. The sources of air pollution can be naturally occurring, such as bioaerosols (pollens/fungi) and/or anthropogenic, such as traffic-related, burning, or industrialised activities. Air pollutants may be classified in many ways but typically divide into two major classes: gaseous and/or particulate (mixture of solid and liquid) forms (**Figure 1.2-2**).

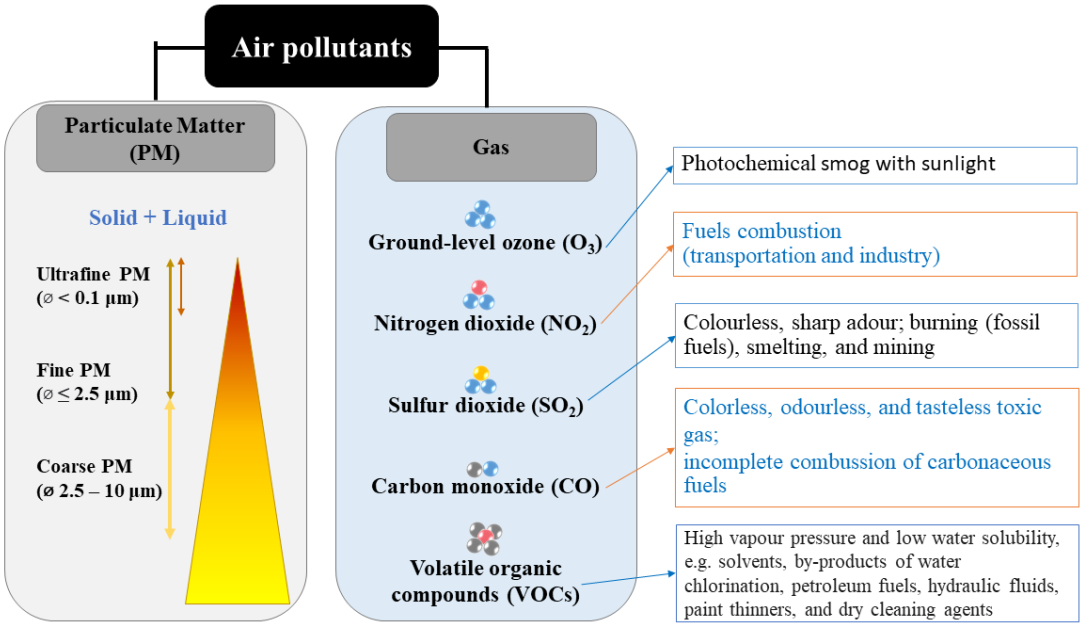


Figure 1.2-2: Schematic to demonstrate the air pollutants classification by the physicochemical characteristic, including particulate matter (PM) and gas

Air pollution (ambient/outdoor and household/indoor) is a global environmental health hazard that has causes approximately 6.7 million premature deaths annually [80]. According to WHO, 2019 ambient air pollution caused the loss of healthy years (Disability-adjusted life years; DALYs) across countries ranging from 249.6 to 2,689 per 100,000 population, with the rate in Thailand being 942.8 per 100,000 population in Thailand (**Figure 1.2-3**). The WHO has categorised air pollution as a risk factor for NCDs such as ischaemic heart disease, stroke, chronic obstructive pulmonary disease, asthma, and cancer [81].

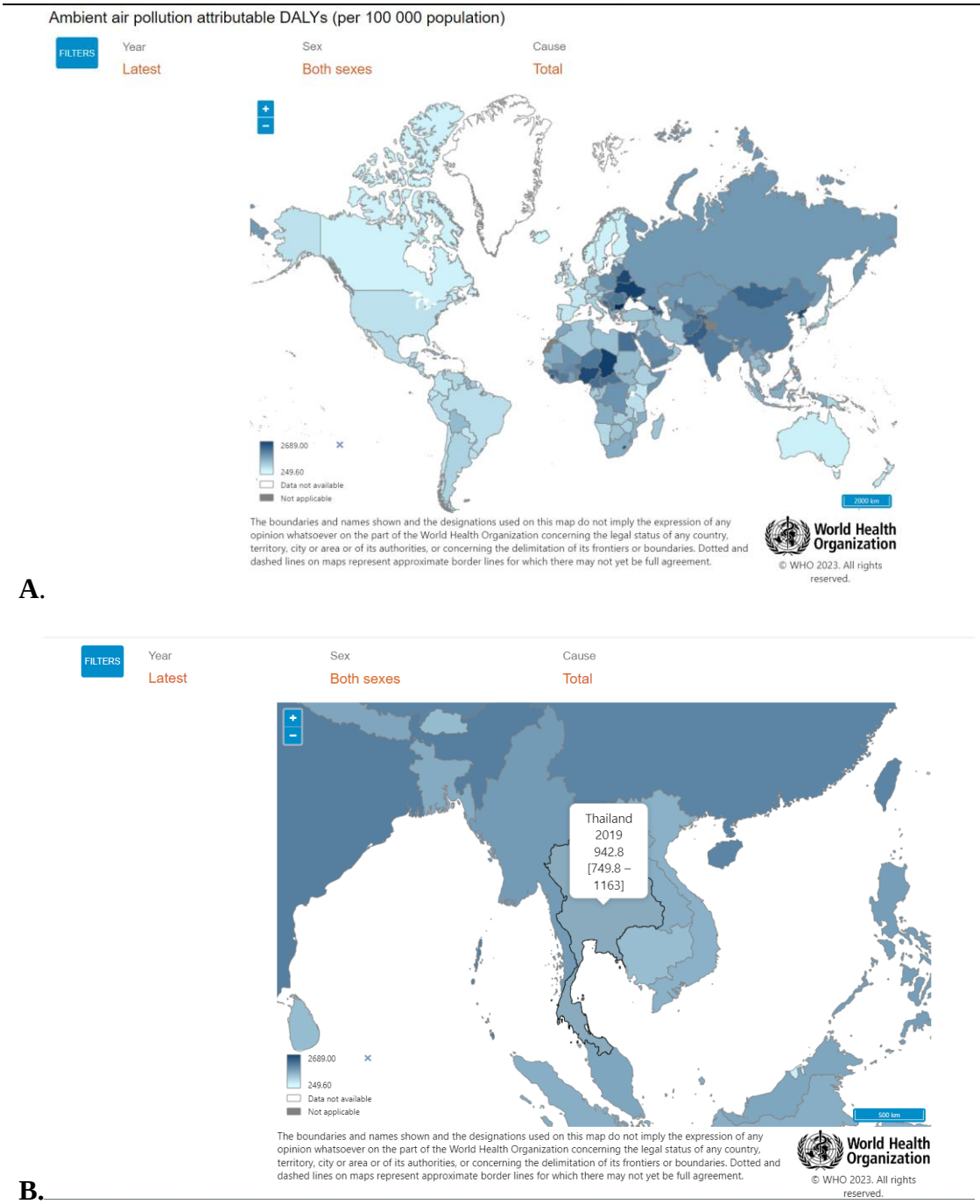


Figure 1.2-3: Ambient air pollution attributable DALYs (per 100000 population) A. Global scale B. Focusing on Thailand.

Source: https://www.who.int/data/gho/data/indicators/indicator-details/GHO/ambient-air-pollution-attributable-dalys-(per-100-000-population)

1.2.1.1 Air pollution in Thailand and association with disease

In Thailand, air pollution has been a significant health and political issue, and there remains a lack of specific overarching regulations to protect against its health effects, such as a "clean air act" [82]. Particulate matter, especially $PM_{2.5}$, NO_2, and volatile organic compounds (VOCs) from agricultural burning, traffic-related activities, construction, and industry, has been demonstrated as pollutants of concern, in Thailand as detailed in **Chapter 2.** Different ministries tackle each pollution source with different laws and regulations (**Table 1.2-1**). Currently thought there is not a single the overarching authority responsible for handling the comprehensive management of air pollution, which has resulted in an inability to tackle the air pollution problem across the country, highlighted in larger cities where the problem can be especially acute, including Bangkok, Chiang Mai, Rayong, and Saraburi. The Thai regulations and national ambient air quality standards (NAAQS) were established 30 years ago but have not been significantly updated since then but only guideline of the ambient $PM_{2.5}$ was updated in 2023. Of particular concern is that they do not adequately consider the health effects on vulnerable groups such as pregnant women, children, and people with underlying diseases.

Table 1.2-1: Laws to regulate air pollution in Thailand.

Laws (established year)	Responsibility/ Function
Enhancement and Conservation of National Environmental Quality Act, B.E. 2535 (1992)	The act is a fundamental environmental law in Thailand. Air pollution control is one of the focuses of the act. It sets out the environmental standards for ambient air quality. Air emission standards for mobile sources, as well as industrial facilities, are prescribed under the act.
Factory Act, B.E. 2535 (1992)	The act regulates industrial premises as a stationary air emission source. Therefore, air emissions from factory chimney stacks shall not exceed the standard concentration limits specified by the act.
Industrial Estate Authority of Thailand Act, B.E.2522 (1979)	The act sets the effluence standard for factories in industrial estates to control pollution load incoming to central wastewater treatment plants of industrial estates.

Like other countries, Thailand has established guidelines to regulate and maintain ambient air quality. These guidelines serve as benchmarks for acceptable levels of pollutants in the air. The specific details of these guidelines are provided in **Table 1.2-2**.

Table 1.2-2: Thailand Ambient Air Quality Standards (latest updated in 2023)

Pollutants	**Standards**					
	Average (mean concentrations)					**Measurement methods**
	1-hr	**8-hr**	**24-hr**	**1-month**	**Annual**	
CO (ppm)	30	9	--	--	--	Non-dispersive infrared
NO_2 (ppm)	0.17	0.07	--	--	--	Chemiluminescence
SO_2 (ppm)	0.3	0.12	--	--	0.04	Pararosaniline
O_3 (ppm)	0.1	0.07	--	--	--	Chemiluminescence
TSP (µg/m^3)	--	--	330	--	100	Gravimetric high volume
PM_{10} (µg/m^3)	--	--	120	--	50	Gravimetric high volume/ beta gauge/ microbalance/ dichotomous air sampler
$PM_{2.5}$ (µg/m^3)	--	--	37.5	--	15	FRM
Pb (µg/m^3)	--	--	--	0.0015	--	Atomic absorption spectrometer

Abbreviations: CO; carbon monoxide, FRM; **federal reference method,** NO_2; nitrogen dioxide, O_3; ozone, SO_2; sulphur dioxide, TSP; total suspended particulate, Pb; lead, PM; particulate matter

1.2.1.2 Environmental air monitoring stations in Thailand

Thailand first introduced air monitoring stations in 1983 and has gradually increased the number of stations to cover all five regions, especially in high traffic (roadside) and industrial areas. Various pollutants are monitored, including particulates, CO, NO_2, SO_2, and ground-level O_3. Initially, the total suspended particulate (TSP) and PM_{10} were monitored and expanded to measure $PM_{2.5}$ since 2001 (two stations in Bangkok and one station in Chiang Mai). In 2020, there were 141 stations (the Pollution Control Department; PCD 69 stations, the Bangkok Metropolitan Administration; BMA 72 stations) (**Figure 1.2-4**), and increasing to 167 stations across Thailand in 2023 (PCD 96 stations, BMA 71 stations) [83]. However, not all stations monitor all these pollutants.

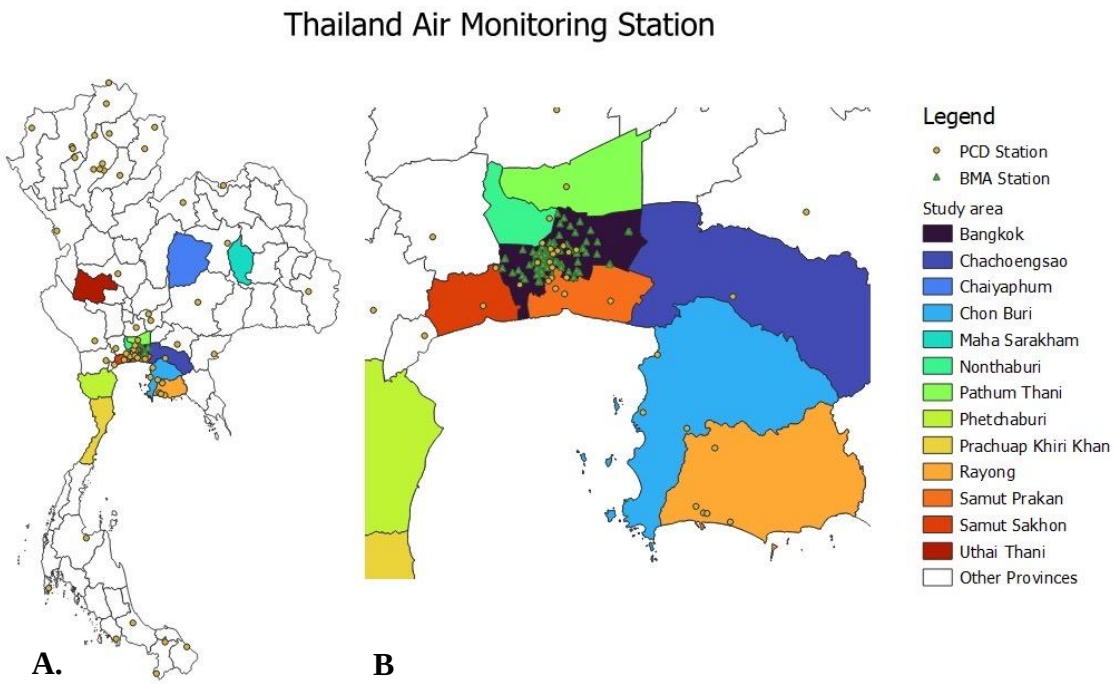


Figure 1.2-4: The map of the air monitoring station in Thailand, 2020 (during the study period), where A. is the air monitoring station for the entire country, and B. is the air monitoring station in the study location.

Abbreviations: PCD, the Pollution Controlled Department, Thailand; BMA, Bangkok Metropolitan Administration

1.2.1.3 Particulate matter 2.5 ($PM_{2.5}$)

Particulate matter 2.5 ($PM_{2.5}$) or fine particulate matter is air pollution with particle size 2.5 μm and smaller. It is a mixture of solid particles and tiny liquid droplets in the air [84]. $PM_{2.5}$ has been recognised as a significant concern for air pollution worldwide due to epidemiological studies of its health impact, e.g. premature death, increased cardiovascular risk, and respiratory illness associated with acute and chronic exposure [85-92]. According to a recent study on the global health burden of ambient $PM_{2.5}$, it was estimated to cause 4.23 (95% confidence interval (CI) 3.0 - 6.14) million excess deaths annually. [85] In Thailand, it was estimated that there are 650 - 38,410 annual fatalities from ambient air pollution and 160 - 5,982 annual fatalities, which could be avoided with a 20-fold reduction in ambient air pollutant concentrations [93]. Average daily (24-hour) concentrations of $PM_{2.5}$ in major Thai cities exceed the safe limits of the WHO and the Thai NAAQS [94]. The health impact of $PM_{2.5}$ exposure, particularly in adults with cardiovascular diseases and respiratory illness, has been well demonstrated. Other diseases, including metabolic and neurocognitive impairment, are also increasing concerns but are still controversial and need more evidence to confirm the association [86]. There has been little research on the health impact on vulnerable groups such as pregnant women, children, and senile people [86, 95].

The links between exposure to air pollution, such as traffic-related air pollution (TRAP), O_3, NO, and SO_x and respiratory allergies (allergic rhinoconjunctivitis and asthma) has been established for direct exposure [96-99]. There is less evidence for the effect of exposures *in utero* and this evidence is reviewed in **Chapter 2**. There is evidence for an association between acute exposure to $PM_{2.5}$ and aggravation of allergic diseases, including asthma and AD in children [100-102]. However, the evidence to support the association between chronic exposure to $PM_{2.5}$ during early life and allergic disease development is still inconsistent [100].

Multiple potential mechanisms of diseases, including metabolic activation, oxidative stress, mutagenicity, genotoxicity, modification of inflammation and immunity processes, and alternation of microbiota, have been proposed [95, 103-108].

1.2.1.4 Method of $PM_{2.5}$ measurement

Generally, air particulate matter can be measured by gravimetric, beta radiation (ray) or beta gauge attenuation, tapered element oscillating microbalance (TEOM), light scattering, or dichotomous air sampler. According to the PCD of Thailand, the reference (standard) method to measure particulate, including $PM_{2.5}$, is the gravimetric method based on the U.S. Environmental Protection Agency (US EPA), which is the federal reference method (FRM) and can detect particulate 0.3 μm at 99%. The accepted equivalent methods are beta ray attenuation, TEOM, and dichotomous air sampler. However, the light scattering method is still helpful for real-time monitoring and can obtain the diffusion or mobility of particle information. The measurement methods used in Thailand for other pollutants, CO, NO_2, SO_2, O_3, and lead, are shown in **Table 1.2-2**.

The Thai NAAQSs were established for particulate matter in 1984 and 1992 for TSP and PM_{10}, respectively. The recommended levels for these and $PM_{2.5}$ are in **Table 1.2-3**. For ambient $PM_{2.5}$, the exposure standard in Thailand was launched in 2009 based on the WHO Air Quality Guidelines (AQG) Global Update 2005 according to the WHO interim target 2 (IT-2) of 50 $\mu g/m^3$ and 25 $\mu g/m^3$ and was recently updated in 2023 with the WHO IT-3 of 37.5 $\mu g/m^3$ and 15 $\mu g/m^3$ for 24-hour-concentration (daily exposure) and annual mean concentration, respectively.

Table 1.2-3: Recommendation levels of ambient air particulate matter in the National Ambient Air Quality Standards (NAAQSs) in Thailand

Particulate	24-hour-concentration ($\mu g/m^3$)	Annual mean concentration ($\mu g/m^3$)	Promulgated year
Total suspended particle (TSP)	330	100	1984 - present
Particulate matter 10 (PM_{10})	120	50	1992 - present
Particulate matter 2.5 ($PM_{2.5}$)	50	25	2009 - 2023
	37.5	15	2023 - present

1.2.1.4.1 Assessment of individual air pollution exposure

Exposure assessment is critical to describe and determine the type(s) and amount of air pollutants individuals are exposed to. It can be done directly or indirectly, as mentioned in **section 1.1.6**, page 10 [109]. The direct approach for air pollution includes individual environment monitoring or biomarker assessment as an exposure proxy. The indirect approach relies on monitoring or measuring biomarkers—monitoring the receptors' environment and then calculating individual exposure from this measurement.

Individual exposure assessment can be conceptualised by the combination of two classification criteria: (i) the characterisation of the location of individual daily activities (point-fixed or trajectory-based) and (ii) the characterisation of air quality (variable or uniform) as shown in **Table 1.2-4**.

Table 1.2-4: Characteristics of the classification criteria for personal exposure assessment [109]

		Individual's activities	
		Point-fixed	Trajectory based
Air quality	Uniform	Subdividing a study area into homogeneous sub-areas and assigning individual daily activities to the residence location	Individual activities are considered for personal exposure assessment (locations and time spent are addressed) and identifying air quality data from the nearest air pollution monitor to these locations.
	Variable	Focused on characterising the spatial and temporal variability in pollutant concentrations during the day, while daily activities are disregard	Combining the spatial-temporal air quality data (direct personal monitoring or modelling) with the time-activity of participant

Measuring individual air pollution can be external or internal exposure at the site of action. Internal exposure, depicted by the cell layer in **Figure 1.2-5**, which pollutants need to cross to enter the bloodstream, can be calculated from external exposure using pharmacokinetic (toxicokinetic) models of ADME. Internal dose measurement focuses on quantifying the amount of a particular substance that has entered the body and is available to interact with biological systems. It provides information about the concentration of the substance in target tissues or organs and can be used to estimate the actual dose received by an individual. Internal dose measurements bridge the gap between exposure assessment and biological effects. For example, some substances to which external exposure occurs may not be absorbed and, therefore, never reach a site of action in the body. Thus, multiple pharmacokinetics represent the actual internal dose of exposure (amount of the contaminants that enter the blood circulation) since it can be altered by several factors affecting internal exposure, such as routes (pathways), frequency, and duration of exposure.

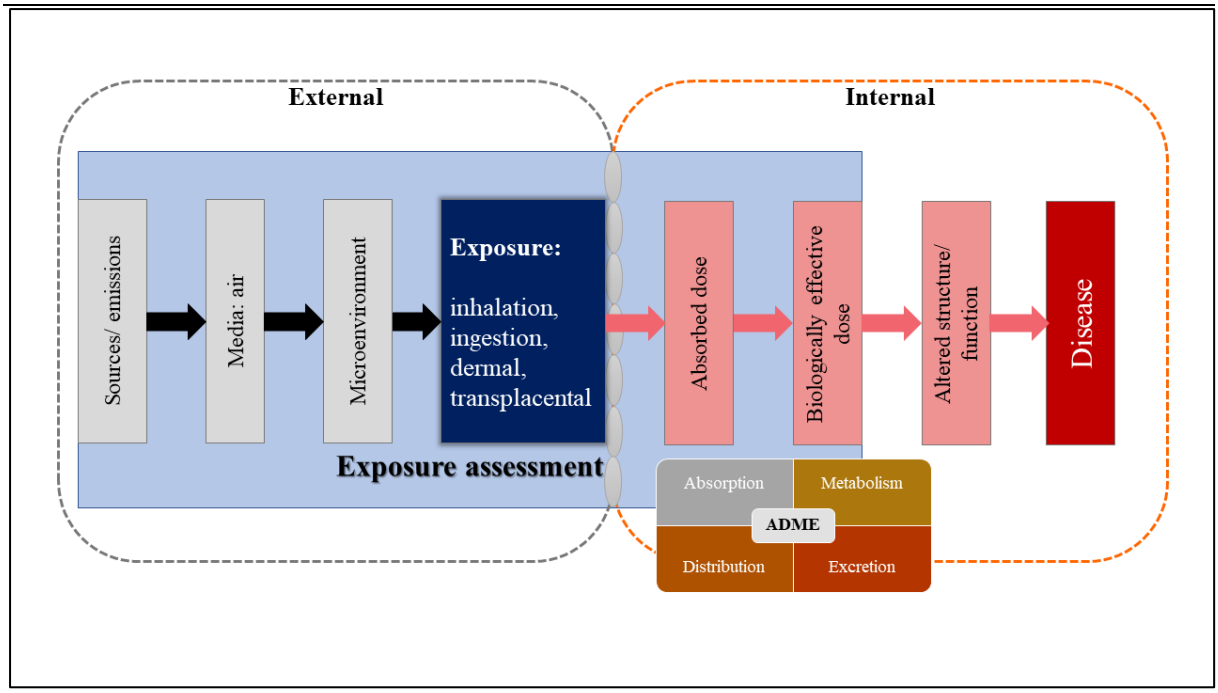


Figure 1.2-5: Exposure assessment of air pollution

Accurate external exposure assessment and assessment of internal exposure provide crucial information about the sources of exposure, which can be multiple (source apportionment). This information helps researchers and policymakers understand the potential risks associated with specific environmental agents and develop appropriate strategies for exposure reduction and prevention; in some cases, exposure assessment can be determined directly for the individual using personal monitoring to measure individual exposure [64]. The indirect approach aims to assess the individual levels of exposure by estimation or modelling based on ambient environmental measurements combined with exposure modelling. However, both direct and indirect approaches measure external exposure and calculation of the internal exposure is often required for accurate risk assessment.

In research studies and risk assessment, it is essential to consider both exposure assessment and internal dose measurement to accurately evaluate the association between environmental

exposures and health outcomes. By integrating information on external exposure levels and internal dose, researchers can gain insights into exposure-response relationships and make informed decisions regarding public health interventions and policies.

The selection of a suitable air pollution assessment approach for studies will depend on exposure duration (short-term or long-term), duration of health effect(s) (acute or chronic), an indicator of the health status (mortality or morbidity), health endpoint(s) of interest (e.g., respiratory diseases), and pollutant of concern [110, 111]. In most cases, internal exposure is the ideal but may not be available so external exposure measurements might be the only option.

1.2.1.4.2 Personal exposure assessment quantifies the individual-level exposure to air pollution.

Individual exposure assessment of air pollution can be conceptualised by the combination of two classification criteria: (i) the characterisation of the location of individual daily activities (point-fixed or trajectory-based) and (ii) the characterisation of air quality (variable or uniform) as shown in **Table 1.2-4**. This concept was proposed by Dias *et al.* [109]. The pros and cons of each approach are summarised in **Table 1.2-5.**

Table 1.2-5*:* Summary of advantages and disadvantages of different air pollution measurement methods, including individual personal exposure assessment.

Approach method	Advantages	Disadvantages	Air concentration measurement	Individual factor
Individual pointed-fixed activities and uniform air quality	- Low cost of implementation - Standard and static	- Exposure error and resulting bias: 1. Underestimation of the inter- and intra-variability of personal exposure within the study population 2. It does not consider microenvironment factors - Inability to account for small-scale spatial variability leading to exposure misclassification. - Blurring of individual time-activity patterns	- Nearest central-site monitoring station, e.g. annual means of air pollution - One central monitoring station - Averaged pollution data from all monitoring stations	Fixed location, e.g. residential address, school address, or combined
Trajectory-based and uniform air quality	- Reduced exposure errors and uncertainties arising from the monitoring networks limited spatial coverage, so it can provide air pollutant concentrations at excellent spatial resolution, capturing its spatial and temporal variation within urban areas. - It can be used to assess periods from hourly to annual averages.	It does not account for personal trajectory and assigns individual daily activities to a point-located position a spatial and variation of individual time-activity patterns.	Modelling techniques: outdoor concentrations with high resolution in time and space, e.g., Land use regression (LUR), air dispersion modelling, spatial interpolation	Fixed location, e.g. residential address, school address, or combined
Individual point-fixed activities and space-variable air quality	Improve spatial resolution of individual's activities	It does not adequately capture the inter-and intra-variability of personal exposure (not considering the spatial variation of air quality) an error of estimation of personal exposure assessment	Estimating microenvironmental concentration of air pollution by a mass-balance indoor model and nearby air quality monitoring station as a proxy for outdoor concentration	Time-activity database (activity survey)
Trajectory-based and space-variable air quality	- Actual exposure values from personal sampling - Consider the microenvironment	- High cost of implementation - Limitation for long-term exposure assessment - Poor compliance with personal sampler-wearing protocols. - Challenge to process and classify spatio-temporal patterns from raw GPS data. - Confidentiality issue	It is directly measured by personal monitoring or indirectly estimated by spatial and temporarily resolved exposure models.	Time-activities diaries or GPS data

1.2.1.5 Estimation of $PM_{2.5}$ concentrations

The concentration of particles that are 2.5 microns or less in diameter ($PM_{2.5}$), comprising solid and liquid substances, can be estimated using ground air monitoring stations, remote sensing (station-free), or combined approaches. Multiple regression modelling, spatial interpolation, and air dispersion modelling are commonly used [87, 112-114]. Each method has advantages and limitations depending on, for example, geographical area, the density of monitoring stations, and available data. The spatial interpolation will be used in this DPhil study due to its relative less complicated and its fundamental is provided in **Section 1.2.1.6**.

1.2.1.6 Using Geographic information system (GIS) in exposure modelling and association with disease estimation

Cholera disease mapping is an early example of data visualisation in environmental health [115]. In 1854, John Snow, widely noted as a pioneer in disease mapping for the study of epidemiology, created the survey map to investigate cholera (waterborne disease) outbreaks in the Soho district of London, England. His map revealed the critical source of the epidemic from contaminated water in the Broad Street pump, leading to the cessation of disease by removing the handle (source elimination) [116]. This concept of visualising data through disease mapping to find the association or causes of diseases is one application of GIS [117]. These tools can be used for data visualisation to display the environmental risk factors and their potentially associated health effects and to determine/predict pollution levels (quantitative) at unknown points (participants' location) from interpolation and geospatial analysis [93, 118-120].

The estimation of $PM_{2.5}$ can be performed by many methods [121-124]. Each method has pros and cons depending on the context in which they were applied, e.g. density of air monitoring stations, factors included in the models, reliability of the data input, duration of exposure assessment, etc. (summarised in **Table 1.2-6**). GIS interpolation is relatively less complex and is increasingly used in air pollution assessment. Moreover, it will also be applied in this DPhil study to estimate $PM_{2.5}$ at participants' residences. GIS interpolation tools can be divided into deterministic, geostatistical methods, and statistical modelling and are mentioned in the following.

1.2.1.6.1 Deterministic interpolation

It includes inverse distance weighted (IDW), Natural Neighbour, Trend surface (TS), and Spline. These methods produce reasonable estimation values at an unsampled point without statistical values (means, standard errors) and can establish a continuous surface of pollution concentration. Compared to other interpolation techniques, they are relatively more straightforward to apply, easy to implement, widely included in (or coupled with) many GIS software packages, and more appropriate in instances where the sampling network is sparse, and errors are assumed to be significant [125, 126]. However, other predictable factors, such as terrain and local patterns, are ignored. In addition, the Lack of data at the algorithm's edges leads to less reliability at the edges, which the algorithm cannot handle.

1.2.1.6.2 Geostatistical interpolation

The common method of geostatistical interpolation is kriging. The assumptions of data characteristics must be fulfilled before applying kriging interpolation, which includes stationarity of data and isotropy, were favourably in some studies [124, 126, 127]. However, the reliability of prediction at the edge is still an issue.

The kriging method can be classified into univariate and multi-variate kriging [125]. Univariate Kriging is a general linear regression model in which the common models are ordinary kriging (OK) and universal kriging. Multi-variate kriging is an extension of univariate kriging, and the common models are cokriging (CoK) and regression kriging (RK).

A significant challenge of geostatistical interpolation in air pollution estimation requires a reasonably dense network of sampling sites [125]. The number of sampling sites varies

depending on the scale of analysis, the scale of variability in the pollutant, local emissions, desired errors in estimates, the topography of the study area, and prevailing meteorological conditions.

1.2.1.6.3 Land use regression (LUR)

LUR is a regression statistical and stochastic modelling approach that aims to predict air pollution levels at specific locations based on land use characteristics rather than directly estimating pollution concentrations at unsampled locations [124, 125]. LUR was first used in the Small Area Variations In Air Quality and Health Study (SAVIAH) to assess the influence of long-term NO_2 exposure on respiratory health in children in Amsterdam by Huddersfield and Prague [128]. In this study, traffic, population density, land use and altitude were compiled in a GIS. Since then, LUR has been used in various urban air pollution studies [124], although it is limited by available information and data variability [129].

Table 1.2-6: Description of each GIS interpolation method for air pollution estimation.

Interpolation techniques	Explanation	Assumption	Advantages	Disadvantages	Application	References
Nearest neighbour (proximity model): Thiessen (Dirichlet/Voronoi) polygons: surrogate measure	• Measuring the proximity of a subject to a pollution source	• Nearness to emission sources proxies for exposure in human populations	• Exploratory analysis for a formative stage of limited knowledge or information on particular issues (little prior evidence) • Quick evaluation of the impact that traffic pollution has on respiratory symptoms	• Misclassification can occur due to ignorance of time-activities patterns, wind patterns, and topography (which may violate the implicit assumption of isotropic dispersion) and terrain and meteorological conditions modify the exposure experience. • Studies relying on self-reported traffic exposure face the potential for recall bias. • Provides relatively crude information	• The association between road proximity and respiratory disease, lung cancer and stroke mortality is often quantified within a buffer of some predefined extent (empirical model)	[125]
Inverse distance weighted (IDW)	• Deterministic method using mathematical functions for interpolation by averaging all the known locations and	• The value of attributed? z at some unvisited point is a distance-weighted average of data points occurring within a neighbourhood or	• Relatively low level of required programming and GIS expertise required	• Not ridge preserving. • Known point sampling should be sufficiently dense to estimate unknown points precisely.	• Interpolates a raster surface[2] (an unknown value) from known points by considering the weighted distance average	[125, 130]

[2] A raster is a type of data structure used in geographic information systems (GIS), remote sensing, and image processing to represent and analyse spatial data. It is a grid-based representation where each cell or pixel in the grid holds a value that corresponds to a specific attribute, such as elevation, temperature, land cover, or any other measurable quantity.

Interpolation techniques	Explanation	Assumption	Advantages	Disadvantages	Application	References
	allocating greater weights to adjacent points by • A linearly weighted combination of a set of sample points • Used to determine the pixel values by a linear combination of sampling points	window surrounding the unvisited point		• Prediction values are less reliable at the edges due to a lack of data • Variability can be exaggerated with too many peak values and depressed with too high a gradient. • It does not consider other factors, e.g., terrain, localised patterns, or meteorological variables.		
Ordinary kriging (OK)	• Geostatistical method using statistical and mathematical functions to create surfaces and assess the prediction uncertainty at the unknown point/area	• There is no global trend in the data, and it suffers the necessary disadvantage of assuming a spatially homogenous variation (called ‘‘the stationarity assumption’’)	• Illustrates the spatial correlation of the surface changes • Provides uncertainty values of the estimation	• Data must be stationary, with no trend. Therefore, air pollution data are not suitable for OK. • Estimation errors can occur due to an assumption violation	• Surface changes	[125]
Universal kriging	• Extension of ordinary kriging that incorporates a drift function to account for a structural component in spatial variation (global trend) of the	• Data have a spatial trend modelled using a simple function of the location coordinates. • Detrended data are locally second-order stationary if covariance	• Provides the ability to interpolate spatially related values when there is a trend in the mean, a property not realised with ordinary kriging	• It is limited to scenarios where the variation exhibited by the attribute of interest can be systematic. • High uncertainty	• It may be of use where trends in the pollution level mix with local variation.	[125, 126]

Interpolation techniques	Explanation	Assumption	Advantages	Disadvantages	Application	References
	pollutant of interest (the sum of a smooth deterministic function). Geographic trend is detected in the data by a polynomial function of locations (an internal trend purely dependent on the locations)	function is used or intrinsically stationary if variogram is used.				
Trend surface (TS)	• A statistical method based on known points in space, fitting a continuous • Mathematical surface and studying the variation regularity of geological variables in the region and local area	• Predicted values can be less than zero, which may not be realistic in some situations, e.g., precipitation amounts	• Can predict values smaller or larger than data minimum or maximum (if actual minimum or maximum data was not collected)	• Highly affected by the extreme values and uneven distribution of observational data points	• Describing broad geographical trends	[121, 130]
Collaborative kriging (CK) or co-kriging	• Uses one or more secondary variables to interpolate the variables of interest related to the main variables. Expressed as a linear combination of the practical sample values	• Stationarity: statistical properties of the variables, such as their mean and covariance, do not vary significantly across the study area • Spatial correlation: The values of one variable at nearby locations are expected to be more	• Correlation between the variables can improve the accuracy of the main predicted values.	• Data compatibility and quality issues can challenge harmonizing and combining the data effectively.	• Estimate and map environmental variables such as air quality, soil contamination, water pollution, or vegetation indices, disease mapping	[121]

Interpolation techniques	Explanation	Assumption	Advantages	Disadvantages	Application	References
		similar than those at distant locations. • Linear relationship: between the primary variable (the variable being estimated) and one or more secondary variables (the correlated variables used to assist in the estimation) • Normality: Variables are usually distributed.				
Spline	• Piece-wise polynomial function equivalent to ruler spline (historical method to achieve the best fitting of smooth curves by eye) • Constructed curve or surface passes through the given data points exactly	• Data continuity: Adjacent spline segments should join smoothly without abrupt slope or curvature changes. • Differentiability: The curve or surface is differentiable at the data points	• It fits data points sufficiently closely while being as smooth as possible	• Not a suitable interpolation method for highly irregular surfaces	• Creating elevation models from point data • Generating contour lines • Interpolating weather data to create spatially continuous maps	[126]
Land use regression	• A regression model that predicts pollution concentrations at a given site based on pollution monitoring data and existing	• Stationarity: statistical properties of the variables, such as their mean and covariance, do not vary significantly across the study area	• The empirical structure of regression mapping allows adaptation to local areas without additional monitoring or data acquisition.	• Area specificity suitable for small-area evaluation • The burden of information gathering of other independent	• Assessment of exposure to traffic-related pollution in an urban area	[125]

Interpolation techniques	Explanation	Assumption	Advantages	Disadvantages	Application	References
	exogenous independent variables, such as surrounding land use, traffic characteristics, and altitude	• Spatial correlation: The values of one variable at nearby locations are expected to be more similar than those at distant locations. • Linear relationship: between the primary variable (the variable being estimated) and one or more secondary variables (the correlated variables used to assist in the estimation) • Normality: Variables usually are distributed. • Homoscedasticity: The spread or dispersion of the prediction errors remains the same regardless of the values of the predictor variables. • Independence of Errors: The errors at one location do not depend on or influence those at other locations.		variables (cost and logical problems) • Land use and/or topography differences across the study area may affect poor correlation between predicted values and measured data.		

1.2.2 Land exposures

While air is a prominent contributor to environmental exposures that can potentially worsen atopic diseases, there are additional sources of exposure, particularly on land, such as heavy metals and pesticides. Therefore, this section highlights certain heavy metals and pesticides utilised in agricultural practices. These substances are worth mentioning due to their potential contribution to environmental exposures.

1.2.2.1 Heavy metals

Heavy metals are naturally occurring; however, human activities, such as mining, industrialisation, agriculture, and therapeutic activities, augment their contamination of the environment [131]. Environmental contamination in soil, water, air, and food chains is the vehicle for bringing heavy metals into the human body. Heavy metals are also found in some cosmetic products [132]. Certain regions have specific activities that can cause some heavy metals to contaminate the environment, such as mining (arsenic; As), coal-fired power plants (mercury; Hg), and battery plants (cadmium; Cd, lead; Pb).

There is a diversity in the definition of "heavy metals". Based on density, heavy metals were those with an elemental density above 5-7 g/cm^3 or relatively high density compared to water [131, 133-136]. With this definition, As and selenium (Se), metalloids with a density less than 5 $g/cm^{3,}$ could not be included in the heavy metals list. However, regarding environmental health sciences, heavy metals are generally considered those metals and metalloids associated with environmental pollution, toxicity and adverse effects on health and biota, and include As and Se [135]. Therefore, the latter definition fits this study's purpose and was applied in this thesis.

Heavy metals can be essential, e.g. copper (Cu), iron (Fe), zinc (Zn), selenium (Se), manganese (Mn) etc. or non-essential to human health. The non-essential and well-known heavy metals toxic to humans are mercury (Hg), lead (Pb), chromium (Cr), nickel (Ni), cadmium (Cd), and arsenic (As). According to the WHO (1 June 2020), As, Cd, Pb, and Hg are among the ten chemicals of primary public health concern [137].

Metals can also be divided into organic and inorganic metals based on metal compounds' chemical nature and bonding characteristics [138]. Organic metals are metal compounds that form bonds with carbon atoms through covalent bonding. The metal atom is typically coordinated with these compounds' organic ligands or organic molecules. Organic ligands can include carbon-based molecules such as aliphatic compounds, aromatic compounds, or organic functional groups. Well-known examples of organic metals regarding adverse health effects include methylmercury (an organic form of mercury) [139] and tetraethyl lead (used in gasoline) [140]. These compounds are often formed through industrial processes, combustion, or biological activity. Human exposure to organic metals primarily occurs through ingesting contaminated food and water sources. For example, methylmercury can accumulate in fish and seafood, common dietary sources. Organic metals can also be inhaled as volatile compounds or absorbed through the skin. Inorganic metals refer to metal compounds that do not form covalent bonds with carbon atoms and are not typically associated with organic ligands. These compounds often involve ionic or metallic bonding between metal and non-metal atoms. Inorganic metals, such as Pb, Hg, As, Cd, and Cr, are toxic and can have adverse health effects [137]. These metals are often found in environmental sources such as contaminated air, water, soil, and food. Inorganic metals can accumulate in the body over time and disrupt normal physiological processes.

1.2.2.1.1 Sources of heavy metals

Heavy metals are naturally occurring; however, human activities (anthropogenic), such as agriculture, mining, electronic waste disposal, construction, and fuel combustion, can contribute to heavy metal pollution. Natural sources include geological processes that release heavy metals through rock and mineral weathering and volcanic activity, which can disperse heavy metals into the atmosphere and water bodies. Industrialised activities like mining, metallurgy, and manufacturing release heavy metals into the air, water, and soil. Agricultural practices involving pesticides, fertilisers, and sewage sludge contribute to soil and water contamination. Improper disposal of electronic waste, batteries, and heavy metal products leads to leaching. Air pollution results from the combustion of fossil fuels in vehicles and power plants, emitting heavy metals like Hg and Pb. Water pollution arises from industrial discharges, stormwater runoff, and poor waste management. Construction, demolition, and consumer products like cosmetics and jewellery release heavy metals, such as Pb and Cd.

1.2.2.1.2 Pathways of heavy metal exposure

Human exposure to heavy metals is mostly via ingestion (Pb, Hg, Cr, As, and Cd) or inhalation (Pb, Hg, Cd, As, Cr, and Ni), and some metals can be absorbed through skin contact (Pb, Hg, and Cd) [141]. Transplacental exposure to As, Hg, Pb, and Se [142-145] and consumption through the breast milk of As, Pb, Cd, and Hg [146-152] have been reported and play a significant role in infants and represent a risk of early life exposure to heavy metals. In addition, bioaccumulation[3] of heavy metals is an important source and pathway, such as organic Hg methyl Hg in fish [139, 153], Cd and As in tobacco leaves, rice, and grain [154, 155].

[3] Bioaccumulation is the gradual build-up of substances in living organisms over time, resulting in higher concentrations. This is a concern when persistent pollutants accumulate in the food chain, potentially impacting ecosystems, and human health.

1.2.2.1.3 Adverse health effects of heavy metal exposure

The adverse effects of heavy metal exposure depend on the type and form of metals, route, dosage, duration, and timing. Modifying factors such as nutritional status, genetic susceptibility, and co-exposure with another hazard also affect the adverse health effects. The organ/system toxicity in humans is summarised in **Table 1.2-7**. The most common organ toxicity is to the gastrointestinal system, and all five heavy metals of concern can cause gastrointestinal disorders. It may be due to the gastrointestinal tract being a major absorption route via ingestion. Hepatic and renal systems are essential for xenobiotic transformation and clearance of heavy metals from the body. They are commonly insulted, leading to dysfunction from heavy metal exposure. Pulmonary, cardiovascular, reproductive, hemopoietic, and neurological disorders are associated with chronic exposure to these heavy metals. The IARC classified As and inorganic As compounds, Cd and Cd compounds, and Cr (VI) compounds as human carcinogens (class 1) [156]. The proposed fundamental toxic mechanism of organ injury from heavy metals is the rising level of oxidative stress in a cell, leading to the destruction of lipids, proteins, and DNA molecules [157, 158].

Table 1.2-7: Organ toxicity of heavy metals of concern (As, Cd, Cr, Pb, Hg)

System/toxicity	As	Cd	Cr	Pb	Hg
Nervous	✓			✓	✓
Respiratory		✓		✓	✓
Gastrointestinal	✓	✓	✓	✓	✓
Hepatic	✓	✓		✓	✓
Renal		✓	✓		✓
Cardiovascular	✓			✓	
Haematology				✓	
Dermatology	✓		✓		
Musculoskeletal		✓		✓	
Metabolic		✓			
Reproductive		✓		✓	✓
Immunology				+/-	
Teratogenic					✓
Carcinogenic	✓ (Skin, lung, bladder)	✓ (Lung)	✓ (Lung)	+/-	✓
References	[156, 157, 159]	[156, 157, 160, 161]	[156, 157]	[156, 157, 160, 162]	[157, 160, 163]

1.2.2.1.4 Heavy metals and allergy

Allergic contact hypersensitivity to heavy metals, a local skin allergic reaction, is well-recognised in children and adults and often is the result of direct contact. Here an introduction is given to summarise what is known and a new analysis of heavy metal exposure and allergic outcomes in children in Thailand is presented in **Chapter 2**.

In European countries, the prevalence of metal contact allergy is 15.7% [164]. The common culprit heavy metals are Cr, cobalt (Co), and nickel (Ni), widely used in costume jewellery, belt buckles, wristwatches, and eyeglasses frames [165-169]. The systemic allergic reactions and immune deterioration due to metal exposures have also been widely examined but showed inconsistent results [75, 78, 170-178]. Chronic Pb exposure in adult workers from storage battery plants could depress T cell numeration and diminish IgG, IgM, and complement levels compared to healthy control groups [175]. The US National Health and Nutrition Examination Survey (NHANES) in the 2005-2006 cycle also demonstrated the association of blood Pb levels

and sIgE levels to common food allergens in adults but not children [75]. Likewise, in a study of South Korean adults, blood Pb, but not Cd nor Hg, was associated with total IgE levels, particularly in dust mite sensitisation groups [177]. Some studies found that children's blood Pb levels were associated with IgE and eosinophils but not asthma [78, 178].

A systematic review and meta-analysis to examine the association between particular heavy metal exposure (Cu, Pb, and Hg) and common allergic diseases in children (food allergy, AD, AR, and asthma) was done by Wang *et al*. in 2022 [179]. They found that only Cu exposure was associated with childhood asthma but not AD nor wheezing in children on meta-analysis. However, Pb and Hg were not associated with any common allergic disease in children. The authors suggested that the variety of study types and differences in the timing of exposure (prenatal vs. postnatal) may influence the metal exposure and allergic outcomes.

1.2.2.2 Pesticides

Some epidemiological studies have made an association of pesticide exposure with allergic outcomes primarily in adult workers. These studies are described briefly here with new in-depth literature analysis of effects in children presented in **Chapter 2,** page 71.

Pesticides are substances used to control, repel, or eliminate pests such as insects, weeds, fungi, and rodents. Several types of pesticides are designed to target specific types of pests (**Table 1.2-8**).

Table 1.2-8: Type of pesticides and explanation

Pesticides	Explanation
Insecticides	These pesticides target insects and control pests that can damage crops, spread diseases, or infest buildings. Insecticides such as organophosphates, pyrethroids, neonicotinoids, and biological insecticides can be further classified.
Herbicides	Herbicides are used to control unwanted plants or weeds. They are commonly used in agriculture, forestry, and landscaping to prevent weed growth that can compete with crops or native plants.
Fungicides	Fungicides control fungal diseases that can damage crops, plants, and structures. They are used to prevent and treat fungal infections in agricultural settings and gardens.
Rodenticides	These are used to control rodents such as rats and mice. They are typically used in agricultural, residential, and industrial settings to prevent damage and disease spread by rodents.
Bactericides	Bactericides target harmful bacteria and are used in agricultural and horticultural practices to prevent the spread of bacterial diseases.
Miticides	Miticides, also known as acaricides, target mites and ticks. They are commonly used in agriculture to protect crops from mite infestations.
Nematicides	Nematicides control nematodes, microscopic worm-like organisms that can damage plant roots and reduce crop yields
Desiccants	Desiccants accelerate the drying of plants or plant parts, making them useful for pre-harvest applications to aid crop drying and harvesting.

1.2.2.2.1 Pesticide exposures

The sources of pesticide exposure depend on the situation and may include occupational, para-occupational, or environmental exposure. Agriculture activities are the primary source of direct and indirect exposures. Direct exposure can happen to farmers, agricultural workers, pesticide applicators, or individuals who live or work near pesticide-treated fields due to direct contact with pesticides during their handling, application, or when in treated areas. Indirect exposure refers to situations where individuals are exposed to pesticides through environmental contamination via food chain residues [180-192] and runoff (contaminated water and soil), leaching and dispersion (contaminated air) from agricultural activities/areas [193-195]. Pesticide exposure can occur through ingestion (intentional or unintentional), inhalation, dermal contact, or transplacental [196, 197]. In addition, breastfeeding infants can be exposed to pesticides by consuming pesticide residues in breast milk [198]. Agricultural-related workers and their family members may be exposed due to participating in farming activities, living within or close to the workplace and/or contamination brought back to the family home on clothing, footwear, etc.

1.2.2.2.2 Adverse health effects of pesticide exposure

Acute pesticide poisoning remains common in low- and middle-income countries including Thailand, for children and adults [199-203]. Chronic exposure is associated with neurotoxicity, cancer, respiratory symptoms, reproductive disorders, and allergic diseases [203-221]. Exposure in vulnerable populations such as pregnant women and children can lead to adverse pregnancy outcomes, congenital defects, and neurodevelopmental and behavioural disorders [219, 222-228]. There is some evidences of immunological disturbance linked to autoimmunity, cancer, and allergy, but this is inadequate to be conclusive [229].

1.2.2.2.3 Pesticides and allergy

Several epidemiological studies in adults have reported a link between pesticide exposure and allergic diseases in farmers in Canada and the US [212, 215, 216]. These are reviewed for children in **Chapter 2** and a brief introduction is provided here. In 2002, Cherry *et al*. conducted a cross-sectional study to examine the association between pesticide use (via telephone interview) and asthma (self-report and later linkage to administrative health records in 2009) in Alberta, Canada [215]. They found that lifetime exposure to phenoxy herbicides is associated with an increased risk of asthma in Alberta grain farmers. The adjusted odds ratio (95% CI) was 1.29 (0.66-2.52), 2.52 (1.25-5.09), and 3.18 (1.54-6.58) for self-reported asthma for short-, medium-, and long-term exposures, respectively, compared to no exposure. A prospective study in the US, which interviewed 22,134 male farmers in Iowa and North Carolina during 2005-2010, found that 19 pesticides were significantly associated with allergic wheeze and 21 pesticides with non-allergic wheeze, and 11 pesticides were associated with both [212]. In this study, allergic wheeze was defined as reporting both wheeze and doctor-diagnosed hay fever and non-allergic wheeze reporting wheeze but not hay fever. The names of those pesticides and the association of allergic outcomes from this study are shown in **Table 1.2-9**. Patel *et al*. also surveyed the use of pesticides and respiratory allergy (asthma and rhinitis) in 11,210 US farm operators in 2011 [216]. They reported that insecticide and herbicide use was significantly associated with lifetime AR (adjusted prevalence OR: 1.4, 95% CI: 1.2 -1.6, $P < 0.001$) and current asthma (adjusted prevalence OR: 1.5, 95% CI: 1.1 -1.8, $P = 0.003$) among farm operators. However, most research has focused on investigating the health effects of pesticides in adults, leaving a significant knowledge gap regarding their impact on children.

Table 1.2-9: The association of pesticide exposure and wheezing among adult male farmers. [212]

Pesticides	Chemical group**	Allergic wheeze	Non-Allergic wheeze
Herbicide			
2-4-D	Phenoxy	✓	
Bantazon	Other	✓	
Simazine	Triazine	✓	
Atrazine	Triazine	✓	✓
Dicamba	Acetic acid	✓	✓
Glyphosate	Organophosphorus	✓	✓
Petroleum Distillates	Petroleum products	✓	✓
Picloram	Acetic acid	✓	✓
Triclopyr	Pyridine	✓	✓
Trifluralin	Dimitroaniline	✓	✓
Clomazone*	Other	✓	✓
Acetochlor	Chloracetanilide		✓
Clorpyralid/Flumetsulam	Acetic acid		✓
Fenoxaprop/Fluazifop	Phenoxy		✓
Imazaquin	Imidazolinone		✓
Mesotrione	Benzoylcyclohexadione		✓
Metoachlor	Chloracetanilide		✓
Nicosulfuron	Urea substitute		✓
Insecticide			
Carbaryl	Carbamate	✓	
Chlorpyrifos	Organophosphorus	✓	
Dimethoate	Organophosphorus	✓	
Zeta Cypermethrin	Pyrethroid	✓	
Malathion	Organophosphorus	✓	✓
Permethrins	Pyrethroid	✓	✓
Pyrethrins	Pyrethroid	✓	✓
Cyfluthrin	Pyrethroid		✓
Fly Spray	Other		✓
Disulfuton*	Organophosphorus		✓
Rodenticide			
Warfarin	--	✓	

*Inverse correlation
** Classified based on http://www.alanwood.net/pesticides.

Studies on insecticides were focused mainly on associations with the risk of occupational asthmatic symptoms. A recent systematic review by Chittrakul *et al*. found that farmers exposed to organophosphates, carbamates, or organochlorines had an increased risk of asthma and asthmatic symptoms with ORs of 1.31 (95%CI: 1.17–1.48, $P = 0.172$), 1.44 (95%CI: 1.08–1.92, $P = 0.031$) and 1.31 (95%CI: 1.19–1.64, $P = 0.131$), respectively [230]. In addition, the report from the 2005-2006 US NHANES examined the association between organophosphate

pesticide exposure (detection of organophosphate pesticide metabolites) and atopic diseases and found reduced odds of food sensitisation and AR, and increased odds of AD in the US population. In this survey, OP metabolites were measured as dialkyl phosphate metabolites (dimethylphosphate, dimethylthiophosphate, dimethyldithiophosphate, diethylphosphate, diethylthiophosphate, and diethyldithiophosphate) in 2,635 urine samples across the US population aged 6-85 years. These dialkyl phosphate metabolite levels indicate ingested OP or environmental exposure to metabolites [219]. However, this study could not find an association between OP metabolite levels and a current diagnosis of asthma.

Similarly to many other countries, pesticides play a significant role in Thailand's agricultural sector, one of the country's primary economic activities. Pesticides are used for agricultural purposes to protect crops from pests and increase agricultural productivity. The Thai government has implemented regulations and measures to control and monitor pesticide use in the country. The Department of Agriculture and the Ministry of Agriculture and Cooperatives have responsibility for the registration, sale, distribution, and use of pesticides. They provide guidelines, training programs, and support to promote the safe and responsible use of pesticides among farmers. However, pesticides are still one of Thailand's significant hazards of concern as the narrative review of the exposure evidences in Thailand in **Chapter 2,** page 71.

1.3 Associating exposure and allergic disease

1.3.1 Studies providing evidence of associations between environmental exposures and allergy

Birth cohort studies are the appropriate type of design to determine the causal relationship between environmental factors (*in utero* or early postnatal period) and the risk of developing

adverse health outcomes in early and later life, particularly chronic disease [231-233]. Many birth cohort studies have been conducted for environmental health research globally (summarised in **Table 1.3-1**). The harmonisation and sharing of data set by networking birth cohort studies between countries has been performed in several continents, such as the Human Early Life Exposure (HELIX) [234-236], the Environmental Health Risks in the European Longitudinal Study of Pregnancy and Childhood (ELSPAC) [237], the Environmental Health Risks in European Birth Cohorts (ENRIECO) project [238, 239], the Life Cycle Project (EU Child Cohort Network) [240], the Birth Cohort Consortium of Asia (BiCCA) [241] to enhance the data comparability, improve statistical power, and address research gaps of rare exposure and outcomes.

Southeast Asia still has a scarcity of birth cohort studies. These are some examples of birth cohort studies conducted e.g. Growing Up in Singapore Towards Healthy Outcomes (GUSTO) [242], The Philippines' Cebu Longitudinal Health and Nutrition Survey (CLHNS) [243], Seremban Cohort Study (SECOST): a prospective study of determinants and pregnancy outcomes of maternal glycaemia in Malaysia [244], MYBIOTA: A birth cohort on maternal and infant microbiota and its impact on infant health in Malaysia [245], a birth cohort study of viral infections in Vietnamese infants and children [246], the Study of Asian Women and their Offspring's Development and Environmental Exposures (SAWASDEE), Thailand [247], and the molecular signature in pregnancy (MSP) cohort of Karen and Myanmar migrant mothers and infants, Thailand [248]. Each study has its unique research objectives, study population, focus areas, limited environmental exposure assessment, and relatively short follow-up time. However, they all contribute valuable insights into maternal and child health, nutritional aspects, some chemical exposure assessment and disease outcomes specific to the region.

However, environmental issues vary by country and may influence various outcomes. The use of information from developed countries that tend to have less exposure to environmental problems may be limited. Thus, environmental research is valuable, particularly prospective and/or birth cohort studies from low- and middle-income countries.

Table 1.3-1: Summary of the prospective birth cohort studies that aim to investigate the association of early life environmental factors and adverse health effects in later life

Birth cohort	Country	Conducted/funded organisation	Number of participants	Study year	Recruitment point	Follow up duration	Aims	References
The ENVIRonmental influence ON early AGEing (ENVIRONAGE)	Belgium	the European Research Council and the Flemish Scientific Research Council	1080 (data in 2015)	2010 – 2015 (recruitment)	At birth	4-6 years, at preadolescent age	To understand human ageing and its interaction with the environment	[249]
Nulliparous Pregnancy Outcomes Study (NuMom2B)	US	NIH	10,037	2010-2013 (recruitment)	The first trimester of pregnancy	Newborn period (at birth)	To determine maternal characteristics, including genetic, physiological response to pregnancy, and environmental factors that predict adverse pregnancy outcomes (APOs)	[250]
CHEAR (Children's Health Exposure Analysis Resource)	US	NIH	NA	2015-2019 (recruitment)	NA	13 years	To create a public database of children's exposures and their health consequences	[251, 252]
ECHO (Environmental Influences on Child Health Outcomes).	US	NIH	NA	2016	NA	NA	To examine the role of environmental factors of prenatal exposures combined with effects of genetic factors and social determinants of health in child health and development outcomes	[251]

Birth cohort	Country	Conducted/funded organisation	Number of participants	Study year	Recruitment point	Follow up duration	Aims	References
Taiwan Birth Panel Study (TBPS)	Taiwan	The Bureau of Health Promotion, Department of Health, Taiwan	486 mother-infant paired	April 2004-January 2005 (enrolment)	First trimester	5 years	To investigate the development of child health and disease through *in utero* and/or early childhood environmental exposures	[253]
Mothers and Children's Environmental Health Study (MOCEH)	South Korea	The National Environmental Health Action Plan (NEHAP)	1,500 pregnant women	Recruitment 2006 – 2010	First trimester	5 years	To examine how exposure to environmental pollutants affects growth, development, and disease.	[254]
The Korean CHildren's ENvironmental Health Study (Ko-CHENS)	South Korea	The Korea Ministry of Environment (MOE), The Republic of Korea and the National Institute of Environmental Research (NIER), Republic of Korea	65,000 pregnant women	Recruitment 2015 - 2019	Before 20 weeks of gestational age	20 years	To investigate the association between environmental exposure and five domains of health outcomes: pregnancy and childbirth, growth and endocrine system, neurodevelopment, social and emotional development, and allergic diseases	[255]
Hokkaido Study on Environment and Children's Health (Hokkaido Study): **1. Sapporo cohort** **2. Hokkaido cohort**	Japan	The Ministry of Health, Labor and Welfare, The Ministry of Education, Culture, Sports, Science and Technology, the Japan Society for the	Sapporo: 514 Pregnant women Hokkaido: 20,926 Pregnant women	Recruitment - Sapporo: July 2002-October 2005 - Hokkaido: February 2003 –	Sapporo: 23-35 weeks of GA Hokkaido: < 13 weeks	17 years	To (1) examine the effects that low-level environmental chemical exposures have on birth outcomes, including birth defects and growth retardation;	[256-258]

Birth cohort	Country	Conducted/funded organisation	Number of participants	Study year	Recruitment point	Follow up duration	Aims	References
		Promotion of Science		March 2012			(2) follow the development of allergies, infectious diseases, and neurobehavioral developmental disorders, as well as perform a longitudinal observation of child development; (3) identify high-risk groups based on genetic susceptibility to environmental chemicals; and (4) identify the additive effects of various chemicals, including tobacco.	
The Japan Environment and Children's Study	Japan	The National Institute for Environmental Studies (NIES), the Ministry of Environment (MOE), and the National Center for Child Health and Development (NCCHD), Japan	100,000 pregnant women	2011-2014	First prenatal examination at cooperating health care provider	≥ 13 years	To evaluate the effects of environmental chemicals on children's health and development.	[259]
The Study of Asian Women and Offspring's Development and Environmental Exposures	Thailand	Chiang Mai University's Research Institute for Health Sciences, Thailand, the Ministry of Public	95 pregnant women (pilot) 394 pregnant women, à 332 maternal-child pairs	March 2011 – February 2012 (pilot) July 2017 – June 2019	Early second trimester (≤16 weeks of pregnancy)	At birth (pilot), 3 years	To investigate the impact of prenatal exposure to pesticides on early indicators of cognitive and motor	[247, 260, 261]

Birth cohort	Country	Conducted/funded organisation	Number of participants	Study year	Recruitment point	Follow up duration	Aims	References
(SAWASDEE): a pilot study		Health of Thailand and Emory University's Rollins School of Public Health					skills, inhibitory control, emotion regulation, and memory	
The Avon Longitudinal Study of Parents and Children (ALSPAC)*	Bristol, UK	the Medical Research Council, the Wellcome Trust, the UK Department of Health, the Department of the Environment, DfEE, the National Institutes of Health, and various medical research charities and commercial companies. The ALSPAC study is part of the WHO initiated European Longitudinal Study of Pregnancy & Childhood	14,062 children at birth	1991 - 1992	Early pregnancy (< 24 weeks)	8-9 years	To investigate the individual's genotype combined with environmental pressures to influence health and development (gene-environment relationships)	[262]
The Danish National Birth Cohort	Denmark	Danish Epidemiology Science Centre	600,000 pregnant women	1999-2000	First trimester	20 years	To study pregnancy complications and diseases in offspring as a function of factors operating in early life, foetal growth, and its determinants.	[263]
The prevention and incidence of asthma and mite allergy (PIAMA) birth cohort	Netherlands	The Netherlands Organisation for Health Research and Development, The	3,963 children	1996 - 1997	At birth	14 years	To investigate the effect of mite-allergen avoidance on the incidence of	[264]

Birth cohort	Country	Conducted/funded organisation	Number of participants	Study year	Recruitment point	Follow up duration	Aims	References
		Netherlands Organisation for Scientific Research, The Netherlands Asthma Fund, the European Union, the National Institute for Public Health and the Environment, The Netherlands Ministry of Spatial Planning Housing and the Environment, The Netherlands Ministry of Health, Welfare and Sport and SAB (Stichting Asthma Bestrijding).					childhood asthma (Intervention study) and to assess lifestyle and environmental risk factors for childhood asthma (Natural History study)	
The Nurse's Health Study	US	NIH	116,911 female registered nurses	1976-2014	Before pregnancy	38 years	To evaluate risks to women's health, including the relationships between oral contraceptives and breast cancer risk; exogenous hormone therapy and breast cancer risk; and smoking, hair dyes and cancer risk and gradually expanded to cardiovascular disease (CVD), type 2 diabetes, fractures, rheumatologic conditions and other	[265-269]

Birth cohort	Country	Conducted/funded organisation	Number of participants	Study year	Recruitment point	Follow up duration	Aims	References
							outcomes of interest in women's health.	
The Maternal-Infant Research on Environmental Chemicals (MIREC) **Follow-up studies** - **MIREC-ID** - **MIREC-CD Plus** - **MIREC-CD3**	Canada	Health Canada, the Ontario Ministry of the Environment, and the Canadian Institutes of Health Research	2,001 pregnant women 400 infants (birth – 6 months)	2008-2011	First trimester	- 6 months (infant development to 6 months) - 3 years (child development at age 3) - 5 years (child development Plus)	To examine potential adverse health effects of prenatal exposure to priority environmental chemicals on pregnancy and infant health	[270]
The Norwegian Mother and Child Cohort Study (MoBa)	Norway	The Medical Birth Registry of Norway (MBRN), the National Institute of Public Health, which was renamed the Norwegian Institute of Public Health (NIPH)	64,136 pregnant women (mother) and 46,438 partners (father)	1999 - 2005	≤ second trimester (18 weeks)	At least 7 years	To examine the association between exposures (including genetic factors) and diseases, aiming at prevention	[271]
EDEN**	France	Two university maternity clinics in Nancy and Poitiers, France and the National Institute of Health and Medical Research (Inserm)	2,002 pregnant women	2003–2006	< 24 weeks gestational age	8 years	To study the pre-and early post-natal determinants of child development and health.	[272-274]
The Health Outcomes and Measures of the Environment (HOME) Study	Cincinnati, Ohio, US	The National Institute of Environmental Health Sciences and the United States Environmental Protection Agency	468 pregnant women (401 delivered, 9 sets of twins)	March 2003 and January 2006	< 19 weeks gestational age	12 years	To determine whether early-life environmental chemical exposures influence children's health.	[275]

Birth cohort	Country	Conducted/funded organisation	Number of participants	Study year	Recruitment point	Follow up duration	Aims	References
The Columbia Center for Children's Environmental Health (CCCEH)	Manhattan, US	Multiple fundings: National Institutes of Health (NIH), Environmental Protection Agency (EPA), Centers for Disease Control and Prevention (CDC), etc.	588 pregnant women, à 700 northern Manhattan pregnant women and newborns	April 1998 - October 2002	At birth	2 years	Examine the effects of prenatal exposure to common toxicants, ETS, Polyaromatic hydrocarbons (PAH), and pesticides on foetal growth, early neurodevelopment, and respiratory health.	[276]

***Collaborated with ELSPAC project, ENRIECO project**
****HELIX project (total n = 32,000) consisted of Born in Bradford (BiB), UK, EDEN, France, INMA, Spain, Kaunas Cohort (KANC), Lithuania, the Norwegian Mother and Child Cohort Study (MoBa), Norway, and Rhea-mother -child Cohort in Crete, Greece**

1.3.2 Adverse Outcome Pathways (AOP)

Exposure to chemicals, whether environmental from the environment or via direct exposure such as drugs, can lead to diseases such as allergic disease through an adverse outcome pathway (AOP). The AOP is a logical sequence of events beginning with chemical interaction with a biological target (molecular initiating event; MIE) leading to a series of events (Key Biological Events; KBE) resulting in an adverse outcome that manifests first at the cell level, then organ, organisms and finally population [277] (**Figure 1.3-1**).

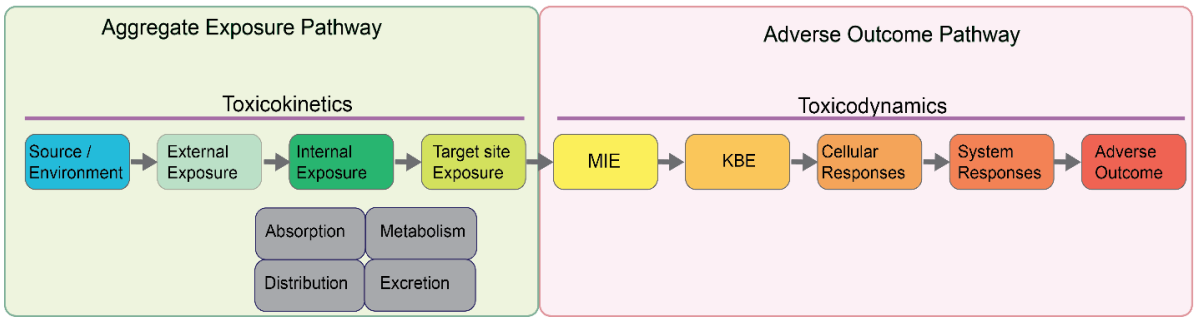


Figure 1.3-1: The genetic concept of an AOP showing some examples of Molecular Initiating Events (MIE) and Key Biological Events (KBE) that connect the exposure pathway to the adverse outcome.

The Mode of Action (MOA) pathway is a similar concept but is chemical-specific. Thus, one MOA per chemical captures the essential events between exposure and adverse outcomes for that chemical. There might be one overall AOP for air pollution exposures, but several MOAs deal with individual chemical contaminants such as particles and gases. MOAs, because they are chemical specific, allow for modifying factors such as genetic susceptibility or developmental stage. Therefore, both AOPs and MOAs act as a framework to organise the events from exposure to the outcome and assess their relevance, leading to greater certainty in the association between exposure and outcome. Some of the Key Biological Events that can link environmental exposures, and atopic adverse outcomes are described in the following sections.

1.3.3 Epigenetics alteration: a potential key biological event (KBE) in environmentally induced allergic diseases

1.3.3.1 What is epigenetics?

Epigenetic mechanisms are biochemical events that control gene expression without DNA sequencing changes [278]. They are fundamental in control, for example, cell differentiation, where each cell has the same genome, but the expression of each gene is different according to the cell requirements [279]. The main epigenetic mechanisms are DNA methylation, histone modifications, and microRNA (miRNA) expression, each described below.

1.3.3.1.1 DNA methylation

DNA methylation adds a methyl group to a DNA base, often at cytosine residues inside CpG dinucleotides [280]. This process is aided by enzymes known as DNA methyltransferases. DNA methylation frequently leads to gene silencing by blocking transcription factors from attaching to gene promoters, limiting gene expression. However, the relationship between DNA methylation and gene expression is complex and can vary based on the location of methylation within the gene. DNA methylation in gene promoter regions, including exons and introns, is generally related to gene suppression, whereas methylation in gene body regions is frequently associated with active gene expression [281]. Gene promoter methylation is reversed by converting the methylation mark to a hydroxy methylation mark [282].

1.3.3.1.2 Histone modification

Histones are the globular proteins that wrap around DNA to form the chromatin complex. They are responsible for posttranslational DNA expression changes via chromatin structure. They are also considered more adaptable forms of gene regulation because they can rapidly respond to changing environmental cues and cellular conditions [283]. This adaptability allows cells to

fine-tune their gene expression profiles to maintain equilibrium and respond to various stimuli. Histone protein modifications such as acetylation, methylation, phosphorylation, and ubiquitination can alter the structure and accessibility of chromatin, impacting gene expression. Histone acetylation, for example, is commonly related to gene activation because it loosens chromatin structure and allows transcription factors to bind to DNA more efficiently. Histone methylation, conversely, can either promote or suppress gene expression depending on the exact location and context of the change [284].

1.3.3.1.3 Non-coding RNA expression

Non-coding RNAs have important functions in controlling gene expression at many levels. The non-coding RNA family comprises miRNAs and long non-coding RNAs (lncRNAs). The microRNAs are tiny RNA molecules that contain 21-23 nucleotides in length and can bind to messenger RNAs (mRNAs). As a result, miRNAs impede their translation into proteins or cause their destruction. This process leads to the downregulation of specific target genes. Long non-coding RNAs (lncRNAs) are longer RNA molecules that are longer than 200 nucleotides in length. Unlike miRNA, they can influence gene expression at multiple levels by interacting with chromatin, transcription factors, and other proteins, regulating gene expression and chromatin structure. Long non-coding RNAs can function as scaffolds, guides, or decoys to influence gene expression in various ways [285].

1.3.3.2 Epigenetics and environment

Epigenetic mechanisms are a fundamental feature of gene expression in mammals, including humans. Epigenetically mediated gene expression occurs throughout the life course and can be perturbed by environmental factors such as chemical exposures, diet, stress, lifestyle, and social determinants of health [286-292]. Environmental factors affecting epigenetic regulation are,

for example, thought to explain the divergences of phenotype often seen in monozygotic twins throughout their lives [293]. Recent evidence from *in vitro*, animal, and human epidemiological studies has suggested that environmental chemicals can alter several epigenetic processes [292]. Some examples of these chemicals are metals (Cd, As, Ni, Cr, Methyl Hg), peroxisome proliferators (trichloroethylene, dichloroacetic acid, trichloroacetic acid), air pollutants (particulate matter, black carbon, benzene), and endocrine disrupting/reproductive toxicants (diethylstilbestrol, bisphenol A, persistent organic pollutants, dioxin). Transmission of these epigenetic alterations and their associated phenotypes across multiple generations (even in the absence of the original exposure) is possible and of increasing concern because this would allow the inheritance of outcomes from exposures in one generation to subsequent generations [286].

1.3.3.3 Epigenetics and chemical exposures

Several chemical exposures, particularly *in utero*, possibly cause the direct effect of epigenetic change or indirectly by creating oxidative stress, altering commensal microbiota, or interfering with the stress hormone [45, 53-56]. In 2009 and 2016, Baccarelli and Bollati [292] and Marczylo *et al*. [286] reviewed evidence to support the effect of environmental chemical exposure on epigenetic alterations, as shown in **Table 1.3-2**. This highlighted that since epigenetic changes do not always lead to phenotypic changes, and can accumulative over time, it is difficult to establish the cause-effect relationships between the environmental factors, epigenetics alteration, and adverse health outcomes. The recent comprehensive review of 2016 by Marczylo *et al*. on epigenetic toxicity induced by environmental factors draws evidence from human cohort studies and rodent models, focusing on its implications for public health and offering substantial evidence on this issue (**Table 1.3-2**).

Table 1.3-2: Summary of evidence supporting environmental chemical exposure and epigenetics alteration in experimental, animal, and human studies from Baccarelli and Bollati, Marczylo *et al*., and Wang *et al*. (focused on lead exposure and epigenetics alteration). [286, 292, 294]

Chemicals	Sources of chemical	Established adverse human health effects	DNA methylation	Histone modification	miRNA expression
Metals					
Arsenic	Drinking water contamination	Non-genotoxic carcinogen[4], skin diseases	✓	✓	✓
Cadmium	Smoking	Non-genotoxic carcinogen, mutagen	✓		
Chromium (VI)	Occupation	Genotoxic carcinogen (lung)	✓	✓	
Lead	Battery, water pipe, lead-based paint, etc	Impair neurological development, behavioural problems, hypertension, cardiovascular diseases, chronic kidney disease, decreased fertility.	✓	✓	✓
Methyl mercury	Seafood	Neurotoxicity	✓		
Nickel	Occupation	Genotoxic carcinogen, cardiovascular disease, respiratory disease	✓	✓	✓
Air pollution					
Particulate matter (PM_{10})	Traffic, construction, industrial activities	Cardiovascular disease, respiratory disease	✓		
Black carbon	Traffic	Cardiovascular disease	✓		
Benzene	Traffic	Genotoxic carcinogen: risk of acute myelogenous leukaemia (AML)	✓		
Formaldehyde	Traffic, construction, industrial activities, household products, e.g., cleaning agents, disinfectants, etc., tobacco smoke, fossil fuels burning, building material	Alzheimer's disease	✓		
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	Military and civil activities	Genotoxic carcinogen, neurotoxicity, immunotoxicity			✓

[4] Non-genotoxic carcinogens are substances that induce cancer through indirect stimulation of hyperplastic responses, without altering DNA, chromosome number or structure.

Chemicals	Sources of chemical	Established adverse human health effects	DNA methylation	Histone modification	miRNA expression
Trichloroethylene (TCE), dichloroacetic acid (DCA), and trichloroacetic acid (TCA)	Groundwater contamination	Teratogen (a cardiac birth defect in children)	✓		
Polycyclic aromatic hydrocarbons (PAHs)	Traffic, fossil fuels, tobacco smoke, industrial activities, volcanic eruption	Peripheral blood lymphocyte chromosomal aberrations	✓		
Persistent Organic Pollutants (POPs)					
Diethylstilbestrol (DES)	Used for miscarriage prevention between 1940 and 1971	Non-genotoxic carcinogen	✓		
Bisphenol A (BPA)	Food and beverage containers, baby bottles, and dental composites	Neurotoxicity, developmental, and reproductive toxicity	✓		
Vinclozolin	Fungicide, residue in fruit and vegetable	--	✓		
Organochlorine pesticides	Pesticides	Neurotoxicity	✓		
Dioxin (Includes polychlorinated dibenzo-*p*-dioxins; PCDDs, polychlorinated dibenzofurans; PCDFs or furans, polychlorinated bisphenols; PCB)	By product of burning and industrial process	Non-genotoxic carcinogen, toxicity to the reproductive, developmental, and immune system			✓
Lifestyle factors					
Cigarette smoke	Smoking	Genotoxic carcinogen (breast and lung cancer), COPD	✓		✓

1.3.3.4 Environment, epigenetics, and allergy

Epigenetic modifications can impact the development and progression of allergic diseases by regulating the expression of genes involved in immune responses, inflammation, and allergic sensitisation [295]. Epigenetic remodelling offers a potential mechanism by which environmental factors such as microbial and dietary exposure, pollution, and smoking mediate their influence on allergy development and/or exacerbation [295]. Microbiota found in farming/rural contexts cause fewer allergy reactions (than their urban counterparts) [296]. Smoking is another example of an air pollutant known to enhance the incidence of allergy illnesses, particularly asthma, and its effects on the epigenetic state have been documented [297]. Allergens and pollutants can influence epigenetic changes via altering DNA methylation and histone modification that contribute to the development of allergies [298].

Environmental exposures during pregnancy can affect the epigenetic programming of the developing foetus, potentially influencing the risk of allergies [299]. Maternal factors such as diet, smoking, stress, and exposure to pollutants can lead to epigenetic modifications in the offspring, which may impact immune development and increase the susceptibility to allergies later in life [300, 301]. Experimental studies indicate that some environmental exposures such as smoking (either active or passive), ozone, allergen, endotoxin, diisocyanate, farm environment, and *Helicobacter pylori* infection can alter the methylation of genes critical to T-helper cell differentiation [CD14, IL-1R, Tumour necrosis factor (TNF), Toll-like receptor (TLR), IL-4, IL-13), which may induce polarisation toward or away from an allergic phenotype and be implicated in asthma [279].

Genetic variations can interact with environmental factors to influence epigenetic changes and the risk of allergies [293]. Certain genetic variants may predispose individuals to be more

susceptible to epigenetic modifications in response to specific environmental exposures, increasing their risk of developing allergies.

It is important to note that the specific epigenetic changes induced by environmental factors and their impact on allergy development are still areas of active research [302]. The interplay between genetics, epigenetics, and the environment in allergic diseases is complex and multifactorial. Further studies are needed to elucidate the precise mechanisms by which environmental factors induce epigenetic changes and how these changes contribute to the development of allergies. Such studies on epigenetics and allergy suggest that epigenetic processes may be helpful as diagnostic tools, assessment of the tolerance after immunotherapy, predicting the success of allergy treatment early, or a target of novel treatment in the future [303].

While some environmental exposures have been associated with the atopic disease through epigenetic mechanisms, many research gaps remain in understanding the effect of *in utero* exposures when substantive changes occur in the epigenome.

1.4 Microbiota/microbiome alteration: a potential link to environmentally induced allergic disease

The microbiome is defined as “a characteristic microbial community occupying a reasonably well-defined habitat which has distinct physio-chemical properties”, and microbiota is “an assembly of microorganisms belonging to different kingdoms [Prokaryotes (Bacteria, Archaea), Eukaryotes (e.g., Protozoa, Fungi, and Algae)]” [304]. The microbiome is exclusive to humans but is also found in animals and the environment as the environmental microbiome.

The connection between human, animal, and environmental health through microbial communities has been proposed in the "one health" concept [305, 306]. The fundamental principle of this concept is the cycling of microorganisms from the environment to plants, animals, and humans and back into the environment. The high biodiversity and interconnectedness between functional units link with a healthy ecosystem [306]. Environmental microbiota can indirectly influence human health by interacting with the human microbiota and shaping the overall microbial diversity and abundance in the environment in which humans live [307]. The alternate is that changes in the human microbiome can also impact the surrounding environment by altering microbial inputs through bodily waste, shedding skin, or oral and respiratory emissions. Disruption of the balance between environmental and human microbiota, such as through environmental pollution or antibiotic use, can have implications for both environmental and human health [308-310].

1.4.1 Microbiome and human diseases

Human microbiota at each body site, such as skin, respiratory tract, gastrointestinal tract, and reproductive system, differ and can influence host (human) health differently [311]. Healthy microbiota can promote the health of the host by producing active molecules, e.g. short-chain fatty acids (SCFA), metabolites of tyrosine or tryptophan to interact with a physiological pathway of the host to regulate organ development, including brain, metabolism, and immunoregulation. These microbial products have been shown to counteract NCDs by upregulation of Treg, downregulation of proinflammatory cytokines production, and induction of hormonal secretion, which acts on anti-inflammatory effects. On the other hand, the disturbing of microbiota (dysbiosis), particularly gut microbiota, can lead to many NCDs, including allergies, autoimmunity, gastrointestinal disorders, obesity, diabetes, metabolic and cardiovascular diseases, cancer, central nervous system dysfunctions such as learning and

memory impairment, and autism, psychological problems such as anxiety and depression, and neurodegenerative disorders like Alzheimer's disease [305]. Not only human microbiota but also environmental microbiota can influence human health. Evidence suggests that exposure to farm environments may have health benefits, particularly in preventing allergies [312].

1.4.2 Early life microbiome and allergy

Early life microbiome, especially the maternal microbiome during pregnancy, may influence foetal and neonatal immune development, leading to allergic diseases in later life, particularly in genetically susceptible populations [313]. The evidence from epidemiological and mechanistic studies suggest that the increase in allergic disease is possibly related to the changing of microbial exposure and diet pattern via altering of the composition of intestinal microbiome and their metabolic products such as short chain fatty acids (SCFAs), and lipopolysaccharide (LPS) or endotoxin [313]. This concept may explain the increasing prevalence of allergic disease worldwide and has been classically demonstrated in several studies, including the Protection Against Allergy: Study in Rural Environments (PASTURE), the Prevention of Allergy Risk Factors for Sensitisation in Children related to Farming and Anthroposophic Lifestyle (PARSIFAL), and the Multidisciplinary Study to Identify the Genetic and Environmental Causes of Asthma in European Community (GABRIEL) Advanced study (GABRIELA) studies [57, 313]. The result from the PASTURE study showed that living in a farm environment was associated with both increased environmental endotoxin levels and decreased incidence of asthma with a change in gene expression of multiple Toll-like receptors (TLR), innate immunity receptors, in cord blood [314]. The findings from two large cross-sectional studies in Europe, PARSIFAL and GABRIELA, also confirmed that living on a farm in early life had a protective effect on allergic outcomes such as rhinoconjunctivitis, wheezing, AD, AR, asthma and allergic sensitisation [296, 315, 316]. Certain environmental chemicals,

including bisphenols, phthalates, POPs, heavy metals, and pesticides, have been found to impact the microbiome's composition and may potentially contribute to allergic outcomes [317].

Genetics, epigenetics, and the microbiome are all linked with the exposome, the sum of all our exposures influencing our phenotype. They all provide a mechanistic linkage between exposure and disease, contributing to key biological events along the AOP. This relationship is depicted in **Figure 1.4-1**.

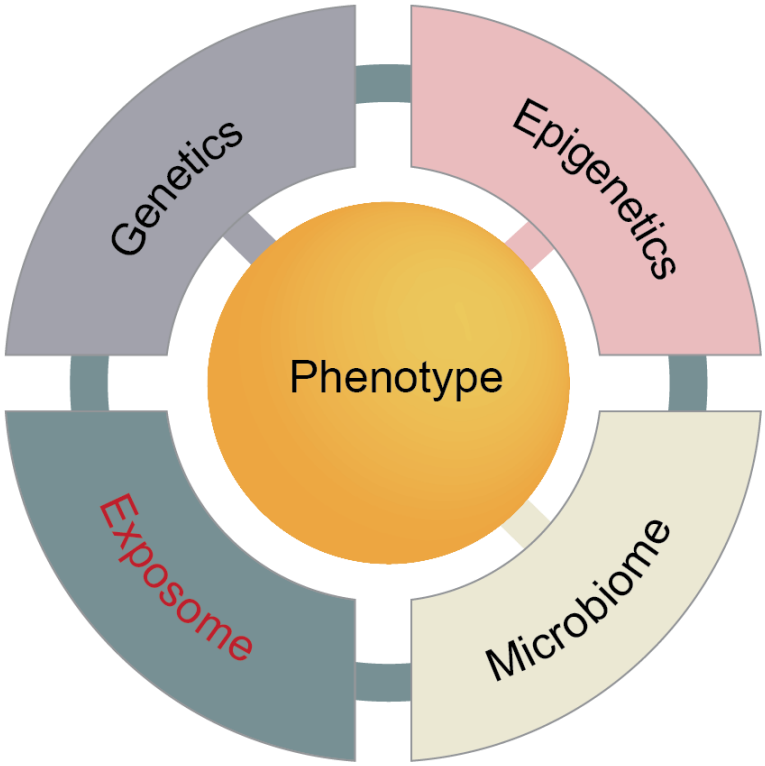


Figure 1.4-1: A schematic depiction of the interaction of genetics, epigenetics, the microbiome and the interaction of the exposome through these to influence phenotype.

1.5 Hypothesis, aims, and objectives

1.5.1 Hypothesis

Early life environmental chemical exposures influence the development of the infant's immune system and impact the child's allergenic health.

1.5.2 Aims

To assess the link between early-life environmental chemical exposures (including metals and air pollution) and allergy in early childhood (allergic skewing, sensitisation, AD, and food allergy).

1.5.3 Scientific objectives

1) To identify early life environmental chemical exposures of concern in Thailand associated with the immune skewing of childhood atopic diseases.

2) To assess the feasibility of conducting a mother-child cohort study in Thailand

3) To assess the association between early-life environmental chemical exposures of metals and $PM_{2.5}$ and atopy in Thai infants (immune skewing, sensitisation, atopic dermatitis, food allergy, and wheezing) by using the integrated strategy of analysis (questionnaires, biomarkers, and spatial analysis).

1.5.4 Summary of the aims of this work

This work therefore seeks to understand if environmental chemical exposures within the urban region of Thailand occurring during pregnancy and early life can induce allergic disease in children. In order to support this, two studies were conducted, firstly a systematic literature

analysis (**Chapter 2**) and secondly a clinical study (**Chapters 3-7** with the study design presented in **Chapter 3**). For the clinical study pairs (dyads) of mothers/children were recruited during the first trimester. Mothers were assessed for health and some chemical exposure during pregnancy and then children followed and assessed similarly for chemical exposure and allergic disease markers to 12 months. These health data were correlated with other exposure data derived directly and from third party sources. Resources limited the size of the study and therefore the power. The outcomes though indicated a potential link between early-life exposure to urban environments and an increased risk of allergies (AD and food allergy).

1.6 Conclusion

This chapter provided the background for the DPhil study, covering key topics such as allergy prevalence, environmental health issues in Thailand, the potential link between environmental exposures and allergies, and exposure assessment. It also established the fundamental aims and objectives of the research. The next chapter will expand on this by reviewing environmental health issues specific to Thai children, helping to identify and prioritise environmental hazards for further investigation in this study.

Chapter 2: Literature review

In this chapter, the thesis extensively explores the environmental health challenges affecting children's well-being in Thailand. Therefore, a narrative review based on systematic literature analysis was conducted, and the findings were published in the paper titled "Children's Environmental Health in Thailand: Past, Present, and Future" in 2018 [73].

By covering this significant research area, the chapter aims to contribute valuable insights into the impact of environmental factors on children's health and well-being, particularly in Thailand. The combination of a narrative and systematic review allows for a comprehensive examination of the available literature and helps draw informed conclusions and implications for future research and policy considerations.

2.1 Introduction

Thailand has experienced various environmental hazards with significant implications for public health [74]. From natural disasters like the 2004 tsunami to increasing occurrences of flooding and drought, the population faces challenges that can have long-lasting impacts on communities and ecosystems. The country also faces diverse chemical pollutants from urbanisation, globalisation, and industrialisation, such as traffic-related emissions, untreated waste, heavy metals, pesticides, and persistent organic pollutants [74].

This literature review addresses children's environmental health in Thailand, focusing on environmental chemicals and air pollution, which are most relevant to children's well-being.

By understanding and mitigating these hazards, policymakers and researchers can work towards creating a healthier and safer environment for the country's youngest and most vulnerable population.

2.2 Objectives

This literature review aims to achieve two objectives:

2.2.1 The conceptualisation of Children's Environmental Health in Thailand

The review seeks to comprehensively understand children's environmental health in Thailand by thoroughly examining epidemiological literature on environmental chemical exposures in Thai children. It involves identifying the types of environmental chemicals and air pollutants that children are exposed to, understanding the prevalence and patterns of exposure, and assessing potential health impacts on children's well-being.

2.2.2 Proposing future directions for research, policy, and practice

The second objective is to propose future directions for addressing children's environmental health in Thailand and similar upper-middle-income countries. It includes outlining potential research priorities, suggesting policy development and implementation strategies, and recommending best practices to improve the health and well-being of children in the context of environmental exposures.

2.3 Methods

The review systematically searched health research databases, including Medline/PubMed, Web of Science, EMBASE, and Scopus. The search strategy used the PICO

(Problem/Patient/Population, Intervention/Indicator, Comparison, Outcome, and (optional) Time element or Type of Study) approach to identify relevant studies, which were "Population" (P) = Thai children, "Indicator" (I) = Exposure (environmental chemical, metal, allergen, or air pollution), "Comparison" (C) = no comparison, "Outcome" (O) = health outcomes. The alternative terms for searching to enhance inclusively and facilitate a comprehensive search in the research context are provided in **Table 2.3-1**. The inclusion criteria encompassed various study designs, and the timeframe was from 1980 to 2017. Both Thai and English language publications were included.

Table 2.3-1: Main concept and alternative terms of PICO search strategy for this review. The asterisks () and question marks (?) are used to widen the search results by including various word endings and spellings. The asterisk () represents an unlimited character number, allowing for variations in word endings or spellings. The question mark (?) represents a limited character number, including different spellings up to the number of question marks used. Adapted from Sinitkul *et al*.[73].

PICO	Main concept	Alternative terms
Population	**Thai**	Thailand
	Child??	Kid?, Pregnan*, "*in utero*", newborn, neonat*, infan*, toddler, preschool, young, teenage*, pupils, student?
Exposure	**Environment**	Chemical, Pollution?, "Air quality", PM, "particulate matter", particle, VOCs, "volatile organic compounds", benzene, "polyaromatic hydrocarbon", PAH, allergen, mold, mould, fungi, cockroach, smok???, metal?, arsenic, lead, cadmium, mercury, plasticizer, POPs, "persistent organic pollutants", "Polychlorinated biphenyl", Dioxins, Furans, "Polybrominated compounds", "Polyfluorinated compounds", "Halogenated hydrocarbons", pesticide?, herbicide?, fungicide?, organophosphate, organochlorine, carbamate, glyphosate, chlorpyrifos, Dichlorodiphenyltrichloroethane, phthalate, BPA, "bisphenol A", Industr*, agricultur*, waste, Hazard???, Toxic
Health Outcomes	**Perinatal**	"Birth weight", prematurity, "birth defects"
	Growth	Weight, height, BMI, "body mass index", obes*
	"Neurodevelopmental disorders"	Development*, autistic?, autism, attention, hyperactive, ADHD, learning, cognitive, intelligen*, IQ, aggressive, pervasi*, Behavior*, Behaviour*
	Allergy	atop??, inflammation, sensitisation, IgE, hypersensitivity, "food allergy", "atopic dermatitis", "allergic dermatitis", wheezing bronchitis, bronchiolitis, hyperresponsive????, hyper-responsive????, asthma, "allergic rhinitis", "perennial rhinitis", hayfever, "hay fever"
	Respiratory	"pulmonary function", "Lung function", "respiratory symptom?", "respiratory infection"
	Endocrinology	Thyroid, diabetes, DM, metabolic?

PICO	Main concept	Alternative terms
	"Cardiovascular diseases"	Coronary, hypertension, "blood pressure", Atheroscler*
	"Kidney diseases"	Renal, "kidney function", "renal function", creatinine
	"Liver diseases"	"Liver diseases", cirrhosis, jaundice
	Poisoning	"acute poisoning", "chronic poisoning"
	Cancer	Malignancy, Leukaemia, Leukemia, Lymphoma, Carcinoma
	Subclinical	"DNA damage", epigenetic, miRNA, Histone, Methylation, Adduct

2.4 Results

As depicted in **Figure 2.4-1**, 343 articles were initially identified from 4 databases. After removing duplicate articles, 273 unique articles were left for further evaluation. Subsequently, the titles and abstracts of these 273 articles were screened, resulting in the selection of 79 articles for critical review. Out of the 79 selected articles, 26 were excluded from the analysis due to restricted access to the entire paper. However, two additional articles, cited in the key article on arsenic exposure, were later included. Therefore, 55 full-text articles were critically reviewed and summarised in **Table 2.4-1**, **Table 2.4-2**, and **Table 2.4-3**. The review was categorised into pesticides, heavy metals, and air pollution.

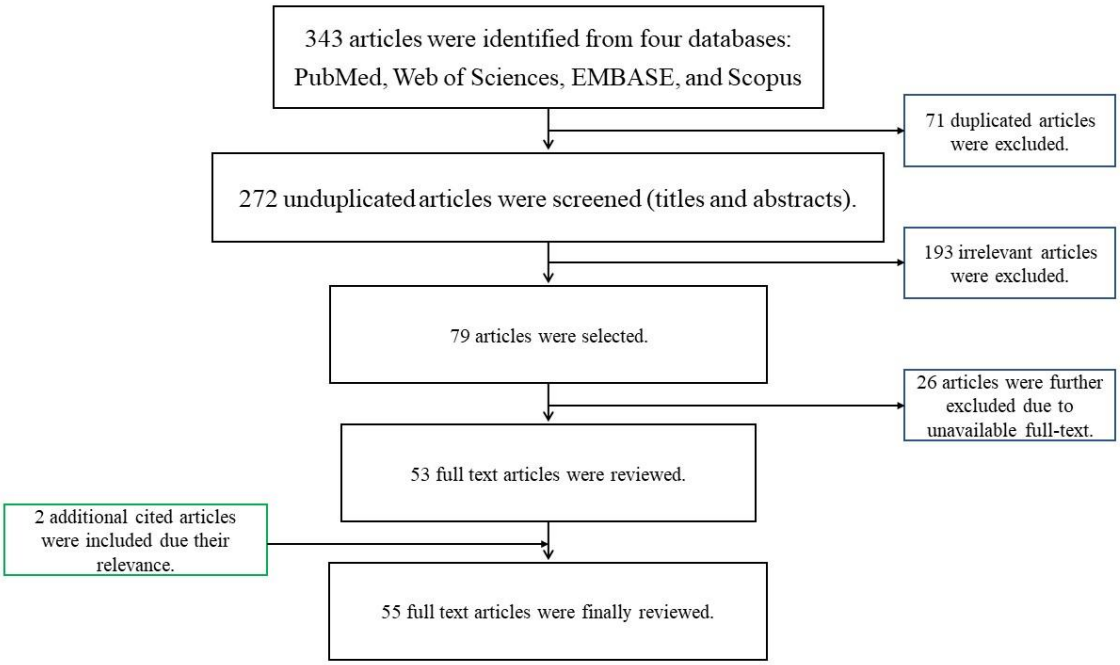


Figure 2.4-1: The protocol to select articles for the literature review of environment and children's health in Thailand.

In most of these fifty-five articles, the exposures investigated were related to pesticides, such as organochlorine, organophosphate, paraquat, glyphosate, or paraquat. Additionally, heavy metals such as lead, arsenic, cadmium, mercury, and air pollution in ambient air pollution or environmental tobacco smoke were also prominent exposures under study. Notably, the sources of these exposures exhibited variation across different geographical regions, highlighting the diverse environmental factors affecting the population under investigation.

Regarding health-related adverse effects, pesticide exposure was associated with neurotoxicity and cognitive impairments. Heavy metal exposure included lead, arsenic, and cadmium, leading to various health outcomes such as renal effects, endocrine disorders, and increased cancer risk. Air pollution sources vary geographically, adversely affecting respiratory health and potential genotoxic and carcinogenic outcomes.

2.4.1 Pesticides

In this review, twelve full-text articles were available that investigated pesticide exposure in Thai children (**Table 2.4-1**), and among them, three studies examined both exposure and subsequent health effects in the same cohorts. Various pesticide metabolites, including those of organochlorine [318, 319], organophosphate [320-325], parathion [326], pyrethroid [321, 325, 327], glyphosate [328], and paraquat [328], were detected in the urine/serum of Thai children, even for pesticides that were officially banned prior to the study periods [318, 319, 326].

Factors associated with pesticide exposure included living close to agricultural farmland, engaging in agricultural activities, insecticide use in the home, and various child behaviours related to pesticide exposure. One study demonstrated the indirect transfer of pesticides from farmers to children through contamination of the home environment [324].

Regarding health outcomes, organochlorine (DDT) or organophosphate (DEP and DAP) exposures were associated with altered thyroid function/hormone levels [318] or neurotoxicity [322], respectively. However, the third study investigating health outcomes found no association between organophosphate or pyrethroid exposure and school children's neurobehaviour [321].

Table 2.4-1: Epidemiological studies on pesticide exposure in Thai children

Pesticides	Authors	Published year	Type of epidemiological study	Age of population	Sample size	Exposure assessment	Sample media	Level measured	Comments
1. Exposure studies (no health outcome assessment)									
Organophosphate	Petchuay *et al.* [323]	2006	Case-controlled	Preschool (2-5 years)	54 (farm: 37, rubber plantation: 17)	DAP metabolites (DMP, DEP, DMTP, DETP, and DEDTP).	Urine	Metabolites	Farm children showed significantly higher DAP concentrations than children outside the farming area, especially during the dry vegetable planting season.
	Panuwat *et al*. [326]	2009	Cross-sectional	School child (10 – 15 years)	306 *A part of a more extensive study that included adult	Parathion metabolites (PNP, DMP, DMAP, DMTP)	Urine	Metabolites • The geometric mean concentrations of PNP, DMP, DMTP, and DMAP were 3.5 ng/ml, 1.9 ng/ml, 1.6 ng/ml, and 29.9 nmol/ml, respectively.	Between 25% and 60% of the PNP (metabolites of #parathion) were detected among farmers and children.
	Hanchenlaksh *et al.* [329]	2011	Cross-sectional	Preschool and school child (2-12 years)	16 *A part of a more extensive study that included adult	DAP metabolites (DEP, DETP, DEDTP, DMP and DMTP)	Urine	Metabolites The geometric mean of DAPs levels was 7.6 mg/g of creatinine.	Exposure for farmers' families appears to occur through transfer from the farmer to family members or contamination of the home environment.
	Rohitrattana *et al.* [330]	2014	Cohort	*in utero* (20-35 years of 28	86, 87, 51 (28 weeks pregnancy, delivery,	• DAP metabolites (DMP, DEP, DETP,	Urine	Metabolites: • The 50th percentile of	The levels of urinary OP metabolites at 28 weeks, delivery,

Pesticides	Authors	Published year	Type of epidemiological study	Age of population	Sample size	Exposure assessment	Sample media	Level measured	Comments
				weeks pregnancy until 2 months post-partum)	two months post-partum)	DEDTP, total DEP, and DAPs) • Interviewed questionnaires (agricultural activities)		DMP, DEP, DETP, DEDTP, total DEP, and DAPs levels (nmol/L: nmole/g creatinine) at different time points were as follows: • 28 weeks pregnancy: 37.7: 48.9, 14.6: 20.8, ND: ND, ND: ND, 45.2: 84.1, 90.1: 160.9 • Delivery: 36.2: 50.5, 14.4: 18.8, 2.4: 6.5, 2.3: 4.5, 64.9: 87.4, 108.9: 190.8 • 2 months postpartum: 38.0: 25.6, 17.3: 14.8, ND: ND, ND: ND, 46.7: 56.1, 92.1: 101.8	and two months postpartum fluctuated based on pesticide exposures at home.

Pesticides	Authors	Published year	Type of epidemiological study	Age of population	Sample size	Exposure assessment	Sample media	Level measured	Comments
	Liu *et al.* [324]	2015	Cross-sectional	Preschool and school child (2- 12 years)	72 * A part of a more extensive study that included adult	• DAP metabolites[%] • Self- administered questionnaires	Urine	Metabolites • The geometric mean of DAPs levels was 3.12 mcg/g of creatinine.	Children in Thai farming families may be exposed to pesticides indirectly through the transfer of pesticide residues from the farmer to the spouse and subsequently to the child or through contamination of the home environment.
Pyrethroid	Rohitrattana *et al.* [327]	2014	Case-controlled	School child (6- 8 years)	53 (Case: controlled = 24: 29)	• PYR metabolites (i.e., 3-PBA DCCA) • Hand wipe samples • Structured interviewed	Urine	Metabolites • The median urinary PYR metabolite levels were not statistically significant between the two groups in the wet or dry seasons.	• A positive correlation (r=0.40–0.46) exists between PYR residues collected from the hands and urinary PYR metabolites. • PYR use in rice farms and households may be a significant source of PYR exposure among children living in the area.
Glyphosate	Kongtip *et al.* [328]	2017	Birth cohort	*in utero* (19-35 years of 28 weeks pregnancy)	82 pairs	• Glyphosate levels • Interviewed questionnaires	• Maternal and cord serum	• The median glyphosate levels in pregnant women's	Factors of glyphosate exposure include working in agriculture or

Pesticides	Authors	Published year	Type of epidemiological study	Age of population	Sample size	Exposure assessment	Sample media	Level measured	Comments
				and neonate				serum were 17.5 ng/ml (range: 0.2 – 189.1 ng/ml), which were significantly higher than those in umbilical cord serum (median 0.2 ng/ml, range: 0.2 – 94.9 ng/ml).	living in families that work in agriculture.
Paraquat	Kongtip *et al.* [328]	2017	Birth cohort	*in utero* (19-35 years of 28 weeks pregnancy) and neonate	82 pairs	• Paraquat levels • Interviewed questionnaires	• Maternal and cord serum	• The median paraquat levels in pregnant women's serum were < 0.4 ng/ml (range: 0.2 – 58.3 ng/ml) and similar to those in umbilical cord serum (median 0.2 ng/ml, range: 0.2 – 47.6 ng/ml).	Factors of paraquat exposure include working in agriculture or living in families that work in agriculture.
Chlorpyrifos	Panuwat *et al.* [325]	2009	Cross-sectional	Student (12 – 13 years)	207	18 specific pesticide metabolites	Urine	Metabolites • A total of 14 metabolites of chlorpyrifos, permethrin, and pyrethroids were found.	• The study classified parents into four occupational groups: farmers, merchants and traders, government and company

Pesticides	Authors	Published year	Type of epidemiological study	Age of population	Sample size	Exposure assessment	Sample media	Level measured	Comments
									employees, and labourers. • The farmers' group showed significantly higher urinary pyrethroid metabolite levels.
Permethrin **Pyrethroids**	Panuwat *et al.* [325]	2009	Cross-sectional	Student (12 – 13 years)	207	18 specific pesticide metabolites	Urine	Metabolites • A total of 14 metabolites of chlorpyrifos, permethrin, and pyrethroids were found.	• The study categorised parents into four occupational groups: farmers, merchants and traders, government and company employees, and labourers. • The farmers' group exhibited significantly higher urinary pyrethroid metabolite levels.
2. **Exposure and health outcome assessment studies**									
[$]Organochlorine	Asawasinsopon *et al.* [318]	2006	Cross-sectional	*in utero*	39 pairs (maternal-neonate)	DDT metabolites:	Maternal and cord serum	Metabolites • p'-DDE was the highest level in maternal and cord serum, with a geometric mean of 1,191	• A negative association was found between cord serum total T4 levels and DDT metabolites.

Pesticides	Authors	Published year	Type of epidemiological study	Age of population	Sample size	Exposure assessment	Sample media	Level measured	Comments
								ng/g lipids in maternal serum and 742 ng/g lipids in cord serum.	
Organophosphate	Kongtip *et al.* [322]	2017	Birth cohort	*in utero*	50 pairs (20-35 years of pregnant women and five months of their children ren)	DAP metabolite: DMP, DEP, DETP, DEDTP	Maternal urine	Metabolites • The median adjusted urinary DAP levels at 28 weeks gestational age of pregnant women were 36.83 nmol/L for DMP, 15.15 nmol/L for DEP, 0.07 nmol/L for DETP, and 0.15 nmol/L for DEDTP.	• Higher total DEP and total DAP metabolite levels during 28 weeks of gestational age in pregnant women were significantly associated with reduced cognitive and motor composite scores on the Bayley-III at five months old in their children.
	Fielder *et al.* [321]	2015	Case-controlled	School child (6-8 years)	53 (Case: controlled = 24:29)	Metabolites of OPs (6 DAP$^{\%}$, TCPy)	Urine	Metabolites • The Rice farm had significantly higher total DAP and TCPy.	• No significant adverse neurobehavioral effects were observed between participant groups during the high and low pesticide use seasons.
Pyrethroid	Fielder *et al.* [321]	2015	Case-controlled	School child (6- 8 years)	53 (Case: controlled = 24:29)	Metabolites of PYR (DCCA)	Urine	Metabolites • The rice farm had significantly	• No significant adverse neurobehavioral effects were observed

Pesticides	Authors	Published year	Type of epidemiological study	Age of population	Sample size	Exposure assessment	Sample media	Level measured	Comments
								higher DCCA levels.	between participant groups during the high and low pesticide use seasons.

[#]**Parathion** was banned in 2007, and data was collected in 2006.

$Organochlorine was banned in early 1983, and data was collected in 2003-2004

[%]**Common 6 DAP metabolites:** DMP, DEP, DMT, DMDTP, DETP, and DEDTP

Abbreviations: DAP; dialkyl phosphate, DCCA; cis/ trans-2,2-(dichloro)-2-dimethylvinylcyclopropane carboxylic acid, DDT; dichlrodiphenyltrichloroethane, DEDTP; diethyl dithiophosphate, DEP; diethyl phosphate, DETP; diethyl thiophosphate, DMAP; dimethylalkylphosphate, DMDTP; dimethyldithiophosphate, DMP; dimethylphosphate, DMTP; dimethylthiophosphate, OPs; organophosphates, PNP; paranitrophenol, p,p'-DDE; 1,1-dichloro-2,2-di(4-chlorophenyl)ethylene, PYR; pyrethroid, TCPy; 3,5,6-trichloropyridinol, 3-PBA; 3-phenoxybenzoic acid

2.4.2 Heavy metals

Twenty-one full-text articles investigated heavy metal exposure in Thai children (**Table 2.4-2**). Among them, nine studies measured both exposure and subsequent health effects in the same cohorts, focusing on lead (3 articles), arsenic (4 articles), cadmium (1 article), and mercury (1 article).

Lead exposure in Thai children was linked to para-occupational activities and household locations, particularly in boatyards, shipyards, and former mining sites [331-340]. Increased lead levels were found in households with members engaged in boat repair [341], and lead contamination persisted in sediment near mining areas [339]. Risk factors for elevated blood lead levels included anaemia, large families, male gender, young age, access to lead-acid batteries, and residing in houses with peeling paint. [331, 332, 340, 342, 343] The health outcomes of chronic lead exposure included cognitive deterioration and dental caries.

Studies on arsenic exposure were concentrated in the Ron Pibul district of Southern Thailand, where former tin mining and smelting activities contaminated drinking water, soil, and the food chain. [344-346] Arsenic exposure was exacerbated by children's risk behaviour, such as playing with soil without handwashing before eating [344]. Arsenic exposure during childhood was associated with increased cancer risk, with specific gene methylation patterns of inflammatory genes (COX2, EGR1 and SOC3) and DNA damage observed [345, 346].

Cadmium exposure in the Northern region of Thailand was primarily a result of past tin mining activities, leading to contamination of land, water, and staple food (rice grown in contaminated soil) [347]. However, the level of cadmium exposure appeared to be decreasing due to the

cessation of mining, leading to reduced environmental contamination [348]. Renal effects associated with cadmium exposure in local Thai children are expected to decrease over time and were confirmed by Swaddiwudhipong *et al.* [347, 349].

Lastly, children near a small-scale gold mine in Phichit demonstrated elevated urinary inorganic mercury levels [350]. However, the calculated hazard quotient suggested that these children were not at risk of nephrotoxicity from inorganic mercury exposure at the observed levels [351].

Table 2.4-2: Epidemiological studies on heavy metal exposure in Thai children

Heavy metals	Authors	Published year	Type of epidemiological study	Population (age)	Sample size	Exposure assessment	Sample media	Level measured	Comments
1. Exposure studies									
Lead	Ruangkanchanasetr *et al.* [342]	1999	Cross-sectional	Infants to school child	511 (infant 84, preschool child 60, school child 377)	• Lead • Questionnaires	Whole blood	• Mean BLL 5.57 ± 2.31 mcg/dL, 4.75 ± 3.25 mcg/dL, 6.72 ± 2.02 mcg/dL, and 9.03 ± 3.65 mcg/dL at birth, two years, kindergartens, and secondary school respectively. • EBLL were 1- 35%.	• Mean BLL and prevalence of EBLL (> 10 mcg/dL) increasing with age. • Risk factors of EBLL: older age group, larger family size, and male gender.
	Chomchai *et al.* [340]	2005	Case-controlled	Infant and preschool child (6 months – 4 years)	296 (exposure = 114, controlled 149)	• Lead • Questionnaires	Whole blood	• The average BLL was 5.65 ± 3.05 mcg/dL. • The overall prevalence of children with EBLL > 10 mcg/dL was 8.1%, while that of the Klong Toey community was 12.5%.	• Predictors factors of EBLL: peeling paint, eating paint chips, and geographical location • Saliva was not suitable for the biomarker of lead exposure
	Thaweboon *et al.* [334]	2005	Cross-sectional	Preschool child (3-6 years)	8 *A part of a more extensive study that included adult	Lead	Salivary and whole-blood	• All children subjects had BLL > 10 mcg/dL • The geometric mean for BLL was found to be 24.03 µg/dl (11.80-46.60 µg/dl), lead in saliva was 5.69 µg/dl (1.82-25.28 µg/dl).	Saliva was not correlated with BLL (γ =-0.025).
	Maharachpong *et al.* [341]	2006	Cross-sectional	Preschool child to adolescent (4-14 years)	319 (from 242 households)	Lead (spatial distribution)	House dust and soil	External biomonitoring • Mean house dust lead was 210 mg/kg (< ATSDR concerning and a dust-lead standard for London (500 mg/kg)).	• The lead content level in soil was exponentially declining with distance from boat-repair yards (point source contamination)

Heavy metals	Authors	Published year	Type of epidemiological study	Population (age)	Sample size	Exposure assessment	Sample media	Level measured	Comments
	Neesanan *et al.* [331]	2011	Retrospective study	Preschool child (3-7 years)	213	• Lead • Interviewed the primary care providers (identify risk factors of EBLL)	• Whole blood	• Mean BLL was 7.71 ± 4.62 mcg/dL (3-25 mcg/dL) 26% had BLL ≥ 10 mcg/dL.	Risk factors of EBLL were male and source of drinking water from either tap or canal.
	Untimanon *et al.* [352]	2011	Case-controlled	Child (< 15 years)	24 (case 22, controlled 2) *A part of a more extensive study that included adult	• Lead • Questionnaire-based interview	• Skin (wipe) • Household floor and dust	• Mean lead contamination in case vs. controlled - Household floor loading: 109.9 vs. 40.1 mcg/m^2 - Household dust content: 434.8 vs. 80.8 mcg/g - Handloading: 64.4 vs. 36.2 mcg/m^2 - Foot loading: 77.8 vs 43.8 mcg/m^2	• Hand lead loading in children was higher than in adults • Skin lead levels were elevated in family members living in a lead-exposed worker's house and related to lead contamination levels.
	Mitchell *et al*. [353]	2012	Cross-sectional	Child (6 months – 14 years)	642	• Lead • Questionnaires	Capillary blood	5.1% had EBLL (cBLL ≥ 10 mcg/dL), with the highest prevalence in children younger than two years.	The risk factors of EBLL were anaemia (Hb < 10 g/dL), exposure to car batteries, and taking traditional medicines.
	Swaddiwudhipong *et al.* [332]	2013	Cross-sectional	Child (1-14 years)	254	• Lead • Questionnaires	• Whole blood • Water and vegetables grown in the area	• The mean BLL was 9.8 ± 5.1 mcg/dL. • The overall prevalence of EBLL (≥10 mcg/dL) was 43.3%	• Lead contamination was found in house floor dust and drinking water in household containers. • 50.8% of EBLL children lived in vented

Heavy metals	Authors	Published year	Type of epidemiological study	Population (age)	Sample size	Exposure assessment	Sample media	Level measured	Comments
									lead-acid batteries, while 23.3% lived in the house without vented lead-acid batteries.
	Swaddiwudhipong *et al.* [354]	2014	Cross-sectional	Child (1-14 years)	695	• Lead • Questionnaires	• Whole blood • Floor dust, drinking water, metal pot	• The geometric mean BLL was 9.16 mcg/dL. • The overall prevalence of EBLL was 47.1%	• The metal pots were safe for cooking rice but might be unsafe for acidic food preparation.
Cadmium	Chaiwonga *et al.* [349]	2013	Cross-sectional	Schoolchild (9-12 years and 13-15 years)	748	• Cadmium • Questionnaires	• Urine	• All urine samples had cadmium of more than 1 mcg/g creatinine (Thai general population is 0.5 mcg/c Cr), with 2% having higher than 5 mcg/g Cr.	The likely exposure sources were dietary (approximately 20-50% of them consume homegrown rice)
2. Exposure and health outcome assessment studies									
Lead	Youravong *et al.* [336]	2006	Cross-sectional	Preschool to school child (6-10 years)	292	Lead	Whole blood	Children with BLL ≥ 10 mcg/dL were 21%.	Lead exposure was *associated* with carries in deciduous teeth but not permanent teeth (cariogenicity) in the dose-response relationship.
	Pusapukdepob *et al.* [339]	2007	Cross-sectional	Child (0-15 years)	126 (case 89, controlled 37) *A part of a more extensive study that included adult	• Lead	• Whole blood and teeth • Soil, vegetables, meat	• Mean BLL in children of case and controlled groups were 25.75 ± 13.89 mcg/dL and 7.68 ± 2 mcg/dL, respectively. • 72% (89 children and nine adults) of the case group had high BLL (> 10 mcg/dL), while there were no cases of	• IQ scores of the case group (vicinity < 30 km from mining) were *significantly lower* than those of the control group (82.7 ± 8.27 vs 96.14 ± 7.8)

Heavy metals	Authors	Published year	Type of epidemiological study	Population (age)	Sample size	Exposure assessment	Sample media	Level measured	Comments
								high BLL in the control group. • Lead concentration in soil vegetables and meat (fish and shellfish) exceeded the recommended standard.	
	Youravong *et al.* [338]	2013	Cross-sectional	Preschool to schoolchild (6-10 years) (same population as [336])	120	• Lead	• Saliva and whole blood	• The salivary lead level low correlated with blood lead level (R^2 = 0.18, p =0.05)	There was no association between salivary lead and dental caries.
Arsenic	Vitayaviraskul *et al.* [344]	2005	Case-controlled	Schoolchild (10 years)	130 (high exposure area 50, low exposure area 50, controlled 30)	• iAs and their metabolites (MMA, DMA) • Questionnaire-based interview	• Urine • Surface soil, ambient air, drinking water, fruit, vegetables, and meat	• The geometric mean of iAs + metabolites were 54.21, 36.61, and 17.50 mcg/g creatinine in high exposure, low exposure, and control groups, respectively. • The average arsenic in the agricultural product was within the standard acceptance of the US FDA at 2 mg/kg. except for the freshwater snail (*Sinotaia ingallsiana*)	• source of exposure was one of the food chains (freshwater snail), water (surface and ground) and soil • Individual risk behaviours of exposure were soil paying without hand washing before eating. • *Probable risk* of developing cancer (between 10^{-5} -10^{-6})
	Intarasunanont *et al.* [355]	2012	Case-controlled	*in utero* and *in vitro* (newborn)	71 (case 55, controlled 16)	• Arsenic • DNA methylation	• Cord blood, nail, and hair • Drinking water and non-drinking water • Cord blood lymphocyte	Arsenic levels in case vs. controlled were - Cord blood: 5.79 ± 0.5 vs 1.97 ± 0.64 mcg/g - Toenails: 1.52 ± 0.38 vs 0.12 ± 0.04 mcg/g - Fingernails: 1.91 ± 0.38 vs 0.08 ± 0.05 mcg/g	*in utero*, arsenic exposure affects DNA methylation, particularly at the p53 promoter region, which may be linked to the mechanism of arsenic carcinogenesis.

Heavy metals	Authors	Published year	Type of epidemiological study	Population (age)	Sample size	Exposure assessment	Sample media	Level measured	Comments
							(DNA methylation in cord blood and *in vitro* lymphocyte treated with arsenic)	- Hair: 0.05 ± 0.01 vs 0.01 ± 0.003 mcg/g.	
	Hinhumpatc *et al.* [345]	2013	Case-controlled (follow-up study)	Schoolchild (5-8 years)	60 (case 40, controlled 20)	• Arsenic • 8-OHdG (DNA damage) and hOGG1 (DNA repair capacity)	• Urine, saliva, toenails, and fingernails • Salivary and urine (8-OHdG (DNA damage) and hOGG1 (DNA repair capacity)	The DNA damage (salivary and urinary 8-OHdG) was increased, but DNA repair capacity (hOGG1) was decreased in the exposure group,	• A follow-up study of prenatally arsenic exposure children and continuing exposure until the age of 5-7 years with matched case-controlled non-exposure, • A potential risk for mutation and cancer from biomarkers.
	Phookphan *et al.* [346]	2017	Case-controlled (follow-up study)	Schoolchild (6-9 years)	81 (case 40, controlled 41)	• Arsenic	Toenails	Arsenic in toenails in exposed and unexposed children was 8.08 ± 1.47 μg/g and 0.76 ± 0.14 μg/g, respectively.	A follow-up study from arsenic exposure *in utero* and continued exposure throughout their early life [355]
Cadmium	Swaddiwudhipong *et al.* [347]	2015	Case-controlled	Schoolchild (7-12 years)	594 (case 301, controlled 293)	• Cadmium • Questionnaire	Urine (Cd, β2-MG and calcium)	19% of children in this study had urinary cadmium ≥ 1 mcg/g creatinine higher in girls and those consuming rice grown in cadmium-contaminated areas.	Significantly higher geometric mean levels of urinary excretion of β2-MG and calcium (early renal effects) were found among children in contaminated areas compared to those in comparison areas.

Heavy metals	Authors	Published year	Type of epidemiological study	Population (age)	Sample size	Exposure assessment	Sample media	Level measured	Comments
Mercury	Umbangtalad *et al.* [356]	2007	Case-controlled	Schoolchild (elementary student)	59 *A part of a more extensive study included adult	• Mercury	• Urine and hair • Water and sediment	• The urinary mercury was 15.82 and 9.95 µg/g creatinine in 'involved' and 'not involved in mining activities', respectively. • Average Hg hair level in all schoolchildren (0.93 µg/g) with no significant difference between each group	The hazard quotient (HQ) based on the inorganic mercury exposure was not at risk (<1).

*__Age group reference__: newborn: birth - 1 month, infant: 1 - 23 months, preschool child: 2 - 5 years, school child: 6 - 12 years, adolescent: 13 - 18 years, child: birth – 18 years

Abbreviations: BLL; blood lead level, cBLL; DMA; dimethylarsinic acid, capillary blood lead level, EBLL; elevated blood lead level, Hb; haemoglobin, iAs; inorganic arsenic, MG; macroglobulin, MMA; monomethylarsonic acid

2.4.3 Air pollution

Most of the seventeen full-text articles investigated exposure to outdoor air pollution and second-hand or environmental tobacco smoke (ETS) and children's health (**Table 2.4-3**). Outdoor air pollution sources varied by geographical area and included traffic-related pollution in urban centres, petrochemical factories in coastal provinces, coal power plants, and agricultural burning activities in the northern region of Thailand.

Traffic-related emissions, including diesel exhaust particles, were reported in urban areas and associated with cancer and non-cancer risks [357-362]. Studies on petrochemical industrial estates focused on VOCs, notably benzene and polyaromatic hydrocarbons (PAHs), showing associations with occupational and environmental health risks in the central region of Thailand [358, 362-365]. Exposure to 1,3-butadiene and PAHs in children near petrochemical industries was linked to cancer risk [362].

Outdoor biomass burning activities, such as forest fires and agricultural burning, were significant sources of air pollution in Northern Thailand, potentially leading to $PM_{2.5}$-bound PAHs. However, evidence of related health outcomes, including respiratory and non-respiratory diseases like cancer, was limited [366].

Two extensive studies assessed Thai children's exposure to second-hand smoke inhalation. One study covering Bangkok and Thailand found a high prevalence of smokers in households with infants, with paternal smoking being significantly associated with specific characteristics [367]. Another study in Kanchanaburi province reported a lower prevalence of second-hand

smoke exposure in teenagers [368]. Only one study investigated ETS exposure and health outcomes, revealing an association between ETS exposure and increased severity of respiratory syncytial virus (RSV) infection in young children [369].

One study examined sensitisation in Thai children with AR, identifying house dust mites as the predominant allergen in Bangkok [370]. Another exposure study collected dust from children's mattresses in Thailand and Singapore, finding differences in dust mite allergen and endotoxin levels between rural Thailand and urban Singapore, associated with air-conditioning use, cleaning practices, and mattress type [371].

Table 2.4-3: Epidemiological studies on air pollution exposure in Thai children

Air pollution	Authors	Published year	Type of epidemiological study	Population (age)	Sample size	Exposure assessment	Media sample	Level measured	Comments
Exposure studies									
Benzene	Ruchirawat *et al.* [357]	2005	Case-controlled	School child (10-12 years, male)	69	• Benzene • t,t-MA levels	• Blood and urine • Ambient air (personal monitoring)	• The benzene levels on the main roads ranged from 16.35 to 49.25 ppb. • Benzene exposure was 4.71 ± 0.25 and 2.10 ± 0.16 ppb in the exposure and control groups, respectively.	School children in Bangkok were exposed in total Benzene more than the control group (living outside the city)
PAHs	Ruchirawat *et al.* [357]	2005	Case-controlled	School child (10-12 years, male)	69	• 1-OHP • PAH	• Urine • Ambient air (personal monitoring)	• Total PAHs on the main roads ranged from 7.10 to 83.04 ng/m3. • Total PAHs was 6.70 ± 0.47 and 1.25 ± 0.24 ng/m^3 in exposure and controlled group, respectively	School children in Bangkok were exposed in total PAH more than the control group (living outside the city)
ETS	Anuntaseree *et al.* [367]	2008	Cross-sectional	Parent of infants	3,256	Questionnaires	na	Prevalence of at least one smoker in the household was 47.2%, paternal smoking in the presence of their infant was 35.1%, maternal smoking 0.3%	• The significant association with paternal smoking is their age of 25-34 years, education less than secondary school, and Muslim fathers. • This study was part of the "Prospective Cohort Study of Thai Children; PCTC.
	Thongthai *et al.* [368]	2008	Cross-sectional	Adolescent (15-19 years)	2,596 *A part of a more extensive study that	Questionnaires	na	Tobacco smoke affects 60% of the population	Smokers were more likely to be male and older, but those exposed to secondhand smoke tended to be female and younger.

Air pollution	Authors	Published year	Type of epidemiological study	Population (age)	Sample size	Exposure assessment	Media sample	Level measured	Comments
					included adult (≥ 15 years, total n = 28,248)				
Aeroallergens	Pumhirun *et al.* [370]	1997	Cross-sectional	School child and Adolescent (10-18 years)	Total 100 *A part of a more extensive study that included adult	Skin prick test 30 aeroallergen	na	Allergic rhinitis patients were mainly sensitised to house dust mites (D. *farina*, D. *pteronyssinus*) and American cockroaches.	• 85% of patients sensitive to house dust mites were positive for both D. pteronyssinus and D. farina (substantial cross-reactivity)
	Lee *et al.* [371]	2006	Cross-sectional	Dust from Young children's home	50	• Endotoxin level • Dust mite	• Dust from the mattress of a young child's home	• Endotoxin level in rural Thailand was higher than in urban Singapore. However, dust mite allergen was higher in urban Singapore. • Endotoxin level was also prominent in the agricultural area despite the farming environment.	• Cleaning practice and mattress type were influencing the endotoxin level. • Air-conditioning which also influenced the dust mite allergen level (positive correlation)
Exposure and health outcome assessment studies									
PM	Aekplakorn *et al.* [372]	2003	Cohort	School child (6-14 years, asthma and non-asthma)	175	• Ambient PM_{10} • Respiratory symptom questionnaires	Ambient air (monitoring station)	A 10-µg/m3 increment was associated with changes in the highest FVC (–6.3 ml, 95% CI: –9.8, –2.8), FEV1 (–6.0 ml, 95% CI: –9.2, 2.7), PEFR (–18.9 ml. sec–1, 95% CI: –28.5, –9.3) and forced	Declines in pulmonary function among asthmatic children were *associated with* increases in PM_{10}.

Air pollution	Authors	Published year	Type of epidemiological study	Population (age)	Sample size	Exposure assessment	Media sample	Level measured	Comments
								expiratory flow 25 to 75% of the FVC (FEF25–75%) (–3.7 ml. sec–1, 95% CI: –10.9, 3.5) in asthmatic children.	
	Preutthipan *et al.* [373]	2004	Cohort	School child	133 (asthma 93, non-asthma 40)	• Ambient PM_{10}	Ambient air (monitoring station)	• PM_{10} levels exceeded 120 mg/m3 (Thai national standard at that time) for 14 days of 31 days records • 4-hr average PM_{10} levels ranged between 46–201 mg/m^3. • PM_{10} levels >120 mg/m3, the daily reported nasal irritation of asthmatic children was significantly higher than when PM^{10} levels were < 120 mg/m^3	• PEFR did not change with different ambient PM_{10} levels in both groups. • Elevated PM_{10} concentrations in Bangkok affect respiratory symptoms of schoolchildren with and without asthma.
	Langkulsen *et al.* [374]	2006	Cross-sectional	School child (10-15 years)	878 (completed PFT 722)	• Ambient PM_{10} • Questionnaire	• Ambient air (monitoring station)	• prevalence of respiratory symptoms increased significantly in the highly polluted area [OR 2.44 (95%CI, 1.21–4.93) and 2.60 (1.38–4.91), in roadside and general areas, respectively].	Increase chronic respiratory symptoms and impaired lung function in high-pollution areas.
SO_2	Aekplakorn *et al.* [372]	2003	Cohort	School child (6-14 years, asthma and non-asthma)	175	• SO2 • Respiratory symptom questionnaires	• Ambient air (monitoring station)	• The ambient SO2 levels were relatively low except for a few days. • The daily 24-hour mean SO2 concentrations were	*No association* between pulmonary function decline and increases in SO_2

Air pollution	Authors	Published year	Type of epidemiological study	Population (age)	Sample size	Exposure assessment	Media sample	Level measured	Comments
								lower than the Thai ambient standards (300 µg/m3).	
Benzene	Navasumrit *et al.* [358]	2005	Case-controlled	School child (12-14 years)	71 (exposure 41, controlled 30)	• trans,trans-muconic acid (MA) • Benzene	• Urine • Ambient air sampling	• Bangkok school children (5.50 ppb) were exposed to significantly higher levels of benzene than provincial school children (2.54 ppb; $p < 0.01$). • Increased DNA damage and decreased repair capacity in the exposed group compared to unexposed children.	Atmospheric levels of benzene in Bangkok were higher at the roadside compared to ambient levels in areas approximately 500 m away.
	Ruchirawat *et al.*[359]	2007	Case-controlled	School child (9-13 years, male)	184 (case 115, controlled 69)	• Benzene • DNA strand breaks and DNA capacity • Genetic polymorphisms of SGTs and CTP450	• Ambient air monitoring • Blood	Benzene of children studying in Bangkok were significantly higher than (3.5 times) student who study in rural school (controlled group)	• DNA strand breaks were significantly higher, but DNA repair capacity was significantly lower in Bangkok children. • Genetic polymorphisms of GSTs and CYP450 enzymes had been detected, involved in the metabolism of benzene and PAHs, but no significant effects on the biomarkers of PAH exposure.
	Buthbumrung *et al.* [361]	2008	Case-controlled	School child	171 (Exposure :	• Benzene	• Blood and urine	• Mean benzene level in ambient air at roadsides adjacent to the Bangkok schools (17.75±2.23	• Children living in high-traffic density areas were exposed to higher levels of benzene than those

Air pollution	Authors	Published year	Type of epidemiological study	Population (age)	Sample size	Exposure assessment	Media sample	Level measured	Comments
				(9-13 years, male)	controlled = 109: 62)	• Benzene and metabolite (MA) levels • 8-OHdG (oxidative DNA damage markers) • DNA isolation and identification of metabolic genotype	• Leukocyte • Air monitoring (personal and area sampling)	ppb) and the rural school (4.49±0.59 ppb) • Level of 9-OHdG in exposure vs-controlled group were 0.25±0.02/105 dG vs 0.08±0.06/105 dG	living in rural areas, which may contribute to oxidative DNA damage.
	Ruchirawat *et al.* [362]	2010	Case-controlled	School child (9-13 years, male)	276 (city: rural = 165: 111) *Part of a larger study that includes adult	• DNA-adduct. • 8-OHdG • DNA strand break and DNA repair capacity • Benzene and 1,3-butadiene, particle-associated PAHs	• PBMCs • Leukocytes • Ambient air monitoring	School children in city and rural respectively, were exposed to; • Benzene - 19.38 and 8.4 mcg/m^3 (city and rural)	• Low levels of benzene exposure, alone or concurrently with other carcinogens, result in early biological effects in the study populations.
PAHs	Ruchirawat *et al.* [375]	2006	Case-controlled	School child (10-12 years, male)	69 (case: controlled = 44: 25)	• PAH-DNA adduct levels. • PAHs	• lymphocytes • Ambient air monitoring	Ambient levels of PAHs are relatively high in Bangkok.	• PAH-DNA adduct levels in lymphocytes were 5-fold higher in Bangkok
	Ruchirawat *et al.* [359]	2007	Case-controlled	School child (9-13 years, male)	184 (case: controlled 115: 69)	• PAHs	Ambient air monitoring	PAHs of children studying in Bangkok were significantly higher than (2 times) students who study in	• DNA strand breaks were significantly higher, but DNA repair capacity was significantly lower in Bangkok children.

Air pollution	Authors	Published year	Type of epidemiological study	Population (age)	Sample size	Exposure assessment	Media sample	Level measured	Comments
								rural schools (controlled group)	• Genetic polymorphisms of GSTs and CYP450 enzymes had been detected, involved in the metabolism of benzene and PAHs, but no significant effects on the biomarkers of PAH exposure.
	Tuntawiroon *et al.* [360]	2007	Case-controlled	School child (8-13 years)	184 (case: controlled = 115: 69)	• 1-HOP • DNA adduct level • Ambient air monitoring	• Urine • Lymphocytes • Ambient air monitoring	The concentration of urinary 1-HOP was significantly higher in Bangkok schoolchildren.	• Bulky carcinogen-DNA adduct levels in peripheral lymphocytes were also significantly higher, and a significantly higher level of DNA strand breaks but lower DNA repair capacity was observed in children in Bangkok.
	Ruchirawat *et al.* [362]	2010	Cross-sectional	School child (9-13 years, male)	276 (city 165, rural 111) *A part from a larger study that includes adult	• DNA-adduct • 8-OHdG • DNA strand break and DNA repair capacity • Benzene and 1,3-butadiene, particle-associated PAHs	• PBMCs • Leukocytes • Ambient air monitoring	School children in city and rural, respectively, were exposed to; • 1,3-butadiene - 2.42 and 0.65 mcg/m^3 (city and rural) • Total PAHs - 4.13 and 1.18 (city and rural) • B[a]P equivalents - 1.50 and 0.43 ng/m^3	Low levels of benzene exposure, alone or concurrently with other carcinogens, result in early biological effects in the study populations.
	Pongpiachan *et al.* [366]	2015	Cross-sectional	Preschool child (risk of $PM_{2.5}$	Risk assessment from environme	$PM_{2.5}$ -bound PAHs	Air monitoring	The average values of Σ3,4-ring PAHs and B[a]P equivalent concentrations in world urban cities were	The cancer risk related to exposure through inhalation appears to be minor, while direct ingestion could

Air pollution	Authors	Published year	Type of epidemiological study	Population (age)	Sample size	Exposure assessment	Media sample	Level measured	Comments
				intake in preschool children of age 0 - 5 years old)	ntal monitoring results			significantly higher than those in samples collected from northern provinces during both sampling periods.	potentially be a significant pathway for children due to their hand-to-mouth activities.
VOCs	Aungudornpukdee *et al.* [364]	2009	Cross-sectional	School child (6-13 years)	2,956	• GIS	• Ambient air (interpolation)	• The distance to the industrial park from residential areas **was not statistically different** between children who had and did not have motor coordination deficits.	The associated factors of visual-motor coordination deficit were gender, monthly parental income, children's age, residential period, and household ETS were the associated factors of
	Singkaew *et al.* [376]	2013	Case-controlled	School child (4-11 years)	6 *A part of a more extensive study that included adult	• VOCs	• Ambient air monitoring	• The average VOC concentration during 2006-2010, which exceeded the Thai annual standard, was 1,2-dichloroethane, 1,3-butadiene, and Benzene.	The lifetime cancer and non-cancer risk in all high-risk groups, including children, were in the *acceptable range* based on the US EPA health risk assessment.
ETS	Sritippayawan *et al.* [369]	2006	Case-controlled	Preschool child (0-5 years)	71	• Cotinine/creatinine, • Questionnaires	• Urine	• 28% of patients had a history of in-house smoking. • The median urinary cotinine level was 0.5 mcg/mg Cr.	ETS exposure increased the risk of desaturation (SpO2 < 9%) in RSV-LRI but was not associated with RSV-LSI itself
DEP: Urban air pollution	Sriyaraj *et al.* [377]	2008	Cross-sectional	School child (6-12 years)	511	Standard ISAAC questionnaires	na	• The prevalence of cigarette smoking for the mother was 4.5%, and for the father, it was 49.3%.	Potential environmental factors positively correlated with the allergic disorder were diesel engine vehicles, antibiotic and paracetamol use, nuts consumption,

Air pollution	Authors	Published year	Type of epidemiological study	Population (age)	Sample size	Exposure assessment	Media sample	Level measured	Comments
								• Mother smoked during the 1st year of the child's life was 5.3%.	having contact with dogs and cats in the first year of life, having contact with farm animals by mother while pregnant, and maternal cigarette smoking. • Prevalence of Rhinitis (24.3% vs. 15.8%), hay fever (23.2% vs. 13.9%), and atopic dermatitis (12.5% vs. 7.2%) were more common in urban areas than in suburban areas. • The prevalence of asthma was not different between both areas (5.5%).

*__Age group reference__: newborn: birth - 1 month, infant: 1 - 23 months, preschool child: 2 - 5 years, school child: 6 - 12 years, adolescent: 13 - 18 years, child: birth – 18 years

Abbreviations: Cr; creatinine, CYP450; cytochrome P450, DEP: diesel exhaust particle ETS; environmental tobacco smoke, FEV1; forced expiratory volume at 1 second, FVC; forced vital capacity, GIS; Geographical Information System, GSTs; glutathione-S-transferases, MA; muconic acid, OR; odds ratios, PAHs; Polyaromatic hydrocarbons, **p**eripheral mononuclear white blood cells; PBMCs, PEFR; peak expiratory flow rate, PFT; pulmonary function test, PM; Particulate matter, SO2: Sulphur dioxide, t,t-MA; t,t-muconic acid, 1-HOP; 1-hydroxypyrene; VOCs; volatile organic compounds, 8-OHdG; 8-oxo-7, 8-dihydro-2'-deoxyguanosine, 95% CI; 95% confidence interval

2.5 Discussion

The comprehensive review and synthesis of these 55 articles offer valuable insights into prevalent exposures within the studied population, shedding light on potential health implications linked to environmental exposures. The findings contribute to a deeper understanding of pesticides, heavy metals, and air pollution risks and their potential impact on public health across different regions. These findings provide valuable insights into the extent and implications of pesticide exposure in Thai children and highlight the need for further research to understand its potential health effects better.

Children's environmental health in Thailand has seen significant changes over the past decades, and the focus has shifted from traditional threats like bacterial contamination and wood smoke to modern hazards such as pesticides, heavy metals, and air pollution. The ban on leaded gasoline in 1993 positively impacted lead exposure, improving air quality and decreasing blood lead levels in children [342, 378]. However, new challenges have emerged due to rapid industrialisation, urbanisation and indoor environments, such as air conditioning and rising moulds.

Pesticides are widely used in agriculture and domestic settings, with organophosphates, carbamates, and pyrethroids commonly employed. Chronic pesticide exposure has been associated with neurotoxicity and cognitive impairments in children. Heavy metal exposure remains a concern, especially in areas with a history of mining and smelting activities. Chronic exposure to arsenic has been linked to increased cancer risk and DNA damage, while cadmium exposure has been associated with renal impairment.

Air pollution remains a significant health concern, particularly in urban and industrial areas. Traffic-related emissions, VOCs from petrochemical industries, biomass burning, and environmental tobacco smoke contribute to poor air quality. The adverse health effects of air pollution in children include respiratory impairments and potential genotoxic and carcinogenic outcomes.

The authors make several recommendations of the papers identified in this literature review to improve children's environmental health in Thailand. They suggest conducting prospective studies that combine exposure and health outcome assessments using state-of-the-art technologies. Regularly monitoring current hazards and focusing on vulnerable populations like pregnant women and children is also recommended. By identifying key pollutants associated with children's health and implementing appropriate policy or behavioural changes, Thailand can work towards safeguarding the health and well-being of its younger population.

2.6 Conclusion

Environmental health in Thailand has made significant strides in recent years, with increased awareness, expanding knowledge, and a growing focus on environmental conservation. However, challenges remain, particularly concerning pesticide, heavy metal, and air pollution exposures, which pose significant health threats to the next generation. To further advance children's environmental health in the country, the following steps are crucial:

2.6.1 Understanding exposure-outcome links

To effectively address environmental health risks, conducting more comprehensive and prospective studies investigating the direct links between environmental exposures and health outcomes in children is essential. Advanced technologies and methodologies can help accurately assess exposure levels and measure health impacts.

2.6.2 Public and policymaker education

Raising awareness among the public and policymakers is of utmost importance. Educational campaigns can be vital in highlighting the risks associated with various environmental pollutants and promoting behaviours and policies that protect children from exposure.

2.6.3 Development of environmental health policies

Establishing robust environmental health policies is critical in mitigating risks and safeguarding children's health. Evidence-based policies that consider the unique vulnerabilities of children can help minimise exposure to harmful environmental contaminants.

2.6.4 Strengthened regulations

Stricter enforcement of existing regulations and implementation of new ones can significantly reduce environmental pollution and protect children's health. Enhanced controls on pesticides, heavy metals, and other hazardous substances are essential in creating a healthier environment.

2.6.5 Enhanced coordination

Collaboration and coordination between various governmental and non-governmental organisations involved in environmental health are essential for success. Multidisciplinary efforts can lead to more effective strategies for protecting children from environmental hazards.

Chapter 3: Study design

This DPhil study represents a nested analysis conducted within the broader framework of the "Assessment of Health Hazard Exposure during Pregnancy and Health Impact among Pregnant Women, Foetuses, and Infants" birth cohort study conducted in Thailand". This chapter presented the overview of the framework of the primary birth cohort to understand the broader context of the study, including study place, eligible criteria, number of participants, ethical approval, discontinuation of participants criteria, funding, preparation of the study, data and specimen collection, and data management. Then, the details of the study design for nested analysis of this DPhil were explained step by step, including the rationale for participants selection for nested analysis, data analysis in terms of exposure, outcome, confounding assessment, and statistical analysis. The primary birth cohort was approved by the Oxford Tropical Research Ethics Committees (OxTREC) (protocol number HCR 17007), the Faculty of Medicine Ramathibodi Hospital, Mahidol University, and the local IRB of Rayong and Samut Prakan Hospital. It was designed to enrol pregnant participants in the first trimester who attend their routine antenatal care at those three hospitals: Ramathibodi, Rayong, and Samut Prakan (details in **section 3.2**). The eligible criteria of participants were detailed in **section 3.4**.

3.1 Overview

The pilot maternal-child prospective study (birth cohort) was designed to collect data from first-trimester pregnant women and their children up to age two in Bangkok, and Samut Prakan, and Rayong provinces to assess the link between early-life environmental chemical exposures (including metals and pollution) and environmentally related diseases/conditions in infant children (0-2 years old). The main objectives of the study, and data collected to address them, are summarised below.

1) to examine the association between lifestyle and environmental exposure(s) in early life and the health outcomes in infant children.

   Data: self-administer questionnaire; SAQ (health hazards at workplace and at home, type of house, home ventilation practise, household chemical products consumption, household ETS, pet owner, visible mould at home, living nearby busy road, and green environment at home), biosamples (blood for heavy metals, urine for cotinine/creatinine, plasma/serum IgE, sIgE, and cytokines measurement), Case Report Form; CRF (physician diagnosed AD, food allergy)

2) to explore the potential mechanism (epigenetics and microbiome) linking them.

   Data: biosample (plasma/serum for miRNA, vaginal and rectal swab for microbiome)

3) to determine the feasibility of birth cohort study focusing on the effect of early life environmental exposures and lifestyle and health-related outcome in Thai children.

   Data: enrolment and retention rate and quantitative data arising from conducting pilot birth cohort study (lesson learned)

A schematic of the study protocol is depicted in **Figure 3.1-1**. However, this DPhil study was a sub-study to follow up with children for up to 12 months. Potential mechanisms, such as epigenetic and microbiome analyses, were not conducted due to time constraints and challenges posed by the COVID-19 pandemic. The study procedures are summarised in **Table 3.1-1**. The data was collected along with the routine clinical follow-up for healthy pregnant women and infants. The participants' information, lifestyle, and focused environmental exposure were consecutively collected from an online SAQ by participants (pregnant women and caregivers of their offspring). The health outcomes in infant children, including congenital anomalies, growth and development, behavioural disorders, and allergic disease, were retrieved from medical records with permission from the participants (informed consent) and transferred to

CRF. The biospecimens, (blood, urine, and vaginal and rectal swabs) were collected, handled, and stored at the Faculty of Medicine, Ramathibodi Hospital, Bangkok. The daily averages of 24-hour $PM_{2.5}$ data was retrieved from the PCD, and the BMA, Thailand, and then the $PM_{2.5}$ level was interpolated to determine the $PM_{2.5}$ level at the participant's residence.

A nested case-control study focusing on infants' atopic outcomes using information from the pilot prospective project was conducted for this DPhil study, where children up to 12 months were eligible for analysis. This design provided the opportunity to analyse lifestyle and environmental factors in early life (*in utero* through infancy) and associated allergic outcomes.

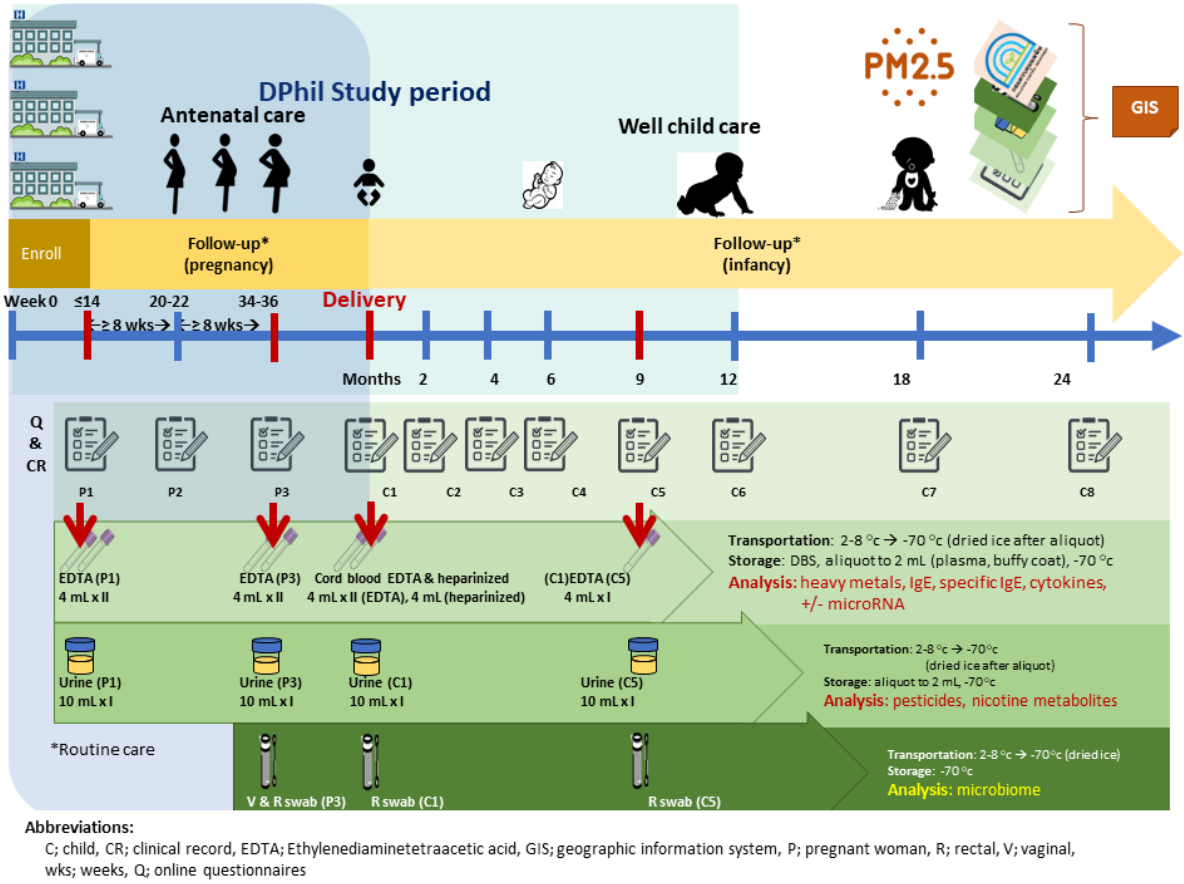


Figure 3.1-1: Study protocol of the pilot maternal-child prospective study (birth cohort) and the subanalysis in the DPhil study

Table 3.1-1: Schedule of study procedures

Procedures	**Visit timing**										
	Visit 1 (P01)	**Visit 2 (P02)**	**Visit 3 (P03)**	**Visit 4 (C01)**	**Visit 5 (C02)**	**Visit 6 (C03)**	**Visit 7 (C04)**	**Visit 8 (C05)**	**Visit 9 (C06)**	**Visit 10 (C07)**	**Visit 11 (C08)**
	Pregnancy period Participant: pregnant women			**Infancy period Participants: infant children**							
	DPhil study										
	Screening and recruitment GA < 14 weeks	Antenatal care GA 20-26 weeks	Antenatal care GA 32-36 weeks	Delivery*** 0 month	Well-child 2 months	Well-child 4 months	Well-child 6 months	Well-child* 9 months	Well-child 12 months	Well-child 18 months	Well-child 24 months
Informed consent	✓										
Eligibility assessment	✓										
Online questionnaires	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓
Electronic clinical record	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Blood collection	✓P (8ml)		✓P (8ml)	✓C** (16 ml), P (8 ml)				✓C (4ml)			
Urine collection	✓P (10ml)		✓P (10ml)	✓P & C (10ml)				✓C (10ml)			
Vaginal swab			✓P								
Rectal swab			✓P	✓P & C				✓C			

***** If the allergic disease is suspected, additional collected blood, urine, rectal swabs and/or faeces will be collected the same as the 9-month-old protocol.
**Cord blood (16ml) of child/children will be collected.
***Participants give birth or are indicated to terminate the pregnancy before 34 weeks for any medical reason; specimens will be collected the same as delivery (0 month).
Abbreviations: C; child/children, GA; gestational age, P; the pregnant woman

3.2 The study locations

The study locations include the Bangkok Metropolitan Region (BMR) and Rayong province. BMR comprises Bangkok, Samut Prakan, Samut Sakhon, Nakhon Pathom, Pathum Thani, and Nonthaburi (**Figure 3.2-1**). Data collection took place at the following hospitals: Ramathibodi Hospital in Bangkok, a medical school and referral centre serving the BMR and central region;

Samut Prakan Hospital, serving as catchment hospitals for Samut Prakan province; and Rayong Hospital, which serves as a catchment hospital for Rayong province. Bangkok is the capital city. Rayong is a coastal province situated in the East, and Samut Prakan is the location of Suvarnabhumi Airport adjacent to Bangkok. All these hospitals were selected due to the focused environmental exposures and their readiness to conduct the birth cohort study. The residential location of participants was in the catchment areas of the 3 hospitals, primarily in industrialised zones (Bangkok, Samut Prakan, and Rayong provinces). Potential sources of environmental exposure are summarised in **Table 3.2-1**. All three provinces contribute to manufacturing (automotive, electronics), logistics, and exports.

In addition, Bangkok has food processing plants, Samut Prakan and Rayong have petrochemicals and heavy industries as part of the Eastern Economic Corridor (EEC) in Rayong, which may contribute to different environmental exposures. Additionally, Bangkok and Samut Prakan have air pollution due to congested traffic and construction.

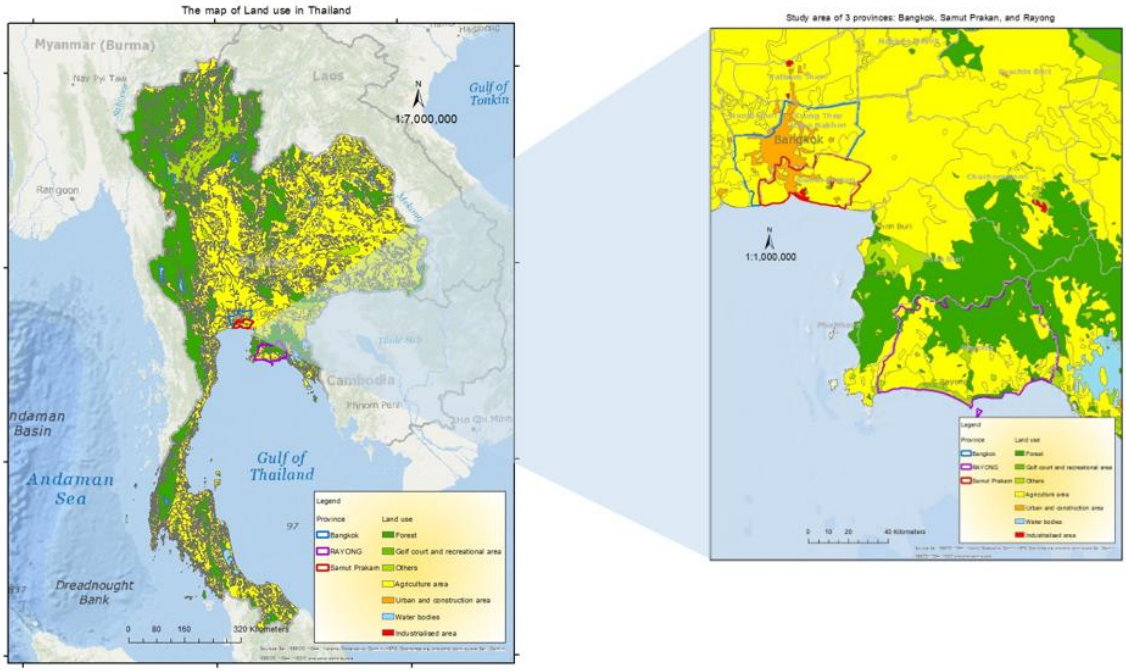


Figure 3.2-1: Study location in Bangkok, and Rayong, and Samut Prakan provinces

Table 3.2-1: Potential sources of environmental exposures in each study area

Aspect	Bangkok	Samut Prakan	Rayong
Dominant industries	Primarily light industries, food processing, textiles, logistics, services	Heavy manufacturing: automotive, electronics, petrochemicals	Heavy industries: petrochemicals, energy production, automotive, steel
Industrial estate focus	Limited large industrial estates within the city (most on the outskirts)	Large industrial estates for manufacturing (automotive, electronics)	Major industrial hub (Map Ta Phut), focusing on petrochemicals and energy
Energy sector	Few energy facilities	Some energy production but more focused on manufacturing	Significant energy production from natural gas, petrochemical refineries, and renewable energy
Environmental issues	Air pollution, traffic emissions, poor waste management	Air and water pollution from industrial emissions and waste	Petrochemical pollution, air, and water quality issues, oil spills
References **https://www.boi.go.th** **http://www.eeco.or.th/en** **http://www.pcd.go.th** **http://worldbank.org**			

The population density in 2018 was 5,278, 1,275, and 197 people/km^2 in, Bangkok, Samut Prakan, and Rayong, respectively (**Table 3.2-2**).

Table 3.2-2: Population, area and population density of the 3 study areas.

Area	Population	Area (km^2)	Density (people/km^2)
Bangkok	8.28 million (10.7 million in 2023)	1,529	5,278 (6,821 in 2023)
Samut Prakan	1.28 million (1.2 million in 2023)	1,004	1,275 (1,195 in 2023)
Rayong	700,000 (720,000 in 2013)	3,552	197 (203 in 2023)
References **http://www.nso.go.th** **http://stat.bora.dopa.go.th** **https://www.eeco.or.th/en** **https://data.worldbank.org**			

3.3 Definitions

The nomenclature variations relevant to the study were carefully considered to establish a definitive framework for conducting assessments. By incorporating both the locally accepted standard usage in Thailand and the international definitions, the approach aimed to guarantee the highest level of precision in collecting data, as outlined below (**Table 3.3-1:**).

Table 3.3-1: The nomenclature and their definitions in this study

Nomenclature	Definitions
Pregnancy	The suspected pregnant women with the evidence to confirm pregnancy by the measurement of human chorionic gonadotropin (hCG) in urine (urine pregnancy test) or in serum (serum pregnancy test) and the detection of foetal heart activity (foetal heart tones) by direct auscultation or by the use of a foetal Doppler ultrasound device or ultrasonography with performed by the obstetricians [379].
Gestational age (GA)	The number of weeks elapsed between the first day of the Last Menstrual Period; LMP (not the presumed time of conception) and the delivery date. It is established by an accurate regular menstrual period or abdominal/transvaginal ultrasonography in irregular periods or by taking medications that alter cycle length (e.g., oral contraceptives, other hormonal preparations, and psychoactive medications) [379]. In this study, "gestational age at enrolment" was calculated from the LMP date. If the LMP date was uncertain, the estimated gestational age from ultrasound was used as a substitute, which is also the routine practice in all three study hospitals in such cases.
Normal pregnancy	A normal pregnancy lasts 40 ± 2 weeks, calculated from the first day of the last regular menses (menstrual or GA) [379]. Periodic antepartum visits for normal pregnancy of 4-week intervals are usually scheduled until 28 weeks, every two weeks until 36 weeks, and weekly until delivery [379].
Trimesters	Pregnancy can be divided into trimesters based on GA, each spanning approximately three calendar months. The first trimester encompasses the period up to $13^{6/7}$ weeks of gestation completion. The second trimester ranges from fourteen weeks (14 weeks' gestation) to

Nomenclature	Definitions
	twenty-seven weeks and six days ($27^{6/7}$ weeks' gestation). The third trimester comprises the time from twenty-eight weeks' gestation through delivery [380].
Abortion	Abortion refers to the termination of pregnancy before reaching 20 weeks' gestation or involving a foetus born with a weight of less than 500 g
Spontaneous abortion	A spectrum of conditions, including threatened, inevitable, incomplete, complete, missed abortion, and septic abortion
Recurrent abortion	The women who undergo repetitive occurrences of spontaneous abortions
Induced abortion	The deliberate termination of a live foetus through surgical or medical means before reaching viability
Premature or preterm infant	This term designates an infant born prior to completing 37 weeks of gestation (equal to or less than $36^{6/7}$ -weeks' gestation).
Early preterm	Infants who are delivered between 20 and $33^{6/7}$- weeks' gestation
Late preterm	Infants delivered between 34 complete weeks' gestation ($34^{0/7}$) and $36^{6/7}$ -weeks' gestation
Post-term pregnancy	Pregnant women whose gestational age (GA) equals or exceeds 42 weeks
Low birth weight (LBW)	Birth weight <2,500 gram
Small for gestational age (SGA)	The birthweight/length ratio (BLR) below the 10th percentile, adjusted for gestational age and sex, was determined based on the INTERGROWTH-21st (IG-21) standard. The BLR percentiles were derived using the Excel-based Newborn Size Calculator available at: https://intergrowth21.com/tools-resources/newborn-size#c4.

According to changing the WHO guideline recommendation on ANC for positive pregnancy experience, the current follow-up ANC in some hospitals has been adapted, as shown in **Table 3.3-2**.

Table 3.3-2: Antenatal care (ANC) schedules

WHO FANC* model	2016 WHO ANC model	Data collection in this study
First trimester (<14 weeks)		
Visit 1: 8-12 weeks	Contact 1: up to 12 weeks	**Visit 1:** up to 14 weeks (Questionnaires and bio-specimens)
Second trimester (14– $27^{6/7}$ weeks)		
	Contact 2: 20 weeks	**Visit 2:** 20-22 weeks. (Questionnaires)
Visit 2: 24-26 weeks	Contact 3: 26 weeks	
Third trimester (≥ 28 weeks)		
Visit 3: 32 weeks	Contact 4: 30 weeks	
	Contact 5: 34 weeks	**Visit 3:** 34-36 weeks. (Questionnaires and bio-specimens)
Visit 4: 36-38 weeks	Contact 6: 36 weeks	
	Contact 7: 38 weeks	
	Contact 8: 40 weeks	

Return for delivery at 41 weeks if not given birth.
Abbreviations: FANC; four-visit focused ANC, WHO; World Health Organisation

3.3.1 Atopic dermatitis or allergic dermatitis (AD) or eczema

Atopic dermatitis is a chronic skin condition characterised by itching, inflammation, and a disturbed skin barrier. The widely used diagnosis tool of AD is Hannifin and Rajka's criteria [381], which was also applied in this study. Hannifin and Rajka's criteria were established to aid in diagnosing AD based on specific clinical and historical features. According to Hannifin and Rajka's criteria, AD diagnosis must be positive ≥ three major and ≥ three minor criteria. The detail of the criteria is shown in **Table 3.3-3**. The major criteria that require careful attention include:

1) Pruritus which refers to the presence of itching or scratching in the last four weeks
2) Typical morphology and distribution, which refers to visible eczema in characteristic locations, such as the flexural areas (inner elbows and knees), face, neck, and wrists in adults, face and extensor surfaces in infants
3) Chronic or relapsing course, which should be continuous or recurrent symptoms for at least six months
4) Personal or family history was the personal or family history of atopic diseases, such as asthma, AR, or AD.

Table 3.3-3: Hannifin and Rajka's diagnostic criteria for atopic dermatitis

Major criteria	**Minor criteria**
1. **Pruritus** 2. **Typical morphology and distribution are flexural lichenification or linearity in adult, facial and extensor involvement during infancy and childhood.** 3. **Chronic or chronically relapsing dermatitis** **4. Personal family history of atopy (asthma, allergic rhinoconjunctivitis, atopic dermatitis)**	1. Xerosis (dry skin) 2. Ichthyosis/palmar hyperlinearity/keratosis pilaris 3. Immediate (type I) skin test reactivity 3. Elevated serum IgE 4. Early age of onset 5. A tendency towards cutaneous infections 6. A tendency towards nonspecific hand or foot dermatitis 7. Nipple eczema 8. Cheilitis

Major criteria	Minor criteria
	9. Recurrent conjunctivitis 10. Dennie–Morgan infraorbital fold 11. Keratoconus 12. Anterior subcapsular cataracts 13. Orbital darkening 14. Facial pallor/facial erythema 15. Pityriasis alba 16. Anterior neck folds 17. Itch when sweating 18. Intolerance to wool and lipid solvents 19. Perifollicular accentuation 20. Food intolerance 21. Course influenced by environmental/emotional factors. 22. White, dermographism/delayed blanch

3.3.2 Allergic diseases risk

Allergic disease risk was defined as any self-reported doctor's diagnosis of allergic diseases (asthma, AR, AD, food allergy) of first-degree relation of the expected child/children (pregnant women, their partner, and/or previous sibling).

3.3.3 Allergic sensitisation

Allergic sensitisation refers to how an individual's immune system reacts abnormally upon exposure to an allergen. This reaction involves the production of allergen-specific Immunoglobulin E (sIgE) antibodies. Upon subsequent exposures to the same allergen, these antibodies can activate mast cells and basophils, releasing diverse inflammatory mediators. This cascade of events leads to allergic symptoms when re-exposed to the allergen.

3.4 Eligible criteria

3.4.1 Inclusion criteria

Participants who satisfy all the following inclusion criteria were enrolled in the study:

1) Thai pregnant women aged ≥ 18 years.
2) Gestational age ≤ 14 weeks who visit antenatal care (ANC) in Bangkok, Samut Prakan province, or Rayong province.
3) Intended to follow up at ANC until the delivery.
4) Intended to follow up with their child/children at the Faculty of Medicine Ramathibodi Hospital, Samut Prakan, or Rayong Hospital or contact the primary care unit of those three hospitals until 24 months of children's age
5) Able to read and write the Thai language fluently.
6) Consent to participate in the study.

3.4.2 Exclusion criteria

Individuals who are HIV positive, have undergone chemotherapy, exhibited immunocompromised conditions, or had autoimmune diseases are excluded from the study to mitigate potential biases in immunological skewing, such as cytokine responses.

3.5 Number of participants

Following the study design of the pilot project, the anticipated number of subjects from the three study area sites – Ramathibodi Hospital, Samut Prakan Hospital, and Rayong Hospital – was set at 151 pregnant women. This number was determined based on the practicality of recruiting participants from three hospitals within a year and considering certain statistical aspects. A common rule of thumb in statistics considers 30 individuals as a substantial sample size. Nonetheless, Browne *et al.* (1995) caution that this might be insufficient if the effect size is small.

In this scenario, prior studies' estimate of biomarker levels was limited. However, the literature review has shown a broad range of exposure prevalence (8.1% – 78.1%). Given the lack of a precise estimate from previous research, we opted to use a prevalence of 50% (0.5) in our sample size calculations for prevalence studies. Among constant margin of error and alpha values, the sample size is maximised when the prevalence is 50%. As a result, we selected an 8% margin of error.

By utilising the formula where Z represents the standard average covariate for a 95% confidence interval (alpha = 0.05), and P and d denote prevalence (50%) and margin of error (8%), respectively, we derived a sample size of 151 pregnant women. Consequently, approximately 50 pregnant women would be recruited from each hospital while acknowledging the possibility of one hospital enrolling more participants than the others. Nevertheless, the study strives for equal numbers to the extent possible.

3.6 Ethical and regulatory consideration

The protocol, participant information sheet, informed consent form, and advertisement/poster were submitted to the OxTREC and local ethics committees for written approval. The Investigator submitted and, where necessary, obtained approval from the above parties for all amendments to the original approved documents.

3.7 Participant confidentiality

The participants were identified only by a participant ID number on all study documents and any electronic database, except for the CRF, where participant initials were added to maintain

the participants' anonymity. All documents were stored securely and only accessible by study staff and authorised personnel. The study complies with the Thailand Personal Data Protection Act, which requires data to be anonymised as soon as it is practical. With the participant's consent, participant clinical data and results from blood analyses stored in the database may be shared with other researchers to use in the future. However, the other researchers will not be given any information that could identify the participant.

3.8 Discontinuation/withdrawal of participants from the Study

The criteria for discontinuation or withdrawal of participants from this study were defined as follows:

3.8.1 Ineligibility (either arising during the study or retrospectively having been overlooked at screening) or,

3.8.2 Significant protocol deviation, or

3.8.3 Significant non-compliance with study requirements, or

3.8.4 Withdrawal of Consent, or

3.8.5 Loss to follow up

Withdrawn participants were substituted to attain the predetermined sample size. The withdrawal did not result in removing their data from the analysis before they opted out of the study. The information provided by withdrawn participants was included in the analysis up until the point of their withdrawal. Baseline characteristics of patients who had to discontinue/withdraw or complete the study during pregnancy and infancy were compared.

3.9 Funding

The Faculty of Medicine, Ramathibodi Hospital, Mahidol University, funded the entire pilot prospective study.

3.10 Preparatory work

Before the study's enrolment in June 2018, extensive preparatory work was undertaken to ensure the seamless execution of the research plan. It included organising a children's and environmental health seminar to foster collaboration among participating hospitals. Additionally, a dedicated effort was made to design and validate questionnaires to collect relevant data. An online database collection and monitoring system were developed to streamline data management. Ethical approval was diligently sought and obtained from all collaborative hospitals and OxTRECs. Furthermore, comprehensive training was provided to research assistants to equip them with the necessary skills and knowledge for the study.

3.10.1 Seminar series on children's and environmental health

Several meetings were held with a diverse team comprising research assistants, nurse practitioners, technicians, obstetricians, and specialist paediatricians. These meetings were conducted at Ramathibodi Hospital, Rayong Hospital, and Samut Prakan Hospital. The aims of these gatherings were 1) to engage in group discussions and in-depth deliberations within the collaborative hospitals and 2) to proactively address and mitigate potential challenges anticipated during the study conduction, for instance, data collection, the handling and storage of specimens, to ensure the smooth progress of the research endeavour. The discussions encompassed both group sessions and in-depth conversations within the collaborative hospitals.

Furthermore, Dr Shoji Nakayama and I also took the initiative to offer sessions on pertinent subjects to disseminate knowledge related to children's environmental health and the associated research methodologies such as GIS, environmental exposure assessment in children, and the importance of environment and children's health. The photographs capturing the ambience of the seminar sessions are presented in **Figure 3.10-1**.



Figure 3.10-1: Capturing the essence of the seminar, these photographs showcase enthusiastic attendees engaging with speakers, encapsulating the dynamic and informative vibe of the event.

3.10.2 Questionnaire designing and validation

The questionnaire was initially developed in a paper-based format, drawing upon existing literature that explored environmental exposures during early life and their potential impact on the health of children aged two years or younger. Expert opinions were sought and integrated

into the initial design to ensure the questionnaire's robustness. Once a consensus was reached within the research team, the questionnaire underwent a validation process involving thirty pregnant women attending the ANC at Ramathibodi Hospital.

Subsequently, the final version of the questionnaire was refined based on the feedback and recommendations obtained from the validation of these thirty questionnaires. This iterative process aimed to enhance the questionnaire's comprehensiveness and suitability for the study's objectives.

3.10.3 Online database collection and monitoring system construction and testing

During the start of this study in 2018, smartphones and internet-connected devices in Thailand were generally used [382, 383]. An online data capture platform was selected to overcome the burden of data entering, validate data at the capture step, and synchronise data collection in this study. The integrated electronic data collection and management (EDCM) systems were developed to achieve the following project requirements.

1) to register and store information of participants (pregnant women and their infants)

2) to collect and store substantial amounts of data in a multi-centre, prospective study manner (multiple visits),

3) to synchronise data from several sources (SAQ, clinical and laboratory records, and biospecimen collection from participants, researchers, and laboratory technicians, respectively),

4) to remotely track and monitor participants' status among researchers and research assistants in real-time (monitor the progress of each component of the study and the project as a whole),

5) to secure data storage (authentication system, e.g., different levels of access, password access function, etc., data encryption, trusted storage location of the central database of the cloud server, daily back-up of data, anti-virus software compatibility),

6) to reposit, manage, and query biospecimens (sample information management system; SIM),

7) the system should be intuitive and user-friendly to augment the participants' retention rate and minimise the coaching burden to use the system (able to be used on desktop computers and mobile phones, with various web browsers),

8) the system should be able to generate the basic updated descriptive statistics to present the real-time progression of the study,

9) exporting function of data in comma-separated values (CSV) format for further analysis in statistical software.

The challenge of selecting an EDCM system in this research setting is its flexibility and limited budget. In-house EDCM provides more flexibility and lower cost than commercial package tool kits such as Kobo Toolbox or Open Data Kit (ODK). In addition, it was chosen for this research regarding the unavailability of free accessibility of EDCM system that fulfilled the project requirement such as Research Data Electronic Capture (REDCap) [384, 385] at Ramathibodi Hospital during the initiation of the study. Therefore, the concept and design were

done and liaised with the data engineer (Dr Ussanai Nithirochananont) to develop this study's in-house integrated online EDCM system. The steps of development consist of

1) literature review of the data management of birth cohort studies and existing database management system,

2) requirement gathering among researchers,

3) matching the requirement with the available database management system for research at the Faculty of Medicine Ramathibodi Hospital, Mahidol University (primary site of the project),

4) analysis of the workflow

5) constructed the system (writing the script),

6) verified the system function to ensure that it can work properly,

7) launched the system at enrolment, and

8) collected feedback from all users to assess the usability, fix the bugs in an actual setting, correct the unforeseen issues which can occur during the implementation, and improve the quality of the web application (loop working).

3.10.3.1 Electronic Data Collection and Management (EDCM)

The construction and rigorous testing of the online EDCM were integral to the study's preparation. This system facilitated data collection, organisation, and real-time data monitoring. Thorough testing was conducted to ensure its functionality and reliability in effectively capturing and managing the study's information. The data inputs were gathered

from participants (SAQ) and researchers (clinical record, biobanking, and research laboratory), as shown in **Figure 3.10-2**.

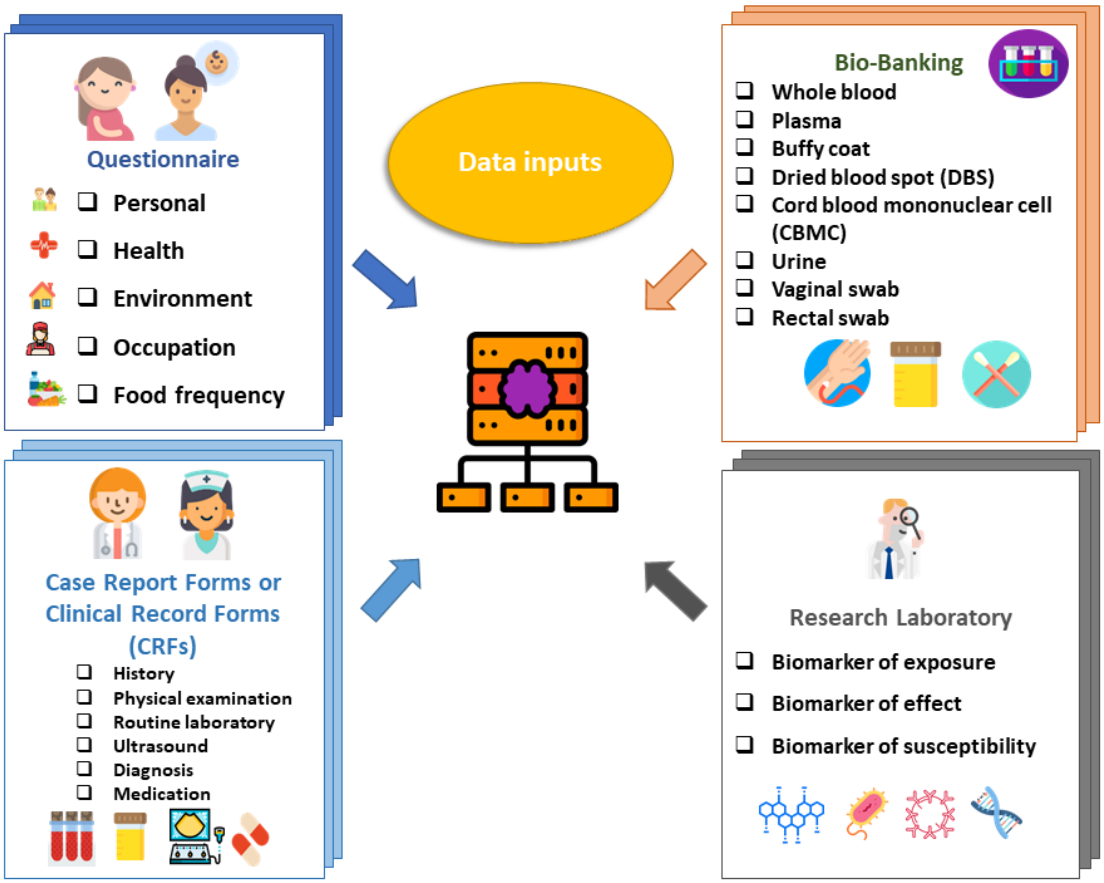


Figure 3.10-2. The data inputs of Electronic Data Collection and Management (EDCM) in this study

3.10.3.2 Web application development

The web application was crafted using Hypertext Markup Language version 5 (HTML5), ensuring compatibility with major web browsers such as Chrome, Internet Explorer, Safari, and Firefox. Hypertext Preprocessor (PHP) and Java Script scripting languages were employed for their dynamic and interactive attributes, capitalising on their flexible and pragmatic nature. The ongoing maintenance of the system has been entrusted to a data engineer.

To store the collected data, MariaDB® was employed as the database system. The Structured Query Language (SQL) commands provided by PHP were utilised to manage various aspects of this web application, including tasks like data addition, deletion, and updates. Furthermore, the development and design of this EDCM system were documented and published in conference proceedings [386].

3.10.4 Training of research assistants and participants at enrolment

In the questionnaire-based study, participants were instructed to complete the information using an online SAQ. They received training on accessing the login system and were introduced to the website's essential functions. Researchers or research assistants were also available to clarify the questionnaires whenever participants required it (**Figure 3.10-3**).

Furthermore, before the study enrolment, research assistants from all study sites underwent comprehensive training to ensure uniformity in their roles and responsibilities. Their performance was periodically assessed and verified throughout the study to maintain synchronisation across the research process.



Figure 3.10-3: Training of research assistants and participants at enrolment.

3.11 Data and specimen collection

3.11.1 Data collection

The data for this DPhil research comprises participant information (from SAQ and health records), 24-hour $PM_{2.5}$ levels, and laboratory results. The specifics of the data collection process are outlined as follows:

3.11.1.1 Self-administer questionnaire (SAQ)

Using an online database collection system, questionnaires capturing participant characteristics, lifestyle, and prenatal environmental exposure were self-administered for pregnant women and infant children during the follow-up period. SAQ in pregnant women consists of five parts: participant's characteristics, health information, lifestyle and environmental exposure, hazards at work, and food frequency questionnaires (FFQ). For infant children, information was participant's and parent’s characteristics, health information, environmental exposure, feeding history, and development.

Pregnant participants were initially assisted in responding by either the researcher or trained research assistants. This assistance ensured that participants could navigate the process and effectively understand the questions and answers. The primary goal was to facilitate their initial engagement with the system.

After the first visit, participants were encouraged to independently complete subsequent SAQ before their scheduled visits within a few days. Throughout the project, assistance was available in person at the outpatient department (OPD) or through phone or the Line application. This approach aimed to maintain consistent and accurate data collection while allowing participants flexibility in their response timing.

3.11.1.2 Health records

Participants' health records that were relevant to the research were obtained by one researcher through the electronic medical record system with proper permissions. Subsequently, the pertinent health data were transferred to the online CRF as required for the study. This process

ensured the integration of accurate and essential health information into the study's data collection system. The health information of pregnant women covered body weight, blood pressure, antenatal concerns, medication usage, labour history, and standard laboratory results. Infant children's health information included perinatal history, growth and developmental milestones, health issues recorded during each well-child visit, allergy-related history, and vaccination records. These comprehensive data sets provided a thorough understanding of the study's health statuses and trajectories of pregnant women and infant children.

3.11.1.3 The 24-hour $PM_{2.5}$ concentrations

The air quality of the ground monitoring stations data was obtained from 1) the Pollution Controlled Department (PCD), the Ministry of Natural Resources and Environmental of Thailand and 2) the Air Quality and Noise Management Division, Environmental Department, the Bangkok Metropolitan Administration (BMA), Thailand. The data was stored in a searchable public database via their official website (http://air4thai.pcd.go.th/ and https://bangkokairquality.com/, respectively). The ambient air concentrations and air quality index, including PM_{10}, $PM_{2.5}$, SO_2, NO, NO_2, CO, and O_3, were measured hourly at the air monitoring station (stationary and mobile). The daily average of 24-hour $PM_{2.5}$ levels was retrieved across the study period from January 2017 – December 2019.

Additionally, the cumulative 24-hour $PM_{2.5}$ concentration at participants' residences was calculated for each trimester during pregnancy and for infants at two-month intervals. The average $PM_{2.5}$ exposure per period was also assessed. The maximum or peak $PM_{2.5}$ exposure during each period was considered; however, the 24-hour $PM_{2.5}$ concentration data provided by the PCD and BMA were not available in a format suitable for analysis.

Geolocations were identified using Google Maps to estimate the 24-hour $PM_{2.5}$ levels at participants' residences. The latitude and longitude coordinates were recorded by choosing the closest point at the subdistrict level whenever possible. This approach aimed to improve the accuracy of geospatial data collection while addressing concerns regarding the confidentiality of participants' precise addresses.

3.11.1.4 Laboratory results

Laboratory analyses were conducted according to the research protocol, encompassing measurements of metals, cotinine, creatinine, total IgE, specific IgE, and cytokine levels. The results of these analyses were entered and securely stored within the online database system in alignment with the established research procedures. Additionally, the raw data could be exported for analysis in CSV format and was further processed within dedicated statistical software tools.

3.11.2 Specimen collection

The protocol for collecting specimens is detailed in **Table 3.11-1**, encompassing blood, urine, vaginal swabs, and rectal swabs, adhering to the guidelines outlined in **Table 3.11-2**.

Table 3.11-1: The specimen collection procedure

Bio-specimen	Tube	Aliquot	Transfer condition before storage	Purposes
Blood (pregnant and cord blood)	EDTA	Plasma, buffy coat, whole blood	2-8 ◦C	miRNA, immunoglobulins, cytokines, organic substances, and metals
Blood (cord blood)	Heparinised	Cord blood mononuclear cells (CBMCs)	2-8 ◦C	In vitro immunological response

Bio-specimen	Tube	Aliquot	Transfer condition before storage	Purposes
Urine	Sterile urine container	Urine	2-8 ◦C	Metal and organic substances
Rectal swab	Cultured swab agar with radiated UV	Not applicable	2-8 ◦C	Microbiome
Vaginal swab	Cultured swab agar with radiated UV	Not applicable	2-8 ◦C	Microbiome

Table 3.11-2: Summary of sample collection and analysis (variables, methods, and specimen)

Variables	Methods	Specimen (period)	Remarks
Exposure assessment (chemicals, metals, and ETS)			
ETS (Nicotine and its metabolites)	LC-MS	Urine (P01, P03, C01, C05)	Analysed in this DPhil
Metals (Cr, Mn, As, Cd, Hg, Pb)	ICP-MS	Blood (P01, P03, C01, C00)	Analysed in this DPhil for P01 and future analysis for P03, C01, C05
[$]Pesticides (OP, carbamates, pyrethroids)	LC-MS	Urine (P1, P3, C01, C05)	Future analysis
Socioeconomic status	Questionnaire	Every visit	Analysed in this DPhil
Potential novel markers/mechanisms			
Microbiome (bacteria)	RT-PCR and NGS	Vaginal swab (P03), and rectal swab (C05)	Analysis in this DPhil
[$]miRNA (epigenetic change)	Microarray	Plasma (P01, P03, C01, C05)	Future analysis
Allergic outcome measurement (response)			
Microbiome (bacteria)	RT-PCR and NGS	Vaginal swab (P03), and rectal swab (C05)	Future analysis
Plasma/serum total IgE	ELISA or Phadia®	Blood (P01, P03, C01, C05)	Analysis in this DPhil
Plasma/serum-specific IgE to common food allergen (cow's milk, soy, egg yolk, egg white, wheat, peanut) and aeroallergen (dust mite, cockroach, fungus)	PhadiaTop® or PhadiaTop infant®	Blood (P01, P03, C01, C05)	Analysis in this DPhil
Th1, Th2, Th17, and Treg-related cytokines, TSLP	ELISA or Cytokine Bead Array	Blood (C05)	Analysis in this DPhil
Atopic dermatitis	Health record	Every visit after the birth	Analysis in this DPhil

Variables	Methods	Specimen (period)	Remarks
Food allergy	Health record and serum-specific IgE	Every visit after the birth	Analysis in this DPhil
Early wheezing	Health record	Every visit after the birth	Analysis in this DPhil

[$]Future work

Abbreviations: As, arsenic; CBC, complete blood count; C01, postnatal child within three days of age; C0**5**, child at well-child care of 12 months of age or when suspected atopic dermatitis or food allergy; ELISA, enzyme-linked immunosorbent assay; ETS, environmental tobacco smoke; GC-MS, gas chromatography-mass spectrometry; Hg, mercury; ICP-MS, Inductively coupled plasma-mass spectrometry; IgE, Immunoglobulin E; LC-MS, Liquid chromatography-mass spectrometry; NGS, next-generation sequencing; OP, organophosphate; Pb, lead; P01, pregnancy first visit; P0**3**, pregnancy at delivery room; RT-PCR; Reverse transcription polymerase chain reaction; Th1, T helper type 1; Th2, T helper type 2; Th17, T helper 17, Treg, regulatory t cell, TSLP; thymic stromal lymphopoietin

3.11.3 Specimen banking

Specimen banking was held at Ramathibodi Hospital Mahidol University with a controlled temperature of -80 ◦C until analysis for at least five years. After that, some of the relevant specimens, including plasma, dried blood spots, vaginal swabs, and nasal swab rectal swabs, were sent to measure the biomarkers at the Centre for Radiation, Chemical and Environmental Hazards (CRCE), UK Heath Security Agency (UKHSA), UK and/or with the controlled temperature by dried ice under the Material Transfer Agreement (MTA) for human specimens.

3.11.4 Biobanking system

The biobanking system was created to organise the large amount and long-duration biospecimen storage. The Nunc® CryoTubes® (ThermoFisher Scientific) Coded Cryobank was used to store the biospecimen under -80 ◦C until analysis. The databases were entered by trained laboratory staff, and the data was backed up daily if there was additional or changed information in the system.

3.12 Data management

The clinical data management system was specifically created for this project. All study data were entered into an online database system and stored in a secured server system with password-protective access and encryption procedures to ensure the confidentiality of the information. Direct access to all trial-related source data/documents was granted to the study team, authorised representatives from the sponsor, research ethics committees, and the regulatory authorities to permit trial-related monitoring, audits, and inspections. The informed consent was obtained and stored in paper form. The study databases were developed according to the validated questionnaire and approved CRF with different access levels between participants and investigators. The laboratory results were recorded separately, stored, and linked to the other study information by study ID number. As for documents that contain patient identifiers, only the site PIs would have access to these documents by password-protective access. The soft copy of data was backed up daily. After the study is closed, the study documents will be kept in accessible storage on request for approximately five years after study completion. The final dataset will be archived in the data repository after publication.

3.13 Data analysis

3.13.1 Exposure assessment

The primary objective of this thesis is to examine the potential links between environmental chemical exposures, such as heavy metals, ETS, and $PM_{2.5}$ levels, and allergic outcomes in infant children. A comprehensive approach was undertaken to achieve this objective, integrating data from SAQ, biomonitoring analyses, and geospatial interpolation of $PM_{2.5}$ concentrations, as detailed in **Figure 3.1-1** and **Table 3.11-2**.

3.13.1.1 Heavy Metals

The measurement of heavy metal concentrations in whole blood for all samples was initially planned to be conducted at the UKHSA. However, the COVID-19 pandemic caused significant disruptions, including the closure of the UKHSA laboratory, making it impossible to complete the analysis there. Consequently, the remaining analyses were shifted to the Department of Toxicology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University. Before proceeding, a validation of results between the two laboratories was conducted for matched participants. Unfortunately, significant differences in metal concentration results were observed, likely due to protocol differences. The protocol had to be adapted to align with the standard procedures at Ramathibodi Hospital. One major change was the digestion method, which shifted from microwave digestion (used at UKHSA) to acid digestion (used at Ramathibodi Hospital).

To ensure the highest precision of results, all available spare samples stored at Ramathibodi Hospital were utilised for the analysis. The targeted heavy metals (arsenic, cadmium, lead, mercury, and manganese) were measured by ICP-MS (Agilent7900) at the Department of Toxicology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University. Please see the details in **Appendix 5**.

3.13.1.2 Environmental tobacco smoke (ETS)

ETS was assessed by SAQ (household ETS) and the measurement of urinary cotinine by liquid chromatography-mass spectrometry (LC-MS) [387] at four-time points: first-trimester (P01), third-trimester (P03), at birth (C01), and 6-12 months (C05). Urinary creatinine, measured by the enzymatic method, was adjusted for urine concentration.

3.13.1.2.1 Self-administer questionnaire (SAQ) of household ETS

The inquiries on ETS exposure within the home environment (household ETS) were directed towards pregnant women. The following question in **Table 3.13-1** was employed to evaluate self-reported household ETS exposure during early life. The table presented the translation of questions about ETS exposure from Thai to English. The questions and corresponding options are provided along with their translations for reference.

Table 3.13-1: The detail of the questionnaire to assess a self-report of household ETS

Roles	**Original questions in the Thai language**	**Translate in English**
Questions	3.5 บริเวณที่อยู่อาศัยของท่านมีคนสูบบุหรี่บ้างหรือไม่ (ตอบได้มากกว่า 1 ข้อ)	3.5 Does your residential area have people who smoke? (You can choose more than one option.)
Options	ไม่มีเลย	None.
	มี ได้แก่ เพื่อนบ้าน/คนข้างบ้าน	Yes, it includes neighbours/people next door.
	มี ได้แก่ ตัวท่านเอง	Yes, it includes me.
	มี ได้แก่ คู่สมรส	Yes, it includes my spouse/partner.
	มี ได้แก่ ญาติ/คนอื่น ๆ ที่อาศัยในบ้าน	Yes, it includes relatives/other individuals residing in the household.
Remark: The system would permit selecting multiple options if the response is not "None".		

3.13.1.2.2 Measurement of urinary nicotine and its metabolites

Liquid chromatography and mass spectrometry (Q Exactive™ Plus Hybrid Quadrupole-Orbitrap™ Mass Spectrometer) was conducted to measure cotinine, nicotine, and 3-hydroxycotinine. The details of the measurement method are in **Appendix 5**. However, since most previous studies employed urine cotinine to indicate ETS, only cotinine was used to interpret the ETS exposure in this study (**Table 3.13-2**). Urine concentration may affect the cotinine level; therefore, urinary creatinine concentration was measured to adjust urine dilution's effect by calorimetric methods according to the company protocol (Sigma-Aldrich®,

MAK080 creatinine assay kit) as shown in **Appendix 5**. A cotinine-creatinine ratio (CCR) of 30 ng/mg creatinine was used to identify ETS [388].

Table 3.13-2: Biomarkers of tobacco exposure (urine cotinine was used to indicate ETS in this study)

Biomarker	Precursor	Specimen	$t_{½}$	Tobacco specific	Other sources
Nicotine*	Nicotine	Blood, urine, saliva, hair	1–2 h	Yes	Nicotine replacement products
Cotinine*	Nicotine	Blood, urine, saliva, hair	16–18 h	Yes	Nicotine replacement products
Anatabine*	Anatabine	Urine	10–16 h	Yes	None
NNAL, NNAL-glucuronides	NNK (TSNA)	Blood, urine	6 weeks	Yes	None
Exhaled CO	Carbon monoxide	Exhaled air	2–6 h	No	Traffic, body formation
Carboxyhemoglobin	carbon monoxide	Blood	4–6 h	No	Traffic, body formation
1-Hydroxypyrene and other polycyclic aromatic hydrocarbon (PAH) metabolites	PAHs	Urine	20 h	No	Traffic, grilled meat, occupation, biomass combustion in homes
Mercopturic acid metabolites	1,3-Butadiene	Urine	–	No	Traffic, combustion products
Mercopturic acid metabolites	Acrolein	Urine	–	No	Traffic, combustion products
Acetonitrile	Acetonitrile	Urine, blood, exhaled air	32 h	No	None
***S*-Phenyl-mercapturic acid**	Benzene	Urine	9 h	No	Traffic, combustion products
Thiocyanate	Hydrogen cyanide	Serum, saliva, urine	7–14 days	No	Diet

NNAL 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNAL-gluc 4-(methylnitrosamine)-1-(3-pyridyl)-1-butanol glucuronide; NNK 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; TSNA tobacco-specific nitrosamines; PAH polycyclic aromatic hydrocarbons
*In smoking cessation studies, anatabine is recommended as nicotine replacement therapy, leading to nicotine and cotinine without tobacco exposure.

3.13.1.2.3 Urinary creatine for cotinine adjustment

The urinary creatinine was measured in all available urine samples to adjust cotinine levels in pregnant women and children to diminish the effect of physiological changes of pregnancy and children on urinary clearance. Creatinine in random urine collection was analysed by a coupled

enzyme reaction, colourimetric detection method with the creatinine assay kit (MAK080, Sigma-Aldrich, Merck), according to the manufacturer's protocol with method validation before analysing all urine samples. The method validation is explained and discussed in **Appendix 5**.

3.13.1.3 The 24-hour $PM_{2.5}$ concentration

The spatiotemporal analysis was conducted to assess both the cumulative concentration and average concentration of $PM_{2.5}$ exposure at participants' residences over specific timeframes and durations, such as each trimester of pregnancy and every three months of infant age.

For pregnant participants, the average daily ambient $PM_{2.5}$ concentration were calculated and adjusted based on the day of pregnancy, using the last menstrual period (LMP) date as a reference. Similarly, for infant participants, the average daily ambient $PM_{2.5}$ concentration were calculated and adjusted using their date of birth as the reference point.

$PM_{2.5}$ exposure during pregnancy was calculated from the first day of pregnancy to the date of birth of the offspring. For children, $PM_{2.5}$ exposure was assessed from birth until 6 months of age. Based on the hypothesis that different exposure periods may influence allergic outcomes in distinct ways, $PM_{2.5}$ exposure was analysed by trimester during pregnancy and at two-month intervals during childhood.

Interpolation

Ambient $PM_{2.5}$ at participants' residences across the study period was estimated by GIS spatiotemporal analysis. The interpolation method was applied regarding GIS analysis's data availability and tool performance.

The general form of the interpolation is defined as

$$\hat{Z}(x_0) = \sum_{i=1}^{N} \lambda_i Z(x_i),$$

where $\hat{Z}(x_0)$ is an unknown value at an un-measured point x_0, $Z(x_i)$. A set of measured values which contains N measurements at location $i =$, and λ_i is the interpolation weight. The calculation of interpolation weight depends on the interpolation method used.

For IDW, interpolation weights can be determined using.

$$\lambda_i = \frac{(1/d_i^P)}{\sum_{i=1}^{N}(1/d_i^P)},$$

where d_i is the distance between the measured point and the predicted point, and p is the distance exponent power. In this interpolation, weights depend on the distance between the measured and predicted points. The measured points closer to the predicted points are assigned a heavier weight than the farther measured points.

For OK, the interpolation weight is determined by

$$\sum_{i=1}^{n} \lambda_i \gamma(x_i - x_j) + \varphi(x_0) = \gamma(x_j - x_0),$$

where $\gamma(x_i - x_j)$ is semivariance between i^{th} and j^{th} sampling points, $\gamma(x_j - x_0)$ is semivariance between measured or sampling point x_i and predicted point x_0, and $\varphi(x_0)$ is a Lagrange multiplier. The two semivariances are derived from the variogram model. The variogram model must be chosen to fit the experimental variogram as much as possible. The combination of all kriging weights has to be constrained so that

$$\sum_{i=1}^{N} \lambda_i = 1$$

to guarantee unbiased estimation of the Kriging method.

The interpolation technique used in this thesis was chosen between IDW and OK. The selection of the interpolation method used the performance test that was applied to the three datasets (January 2018, January 2019, and January 2020). Performance tests were done by the cross-validation method to determine minimum, maximum, and root mean square error (RMSE) values and compared with the actual $PM_{2.5}$ data from ground monitoring stations. The interpolation in method selection and prediction of the entire dataset steps were performed in the QGIS Geographic Information System version 3.34.9 (Open-Source Geospatial Foundation Project. http://qgis.org).

3.13.2 Outcome assessment

The primary outcomes investigated in this DPhil thesis encompass a range of elements. These include allergic diseases, namely AD and food allergy, and early wheezing. Additionally, the study explored allergic sensitisation, as determined by both total IgE and sIgE levels directed towards common allergens. Lastly, the levels of cytokines were also a critical aspect of the investigation. Further explanations for each of these components are provided in the subsequent sections.

3.13.2.1 Allergic diseases

This DPhil study focused on the incidence of allergic diseases in infant children, including AD, food allergy, and early wheezing, commonly observed in this age group. The diagnoses were made by specialists such as Paediatric allergists, Paediatric dermatologists, and Paediatricians when deemed appropriate. Infant participants were monitored through structured interviews conducted during each visit, which occurred every few months. Additional assessments took place when clinical signs of allergic diseases were suspected. The Paediatricians carefully documented the responses and diagnoses within the online clinical record form of the original pilot prospective study. The comprehensive records captured details such as suspected symptoms, potentially allergenic foods (in cases of suspected FA) and causes of wheezing. The criteria for diagnosing food allergy and AD followed established standard practices in this DPhil study.

3.13.2.2 Allergic sensitisation

Allergic sensitisation is when an individual's immune system becomes hypersensitive to a specific allergen, producing allergen-specific antibodies, particularly IgE. In this study, the measurement of both total IgE and sIgE levels directed towards common allergens was conducted by widely recognised definitions and practices. Elevated IgE levels have been associated with an increased risk of allergy development (atopy) [389]. However, it is important to note that other factors, such as parasitic infections, myeloma, and some primary immune deficiencies, can also have IgE levels elevation. However, these elevations are not specific and should be interpreted cautiously, as they can be attributed to various underlying causes [390]. Specific IgE to allergens seems to have a more pronounced association with allergic diseases [391]. The correlation between total IgE and sIgE is presented in **Table 3.13-3** [390].

Table 3.13-3: Associations between Total IgE and specific IgE measurements.

	Normal total IgE level	**Elevated total IgE level**
Absent sIgE	Non-allergic patient	Non-allergic elevated IgE conditions
Present sIgE	Patients with sensitisation(s)	Patients with sensitisation(s)
Abbreviation: sIgE, specific IgE		

3.13.2.2.1 Measurement of specific IgE (sIgE) to a common allergen in serum/plasma of infant children

The presence of sIgE to common allergens was investigated by measuring the sIgE to a common allergen in the infant group, which were the food allergens: cow's milk, hen's egg (egg white), fish, peanut, wheat, and soybean [392]. Similarly to ImmunoCap™100, ImmunoCAP™ Phadiatop™ Infant (Phadiatop™ Infant, Pharmacia Diagnostics AB, Uppsala, Sweden) was selected for use in this study due to its practicality and established acceptability as a method to detect common food allergens. In clinical practice, using Phadiatop™ Infant could be recommended as an additional tool to enhance the clinical evaluation for distinguishing IgE-mediated diseases among young children presenting symptoms akin to allergies [393, 394]. However, it is also employed to determine allergic sensitisation, a biomarker indicating the risk of developing allergic diseases [391].

The Results were reported as Pharmacia Arbitrary Units per litre (PAU/L), indicating the degree of sensitisation. The specific IgE levels were classified into seven classes, as shown in **Table 3.13-4**. The cut-off value of sensitisation was ≥ 0.35 PAU/L and coded as positive in all dichotomous analyses.

Table 3.13-4: Classification of sIgE levels by ImmunoCAP™ Phadiatop™ and Phadiatop™ Infant

Specific IgE levels (PAU/L)	Classification	Sensitisation
< 0.35	Class 0	Negative
0.35 – 0.69	Class 1	Positive
0.70 – 3.49	Class 2	
3.5 – 17.49	Class 3	
17.5 – 49.99	Class 4	
50 – 99.99	Class 5	
≥ 100	Class 6	

3.13.2.3 Plasma total IgE in infant children

Total IgE levels were assessed in cord serum/plasma at 6-12 months of age and when there were suspicions of allergic diseases before 12 months. The measurement was conducted using the automated fluorescence enzyme immunoassay with the ImmunoCAP™ 100 (Thermo Fisher Scientific, Phadia, GmbH, Freiburg, Germany), adhering to the manufacturer's protocol. This comprehensive quantitative test offers precise measurements of circulating IgE levels across a broad measuring range spanning from 2 to 5000 kU/L. It helps identify allergic sensitisation and the potential presence of allergic conditions. It is also in line with clinical practices at Ramathibodi Hospital and is recognised as a standard procedure for measuring total IgE levels.

In brief, for IgE analysis, stored plasma/serum was thawed at 2-8 °C overnight. Approximately 40 μL of plasma/serum was required for each analysis. The analyses were conducted at the Department of Toxicology, Ramathibodi Hospital, Mahidol University, Thailand. The laboratory personnel conducting the analyses were blinded to the participant's status. The results were stored in the online database system of the pilot prospective study and then retrieved for statistical analysis. The cut-off value of plasma/serum IgE levels as a clinical diagnostic indicator of atopic sensitisation is age-dependent, as indicated in **Table 3.13-5**.

Table 3.13-5: Cut-off value of plasma/serum total IgE levels

Population	The cut-off value of plasma/serum total IgE levels (kU/l)		References
Pregnant women	< 100 100 - < 150 ≥ 150	normal high very high	[395, 396]
Children at birth	< 2 ≥ 2	normal high	+1SD Y20ImmunoCAP™ Total IgE Directions for Use 52-5292-EN/08
Infant children (≤ 12 months)	< 10 ≥ 10	normal high	[397] +1SD Y20ImmunoCAP™ Total IgE Directions for Use 52-5292-EN/08

3.13.2.4 Cord plasma/serum IgA

Cord plasma/serum was measured to exclude maternal blood contamination using an ELISA for quantitative detection of human IgA (IgA Human Uncoated ELISA Kit, Invitrogen®, Austria) as the company protocol. The details are in **Appendix 5**. The concentrations exceeding 10 mg/ml indicated maternal blood contamination and would be excluded from the cord blood analysis for IgE and sIgE [398].

3.13.2.5 Cytokine concentrations

Plasma/serum samples obtained from infant children aged 6-12 months were used to measure the proinflammatory and allergic cytokines. The selection of specific cytokines was based on their detection in human plasma and their relevance to the atopic disease outcomes investigated in this research, including AD, FA, and early wheezing. The evaluation of cytokine levels encompassed Th1, Th2, Th17, Treg, and TSLP-related cytokines and was performed using the BD™ Cytometric Bead Array (BD™ CBA) and ELISA (abcam®) methods. An overview of representative Th1, Th2, Th17, Treg, and proinflammatory cytokines was demonstrated in **Table 3.13-6**. The Cytometric Bead Array (CBA) method was chosen for its ability to conduct multiple analyses on a single sample, minimal sample volume requirement, rapid processing

of multiple samples on a single platform, and results comparable to well-established methods like ELISA [399]. In addition to their detection performance, accessibility within Ramathibodi Hospital is also considered.

For specific cytokines such as IL-21, IL-22, IL-33, and TSLP, which were not available for measurement using the CBA technique, ELISA (abcam®) was utilised following the manufacturer's protocol. The sensitivity of each cytokine analysis was also documented in **Table 3.13-6**. In cases where the measured concentration was lower than the sensitivity limit (LOD) of the kits, the value was replaced by LOD/$\sqrt{2}$. Samples were stored at -80°C until analysis and thawed overnight at 2-8°C. Given the limited sample volumes, a two-fold dilution was applied to all samples before analysis.

Table 3.13-6: Targeted plasma/serum cytokines to measure in this study.

Cytokine	Representative	Explanation	Test methods	Catalogue number	Limit of detection limit or assay range	References
IL-1*β*	Proinflammatory	Principal mediators of the inflammatory response	BD™ CBA Human IL-1β Flex Set	558279	10-2,500 pg/ml	[400, 401]
IL-2	Th1/Th2/Th17	Non-specific T cell cytokine	BD™ CBA Th1/Th2/Th17 kit	560484	2.6 pg/ml	
IL-4	Th1/Th2/Th17 Anti-inflammatory	Th2 polarisation, AD-related cytokine	BD™ CBA Th1/Th2/Th17 kit	560484	4.9 pg/ml	[401, 402]
IL-5	Th2	Th2 polarisation, AD-related cytokine	BD™ CBA Human IL-5 Flex Set	558278	10-2,500 pg/ml	[402]
IL-6	Th1/Th2/Th17, proinflammatory	Potent inducer of the acute-phase response, as well as specific cellular and humoral immune responses, including B cell differentiation and T cell activation, including T helper 17 (TH17) cells	BD™ CBA Th1/Th2/Th17 kit	560484	2.4 pg/ml	[401]
IL-8 (CXCL8)	Proinflammatory	Proinflammatory chemokine and cytokine play a role in immune responses and inflammation. It is produced by macrophages, neutrophils, and endothelial cells in response to infection or tissue damage. IL-8 functions as a chemoattractant, recruiting immune cells, particularly neutrophils, to sites of inflammation. It also contributes to the activation of immune cells and the regulation of immune responses.	BD™ CBA Human IL-8 Flex Set	558277	10-2,500 pg/ml	[401]
IL-10	Tregs, anti-inflammatory	Anti-inflammatory cytokine that controls immune responses by suppressing inflammation and regulating immune cell activation.	BD™ CBA Th1/Th2/Th17 kit	560484	4.5 pg/ml	[401, 403, 404]
IL-12/IL-23p40	Proinflammatory	Linking innate and adaptive immune responses, produced by phagocytic cells, induces the production of IFN-γ and regulates Th1, type 1 inflammatory responses.	BD™ CBA	560154	40-10,000 pg/ml	[401]
IL-13	Th2 Anti-inflammatory	Th2 polarisation, AD-related cytokine	BD™ CBA Human IL-13 Flex Set	558450	10-2,500 pg/ml	[401, 402]

IL-22	Th22 and **AD-related cytokine**	IL-22, a cytokine linked to Atopic Dermatitis (AD), is involved in inflammation responses via the Jak/STAT and MAPK signalling pathways. IL-22 is generated by NK cells and Th1-type T cells, triggering pro-inflammatory cytokine production in human keratinocytes.	ELISA, Human IL-22 SimpleStep ELISA® Kit	ab216170	Sensitivity: 2.61 pg/ml Range: 13.12 pg/ml - 840 pg/ml	[405]
IL-31	Th2 polarisation, **AD-related cytokine**	IL-31 triggers STAT3 activation and possibly STAT1 and STAT5 via the IL-31 receptor of IL31RA and OSMR. IL-31's role might extend to skin immunity.	ELISA, Human IL-31 SimpleStep ELISA® Kit	ab235637	Sensitivity: 1.5 pg/ml Range: 117.2 pg/ml - 7500 pg/ml	[402]
IL-33	Proinflammatory, **food allergy-related cytokine**	IL-33, a proinflammatory cytokine belonging to the IL-1 family, has implications for priming subsequent allergic responses and enhancing both mucosal and systemic immune reactions. It exerts its effects by binding and signalling through the IL-1RL1/ST2 receptor. IL-33 is produced as a pro-protein and is widely expressed in various tissues.	ELISA, Human IL-33 SimpleStep ELISA® Kit	ab223865	Sensitivity: 4.7 pg/ml Range: 11.72 pg/ml - 750 pg/ml	[401, 406]
TNF	Th1/Th2/Th17, Inflammatory	Principal mediators of the inflammatory response have a critical role in orchestrating the local inflammatory response through cell activation and initiation of a cytokine cascade.	BD™ CBA Th1/Th2/Th17 kit	560484	3.8 pg/ml	[401]
IFN-γ	Th1/Th2/Th17, Inflammatory	Critical mediator of T cell recruitment and phagocyte-mediated clearance of pathogens	BD™ CBA Th1/Th2/Th17 kit	560484	3.7 pg/ml	[401]
IL-17A	Th17	Th17-associated molecules, AD-related cytokine	BD™ CBA Th1/Th2/Th17 kit	560484	18.9 pg/ml	[402]
TSLP	Epithelial cytokine, f**ood allergy-related cytokine**,		ELISA, –TSLP Human SimpleStep ELISA® Kit	ab192149	Sensitivity: 3 pg/ml Range: 15.6 pg/ml - 1000 pg/ml	[407]

Abbreviations: AD, atopic dermatitis; CBA, Cytometric Bead Array; ELISA, enzyme-linked immunosorbent assay; IFN-γ, interferon-gamma, IL, Interleukin; OSMR, Oncostatin M Receptor; STAT, Signal Transducer and Activator of Transcription; TSLP, Thymic stromal lymphopoietin; Tregs, regulatory T cell;

3.13.3 Confounding assessment

Several potential confounding factors were carefully considered in this study, which aimed to investigate the association between early-life environmental exposures and allergic outcomes in children. Confounding factors could independently influence both the exposure and the outcome, leading to misinterpreting the observed association. The following potential confounders were addressed to ensure the validity of our findings:

3.13.3.1 Socioeconomic status (SES)

Socioeconomic factors, such as family income, education, and occupation, can impact environmental exposures and health outcomes. The relevant information on participants' socioeconomic backgrounds was included in the analysis to control for potential effects.

3.13.3.2 Family history of allergies

Family history of allergies, especially among first-degree relatives (father, mother, and siblings), has been linked to an increased risk of allergic outcomes in children. Data on maternal and paternal allergic histories were gathered through the SAQ to address genetic predisposition. Additionally, the allergic sensitisation of pregnant women was assessed by measuring total IgE levels in the first and third trimesters and sIgE levels in the first trimester. These measures aimed to account for potential confounding factors related to genetic susceptibility.

3.13.3.3 Plasma total IgE in pregnant women

The total IgE in plasma of pregnant women in first and third trimester ELISA measured pregnant participants with the IgE Human Uncoated ELISA Kit, Invitrogen® (Thermo Fisher

Scientific) adapted based on the manufacturer's protocol and protocol optimisation prior to actual analysis (detailed in **Appendix 5**). The analytical range is 7.8-500 ng/ml, with a sensitivity of 7.8 ng/ml (3.25 KU/L).

3.13.3.4 Plasma sIgE in pregnant women (sensitisation)

In first-trimester pregnant women, plasma sIgE to common aeroallergens was assessed using ImmunoCAP™ Phadiatop™ (Phadiatop, Pharmacia Diagnostics AB, Uppsala, Sweden). This test includes sIgE measurement to a mixture of representative aeroallergens, including grasses (Timothy), trees (birch), weeds (ragweed), cats, dogs, house dust mites (Dermatophagoides), and moulds. The amount of plasma/serum required for this measurement was 50 μL. As mentioned in **Appendix 5**, the sample preparation, measuring methods, result reporting, and interpretation were consistent with the ImmunoCAP™ Phadiatop®.

3.13.3.5 Breastfeeding

Breastfeeding and early-life nutrition can impact immune system development and allergic outcomes. Since breastfeeding can decrease risk of allergic diseases, sensitisation, and elevated total IgE in children [408]. Therefore, data on breastfeeding considered potential confounders practices were collected via SAQ and were included for analysis.

3.14 Statistical analysis

Data analysis was performed using Microsoft Excel, the Statistical Products and Service Solutions package for Windows (version 29.0.2.0) (SPSS, Chicago, IL).

3.14.1 Descriptive

A descriptive statistical analysis was conducted to summarise the data. For normally distributed data, means and standard deviations (SD) were utilised, whereas skewed data were summarised using medians and interquartile ranges (IQR) or min-max. Categorical data were summarised through frequency counts and percentages.

3.14.2 Baseline comparisons

Depending on the distribution, continuous data were compared between significant groups using either a t-test or the Mann-Whitney test when applicable. The associations between categorical variables were assessed using Chi-squared tests or Fisher's exact test. For evaluating linear relationships between continuous/ordinal variables, Spearman's correlation coefficients were applied due to the non-normal distribution of data in this DPhil study. A two-sided 95% confidence interval (CI) was calculated and reported for the endpoint parameter for each allergic outcomes (allergic diseases, sensitisation, and elevated total IgE concentration in infant children). Log-transformations were applied to address variables with non-normal distributions, however, if the data still cannot correct to normal distribution after logarithm procedure were done, the original data would apply by using nonparametric statistical test to explore any statistically significant.

3.14.3 Interpolation of $PM_{2.5}$

The concentration of ambient $PM_{2.5}$ at participant's residence were estimated from actual address, adjusted by the date of Estimated date of confinement (EDC) for pregnant women (pregnancy exposure) and children's birth date (children exposure). The average 24-hour $PM_{2.5}$ concentration by trimester and every 2 months of children age were grouping. Additionally,

accumulative 24-hour $PM_{2.5}$ for each trimester, and every 2 months of children age were also compared between children with and without allergy groups. The association analysis of $PM_{2.5}$ exposure and allergic outcomes were done by using $PM_{2.5}$ concentration in continuous and categorised data at ≥ 25 $\mu g/m^3$ were performed to examine the association of $PM_{2.5}$ exposure in early life (*in utero* and first 6 months of infancy period). The endpoint of $PM_{2.5}$ association was 6 months of children due to the average allergic diseases' occurrence around this age and minimum requirement to follow up and included in this DPhil analysis.

3.14.4 Association of environmental exposure with outcomes

3.14.4.1 Univariate analysis

In the initial phase of univariate analysis, a significance level of 0.10 (α) was adopted to identify potential associations for further exploration in the multivariate analysis. This threshold was chosen to mitigate the risk of overlooking potentially meaningful associations, particularly given the relatively small sample size, which might limit the statistical power to detect true effects. Using binary outcomes (e.g., presence or absence of allergic diseases, sensitisation, and elevated total IgE concentrations at 10 kU/L in children aged 6–12 months), binary logistic regression models were employed to examine the associations between the potential variables and each allergic outcome.

3.14.4.2 Multivariable analysis

A multivariable logistic regression model was applied to investigate the relationships between multiple early-life environmental exposures (*in utero* and early infancy) and allergic outcomes, accounting for potential confounders. This analysis was conducted in three sequential steps:

Model 1: All variables that yield $P \leq 0.1$ from the univariate analysis for each outcome were included in the multivariable logistic regression model. To avoid collinearity, variable correlations within similar groups, such as CCR and household ETS, were assessed if indicated, and a representative factor was selected instead of including all related factors in the analysis. The forward likelihood ratio (LR) test was used for variable entering. This approach was chosen due to the small sample size, which necessitated a robust method capable of handling numerous independent variables while maintaining model stability and fit.

Model 2: Variables retained in Model 1 were reanalysed using the enter method for variable entering. This approach aimed to enhance statistical power by recovering samples excluded during the forward LR procedure, ensuring a more comprehensive analysis.

Model 3: To address imbalances in outcome prevalence across data collection sites, a multilevel multivariable logistic regression model was applied. This model included site of data collection as a random effect to account for clustering. Additionally, the sequential Bonferroni correction was applied to minimise the risk of false positives due to multiple comparisons. Statistical significance was determined by a P-value ≤ 0.05, accompanied by 95% confidence intervals that did not include 1.

This sequential modelling approach ensures a robust exploration of early-life environmental exposures and their associations with allergic outcomes while accounting for confounding factors, clustering effects, and issues related to multiple comparisons.

3.15 Conclusion

This chapter describes the study design for this DPhil research, drawing on key insights from the Introduction (**Chapter 1**) and Literature Review (**Chapter 2**) while considering practical and feasible approaches within the Thai context. It aims to optimise the research process while ensuring the study effectively addresses the thesis question. Additionally, this chapter covers essential ethical considerations and the procedures for handling biological samples, ensuring compliance with research standards and ethical guidelines.

Chapter 4: Overview results of the birth cohort study

This chapter presents the outcomes of the cohort's comprehensive clinical assessments, including the overview of the number of participants during the enrolment process, participants' characteristics, lifestyle, environmental exposures, and incidence of allergic diseases in infant children. The assessments were conducted on pregnant women and their infant children, focusing on elucidating potential associations between various exposures and allergic outcomes. It provided the foundation for the subsequent analyses, enabling the investigation of potential associations between exposures, biomarkers, and allergic outcomes in later chapters. By presenting the detailed clinical assessment results, this chapter contributes to a comprehensive understanding of the complex dynamics between environmental exposures and allergic outcomes within the cohort.

4.1 Enrolment process: participant overview

This section provides an overview of the participant numbers and their progression throughout the enrolment process. The cohort study had gradually enrolled participants since 6 June 2018 in Ramathibodi Hospital and a month later in Rayong Hospital and Samut Prakan Hospital, and it was expected to finish the enrolment within six months. However, the recruiting rate was slow due to the inclusion criteria to limit the age of pregnant women between 18-34 years old. Therefore, the age of pregnant women was extended to ≥ 18 years old with no upper limit. In addition, the enrolment for this project was finished in August 2019 (**Figure 4.1-1**). Over the 14-month enrolment period, there were 203 pregnant participants comprising 151, 49, and 3 from Ramathibodi, Rayong, and Samut Prakan Hospitals, respectively (**Figure 4.1-2**). Of these enrolled participants, 55 discontinued or withdrew from the study before delivery (40 were ineligible, 7 withdrew consent, 5 lost to follow-up, and 3 significant protocol deviations).

Ineligible women after enrolment occurred from the overlooked at screening e.g. GA > 14 weeks at enrolment date (2 pregnant women), serology positive for HIV (2 pregnant women), and change the ANC hospital (36 pregnant women). All retained participants finished their pregnancy by either miscarriage (n = 17), termination of pregnancy due to medical reasons (n = 6) or delivery (n = 125). Among delivered newborns, there were four pairs of twins, and 129 newborn infants were enrolled in the infancy period of this study.

Among all delivered newborn infants, 39 were discontinued from the study after delivery or during the infancy period, and two of them died during the perinatal period, one from a complication of Trisomy-13 (Ramathibodi Hospital) and one from meconium aspiration syndrome with respiratory failure (Rayong Hospital). Ineligibility occurred as the infant/s migrated to other provinces and could not follow up per protocol (15 infants) and it is common in Thailand: parents still work in the city but the children are raised upcountry by grandparents. Parent of fourteen children included 2 pairs of twins withdrew consent to follow up before 6 months. The 2nd born twins were excluded from the DPhil study to minimise selection bias from same exposure. Therefore, eighty-six infants completed the study at 6-12 months of age and included in this DPhil study. The variation of enrolment across study period also observed (**Figure 4.1-1),** thus, the season of data collection and birth season were included in the analysis. The detailed enrolment and follow-up results are demonstrated in **Figure 4.1-2**.

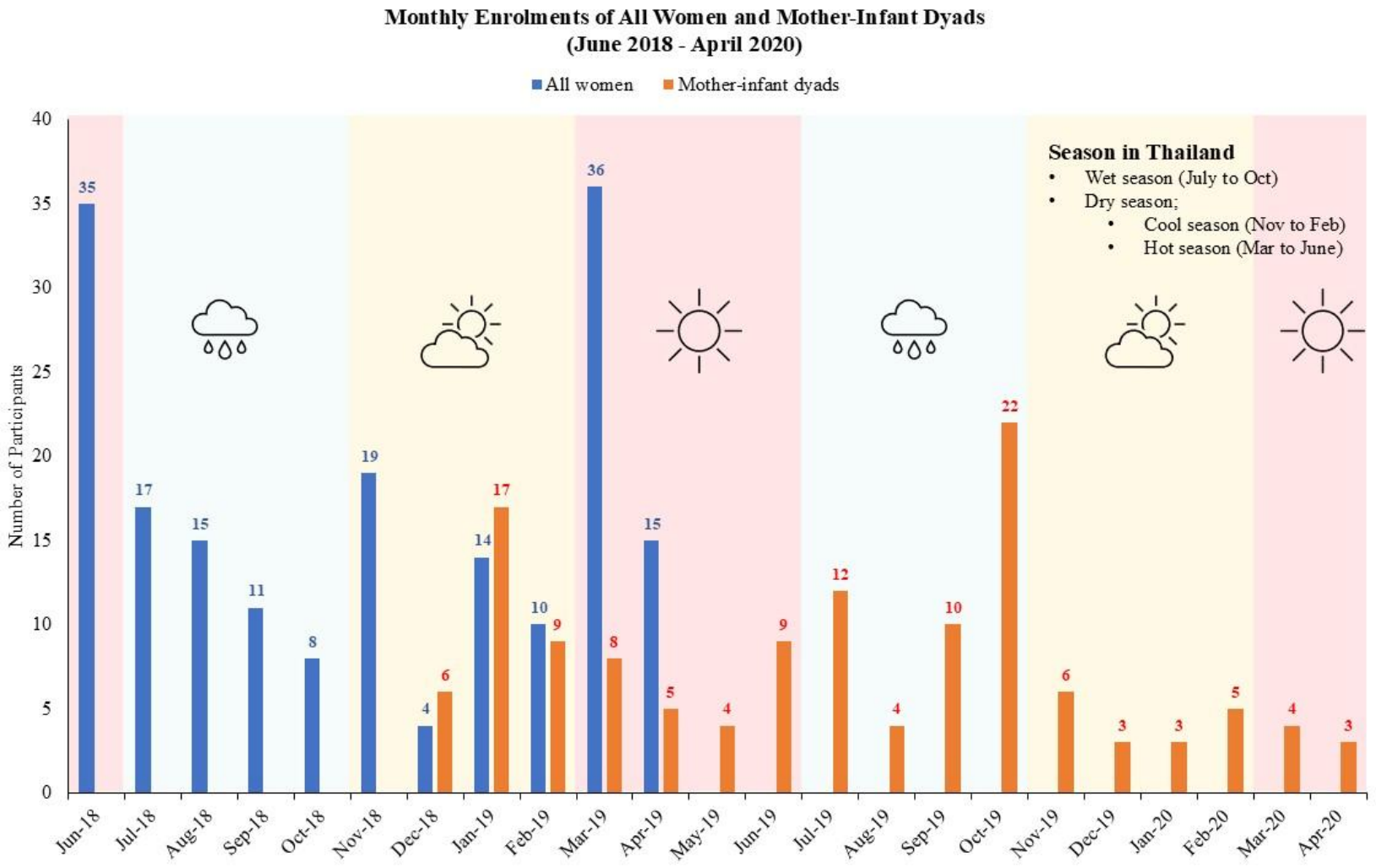


Figure 4.1-1: The enrolments for "All women" and "Mother-infant" dyads per month.

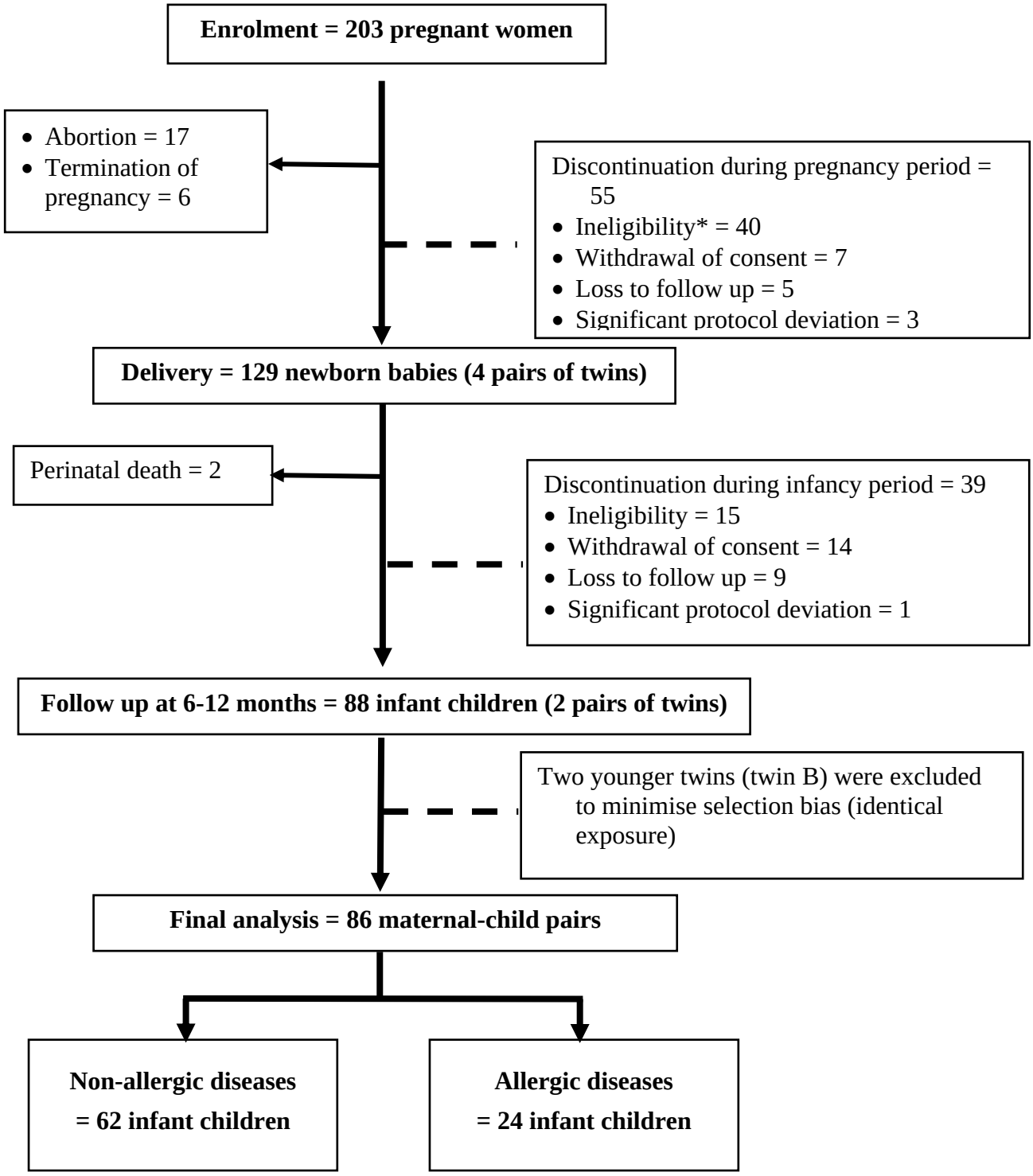


Figure 4.1-2: Flowchart of the study, *the ineligibility occurred by gestational age at enrolment > 14 weeks (by ultrasound), seropositive to HIV, unable to follow up before their child age 2 years for some reason.

4.2 Baseline characteristics of pregnant participants

The pregnant characteristics consist of 1) a comparison between the pregnant women who were included (Dyad) and were not included (Non-dyad) groups in this DPhil, 2) the lifetime prevalence of allergic diseases in pregnant participants, partners, and previous child/children and 3) a comparison between pregnant women whose their children develop allergic diseases (Allergy) and not develop allergic diseases (No allergy) groups. The "lifetime prevalence" in this thesis means the phase life to date of participants opt-in the study.

4.2.1 Pregnant participants comparing between dyads and non-dyads

The baseline characteristics of pregnant participants by comparing the dyads and non-dyad groups before were detailed in **Table 4.2-1** and **Table 4.2-2**.

Overall, the site of data collection, pregnant participant's education level, and pregnant participant's occupation were different between dyad and non-dyad groups as shown in **Table 4.2-1**. Other factors, including age at enrolment, marital status, gestational age at enrolment, gravida, family income, and number of family members, did not show statistically significant differences between the two groups. Furthermore, health status and allergic history also did not exhibit statistical significance **Table 4.2-2**.

Table 4.2-1: Demographic characteristics of pregnant participants at enrolment of the first trimester, comparing "Dyad" vs. "Non-dyad" group.

Variables; n (%)	Overall N = 203	Non-dyad N = 117	Dyad N = 86	*P*
Site				
Ramathibodi Hospital	151 (74.38)	75 (64.1)	76 (88.4)	**< 0.001**[$]
Rayong Hospital	49 (24.14)	39 (33.3)	10 (11.6)	
Samut Prakan Hospital	3 (1.48)	3 (2.6)	0 (0.00)	
Pregnancy age at enrolment				
18 - < 35 years	183 (90.15)	107 (91.5)	76 (88.4)	0.467[#]
≥ 35 years	20 (9.85)	10 (8.5)	10 (11.6)	
Marital status				
Married	191 (94.09)	107 (91.5)	84 (97.7)	0.063[#]
Divorce/ widowed	12 (5.91)	10 (8.5)	2 (2.3)	
Mean gestational age at enrolment (SD); weeks	9.5 (2.93)	9.29 (3.5)	9.91 (1.9)	0.142
Gravida (G)				
G1 (primigravida)	93 (45.8)	56 (47.9)	37 (43.0)	0.260[#]
G2	69 (34.0)	42 (35.9)	27 (31.4)	
≥ **G3**	41 (20.2)	19 (16.10)	22 (25.6)	

Variables; n (%)	Overall N = 203	Non-dyad N = 117	Dyad N = 86	*P*
Household monthly income (Thai Baht)				
≥ 20,000	61 (30.50)	40 (35.1)	21 (25.6)	0.157[#]
< 20,000	135 (66.5)	74 (64.9)	61 (74.4)	
Median (Min-Max)	30,000 (1,500 – 500,000)	30,000 (1,500 – 500,000)	30,000 (5,000 – 150,000)	0.874[ł]
Number of family member (excluding expected child/ children)				
< 4	109 (53.7)	64 (54.7)	45 (52.3)	0.737[#]
≥ 4	94 (46.3)	53 (45.3)	41 (47.7)	
Median (Min-Max)	3 (2 – 10)	3 (2 – 10)	3 (2 – 10)	0.804[ł]
Pregnant participant education				
Below undergrad	104 (51.2)	69 (59.0)	35 (40.7)	**0.036**[##]
Undergrad	77 (37.9)	37 (31.6)	40 (46.5)	
Graduate	22 (10.8)	11 (9.4)	11 (12.8)	
Pregnant participant occupation				
Unemployed, housewife, student, freelancer	68 (33.5)	49 (41.9)	19 (22.1)	**0.003**[##]
Medical personal, industry, hairdresser	13 (6.4)	4 (3.4)	9 (10.5)	
Teacher, officers or employees either in government or private sector	92 (45.3)	44 (37.6)	48 (55.8)	
Merchant, personal business	30 (14.8)	20 (17.1)	10 (11.6)	

[#]Pearson Chi-square
[$]Fisher's Exact Test
[ł]Median test

Table 4.2-2: Background of health, atopic status, and medication use at enrolment (1st trimester of pregnant women), comparing "Non-dyad" vs. "Dyad" groups.

Variables; n (%)	Overall N = 203	Non-dyad N = 117	Dyad N = 86	*P*
Underlying disease of pregnant participants				
No underlying disease	134 (66.0)	79 (32.5)	55 (64.0)	0.596#
Common allergic diseases	28 (13.8)	14 (12.0)	14 (16.3)	0.379#
Allergic rhinitis	25 (12.3)	12 (10.3)	13 (15.1)	0.298#
Asthma	6 (3.0)	3 (2.6)	3 (3.5)	0.700$
Atopic dermatitis	3 (1.5)	1 (0.9)	2 (2.3)	0.575$
Food allergy	2 (1.0)	2 (1.7)	0 (0.00)	0.509$
Diabetes Mellitus (DM) type 2	2 (1.0)	1 (0.9)	1 (1.2)	1.000$
Hypertension	0 (0.00)	0 (0.00)	0 (0.00)	--
Other diseases	24 (11.8)	17 (14.5)	7 (8.1)	0.163#
Underlying disease of pregnant participants' spouse				
No underlying disease	168 (82.8)	96 (82.1)	72 (83.7)	0.#756#
Common allergic diseases	19 (9.4)	168 (6.8)	11 (12.8)	0.150#
Allergic rhinitis	15 (7.4)	7 (6.0)	8 (9.3)	0.372#
Asthma	1 (0.5)	1 (0.9)	0 (0.0)	1.000$
Atopic dermatitis	3 (1.5)	0 (0.0)	03 (3.5)	0.074$
Food allergy	3 (1.5)	1 (0.9)	2 (2.3)	0.575$
Atopic history of participant's previous child/children				
No underlying disease or no previous child	118 (58.1)	70 (59.8)	48 (55.8)	0.567#
Common allergic diseases	7 (3.4)	4 (3.4)	3 (3.5)	1.000$
Allergic rhinitis	2 (1.0)	1 (0.9)	1 (1.2)	1.000$
Asthma	2 (1.0)	1 (0.9)	1 (1.2)	1.000$
Atopic dermatitis	2 (1.0)	1 (0.9)	1 (1.2)	1.000$
Food allergy	2 (1.0)	1 (0.9)	1 (1.2)	1.000$
Medication taken within three months before enrolment				
No medication/ vitamin taking	149 (73.4)	89 (76.1)	60 (69.8)	0.315#
Antihistamine	17 (8.4)	10 (8.5)	7 (8.1)	0.918#

Variables; n (%)	Overall N = 203	Non-dyad N = 117	Dyad N = 86	*P*
Vitamin	16 (7.9)	9 (7.7)	47 (8.1)	0.907[#]
Folate	23 (11.3)	12 (10.3)	11 (12.8)	0.573[#]
Others[*]	10 (4.9)	5 (4.3)	5 (5.8)	0.746[$]
Exercise activity within the past three months				
Never	89 (43.8)	53 (45.3)	36 (41.9)	0.590[#]
Sometimes (<1-3 times a month)	91 (44.8)	53 (45.3)	38 (44.1)	
Regularly (≥1-3 times a week)	23 (11.3)	11 (9.4)	512 (14.0)	

*Other medication intake included paracetamol, herb, vitamin C, multivitamin, docosahexaenoic acid (DHA), and green tea tablet
[#]Pearson Chi-square
[#]Wilcoxon Mann-Whitney U test
[$]Fisher's Exact Test
[#]Pearson Chi^2

4.2.2 Lifetime prevalence of allergic diseases in pregnant participants, partners, and previous child/children

The lifetime prevalence; which is the life to date of the enrolment in this study, of allergic diseases assessed by SAQ in the first trimester (P01) was demonstrated in **Table 4.2-2**. Among the pregnant women, 13.8% reported having common allergic diseases, while 9.4% of partners and 3.4% of previous children had similar conditions. The specific allergic diseases included AR (12.3% among pregnant women, 7.4% among partners, and 3.4% among previous children), asthma (3.0% among pregnant women, 0.5% among partners, and 1.0% among previous children), atopic dermatitis (1.5% among pregnant women, 1.5% among partners, and 1.0% among previous children), and food allergy (1.0% among pregnant women, 1.5% among partners, and 1.0% among previous children).

4.2.3 Baseline characteristics comparing allergic vs non-allergic children

Comparison between allergic vs. non-allergic children, most of baseline characteristics of pregnant participants were not statistically different between both groups except site of data collection, mean body mass index (BMI) before pregnancy, gravida, pregnant participant's occupation, underlying diseases of sibling, and vitamin intake during 1st trimester of pregnancy (**Table 4.2-3**). The prevalence of children allergy was higher in Ramathibodi Hospital than in Rayong Hospital (31.6% vs 0%), therefore, the site adjustment was conducted in the final Model analysis. Preeclampsia has been reported to be associated with the development of asthma, eczema, and allergy development in children [409] but the data was not collected in this DPhil study, thus it can be considered a study limitation.

Table 4.2-3: Comparison of non-allergic infant (No allergy) vs allergic infant (Allergy) groups (n = 86)

variables	Total N = 86	No allergy N = 62	Allergy N = 24	*P*
Site				
Ramathibodi Hospital	76 (88.4)	52 (83.9)	24 (100.0)	**0.056$**
Rayong Hospital	10 (11.6)	10 (16.1)	0 (0.0)	
Maternal age at pregnancy				
< 35 years	76 (88.4)	55 (88.7)	21 (87.5)	1.000$
≥ 35 years	10 (11.6)	7 (11.3)	3 (12.5)	
Mean Body Mass Index (BMI) before pregnancy; kg/m^2	23.2 (4.1)	22.7 (3.8)	24.6 (4.7)	**0.067#**
Maternal marital status				
Married	84 (97.7)	60 (96.8)	24 (100.0)	1.000$
Prefer not to say	2 (2.3)	2 (3.2)	0 (0.0)	
Birth status (gestational age at birth)				
Preterm (GA < 37 weeks)	9 (10.5)	7 (11.3)	2 (8.3)	1.000$
Term (GA ≥ 37 weeks)	77 (89.5)	55 (88.7)	22 (91.7)	
Birth weight (gram)				
< 2,500 (low birth weight; LBW)	9 (10.7)	4 (6.7)	5 (20.8)	0.111$
≥ 2,500	75 (89.3)	56 (93.3)	19 (25.3)	
Weight/Length ratio (WLR) at birth; percentile				
< 10 (small for gestational age; (SGA)	12 (14.5)	6 (10.2)	6 (25.0)	0.096$
≥ 10	71 (85.5)	53 (89.8)	18 (75.0)	
Season of birth				
Wet (June – Oct)	43 (50.0)	33 (53.2)	10 (41.7)	0.568#
Cool (Nov– Feb)	32 (37.2)	21 (33.9)	11 (45.8)	
Hot (March – May)	11 (12.8)	8 (12.9)	3 (12.5)	
Gravida (G)				
G1	37 (43.0)	23 (37.1)	14 (58.3)	**0.057#**
G2	27 (31.40	19 (30.6)	8 (33.3)	
≥ G3	22 (25.6)	20 (32.3)	2 (8.3)	
Monthly family income (THB)				

variables	Total N = 86	No allergy N = 62	Allergy N = 24	*P*
≤ 20,000	21 (25.6)	17 (28.3)	4 (18.2)	0.351#
< 20,000	61 (74.4)	43 (71.7)	18 (81.8)	
Number of family members during pregnancy				
< 4	45 (52.3)	34 (54.8)	11 (45.8)	0.453#
≥ 4	41 (47.7)	28 (45.2)	13 (54.2)	
Pregnant participant education				
Below undergrad	35 (40.7)	26 (41.9)	9 (37.5)	0.917#
Undergrad	40 (46.5)	28 (45.2)	12 (50.0)	
Graduate	11 (12.8)	8 (12.9)	3 (12.5)	
Pregnant participant's occupation				
Unemployed, housewife, student, freelancer	19 (22.1)	18 (29.0)	1 (4.2)	**0.009#**
Medical personal, industry, hairdresser	9 (10.5)	3 (4.8)	6 (25.0)	
Teacher, officers or employees either in government or private sector	48 (55.8)	34 (54.8)	14 (58.3)	
Merchant, personal business	10 (11.6)	7 (11.3)	3 (12.5)	
Underlying diseases of pregnant participants				
No underlying disease	134 (66.0)	79 (67.5)	55 (64.0)	0.596#
Common allergic diseases	28 (13.8)	14 (12.0)	14 (16.3)	0.379#
Allergic rhinitis	25 (12.3)	12 (10.3)	13 (15.1)	0.298#
Asthma	8 (3.9)	5 (4.3)	3 (3.5)	1.000$
Atopic dermatitis	3 (1.5)	1 (0.9)	2 (2.3)	0.575$
Food allergy	2 (1.0)	2 (1.7)	0 (0.0)	0.509$
Diabetes Mellitus (DM) type 2	2 (1.0)	1 (0.9)	1 (1.2)	1.000$
Hypertension	0 (0.0)	0 (0.0)	0 (0.0)	-
Other diseases	21 (10.3)	14 (12.0)	7 (8.1)	0.376#
Underlying disease of pregnant participants' spouse				
No underlying disease	68 (79.1)	49 (79.0)	19 (79.2)	0.989#
Common allergic diseases	11 (12.8)	7 (11.3)	4 (16.7)	0.491$
Allergic rhinitis	8 (9.3)	6 (9.7)	2 (8.3)	1.000$
Asthma	1 (1.2)	0 (0.0)	1 (4.2)	0.279$

variables	Total N = 86	No allergy N = 62	Allergy N = 24	*P*
Atopic dermatitis	3 (3.5)	1 (1.6)	2 (8.3)	0.187[$]
Food allergy	2 (2.3)	1 (1.6)	1 (4.2)	0.483[$]
Underlying disease of participant's previous child/children				
No underlying disease or no previous living child	72 (83.7)	49 (79.0)	23 (95.8)	**0.100[$]**
Common allergic diseases	7 (3.4)	4 (3.4)	3 (3.5)	1.000[$]
Allergic rhinitis	1 (1.2)	1 (1.6)	0 (0.0)	1.000[$]
Asthma	1 (1.2)	0 (0.0)	1 (4.2)	0.279[$]
Atopic dermatitis	1 (1.2)	1 (1.6)	0 (0.0)	1.000[$]
Food allergy	1 (1.2)	1 (1.6)	0 (0.0)	1.000[$]
Medication taken within three months before enrolment				
No medication/ vitamin taking	60 (69.8)	45 (72.6)	15 (62.5)	0.361[#]
Antihistamine	7 (8.1)	5 (8.1)	2 (8.3)	1.000[$]
Vitamin	7 (8.1)	2 (3.2)	5 (20.8)	**0.017[$]**
Folate	11 (12.8)	8 (12.9)	3 (12.5)	1.000[$]
Others[*]	5 (5.8)	4 (6.5)	1 (4.2)	1.000[$]
Exercise activity within the past three months				
Never	36 (41.9)	24 (38.7)	12 (50.0)	0.515[#]
Sometimes (<1-3 times a month)	38 (44.2)	28 (45.2)	10 (41.7)	
Regularly (≥1-3 times a week)	12 (14.0)	10 (16.1)	2 (8.3)	
Pregnancy complication				
GDM	5 (5.8)	2 (3.2)	3 (12.5)	0.130[$]
Preeclampsia and eclampsia	0 (0.0)	0 (0.0)	0 (0.0)	--
Preterm labour	1 (1.2)	1 (1.6)	0 (0.0)	1.000[$]

*Other medication intake included paracetamol, herb, vitamin C, and green tea tablet
[#]Pearson Chi square
[$]Fisher's Exact Test
[#]Independent samples t-test

4.3 Participants' residence and air monitoring station in Thailand during the study period

During the study, a comprehensive investigation explored the dynamics between environmental factors and allergic outcomes among Thai infants. This endeavour encompassed an in-depth analysis of the study area, participants' residences, and the positioned air monitoring stations across Thailand (**Figure 4.3-1** and **Figure 4.3-2**).

The study provinces were selected to encompass a representative cross-section of urban and suburban regions within Thailand. This geographic diversity facilitated an understanding of the potential impact of environmental exposures on infant allergy across varying urbanisation levels and socioeconomic contexts—such an inclusive approach aimed to capture a comprehensive snapshot of the multifaceted dynamics.

Participating residences were distributed across these chosen study areas, ensuring a broad and diverse representation of infants and families. This approach included various lifestyles, living conditions, and potential environmental stressors that might influence infant allergy. By incorporating a range of residential environments, the study aimed to yield insights that could be generalised to broader populations while accounting for localised variations.

Integral to this investigation were positioned air monitoring stations across Thailand. These stations systematically collected data on air quality, pollutant levels, and other relevant environmental variables. Placed in urban, suburban, and rural locations, these stations played a pivotal role in quantifying the extent of exposure to air pollutants among the participants.

This data, in turn, facilitated the examination of potential correlations between environmental exposures and the health outcomes of allergic diseases, sensitisation, and elevated IgE concentration in infants.

The comprehensive overview of the study provinces, participants' residences, and the distributed air monitoring stations established a robust foundation for uncovering the complicated interplay between environmental factors and the health outcomes of interest. This holistic approach allowed for exploring potential associations while acknowledging the dynamic contextual factors that shape the health of Thai infants across various regions of the country.

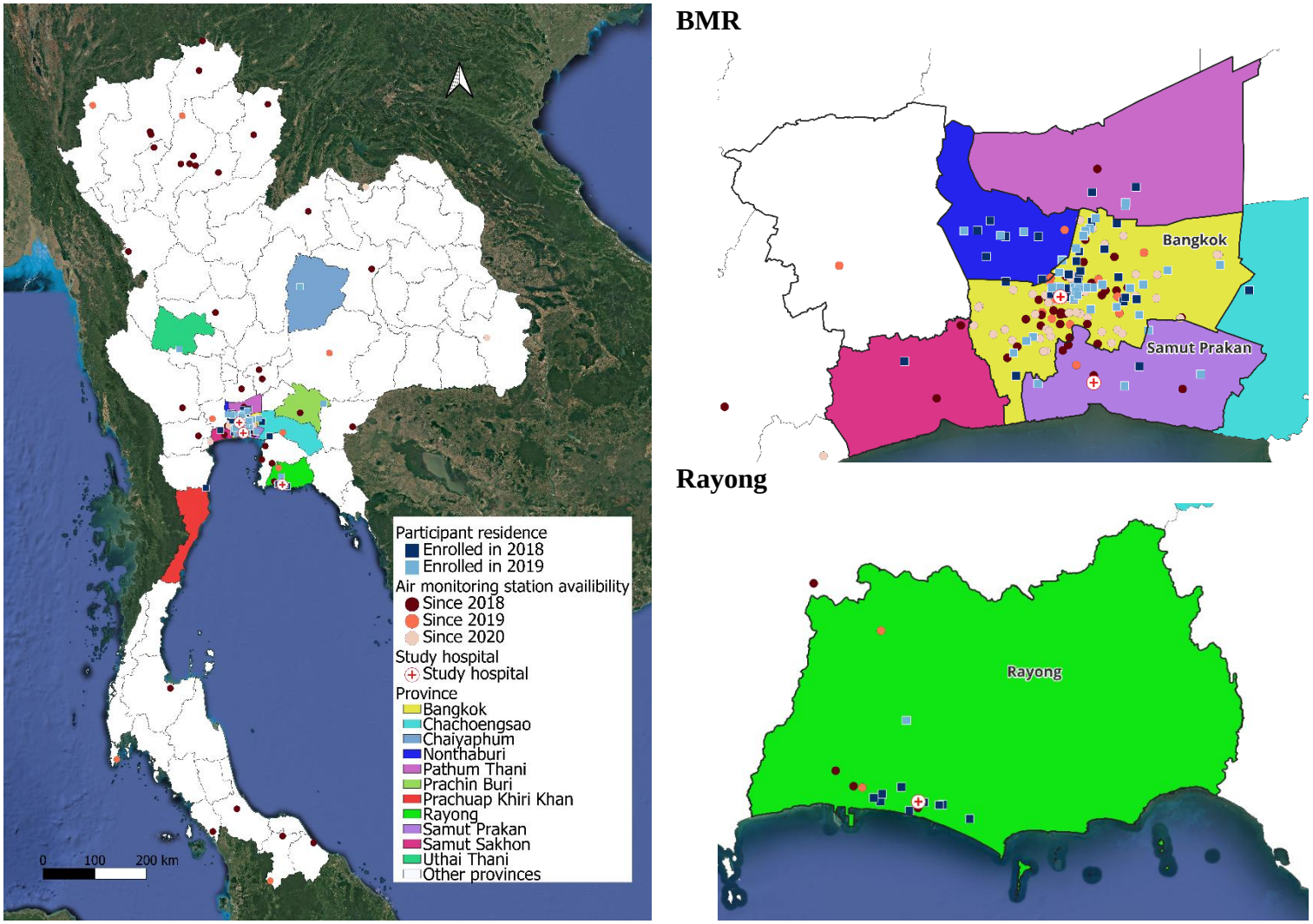
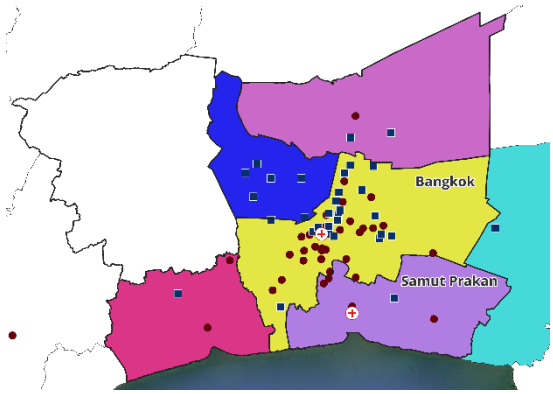


Figure 4.3-1: Overview of the study area, participant's residences, study hospitals and air monitoring stations over the study period. The total of 76 air monitoring stations contributed to data for this study (seventy and six for Ramathibodi and Rayong Hospitals, BMR; Bangkok Metropolitan Region)

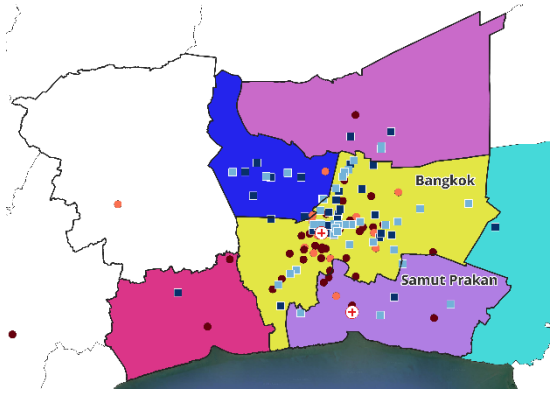
2018, BMR



2018, Rayong



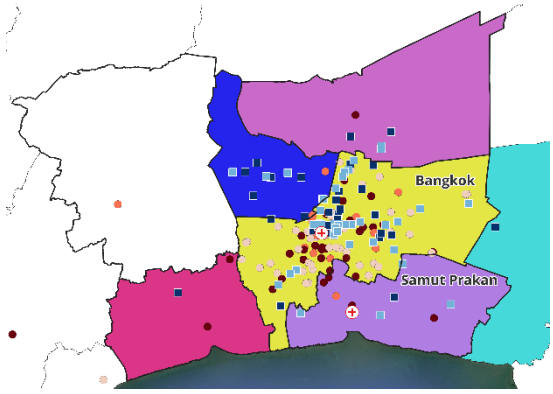
2019, BMR



2019, Rayong



2020, BMR



2020, Rayong



Figure 4.3-2: The map of study provinces by year and province. BMR; Bangkok Metropolitan Region.

4.4 Discussion

4.4.1 Interpretation of the outcome

4.4.1.1 Attrition bias

In a birth cohort study, attrition bias is the potential bias that arises when participants drop out or are lost to follow-up throughout the study [410]. It can occur when specific characteristics or factors influence the likelihood of participants dropping out or being lost to follow-up. It can result in a non-random loss of participants, leading to an unrepresentative sample and potential bias in the study findings. The reasons for attrition can be diverse, including participants moving away, loss of contact, withdrawal of consent, non-compliance, or other personal circumstances. The impact of attrition bias on a birth cohort study depends on the extent and nature of the attrition, the variables being studied and the specific research questions [411]. If participants who drop out differ systematically from those who remain in the study, it can introduce bias by affecting the distribution of key variables or outcomes of interest. This bias can undermine the validity and generalisability of the study's results.

Several strategies were implemented to mitigate attrition bias in the birth cohort study. These included efforts to minimise participant loss through consistent contact and engagement, providing incentives or support to encourage continued participation, and using appropriate statistical techniques to investigate potential biases by comparing the characteristics of those who drop out with those who remain in the study. In this DPhil study, the attrition bias also be an issue even though several strategies by consistent contact and providing incentives already applied. The appropriate statistical techniques have been considered since the overview result found that the significant different factors between Dyads and Non-dyads were site of data collection (Hospital), maternal education, and maternal occupation which will be adjusted in

the analysis to minimise the attrition bias. Accounting for the limitation of small sample size, the sensitivity analyses and missing data imputation was not conducted. This approach may be considered for future projects with larger datasets to address the missing data issue.

4.4.1.2 External validity and generalisability

Birth cohort studies often recruit participants from specific geographic areas or demographic groups, which may limit the generalisability of findings to broader populations [231]. In this study, which is not a population-based birth cohort study, most participants live in the city. For this reason, this sample will not fully represent the diversity of the entire Thai population, given the variations in socioeconomic status, geographical location, housing type and health behaviours that can influence environmental exposure patterns and health outcomes. As such, when interpreting the study's results, it was essential to consider this potential limitation and its impact on the broader applicability of the findings.

4.4.2 Integrated clinical research informatics system

The Integrated clinical research informatics system was a necessary but complicated tool. It was pivotal in simplifying and overseeing the complex web of data and processes fundamental to this birth cohort study. It guaranteed the precision, security, and availability of data, thereby making a substantial contribution to the study's accomplishment and the emergence of invaluable insights into the well-being of pregnant women and infants.

Nonetheless, our study faced substantial challenges, primarily from resource constraints, notably in funding. These limitations posed hurdles and necessitated creative solutions to ensure the project's continuity and success. Moreover, the experience of the system developers,

as well as the technology's acceptability among users [412], significantly influenced the system's usability and feasibility during its development phase. Overcoming these challenges required resourcefulness and collaboration within the research team, highlighting the importance of adaptability in the face of real-world research constraints.

4.5 Conclusion

This chapter provides an overview of the research findings, detailing the enrolment process of the birth cohort study and comparing the baseline characteristics of maternal-infant dyads and non-dyads to assess potential attrition bias in this DPhil research. It also establishes the groundwork for examining demographic and key environmental factors (**Chapter 5**) in early life that may contribute to immunological alterations in infants and/or allergic outcomes (**Chapter 6**). Additionally, the spatial distribution of air monitoring stations, participant residences, and the study hospital is presented to enhance the understanding of geographic variations and $PM_{2.5}$ exposure estimates at participants' residences.

Chapter 5: Exposure assessment result

This chapter presents critical exposure assessment results, focusing on key areas of interest: evaluating home environments during pregnancy, assessing lifestyle-related health hazards, measuring exposure to ETS, evaluating heavy metals exposure during the first trimester of pregnancy, and estimating $PM_{2.5}$ levels at participants' residences. These assessments provide valuable insights into environmental factors influencing infants' immunological and allergic outcomes.

By examining home environments and health hazards at work and home (related to lifestyle), we may identify potential exposure impacting infant immunological and allergic outcomes. The assessment of ETS exposure reveals its health effects while investigating heavy metals exposure during early pregnancy highlights potential risks. Estimating $PM_{2.5}$ levels during this sensitive period enhances our understanding of air quality effects in early life on children’s health.

These findings contribute to our understanding of study participants' environmental exposures and their implications for maternal and infant health, particularly regarding immunological and allergic outcomes, which are the focus of this study. They establish a foundation for exploring the intricate relationship between environmental factors and health outcomes, guiding future research and informing public health initiatives.

The exposure assessment was assessed in pregnant women and infant children. It consisted of heavy metals in the whole blood of pregnant women's first trimester, nicotine from four

consecutive urine of pregnant women (first and third trimester), and infant children (at birth and 6-12 months).

5.1 The home environment during the pregnancy period

Comparing infants with and without diagnosed allergies (**Table 5.1-1**), differences were observed in the type of residence, home ventilation systems, and temperature controlled measures across the three trimesters of pregnancy, particularly regarding the use of air conditioning (AC) and opening windows or doors during the first and third trimesters. Additionally, recent furnishing or renovation of the house during the first trimester and pet ownership status during the second trimester were significantly different between the two groups. Consequently, the home environment during pregnancy was further analysed using univariate and multivariable logistic regression models where appropriate.

Table 5.1-1: Home environment during pregnancy period (first, second, and third trimester) comparing between infant without diagnosed allergy; AD and/or food allergy group (No allergy) and infant with diagnosed allergy (Allergy) group, N = 86

Variables; n (%)	First trimester			Second trimester			Third trimester		
	No allergy	Allergy	*P*	No allergy	Allergy	*P*	No allergy	Allergy	*P*
	N = 62	N = 24		N = 62	N = 24		N = 62	N = 24	
Type of residence									
Detached house	27 (43.5)	13 (25.0)	**0.078**[#]	21 (34.4)	12 (50.0)	**0.074**[#]	21 (35.0)	8 (33.3)	**0.051**[#]
Flat/ condominium/ apartment	30 (48.4)	6 (54.2)		34 (55.7)	7 (29.2)		32 (53.3)	8 (33.3)	
Row house/ townhouse/ attached house	5 (8.1)	5 (20.8)		6 (9.8)	5 (20.8)		7 (11.7)	8 (33.3)	
Ventilation and temperature control system									
Open windows/ doors	47 (75.8)	10 (41.7)	**0.003**[#]	40 (65.6)	12 (50.0)	0.185[#]	48 (80.0)	14 (58.3)	**0.041**[#]
Air conditioner (AC)	38 (61.3)	20 (83.3)	**0.050**[#]	39 (63.9)	21 (87.5)	**0.032**[#]	40 (66.7)	22 (91.7)	**0.019**[#]
Fan	50 (80.6)	18 (75.0)	0.564[#]	50 (82.0)	19 (79.2)	0.766[$]	49 (81.7)	21 (87.5)	0.748[$]
Mist fan (fan foggy)	1 (1.6)	0 (0.0)	1.000[$]	0	0	--	0	1 (4.2)	0.286[$]
Ventilation fan (exhaust fan)	3 (4.8)	1 (4.2)	1.000[$]	1 (1.6)	1 (4.2)	0.487[$]	2 (3.3)	1 (4.2)	1.000[$]
Air purifier	1 (1.6)	1 (4.2)	0.483[$]	5 (8.2)	4 (16.7)	0.263[$]	3 (5.0)	4 (16.7)	0.099[$]
Visible mould in the house									
Noticeable	29 (46.8)	12 (50.0)	0.726[#]	27 (44.3)	9 (37.5)	0.848[#]	27 (45.0)	13 (54.2)	0.749[#]
Do not noticeable	18 (29.0)	5 (20.8)		20 (32.8)	9 (37.5)		18 (30.0)	6 (25.0)	
Uncertain	15 (24.2)	7 (29.2)		14 (23.0)	6 (25.0)		15 (25.0)	5 (20.8)	
Pet owner									
Any pet	26 (41.9)	8 (33.3)	0.464[#]	25 (41.0)	4 (16.7)	**0.033**[#]	22 (36.7)	5 (20.8)	0.160[#]
Dog	16 (25.8)	4 (16.7)	0.368[#]	16 (26.2)	3 (12.5)	0.171[#]	13 (21.7)	3 (12.5)	0.539[#]
Cat	13 (21.0)	3 (12.5)	0.539[$]	9 (14.8)	0 (0.0)	0.056[$]	9 (15.0)	0 (0.0)	0.054[$]
Rabbit	1 (1.6)	1 (4.2)	0.483[$]	1 (1.6)	0 (0.0)	1.000[$]	2 (3.3)	0 (0.0)	1.000[$]
Another pet except dog, cat, rabbit*	0 (0.0)	1 (4.2)	0.279[$]	5 (8.2)	1 (4.2)	0.671[$]	3 (5.0)	3 (12.5)	0.346[$]

Variables; n (%)	First trimester			Second trimester			Third trimester		
	No allergy	Allergy	*P*	No allergy	Allergy	*P*	No allergy	Allergy	*P*
	N = 62	N = 24		N = 62	N = 24		N = 62	N = 24	
Household ETS									
Present of household ETS	46 (74.2)	17 (70.8)	0.752[#]	46 (75.4)	17 (70.8)	0.665[#]	46 (76.7)	17 (70.8)	0.577[#]
ETS from the neighbour (s)	23 (37.1)	9 (37.5)	0.972[#]	28 (45.9)	10 (41.7)	0.724[#]	31 (51.7)	11 (45.8)	0.629[#]
Self-tobacco smoke	0 (0.0)	0 (0.0)	--	0 (0.0)	0 (0.0)	--	0 (0.0)	0 (0.0)	--
ETS from spouse	16 (25.8)	5 (20.8)	0.630[#]	12 (19.7)	4 (16.7)	1.000[$]	13 (21.7)	2 (8.3)	0.212[$]
ETS from relative (s) or housemate (s)	12 (19.4)	5 (20.8)	1.000[$]	10 (16.4)	5 (20.8)	0.753[$]	8 (13.3)	6 (25.0)	0.209[$]
Living near heavy traffic area (< 50 m)	25 (40.3)	9 (37.5)	0.810[#]	19 (31.1)	6 (25.0)	0.576[#]	15 (25.0)	5 (20.8)	0.685[#]
Present of green environment at home	52 (83.9)	20 (83.3)	1.000[$]	49 (80.3)	20 (83.3)	1.000[$]	47 (78.3)	22 (91.7)	0.212[$]
Using bathroom cleaning liquid	4 (6.5)	2 (8.3)	0.670[$]	6 (9.8)	2 (8.3)	1.000[$]	6 (10.0)	2 (8.3)	1.000[$]
Using bleach for washing	53 (85.5)	20 (83.3)	0.750[$]	51 (83.6)	19 (79.2)	0.753[$]	51 (85.0)	20 (83.3)	1.000[$]
Using disinfectant for cleaning other household products	31 (50.0)	8 (33.3)	0.164[#]	32 (52.5)	9 (37.5)	0.214[#]	29 (48.3)	9 (37.5)	0.367[#]
Using any household chemical products	36 (58.1)	16 (66.7)	0.464[#]	35 (57.4)	13 (54.2)	0.788[#]	36 (60.0)	15 (62.5)	0.832[#]
Recently furnished/ renovated house	7 (11.3)	8 (33.3)	**0.025[$]**	10 (16.4)	4 (16.7)	1.000[$]	12 (20.0)	7 (29.2)	0.364[#]

*Another pet were birds, chicken, sugar glider, and goldfish
[#]Pearson Chi-Square
[$]Fisher exact test

5.2 Health hazards related to lifestyle factors

There was no statistical significance in all health hazards related to lifestyle factors (**Table 5.2-1**) and no significant difference in household ETS exposure between infants with and without allergies. The eating patterns and exercise habit in pregnancy were also examined. However, the differences between infants with and without allergies were not found (data not shown).

Table 5.2-1: The health hazards related to lifestyle (at work and/or home) during the pregnancy period (first, second, and third trimester) comparing between infants without diagnosed allergy; AD and/or food allergy group (No allergy) and infant with diagnosed allergy (Allergy) group, N = 86.

Variables; n (%)	First trimester			Second trimester			Third trimester		
	No allergy	Allergy	*P*	No allergy	Allergy	*P*	No allergy	Allergy	*P*
	N=62	N=24		N=62	N=24		N=62	N=24	
Moving vehicle/ boat	14 (22.6)	8 (33.3)	0.305[#]	13 (21.3)	6 (25.0)	0.713[#]	12 (20.3)	5 (20.8)	1.000[$]
Nightshift duty (10 PM-6 AM)	8 (12.9)	4 (16.7)	0.731[$]	7 (11.5)	4 (16.7)	0.496[$]	8 (13.6)	2 (8.3)	0.716[$]
The heavy lifting of ≥ 15 Kg	13 (21.0)	5 (20.8)	0.989[#]	9 (14.8)	6 (25.0)	0.344[$]	10 (16.9)	3 (12.5)	0.748[$]
Long-standing	22 (35.5)	10 (41.7)	0.595[#]	19 (31.1)	5 (20.8)	0.342[#]	17 (28.8)	8 (33.3)	0.684[#]
Excessive bending	35 (56.5)	13 (54.2)	0.848[#]	35 (57.4)	14 (58.3)	0.936[#]	37 (62.7)	13 (54.2)	0.471[#]
Excessive stair climbing > 4 times/day	33 (53.2)	14 (58.3)	0.670[#]	31 (50.8)	15 (62.5)	0.331[#]	29 (49.2)	14 (58.3)	0.448[#]
Vibration	11 (17.7)	4 (16.7)	1.000[$]	11 (18.0)	2 (8.3)	0.334[$]	8 (13.6)	4 (16.7)	0.737[$]
Significant heat	15 (24.2)	6 (25.0)	0.938[#]	13 (21.3)	4 (16.7)	0.768[$]	12 (20.3)	3 (12.5)	0.536[$]
Loud noise	9 (14.5)	4 (16.7)	0.750[$]	7 (11.5)	5 (20.8)	0.306[$]	8 (13.6)	7 (29.2)	0.119[$]
Aerosol/ vapour substances	10 (16.1)	4 (16.7)	1.000[$]	8 (13.1)	3 (12.5)	1.000[$]	6 (10.2)	5 (20.8)	0.283[$]
Dust, fume, metal, or fibre	14 (22.6)	3 (12.5)	0.376[$]	10 (16.4)	2 (8.3)	0.495[$]	9 (15.3)	2 (8.3)	0.497[$]
Exhaust system	24 (38.7)	12 (50.0)	0.341[#]	26 (42.6)	9 (37.5)	0.666[#]	21 (35.6)	8 (33.3)	0.845[#]
Chemical exposure	8 (12.9)	3 (12.5)	1.000[$]	12 (19.7)	3 (12.5)	0.540[$]	13 (22.0)	5 (20.8)	0.904[#]

[#]Pearson Chi-Square
[$]Fisher exact test

5.3 Environmental tobacco smoke (ETS)

ETS in this study were measured in two methods to represent different patterns of ETS. Self-reported SAQ evaluated household ETS during the pregnancy period (first, second, and third trimester), and secondly, total ETS exposure by measurement of cotinine in urine with/without creatinine correction.

5.3.1 Comparison of environmental tobacco smoke (ETS) exposure and urine cotinine/creatinine ratio (CCR)

The comparison of household self-reported ETS exposure, as assessed via the SAQ, and urine cotinine/creatinine (CCR) ratio was conducted. However, no significant differences were found between the CCR groups and the household ETS groups (**Table 5.3-1**).

Table 5.3-1: The comparison of household ETS exposure and urinary cotinine/creatinine ratio in pregnant participants.

Trimester	Household ETS by SAQ	CCR ≤ 30 ng/mg Cr n (%)	CCR > 30 ng/mg Cr n (%)	*P*
1st trimester (N = 86)	Yes	61 (72.6)	2 (100.0)	1.000
	No	23 (27.4)	0 (0.0)	
3rd trimester (N = 82)	Yes	60 (76.9)	2 (50.0)	0.249
	No	18 (23.1)	2 (50.0)	

Fisher's Exact Test used for *P*-value calculation.

5.3.2 Household self-reported ETS

According to the information from SAQ, the prevalence of non-exposure to household ETS increased from first to third trimester (21.4% to 28.8%). The prevalence of household ETS during pregnancy was 74.2%, 75.4%, and 76.7% in the first, second, and third trimester, respectively (**Table 5.1-1**). None of the pregnant participants was an active smoker. There was

a similar percentage of sources of exposure across three trimesters, which were the most from a neighbour (s), spouse, and housemate (s).

5.3.3 Total ETS exposure (urine cotinine-creatinine ratio; CCR)

Urine CCR across the pregnancy period showed significant fluctuations, characterised by a notable increase from the first to the third trimester, a subsequent decrease at birth, and a substantial rise by 6–12 months of age (**Table 5.3-2**).

5.3.4 Comparison urine cotinine/creatinine ratio (CCR) between allergy and no allergy infants

The overall data showed the significant different of CCR across time point, however, there was not statistically significant ($p < 0.05$) after subgroup analysis by allergy status. The comparison of urine CCR for four consecutive periods was also shown in **Table 5.3-2**. The result showed that there was no statistical significance existed between allergy and no allergy groups for all visits.

Table 5.3-2: The comparison of urine cotinine/creatinine (CCR) ratio of pregnant women in the first trimester (P01) and third trimester (P03) and children at birth (C01) and at 6-12 months (C05) in children without diagnosed allergic diseases (No allergy) and with diagnosed allergic diseases (Allergy), N = 59.

Variables	Pregnancy 1st trimester			Pregnancy 3rd trimester			Children at birth			Children at 6-12 months		
	No allergy (N = 62)	Allergy (N = 24)	*P*	No allergy (N = 61)	Allergy (N = 23)	*P* [a]	No allergy (N = 47)	Allergy (N = 22)	*P* [a]	No allergy (N = 56	Allergy (N = 21)	*P* [a]
CCR; ng/mg creatinine, median (IQR)	0.181 (0.490)	0.214 (0.330)	0.765[a]	0.346 (0.960)	0.207 (1.000)	0.511[a]	0.074 (0.510)	0.061 (0.290)	0.607[a]	0.496 (7.95)	0.048 (1.95)	0.084[a]
CCR > 30 ng/mg creatinine, n (%)	2 (3.2)	0 (0.0)	1.000[$]	3 (4.8)	1 (4.2)	1.000[$]	0 (0.0)	0 (0.0)	--	6 (9.7)	2 (8.3)	1.000[$]

[a]Independent-Samples Mann-Whitney U test
[$]Fisher's Exact Test
The CCR was not normal distribution even the log transformation was applied, therefore, crude data was used regards to the clinical relevance instead of log-transformed data.

5.4 Heavy metals in whole blood of first-trimester pregnant women

The analysis of heavy metal concentrations in whole blood from seventy-seven first-trimester pregnant women in this DPhil study is summarised in **Table 5.4-1**. Cd concentration was above the detection limit in 14 samples (18.2%), with 12 samples (21.8%) from the no-allergy group and 2 samples (9.1%) from the allergy group. The median concentrations of most heavy metals were higher in the no-allergy group compared to the allergy group, except for Cr that was higher in the allergy group than in the no-allergy group, but no statistical difference.

Despite these observations, no statistically significant differences were found between the no-allergy and allergy groups for any of the six metals analysed, except for Pb exposure [median (IQR) 11.649 (6.800) vs 9.275 (4.498) µg/L, $P = 0.019$ for no allergy vs allergy group].

Table 5.4-1: The heavy metals in whole blood of pregnant women, first-trimester. The measured heavy metals were chromium (Cr), manganese (Mn), arsenic (arsenic), cadmium (Cd), mercury (Hg), and lead (Pb).

Metals (µg/L)		No allergy (N = 55)	Allergy (N=22)	*P*
Cr	Median (IQR)	0.310 (0.785)	0.540 (1.308)	0.071 [a]
	Mean ± SD	0.519 ± 0.683	0.795 ± 0.747	
Mn	Median (IQR)	11.540 (4.970)	11.468 (5.065)	0.848 [a]
	Mean ± SD	12.505 ± 5.633	12.058 ± 4.283	
As	Median (IQR)	1.770 (1.450)	1.423 (0.998)	0.191 [a]
	Mean ± SD	2.074 ± 1.030	1.924 ± 1.468	
Cd	Median (IQR)	0.251 (0.000)	0.251 (0.000)	0.164 [a]
	Mean ± SD	0.327 ± 0.182	0.266 ± 0.051	
Hg	Median (IQR)	1.470 (1.510)	1.275 (1.174)	0.539 [a]
	Mean ± SD	1.844 ± 1.257	1.905 ± 1.798	
Pb	Median (IQR)	11.640 (6.800)	9.715 (4.498)	**0.019** [a]
	Mean ± SD	15.245 ± 16.468	10.093 ± 3.966	

[a] Independent-Samples Mann-Whitney U Test

5.5 Particulate matter 2.5 ($PM_{2.5}$) estimations by GIS

This section presented the interpolation of daily average ambient 24-hour $PM_{2.5}$ by month during the study period to give a whole picture of it spatiotemporal variation across Thailand. Then, the estimated average $PM_{2.5}$ exposure at participants' residences was presented.

5.5.1 $PM_{2.5}$ in Thailand during the study period (2018-2020)

The interpolation of average 24-hour $PM_{2.5}$ concentrations by month across Thailand during the study period (2018–2020) is illustrated in **Figure 5.5-1**. Annual temporal variations in ambient $PM_{2.5}$ concentrations were evident, with the lowest levels observed during the rainy season (June–August) and the highest levels during the winter season (January–February). Regional differences in exposure levels were also apparent. In the Central and Eastern regions, where the study sites were located, average 24-hour $PM_{2.5}$ concentrations peaked between December and February (37.6–75 μg/m³, shown in orange) and dropped to their lowest levels in June–August (<15 μg/m³, shown in blue). Notably, during the COVID-19 lockdown period (April to July 2020), 24-hour $PM_{2.5}$ concentrations appeared to improve compared to the same months in 2018 and 2019, as indicated by a greater extent of areas with lower $PM_{2.5}$ levels. However, this observation may also have been influenced by other factors, such as meteorological conditions, which were beyond the scope of this DPhil study to investigate.

These spatiotemporal variations highlight the importance of considering seasonal effects in the analysis. It should also be noted that the study's focus on the Central and Eastern regions may limit the generalisability of findings to populations in other areas of Thailand.

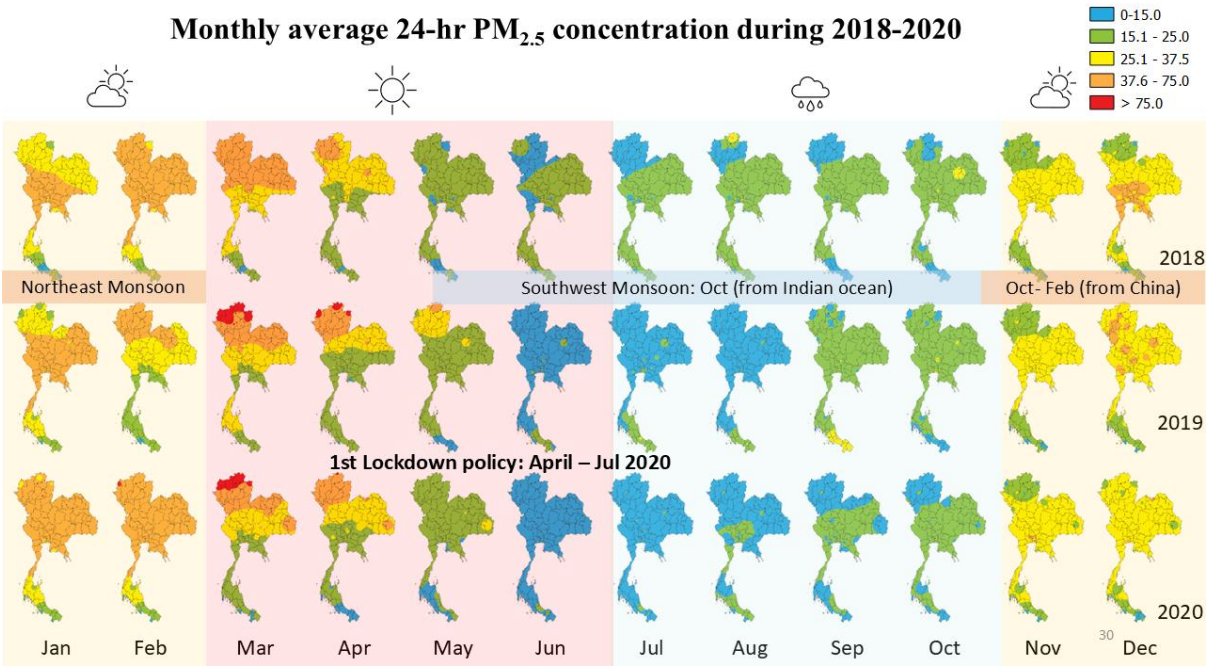


Figure 5.5-1. Spatiotemporal interpolation of monthly 24-hour $PM_{2.5}$ concentration in Thailand during the study period (Jan 2018 - Dec 2020)

5.5.2 The estimation of $PM_{2.5}$ exposure at the residential level during pregnancy and infancy period

Overall, the average 24-hour $PM_{2.5}$ exposure was above 15 $\mu g/m^3$ throughout pregnancy and infancy in allergy and no-allergy children groups. Across various trimester of pregnancy, no significant differences in $PM_{2.5}$ concentrations both average and accumulative were observed between infants without allergies (No allergy group) and those with allergies (Allergy group), shown in **Table 5.5-1**.

In infancy, the analysis reveals no remarkable distinctions in $PM_{2.5}$ concentrations across different intervals excepts for during first 2 months of infant children (0-2 months) which the "Allergy" group had higher concentration of both average and accumulation of 24-hour $PM_{2.5}$

than "No allergy" group [median (IQR) 26.936 (18.463) vs 32.386 (13.771) μg/m^3 for No allergy vs Allergy group] (**Table 5.5-1**).

Table 5.5-1: Comparison of $PM_{2.5}$ concentrations between infant with (Allergy) or without allergic diseases (No Allergy) groups across pregnancy and infancy periods

$PM_{2.5}$ (μg/m^3)		**No allergy (N=62)**	**Allergy (N=24)**	***P*** [b]
Pregnancy period				
24-hour average				
1st Trimester	Median (IQR)	25.955 (14.900)	23.029 (14.079)	0.464
	Mean ± SD	26.974 ± 8.995	25.760 ± 7.493	
2nd Trimester	Median (IQR)	23.108 (11.913)	21.982 (10.887)	0.402
	Mean ± SD	25.656 ± 7.334	24.219 ± 6.913	
3rd Trimester	Median (IQR)	23.580 (17.559)	29.58 (20.494)	0.277
	Mean ± SD	27.076 ± 10.501	28.846 ±10.731	
Accumulative				
1st Trimester	Median (IQR)	2,543.563 (1,460.190)	2,256.833 (1,379.787)	0.464
	Mean ± SD	2,643.465 ± 881.546	2,524.474 ± 734.349	
2nd Trimester	Median (IQR)	2,264.621 (1,167.433)	2,154.203 (1,066.924)	0.402
	Mean ± SD	2,514.79 ± 718.758	2,373.504 ± 677.471	
3rd Trimester	Median (IQR)	1,845.942 (1,372.504)	2170.387 (1,675.503)	0.500
	Mean ± SD	2,076.274 ± 868.936	2,306.666 ± 881.313	
Infancy period				
24-hour average				
0-2 months	Median (IQR)	26.936 (18.463)	32.396 (13.771)	**0.056**
	Mean ± SD	26.376 ± 10.339	31.032 ± 8.951	
2-4 months	Median (IQR)	23.957 (20.642)	24.348 (15.795)	0.464
	Mean ± SD	27.337 ± 11.693	29.001 ±9.483	
4-6 months	Median (IQR)	26.691 (15.121)	20.570 (15.868)	0.703
	Mean ± SD	26.498 ± 10.505	25.223 ± 10.035	
Accumulative				
0-2 months	Median (IQR)	1,508.563 (1,033.911)	1,814.170 (771.176)	**0.056**
	Mean ± SD	1,477.037 ± 578.957	1,737.807 ± 501.275	
2-4 months	Median (IQR)	1,341.584 (1,155.953)	1,363.494 (884.504)	0.464
	Mean ± SD	1,530.855 ± 654.835	1,624.083 ± 531.066	
4-6 months	Median (IQR)	1,494.687 (846.793)	1,151.923 (888.633)	0.703
	Mean ± SD	1,483.870 ± 588.283	1,412.477 ± 561.963	

[a] Independent-Samples Mann-Whitney U Test

5.6 Discussion

The chemical exposures in pregnant and infants were evaluated by SAQ (cleaning household products, VOCs from recently furnished in the house, and household ETS) and specific chemical measurements [heavy metals, CCR (estimated ETS), and ambient $PM_{2.5}$].

5.6.1 Volatile organic compounds (VOCs) from recently furnished furniture in the home, cleaning household products

The major indoor air pollutants are VOCs and PM. Indoor VOCs are commonly emitted from paints, lacquers, cleaning liquids products, furnishings, copiers, printers, glues, adhesives or permanent markers [413]. This study evaluated the indoor VOCs during pregnancy by self-reported (SAQ) across each trimester to assess the recently furnished home and household cleaning product that represented routine and relatively chronic exposure. Over half of the participants (55.8 – 60.5%) reported using household cleaning chemical products, including bleach, disinfectants, and bathroom cleaning liquids, across three trimesters of pregnancy (**Table 5.1-1**). The self-reported of the recently furnished home were 16.3 – 22.1%, varying across trimesters. These represented substantial prenatal exposure to VOCs and raised awareness of health effects, particularly to immunological alteration or allergic diseases in later life.

Since the objective of this study was to explore environmental factors potentially associated with allergic diseases in children, various environmental exposures were collected. However, the study was not specifically designed to measure indoor PM levels, which can arise from household activities such as cooking, burning incense or candles, and cleaning practices. Additionally, the SAQ was carefully designed to balance the amount of information collected

with minimising participant burden. As a result, questions specifically addressing sources of indoor air pollution were not included. This limitation constrains the ability to fully interpret the effects of indoor air pollution on children's allergic outcomes.

5.6.2 Environmental tobacco smoke (ETS) in Thai pregnant women

5.6.2.1 Self-reported household ETS exposure

In this study, the prevalence of self-reported household ETS exposure is 71.2 -78.6%, decreasing along the gestational age. The sources of household ETS were commonly from neighbour (s), spouse, and housemate, respectively. This result is compatible with ETS exposure during pregnancy in Thai people, which was reported by 88.2%, and urine tests for cotinine by direct barbituric acid (DBA) method were positive in 93.9% (479). Apart from households, common places of ETS exposure in Thailand were street/bus stops, households, and commercial shops/restaurants. The prevalence of ETS exposure from both previous studies and this study is markedly higher than the smoking prevalence in Thai people aged ≥ 15 years, approximately 20% [414], and reflects that tobacco smoke from one person widely affects the public perspective.

5.6.2.2 ETS exposure via CCR measurement

The total ETS exposure with the cut-off of 30 ng/mg creatinine of CCR was found for only 6 for all visits in pregnant period. This finding contradicts a high rate of self-reported household ETS exposure (**Table 5.3-1**). Although household ETS were observed, it may reflect the low internal exposure to ETS, with genetic influence that should be considered and needed further clarification. Additionally, the differing nature of these measures, which represent distinct

aspects of ETS exposure, as well as inherent limitations in interpreting them together may explain this discordant finding.

Interestingly, among pregnant participants who reported household ETS exposure, more than 70% had a CCR ratio of ≤30 ng/mg creatinine during both the first and third trimesters. This finding suggests that a CCR ratio cutoff of ≤30 ng/mg creatinine may be insufficient to detect secondhand smoke exposure or household ETS in this population. Several factors could contribute to this limitation, including genetic variations affecting nicotine metabolism, which may result in lower detectable cotinine levels.

The CCR fluctuated across pregnancy and infancy, likely reflecting variations in ETS exposure due to physiological, behavioural, and situational factors. Increased indoor exposure during certain pregnancy periods or postpartum lifestyle changes may contribute to these fluctuations.

Metabolic changes during pregnancy, including increased glomerular filtration rate (GFR), increase nicotine metabolism, and altered hormonal regulation, can enhance renal clearance, potentially lowering urinary cotinine levels despite similar exposure [415, 416]. Meanwhile, decreased creatinine excretion due to fluid retention and reduced muscle mass may further affect CCR values, particularly in the third trimester [417]. These physiological changes challenge the use of a single CCR cutoff for ETS exposure assessment, highlighting the need for trimester-specific thresholds.

Beyond physiological factors, the observed increase in CCR at 6–12 months of age may indicate heightened ETS exposure during infancy, potentially due to changes in household smoking behaviours or increased infant mobility, which may bring them into closer proximity to smoking environments. While nicotine transfer through breast milk has been identified as a potential indirect source of exposure in infants of smoking mothers [418], this mechanism is unlikely to account for the observed CCR increase, as no cases of maternal smoking were reported in the SAQ. Further research is warranted to examine the associations between CCR patterns, maternal smoking behaviours, household smoking restrictions, and genetic variations in cotinine metabolism.

5.6.3 Heavy metal exposure in pregnant women

The analysis of heavy metal concentrations in the whole blood of first-trimester pregnant women (P01) in this birth cohort study revealed that all six potentially concerning heavy metals (As, Cd, Cr, Mn, Hg, and Pb) were detected at very low concentrations. Notably, no statistically significant differences were observed between infants without allergies and those with allergies, except for Pb exposure, which was higher in the non-allergy group [median (IQR): 11.649 (6.800) vs. 9.275 (4.498) μg/L, P = 0.019]. Interestingly, although the mean Pb concentration was higher in the non-allergy group, it remained well below the US Centers for Disease Control and Prevention (CDC) recommendation of threshold of concern in pregnant women (50 μg/L or 5 μg/dL) [419]. This finding highlights the need for further investigation into the potential relationship between lead exposure and atopic outcomes.

Cadmium was detected only 18.2% of pregnant participants in the dyad with not statistically significant different between allergy and no allergy groups. This low rate of detection of Cd is

consistent with the fact that none of the pregnant participants in this cohort were active smokers since tobacco smoking is a well-known significant source of environmental Cd exposure [171].

5.6.4 Ambient $PM_{2.5}$ exposure

During the study period from January 2018 to December 2020, the average 24-hour $PM_{2.5}$ exposure in pregnant women and infants consistently exceeded the latest WHO air quality guideline of 15 µg/m³ [81]. The mean (min, max) $PM_{2.5}$ concentrations for each evaluation period, from the first trimester of pregnancy to infancy at six months, were 26.64 (9.42, 44.10), 25.26 (14.10, 42.39), 27.57 (12.03, 48.35), 27.69 (9.08, 49.90), 27.81 (9.86, 54.12), and 26.14 (11.35, 51.62) µg/m³, respectively. These findings underscore the persistent issue of ambient $PM_{2.5}$ pollution in Thailand, highlighting the need for continued mitigation efforts.

Additionally, both average 24-hour and cumulative $PM_{2.5}$ concentrations were significantly higher in the allergy group at $P < 0.1$, warranting further investigation into the association between $PM_{2.5}$ exposure and allergic outcomes.

It is important to note that the study period coincided with Thailand's COVID-19 pandemic and related control measures. Starting with partial lockdown measures from March 26, 2020, followed by a national lockdown policy from April to May 2020, and subsequent gradual relaxation in June 2020 [420, 421]. Various sectors, including industries, transportation, and recreation, were temporarily shut down during the tight lockdown policy was enforced. Several studies have highlighted the correlation between these lockdown policies and reduced air pollution concentration, particularly in urban areas like Bangkok. For instance, Dejchanchaiwong and Tekasakul observed an 11.1% decrease in $PM_{2.5}$ concentrations at road

sites in Bangkok during the lockdown [422]. Similarly, Wetchayont *et al.* reported a 20.56% increase in $PM_{2.5}$ concentrations before the lockdown (pre-March 26, 2020) and subsequent reductions of -15.79% and -23.34% during the lockdown (March 26, 2020 - May 30, 2020) and the new normal period (June 1, 2020 - September 30, 2020), respectively [421]. As a result, the $PM_{2.5}$ exposure concentration observed in this study, may partially represent the $PM_{2.5}$ exposure in Thai pregnant women and infants and may reflect the impact of COVID-19 lockdown measures on air quality. Future research, encompassing larger and longer-term studies, is warranted to provide a more comprehensive understanding of $PM_{2.5}$ exposure in pregnant women and infants.

5.7 Conclusion

This chapter presented the exposure assessment result in early life of the pregnant-infant dyads (first trimester of pregnant women to infant children), collected data from SAQ, biomarkers, and $PM_{2.5}$ interpolation, including home environment, health hazards related to lifestyle factors, ETS, heavy metals, and ambient $PM_{2.5}$. To draw a picture of highlighted hazards that may link to the immunological alteration and/or allergic outcomes in infant children (**Chapter 6**).

Chapter 6: Outcome assessment result

This chapter is divided into three key parts, each offering insights into the relationship between environmental exposures and allergic outcomes. These three parts are immunological, specific IgE and total IgE, and atopic disease assessments. It will be a bridge between the exposure assessment chapter and the understanding of how environmental exposures influence allergic outcomes.

In the first part, the concentration of plasma/serum cytokines of infants aged 6-12 months was presented by comparing allergy and no allergy groups. The differences observed in cytokine profiles between these two groups may offer valuable clues into the immunological underpinnings of allergic diseases and how environmental factors may shape these patterns.

The second part is the sensitisation evaluation by and sIgE to common allergens (inhalants for pregnant women and food for infants) and total IgE. These assessments illuminate the atopic status across critical periods, offering insights into the early sensitisation patterns, IgE concentration, and the potential influence of prenatal exposures on allergic outcomes.

Lastly, the outcome of allergic diseases included food allergies, AD, and early wheezing. The cumulative incidences of these key allergic conditions were used.

6.1 Immunological assessment (cytokine profiles of infant children)

Of the children born to the mothers in the dyads, 61 were measured for cytokine concentrations. Nineteen available plasma samples were diagnosed with allergic diseases before 12 months. The median (IQR) age of blood collection was 9.29 (1.55) and 9.30 (0.61) months for no allergy and allergy group, respectively. IL-1β, IL-4, IL-5, and IL-13 concentrations were detected above LOD in 6.6 – 59.0% of samples, and the rest were detected at above LOD more than 90% of samples. However, for IL-4, and IL-5, the allergy group had higher detection rate and measured at higher concentration than no allergy group. Among the detected cytokines ≥ 50% of samples, IL-4, IL-6, IFN-γ, TNF-α, and TSLP were prominent in the allergy children group. However, they were not statistically significant. Median IL-8 and IL-12/23p40 were significantly higher in no allergy group than allergy group. The detailed plasma cytokines results are shown in **Table 6.1-1**.

Table 6.1-1: Plasma/serum cytokines concentration of children at 6-12 months, N = 61

Cytokines (pg/ml)		No allergy (N = 42)	Allergy (N = 19)	*P*	Number of detectable (%)
IL-1β	Median (IQR)	0.000 (0.558)	0.000 (0.954)	0.451	27 (44.3)
	Mean ± SD	0.822 ± 1.800	0.968 ± 2.615		
IL-2	Median (IQR)	1.080 (0.680)	1.050 (0.860)	0.882	61 (100.0)
	Mean ± SD	1.479 ± 0.680	1.697 ± 1.778		
IL-4	Median (IQR)	0.290 (0.770)	0.430 (1.490)	0.329	36 (59.0)
	Mean ± SD	0.575 ± 1.069	0.918 ± 1.437		
IL-5	Median (IQR)	0.000 (0.133)	0.046 (0.401)	0.170	25 (41.0)
	Mean ± SD	0.263 ± 0.768	0.675 ± 2.072		
IL-6	Median (IQR)	2.270 (4.620)	2.980 (7.420)	0.461	54 (93.1)
	Mean ± SD	28.211 ± 104.429	7.011 ± 12.067		
IL-8	Median (IQR)	5.914 (7.354)	4.106 (7.792)	**0.066**	61 (100.0)
	Mean ± SD	95.294 ± 321.785	94.354 ± 372.825		
IL-10	Median (IQR)	2.340 (1.630)	2.250 (1.860)	0.431	61 (100.0)
	Mean ± SD	2.787 ± 2.773	2.847 ± 2.775		
IL-12/IL-23p40	Median (IQR)	40.345 (28.542)	30.676 (15.396)	**0.060**	61 (100.0)
	Mean ± SD	40.345 ± 18.478	31.393 ± 10.963		
IL-13	Median (IQR)	0.000 (0.000)	0.000 (0.000)	0.404	4 (6.6)
	Mean ± SD	0.365 ± 2.201	0.356 ± 1.412		
IL-17α	Median (IQR)	7.340 (6.560)	5.020 (5.140)	0.127	55 (90.2)
	Mean ± SD	7.774 ± 5.141	5.978 ± 3.138		
IL-22	Median (IQR)	3.300 (3.395)	3.800 (3.995)	0.460	61 (100.0)
	Mean ± SD	4.729 ± 5.626	5.276 (4.479)		
IL-31	Median (IQR)	50.444 (41.111)	49.429 (75.492)	0.570	61 (100.0)
	Mean ± SD	55.668 ± 34.836	93.345 ± 154.954		
IL-33	Median (IQR)	18.148 (16.311)	14.074 (15.077)	0.852	61 (100.0)
	Mean ± SD	23.378 ± 26.533	24.219 ± 5.626		
IFN-γ	Median (IQR)	2.520 (1.510)	2.950 (1.500)	0.326	61 (100.0)
	Mean ± SD	3.309 ± 3.770	3.690 ± 4.067		
TNF-α	Median (IQR)	0.940 (1.640)	1.450 (1.830)	0.332	57 (95.0)
	Mean ± SD	2.226 ± 5.590	2.936 ± 4.423		
TSLP	Median (IQR)	7.925 (11.859)	11.675 (13.615)	0.888	61 (100.0)
	Mean ± SD	12.319 ± 11.951	12.411 ± 9.898		
Independent-Samples Mann-Whitney U Test					

6.2 Specific IgE (sIgE) and total IgE in pregnant women and infant children

Overall, there were no significant differences in all measured variables (sIgE and total IgE) between individuals with (allergy group) and without allergies (no allergy group), as shown in **Table 6.2-1** and **Figure 6.2-1**. Notably, the median concentration for total IgE of pregnant women both first and third trimester in the no-allergy group were higher than allergy group (IgE P01 and IgE P03). On the contrary, total IgE and specific IgE in children at 6-12 months (IgE C05 and sIgE C05) were higher in the allergy children group than in the no-allergy group. Total serum/plasma IgE in cord blood (IgE C01) was lower than the LOD of 2 kU/L for almost samples (66 from 69 samples). The study also examined the atopic status by comparing acceptable age-appropriate IgE and sIgE concentration for common allergens between groups of children with allergies and those without allergies. The results indicated that no statistically significant difference was observed for any variables except the number of elevated total IgE in third trimester pregnant women that allergy group was significantly higher than no allergy group (16.7% vs 4.8%, $P = 0.091$)

Table 6.2-1: Comparing the plasma/serum specific IgE (sIgE) and total IgE (IgE) concentration in pregnant women (first and third trimester) and infant children (at birth and 6-12 months) between no allergy and allergy children's groups.

Variables		No-allergy N = 62	Allergy N = 24	*P*
Specific IgE (sIgE); PAU/L				
Pregnancy 1st trimester	Median (IQR)	0.090 (0.373)	0.090 (36.880)	0.761[b]
(N = 62, 24)	Mean ± SD	2.179 ± 5.776	2.606 ± 7.615	
	≥ 0.35 PAU/L; n(%)	*8 (12.9)*	*6 (25.0)*	*0.187[$]*
Pregnancy 2nd trimester	Median (IQR)	--	--	--
	Mean ± SD	--	--	
	≥ 0.35 PAU/L; n(%)	--	--	--
Children at birth	Median (IQR)	0.010 (0.010)	0.010 (0.040)	0.896[b]
(N = 50, 23)	Mean ± SD	0.026 ±0.046	0.187 ± 0.012	
	≥ 0.35 PAU/L; n(%)	*0 (0.0)*	*0 (0.0)*	--
Children at 6-12 months	Median (IQR)	0.100 (0.230)	0.240 (2.165)	0.281[b]
(N = 51, 17)	Mean ± SD	0.297 ± 0.660	4.138 (8.250)	
	≥ 0.35 PAU/L; n(%)	*9 (14.5)*	*6 (25.0)*	*0.342[$]*
Total IgE (IgE); kU/L				
Pregnancy 1st trimester	Median (IQR)	24.039 (59.801)	13.034 (71.083)	0.927[b]
(N = 62, 24)	Mean ± SD	46.430 ± 71.582	68.027 ± 119.072	
	≥ 100 kU/L; n (%)	*3 (4.8)*	*4 (16.7)*	*0.302[$]*
Pregnancy 3rd trimester	Median (IQR)	21.573 (37.924)	11.975 (43.204)	0.736[b]
(No = 61, 23)	Mean ± SD	33.935 ± 41.286	44.805 ± 74.129	
	≥ 100 kU/L; n (%)	*3 (4.8)*	*4 (16.7)*	***0.091[$]***
Children at birth	Median (IQR)	0.589 (0.000)	0.589 (0.000)	0.779
(N = 50, 19)	Mean ± SD	2.749 ± 8.711	0.589 ± 0.000	
	≥10 kU/L; n (%)	*2 (100.0)*	*0 (0.0)*	*1.000[$]*
Children at 6-12 months	Median (IQR)	9.146 (16.597)	16.083 (50.629)	0.141[b]
(N = 50, 19)	Mean ± SD	12.138 ± 10.982	38.261 ± 60.131	
	≥10 kU/L; n (%)	*23 (37.1)*	*11 (45.8)*	*0.457[#]*

[b] Independent-Samples Mann-Whitney U Test
[#] Chi-Square Tests
[$] Fisher's Exact Test

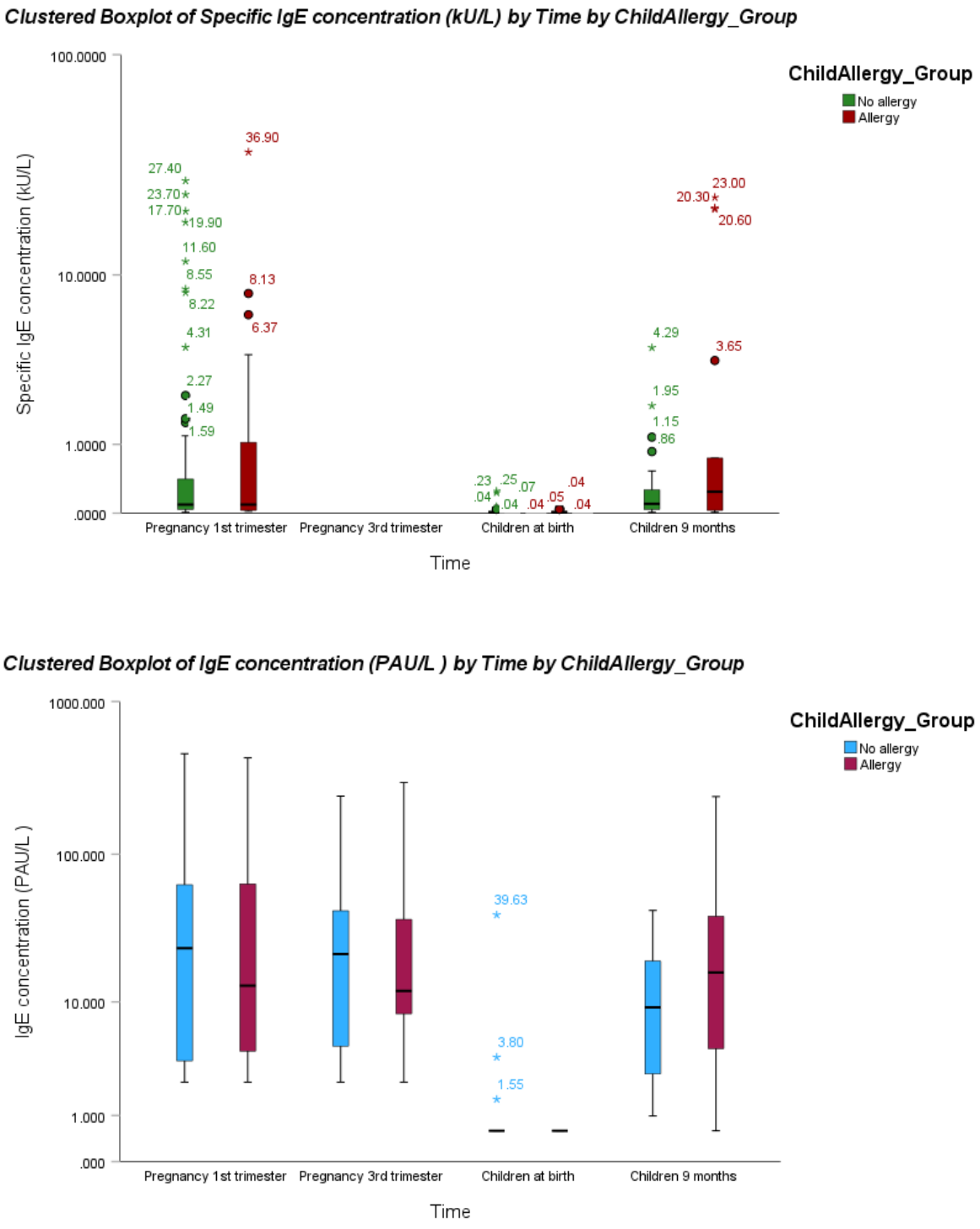


Figure 6.2-1: Specific IgE (sIgE); upper, and total IgE (IgE); lower, in pregnancy and children across the study period of dyad group. Both sIgE and IgE concentrations were log-transformed using log10 to improve visualisation.

6.3 Allergic diseases in infants

Among the newborn deliveries, eighty-six infants successfully participated in the study until they reached the age of 6-12 months. Out of this group, twenty-four infants (27.9%) were identified as having developed allergic diseases before nine months. Specifically, there were seven cases of AD (8.1%), six cases of food allergies (7.0%), and eleven cases (12.8%) of a combination of food allergy and AD (**Figure 6.3-1**). All these infants with atopic conditions received their diagnoses from paediatricians and had their diagnoses subsequently confirmed by either dermatologists or allergists.

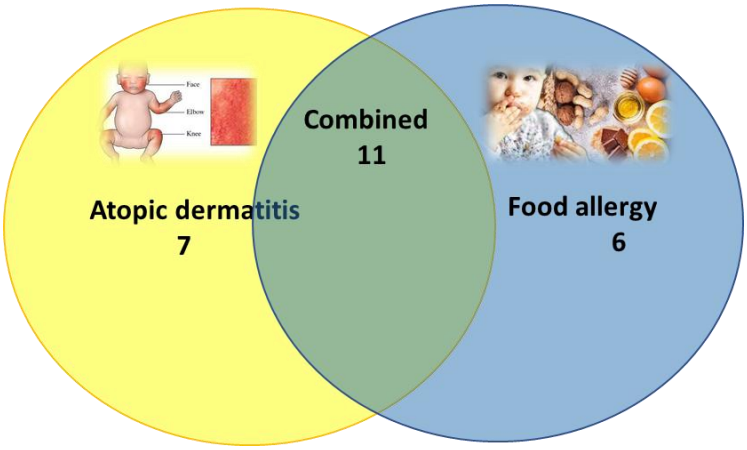


Figure 6.3-1: Allergic diseases in infant children of this study

The details of each allergic infant are demonstrated in **Table 6.3-1**. The average age (SD) of diagnosis AD and FA were 6.8 (2.8) and 8.5 (2.9) months, respectively. The most common food allergies were cow's milk and hen's egg (**Figure 6.3-2**), accounting for 60% of the culprit foods. Among food-allergic infants, seven of 17 infants were allergic to multiple foods.

For respiratory allergies, including early wheezing and AR, three cases of early wheezing were identified. However, all were attributed to respiratory infections, such as acute bronchiolitis caused by respiratory syncytial virus (RSV). Additionally, none of these cases responded to bronchodilator treatment. AR was not observed in this study. Consequently, these three cases were not included in the allergy group.

Table 6.3-1: The detailed characteristics of allergic infants in this study (n = 24)

No.	AD	Age of AD diagnosis (years)	FA	Type of FA	Symptom (s) of FA	Age of FA diagnosis (years)	Culprit food (s)	PhadiaTop® Infant (PAU/L)	Total IgE at 6-12 months (kU/L)
1	Y	0.41	Y	IgE	Urticaria	0.79	Legumes, sesame, peanut and soy	0.24	na
2	Y	0.79	Y	IgE, mixed	Urticaria, AD	0.79	Cow's milk, egg yolk, wheat	20.6	151
3	N	0.51	N	na	na	na	na	0.69	19
4	Y	0.76	Y	Mixed, non-IgE	AD, colitis (mucous-bloody stool)	0.34	na	0.01	59.6
5	N	na	Y	IgE, mixed	Urticaria, AD	1.07	Cow's milk, egg, and shrimp	na	< LOD
6	Y	1.04	Y	IgE., mixed	Urticaria, AD	1.04	na	na	na
7	Y	0.49	N	na	na	na	na	na	na
8	Y	0.37	N	na	na	na	na	na	na
9	Y	0.26	Y	Mixed	AD	0.31	Egg white, egg yolk	na	30.6
10	N	na	Y	IgE	Urticaria	0.79	Egg white	0.03	66.9
11	Y	0.62	N	na	na	na	na	0.07	6.4
12	Y	0.77	N	na	na	na	na	0.51	38.6
13	Y	0.36	Y	Mixed	AD	0.36	na	0.24	60.1
14	Y	0.34	Y	Mixed	AD	0.34	na	0.02	< LOD
15	Y	0.77	Y	IgE, mixed, non-IgE	Urticaria, AD, vomiting/diarrhoea	0.77	Cow's milk, egg white, egg yolk, fish (Tilapia)	23	574
16	Y	0.35	Y	IgE, mixed	Urticaria, AD	0.64	Cow's milk, wheat, egg yolk, and soy	na	174
17	N	na	Y	Mixed	AD	0.77	Shrimp	na	na
18	Y	0.76	N	na	na	na	na	0.02	22.4
19	N	na	Y	IgE, mixed	Urticaria, AD	0.67	Cow's milk	0.14	7.49
20	N	na	Y	IgE	Urticaria	0.92	Cow's milk	20.3	345
21	Y	0.82	Y	Mixed	AD	0.53	na	3.65	129
22	Y	0.77	Y	Mixed	AD	na	na	0.03	2.7
23	N	na	Y	IgE	Urticaria	0.71	Cow's milk	0.06	15
24	Y	0.25	Y	IgE, mixed	Urticaria, AD	0.36	Cow's milk, egg yolk	0.74	40.1

Abbreviations: AD, atopic dermatitis or allergic dermatitis; FA, food allergy; N, no; na, not applicable or not available; Y, yes

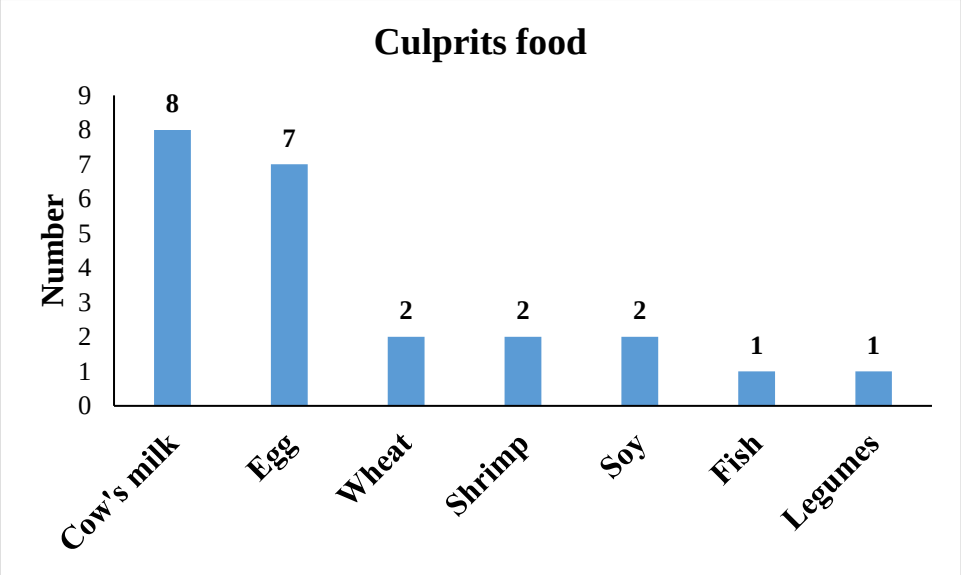


Figure 6.3-2: The number of food type for food allergies (culprits food) in this study

6.4 Discussion

6.4.1 Immunological outcomes (cytokines concentration in infant children)

The median (IQR) age of blood collection was similar between no allergy and allergy group [9.29 (1.55) vs 9.30 (0.61) months for no allergy and allergy group, respectively], therefore, the variation of age might not be an issue for interpretation of the result from this study. However, regards to the low-rate detection at above LOD of IL-1β, IL-4, IL-5, and IL-13 concentrations at <50% of samples, the interpretation of these cytokines may need caution, or it might represent the low secretion of this cytokines in infant children of this study. However, IL-4 and IL-5 seemed to be measure at higher concentration than in allergy group than no allergy group, compatible with the role of IL-4 and IL-5 in Th2 that drive allergic diseases.

IL-6, TNF-α, and TSLP were prominent in the allergy children group that supported by several research that these cytokines were associated with atopic dermatitis and food allergy [20, 423]. However, IFN-γ that seemed to be counteracted with allergy by augment the allergen tolerance that contradict to the result in this DPhil study and needed to be clarify by the larger study. However, they were not statistically significant.

IL-8, a proinflammatory cytokines that play a significant role by attracting neutrophil to the inflamed skin and contributing of atopic dermatitis [424] and can be produces more in food allergy infants, was significantly higher in no allergy group than allergy group [425].

However, for other cytokines associated with allergic diseases, particularly AD and food allergy—such as IL-31, IL-33, and IL-17α—this study found lower concentrations in the

allergy group compared to the no-allergy group but not reach statistically significant. This finding could potentially be influenced by the severity of the disease at the time of sample collection, which might have affected cytokine levels. Further studies are needed to clarify these observations and determine their significance in the context of allergic disease mechanisms.

This study found higher concentration of IL-12/IL-23p40, significant at $P = 0.060$ in the non-allergy group compared to the allergy group. IL-12/IL-23p40, often called p40, is a subunit of two important cytokines, IL-12 and IL-23. They belong to the pro-inflammatory cytokines IL-12 family (IL-12, -23, -27, -35, and -39). IL-12 and IL-23 derived the majority from DCs and macrophages. These cytokines play crucial roles in the immune system, particularly in regulating the differentiation and function of T cells and activated NK cells, which are central to innate and adaptive immunity against microbes, viruses, and parasites [426]. IL-12 stimulates IFN-γ production and drives Th1, essential in cell-mediated immunity inflammation, defence against viruses and bacteria, and immune intolerance.

In contrast to IL-12, IL-23 also influences Th17 differentiation, a subtype of T lymphocyte implicated in chronic inflammatory/autoimmune-mediated diseases [427]. IL-12/IL-23p40 was demonstrated as proinflammatory cytokines important in immune defence mechanisms and autoimmune/inflammatory diseases. Since underproduction can lead to infection susceptibility in preterm neonates [428], and overproduction can lead to inflammatory bowel disease (IBD) [429]. It is also significant in the promising targeted therapy of allergic diseases [430]. The significantly higher concentration of IL-12/IL-23p40 in the non-allergy group than in the allergy group suggest a potential role for this cytokine subunit in modulating allergic

responses, which may be through the Th1 skewing. However, this study did not find the differences in Th1 cytokines (IFN-γ and TNF-α) to confirm this potential mechanism.

Further research would be needed to understand this difference's precise mechanisms and implications, but it could indicate a regulatory role for IL-12/IL-23p40 in reducing the risk of allergies, especially AD and food allergies. This hypothesis was supported by the promising result of a phase II study of the novel biological agent of an anti-IL-12/IL23p40 monoclonal antibody (Ustekinumab) that diminishes Th1 and Th17/Th22 responses in adult AD [430]. However, the clinical data still need to be evaluated in the children group [431], and there is still a lack of evidence of role of IL-12/IL-23p40 food allergy children.

The study aimed to explore environmental factors influencing immunological changes potentially linked to the development of allergic diseases in children. However, this study was limited in its ability to elucidate the mechanisms driven by cytokine changes prior to the onset of allergic diseases due to its design and inadequate plasma samples of umbilical cord. Specifically, cytokine concentrations were not measured before the occurrence of disease outcomes, which constrains the ability to provide mechanistic evidence of how environmental interactions might drive allergic disease development through cytokine-mediated pathways. Nevertheless, the associations identified through univariate logistic regression analysis offer valuable insights that can guide future investigations to confirm this hypothesis.

6.4.2 Specific IgE (sIgE) and total IgE in pregnant women and children

Overall, there were no significant differences in all measured variables (sIgE and total IgE) between individuals with allergies (allergy group) and those without allergies (no-allergy

group) except number of elevated IgE in third trimester that higher rate in allergy group, as shown in **Table 6.2-1** and **Figure 6.2-1**. Notably, the median total IgE concentrations of pregnant women during both the first and third trimesters (IgE P01 and IgE P03) were higher in the no-allergy group compared to the allergy group, which contrasts with the typical association of elevated IgE with allergic conditions. This finding may reflect differences in maternal immune regulation during pregnancy, atopic status of pregnant women, or environmental factors that modulate IgE levels [432]. Conversely, total IgE and specific IgE levels in children aged 6–12 months (IgE C05 and sIgE C05) were higher in the allergy group, consistent with the expected role of IgE in allergic sensitisation. Total IgE in cord blood (IgE C01) was below the detection limit of 2 kU/L in nearly all samples (66 of 69), suggesting that cord blood IgE is not a reliable predictor of allergic outcomes in this cohort.

While no statistically significant differences were observed for most variables, the proportion of elevated total IgE levels in third-trimester pregnant women was higher in the allergy group compared to the no-allergy group (16.7% vs. 4.8%, $P = 0.091$). These findings contradict with previous report that prenatal IgE associated with AD in their offspring especially in the non-atopic pregnant women [433]. Although this result did not reach conventional significance, it suggests a potential link between maternal IgE levels and the child's allergic outcomes, warranting further investigation. These findings highlight the complexity of using IgE as a biomarker for allergic diseases, given the influence of genetic predisposition, environmental exposures, and immune modulation. Future studies should explore longitudinal changes in IgE levels and incorporate additional immune markers, such as cytokines, to better elucidate the mechanisms underlying allergic diseases.

6.4.3 Incidence of allergic diseases in the study population

In this study, the incidence of allergic diseases in infants was 27.9%, with AD accounting for 20.9% and food allergy for 7.0% of cases. Notably, among infants with AD, 55.0% also had concurrent food allergies. These findings suggest that the incidence rates observed in this study are lower than the reported global prevalence rates, where AD affects approximately 39% of children under 18 years old, and food allergy affects around 35% within the same age group [35-38]. However, a different picture emerges when comparing the study's results to the prevalence of allergic diseases in Thai children. The incidence rates in this study align more closely with the prevalence of AD, as reported in the ISAAC survey from 2003, which documented a prevalence of 16.7% for AD among Thai children [36]. Furthermore, when considering parent reports of food allergy in northern Thailand in 2019 [39], the prevalence of 5.5% is consistent with the food allergy incidence rate in this study.

The lower incidence of AD and food allergy observed in this study, compared to the global prevalence, may be attributed to several factors. First, the study focused on collecting prospective data from an infant group, and this age of assessment could have influenced the results. The cumulative incidences of these conditions in older children might differ and affect this observed difference. Additionally, several other factors could contribute to this variation. Genetic variations among populations, differences in environmental exposures, and diverse lifestyles can all impact the prevalence of allergic diseases. Furthermore, the assessment method used in this study, which involved physician-diagnosed allergic diseases, differs from the global prevalence estimates typically based on the International Study of Asthma and Allergies in Childhood (ISSAC) criteria and self-reported data. These variations in data collection methods can influence the reported prevalence rates.

However, when explicitly comparing the results within the Thai population, it is notable that the incidence was similar despite the differences in allergy evaluation methods. It suggests that ethnic influences may play a role in shaping the observed prevalence rates in different countries. Moreover, the study's findings align with previously reported incidence rates of allergic diseases in Thai children. This consistency enhances the confidence in the generalisability of the results to the broader Thai population. It implies that the study's findings align with existing data on allergy incidence in Thai children, strengthening the reliability of the conclusions drawn from this research.

6.5 Conclusion

This chapter presents infant outcomes, including immunological profiles, sensitisation, IgE concentrations, and the occurrence of allergic diseases. Given that immunological alterations, sensitisation, and IgE concentrations can act as both outcomes and contributing factors in the development of allergic diseases, comparisons between allergy and non-allergy groups are provided. These analyses form the basis for further exploration of the association between early life environmental factors, immunological changes, and allergic outcomes in infants and young children (**Chapter 7**).

Chapter 7: The association of *in utero* chemical exposures and infant allergies

This chapter explores the potential association between early-life chemical exposures and allergy incidence in infant children. The exploration considered the influence of lifestyle factors and socioeconomic status (SES) as potential confounding variables or co-factors that might affect the observed relationship. The analysis is primarily based on the outcomes of univariate assessments of specific chemical exposures, including heavy metals, ETS, and $PM_{2.5}$, about allergic outcomes among infants. These allergic outcomes encompass a range of factors, such as the development of allergic diseases, allergic sensitisation in infant (sIgE to common food allergen ≥ 0.35 PAU/L), and elevated infant total IgE concentration (total IgE ≥ 10 kU/L).

The analysis was carried out in a stepwise manner for univariate analysis. Firstly, a univariate analysis was conducted by selecting the clinically significant factors from the literature and the exposure assessment result in **Chapter 5** with $P \leq 0.1$, where each chemical exposure was individually assessed for its association with the allergic outcomes in infants. It involves examining whether there was a statistically significant relationship between each exposure and the occurrence of allergic diseases, sensitisation, and elevated IgE concentration in infants.

A multivariable analysis was performed following the univariate analysis to investigate further the significant factors identified in the previous step. Specifically, factors that show statistically significant evidence with $P \leq 0.1$ were considered for inclusion in the multivariable analysis.

This analysis helped to account for potential confounding variables and assess the independent association between chemical exposures and allergic outcomes.

The blood concentration of various heavy metals in pregnant women was measured during the first trimester (P01) to assess heavy metal exposure. Maternal and infant total IgE concentration was measured multiple times: in the first (P01) and third trimesters (P03) of pregnancy, at delivery (C01), and when the infants were between 6 and 12 months old (C05). Additionally, sIgE concentration was measured in pregnant women during the first trimester (P01), in newborn infants at birth (C01), and again when the infants reached 6 to 12 months of age (C05).

Cord blood was analysed for the presence of IgA to ensure result accuracy, which indicates maternal blood contamination. IgA concentration equal to or exceeding 6 mg/dL indicate maternal blood contamination, potentially affecting the analysis [434].

Moreover, the study explores the correlation between lifestyle factors, socioeconomic status, $PM_{2.5}$ exposure, metal exposures, and allergic outcomes (allergic diseases, infant total IgE concentration, and infant sIgE concentration). This correlation analysis helps understand how these factors might interact and contribute to the observed outcomes.

The chapter thus employed a comprehensive approach to investigating the relationship between *in utero* chemical exposures and infant allergies. Additionally, the study explored the broader context by examining correlations with lifestyle factors and socioeconomic status.

7.1 The correlation between chemical environmental exposure and cytokines concentration in infant children

The associations between chemicals and cytokines revealed distinct patterns when grouped by chemical types of heavy metal exposure, ETS exposure, and $PM_{2.5}$ exposure as shown in **Table 7.1-1**. The visualisation of the correlation coefficient of each cytokine concentration and each chemical (heat map) also presented in **Figure 7.1-1**.

7.1.1 Spearman's correlation of heavy metal concentration in pregnant women and plasma/serum cytokines in infant children

The correlation analysis of heavy metal concentrations in pregnant women with cytokine concentrations in infant children, as detailed in **Table 7.1-1**, reveals diverse associations. Significant positive and inverse correlations (correlation coefficients ranging from 0.2 to 0.4) were observed between various cytokines - such as IL-5, IL-6, IL-10, IL-31, IFN-γ, and TSLP – and different heavy metals. In vice versa, all examined heavy metals, except Cd and Pb, showed the significant with infant cytokines concentration.

There are different linkages of cytokines with disease states and so these were tested individually for association with heavy metals. Among the Th1 cytokines, only As demonstrated a significant positive correlation with IFN-γ (coefficient 0.225, $P = 0.050$). In the Th2 cytokine group (IL-5 and IL-10), Cr exhibited significant positive correlations with IL-10 (coefficient 0.288, $P = 0.016$), and Mn significant positive correlated with IL-5 (coefficient 0.285, $P = 0.018$).

For atopic dermatitis related cytokines, Hg was positively correlated with IL-31, and TSLP (coefficient 0.223, and 0.270, $P = 0.045$, and 0.023, respectively), As with TSLP (coefficient 0.287, $P = 0.017$) while Cr was significantly inverse correlated with both IL-31 and TSLP (coefficient -0.366, and -0.332, $P = 0.003$, and 0.007, respectively). Similarly, for inflammatory cytokines, Hg demonstrated a negative association with IL-6 (coefficient -0.353, $P = 0.005$).

Table 7.1-1: The correlation between heavy metal concentration in first-trimester pregnant women (P01), urine cotinine/creatinine ration (CCR) at each visit in pregnant women (P01, P03) and children (C01, C05), average daily $PM_{2.5}$ during each visit in pregnant women (P01, P02, P03) and children (C02, C03, C04), and plasma/serum cytokines concentration in infant children (C05), the Spearman's correlation coefficients for each pair of variables are presented.

Cytokines	Heavy metal (P01) N = 55						CCR N = 48				Average daily $PM_{2.5}$ N = 61					
	As	**Cd**	**Cr**	**Hg**	**Mn**	**Pb**	**P01**	**P03**	**C01**	**C05**	**P01**	**P02**	**P03**	**C02**	**C03**	**C04**
IL-1β	-0.148	-0.115	0.045	-0.041	0.032	0.085	**0.235 ***	0.034	0.017	1.000	-0.144	0.116	0.209	-0.110	-0.110	-0.143
P	0.141	0.202	0.372	0.384	0.409	0.269	**0.034**	0.193	0.488	0.321	0.134	0.186	0.053	0.200	0.200	0.135
IL-2	0.049	-0.027	0.221	0.013	-0.057	0.041	0.059	0.170	**0.251 ***	-0.028	-0.092	**0.362 ****	0.119	**-0.242 ***	**-0.313 ****	-0.123
P	0.362	0.422	0.052	0.464	0.339	0.383	0.326	0.100	**0.031**	0.418	0.241	**0.002**	0.181	**0.030**	**0.007**	0.173
IL-4	-0.141	0.027	0.068	-0.011	0.207	0.043	**0.321 ****	0.116	-0.079	0.209	-0.059	0.100	0.159	-0.018	-0.023	-0.026
P	0.153	0.422	0.310	0.468	0.065	0.377	**0.006**	0.192	0.283	0.058	0.327	0.220	0.110	0.446	0.430	0.421
IL-5	0.083	0.006	-0.079	0.191	**0.285 ***	-0.037	0.096	0.023	0.055	0.100	-0.114	0.069	0.132	-0.072	-0.067	-0.144
P	0.274	0.481	0.284	0.081	**0.018**	0.396	0.276	0.119	0.173	0.451	0.190	0.297	0.156	0.291	0.303	0.134
IL-6	-0.118	0.034	0.149	**-0.353 ****	0.041	0.023	0.014	-0.119	0.006	-0.149	**-0.257 ***	0.088	**0.297 ***	0.087	-0.127	-0.123
P	0.203	0.407	0.145	**0.005**	0.387	0.434	0.457	0.191	0.483	0.139	**0.026**	0.254	**0.012**	0.259	0.172	0.179
IL-8	-0.087	0.122	0.164	-0.209	-0.028	-0.041	0.077	-0.156	-0.128	-0.016	-0.178	0.054	**0.275 ***	0.091	-0.061	-0.097
P	0.264	0.187	0.116	0.063	0.419	0.382	0.276	0.119	0.173	0.451	0.085	0.341	**0.016**	0.244	0.319	0.229
IL-10	-0.059	0.007	**0.288 ***	-0.048	-0.033	-0.063	0.201	0.046	0.081	0.005	-0.080	0.204	0.174	-0.129	-0.158	-0.035
P	0.333	0.480	**0.016**	0.364	0.405	0.324	0.060	0.364	0.277	0.486	0.269	0.058	0.089	0.160	0.112	0.394
IL-12/IL-23p40	0.043	-0.055	-0.017	0.147	-0.096	0.186	0.104	0.122	0.055	0.163	-0.144	0.116	0.209	-0.110	-0.110	-0.143
P	0.378	0.346	0.451	0.142	0.243	0.087	0.240	0.205	0.277	0.134	0.134	0.186	0.053	0.200	0.200	0.135

Cytokines	Heavy metal (P01) N = 55						CCR N = 48				Average daily $PM_{2.5}$ N = 61					
	As	**Cd**	**Cr**	**Hg**	**Mn**	**Pb**	**P01**	**P03**	**C01**	**C05**	**P01**	**P02**	**P03**	**C02**	**C03**	**C04**
IL-13	-0.123	-0.005	0.017	0.069	0.204	0.074	**0.215 ***	-0.029	0.075	**0.296 ***	-0.139	0.128	0.117	-0.097	-0.114	-0.120
P	0.186	0.487	0.450	0.308	0.067	0.296	**0.048**	0.414	0.290	**0.012**	0.142	0.164	0.185	0.228	0.190	0.178
IL-17α	-0.070	0.106	0.198	-0.161	-0.110	-0.086	0.187	0.184	-0.158	0.084	-0.124	-0.167	0.176	0.206	0.158	-0.020
P	0.305	0.220	0.073	0.121	0.212	0.266	0.102	0.105	0.142	0.284	0.170	0.099	0.087	0.056	0.112	0.439
IL-22	-0.030	-0.092	-0.094	0.021	-0.013	0.050	-0.041	0.053	0.093	**-0.374 ****	**0.265 ***	-0.039	**-0.267 ***	-0.019	0.045	**0.231 ***
P	0.415	0.251	0.247	0.440	0.462	0.360	0.378	0.345	0.248	**0.002**	**0.019**	0.384	**0.019**	0.442	0.365	**0.037**
IL-31	0.108	-0.163	**-0.366 ****	**0.232 ***	0.017	0.099	0.154	**-0.302 ***	0.011	0.166	-0.188	-0.122	0.139	0.094	0.013	**-0.314 *****
P	0.217	0.117	**0.003**	**0.045**	0.452	0.237	0.118	**0.010**	0.469	0.106	0.073	0.175	0.143	0.235	0.462	**0.007**
IL-33	-0.184	-0.051	0.192	-0.027	-0.017	-0.124	**-0.213 ***	0.157	-0.006	-0.108	**0.368 ****	0.022	**-0.248 ***	-0.173	0.114	**0.422 *****
P	0.090	0.356	0.080	0.421	0.450	0.183	**0.050**	0.117	0.483	0.209	**0.002**	0.432	**0.027**	0.092	0.192	**<0.001**
IFN-γ	**0.225 ***	0.158	-0.132	0.167	0.147	-0.017	-0.041	0.108	0.157	-0.114	-0.017	0.057	0.056	-0.035	-0.028	0.036
P	**0.050**	0.124	0.168	0.112	0.141	0.451	0.391	0.233	0.143	0.221	0.447	0.332	0.334	0.394	0.414	0.391
TNF	0.163	-0.045	-0.012	0.047	0.145	-0.084	0.198	-0.092	0.148	-0.169	**-0.227 ***	0.182	0.198	-0.085	-0.210	-0.194
P	0.120	0.373	0.464	0.367	0.148	0.272	0.064	0.246	0.141	0.105	**0.040**	0.082	0.065	0.259	0.054	0.069
TSLP	**0.287 ***	-0.121	**-0.332 ****	**0.270 ***	0.016	0.012	**0.376 *****	**-0.324 ****	**-0.231 ***	**0.309 ****	**-0.317 ****	-0.204	0.210	0.199	0.012	**-0.389 ****
P	**0.017**	0.190	**0.007**	**0.023**	0.453	0.465	**0.001**	**0.006**	**0.044**	**0.009**	**0.006**	0.057	0.052	0.062	0.463	**0.001**

*** *P* ≤ 0.05, ** *P* ≤ 0.01, *** *P* ≤ 0.001**

Remarks; P01, pregnancy 1st trimester: P02, pregnancy 2nd trimester: P03, pregnancy 3rd trimester: C01, children at birth: C02, children at 2 months: C03, children at 4 months: C04, children at 6 months.

Cytokines	Heavy metal (1st trimester; P01) N = 55						CCR N = 48				Average daily $PM_{2.5}$ N = 61					
	As	Cd	Cr	Hg	Mn	Pb	P01	P03	C01	C05	P01	P02	P03	C02	C03	C04
IL-1β	-0.148	-0.115	0.045	-0.041	0.032	0.085	**0.235**	0.034	0.017	1.000	-0.144	0.116	0.209	-0.11	-0.11	-0.143
IL-2	0.049	-0.027	0.221	0.013	-0.057	0.041	0.059	0.170	**0.251**	-0.028	-0.092	**0.362**	0.119	**-0.242**	**-0.313**	-0.123
IL-4	-0.141	0.027	0.068	-0.011	0.207	0.043	**0.321**	0.116	-0.079	0.209	-0.059	0.1	0.159	-0.018	-0.023	-0.026
IL-5	0.083	0.006	-0.079	0.191	**0.285**	-0.037	0.096	0.023	0.055	0.1	-0.114	0.069	0.132	-0.072	-0.067	-0.144
IL-6	-0.118	0.034	0.149	**-0.353**	0.041	0.023	0.014	-0.119	0.006	-0.149	**-0.257**	0.088	**0.297**	0.087	-0.127	-0.123
IL-8	-0.087	0.122	0.164	-0.209	-0.028	-0.041	0.077	-0.156	-0.128	-0.016	**-0.178**	0.054	**0.275**	0.091	-0.061	-0.097
IL-10	-0.059	0.007	**0.288**	-0.048	-0.033	-0.063	0.201	0.046	0.081	0.005	-0.08	0.204	0.174	-0.129	-0.158	-0.035
IL-12/IL-23p40	0.043	-0.055	-0.017	0.147	-0.096	0.186	0.104	0.122	0.055	0.163	-0.144	0.116	0.209	-0.11	-0.11	-0.143
IL-13	-0.123	-0.005	0.017	0.069	0.204	0.074	**0.215**	-0.029	0.075	**0.296**	-0.139	0.128	0.117	-0.097	-0.114	-0.12
IL-17α	-0.07	0.106	0.198	-0.161	-0.11	-0.086	0.187	0.184	-0.158	0.084	-0.124	-0.167	0.176	0.206	0.158	-0.02
IL-22	-0.03	-0.092	-0.094	0.021	-0.013	0.05	-0.041	0.053	0.093	**-0.374**	**0.265**	-0.039	**-0.267**	-0.019	0.045	**0.231**
IL-31	0.108	-0.163	**-0.366**	**0.232**	0.017	0.099	0.154	**-0.302**	0.011	0.166	**-0.188**	-0.122	0.139	0.094	0.013	**-0.314**
IL-33	-0.184	-0.051	0.192	-0.027	-0.017	-0.124	**-0.213**	0.157	-0.006	-0.108	**0.368**	0.022	**-0.248**	-0.173	0.114	**0.422**
IFN-γ	**0.225**	0.158	-0.132	0.167	0.147	-0.017	-0.041	0.108	0.157	-0.114	-0.017	0.057	0.056	-0.035	-0.028	0.036
TNF	0.163	-0.045	-0.012	0.047	0.145	-0.084	0.198	-0.092	0.148	-0.169	-0.227	0.182	**0.198**	-0.085	-0.21	-0.194
TSLP	**0.287**	-0.121	**-0.332**	**0.270**	0.016	0.012	**0.376**	**-0.324**	**-0.231**	**0.309**	-0.317	-0.204	**0.21**	0.199	0.012	**-0.389**

Figure 7.1-1: The colour gradient (heat map) to show the level of correlation coefficient of each cytokine concentration and each chemical; heavy metal concentration, urine cotinine/creatinine ratio, and average daily $PM_{2.5}$ concentration. The colour indicates where each cell value falls within that range of positive correlation (green) and inverse correlation (red).

7.1.2 Spearman's correlation of urine cotinine-creatinine ratio (CCR) and cytokine concentration

The associations between the urine CCR across different trimesters of pregnancy and in children were explored and associated with infant cytokine concentrations (**Table 7.1-1** and **Figure 7.1-1**).

7.1.2.1 CCR in pregnant women and cytokine concentration in infant children

During a birth cohort study, cytokines were measured in infant children at 6 – 12 months, while CCR concentrations were assessed in pregnant women during the 1st and 3rd trimesters and in infant children at birth and 6-12 months. Positive correlations were observed between maternal CCR concentrations in 1st trimester and several infant cytokines, including proinflammatory (IL-1β with coefficient 0.235, $P = 0.034$), Th2-skewed (IL-4 and IL-13 with coefficient 0.321 and 0.215, $P = 0.006$ and 0.048, respectively), and epithelial related (TSLP with coefficient 0.376, $P = 0.001$) immune responses in infants. Inverse correlations were also noted with IL-31, coefficient -0.302, $P = 0.010$ (with 3rd trimester CCR), IL-33, coefficient -0.213, $P = 0.050$ (with 1st trimester CCR), and TSLP cytokines, coefficient -0.324, $P = 0.006$ (with 3rd trimester CCR) often associated with allergic inflammation and epithelial cell activation as known as AD-related cytokines.

7.1.2.2 CCR and cytokine concentration in infant children

In newborn infants, CCR concentrations measured in cord blood at birth showed positive correlations with IL-2 (coefficient 0.251, $P = 0.031$). Conversely, an inverse correlation was observed between cord blood CCR and TSLP (coefficient -0.231, $P = 0.044$). These findings align with patterns observed during pregnancy, where maternal CCR concentrations showed

dynamic correlations with cytokines, including a bidirectional relationship with TSLP—positive in the 1st trimester and negative in the 3rd trimester.

At 6–12 months of age, CCR concentrations measured during infancy demonstrated positive correlations with IL-13 (coefficient 0.296, $P = 0.012$), and TSLP (coefficient 0.309, $P = 0.009$), the cytokines associated with Th2-skewed immune responses and allergic diseases. Additionally, CCR at 6–12 months showed an inverse correlation with IL-22 (coefficient -0.374, $P = 0.002$), a cytokine involved in epithelial barrier integrity and tissue repair, pointing to its complex role in modulating barrier-protective and inflammatory responses during early childhood.

7.1.3 Spearman's correlation of ambient $PM_{2.5}$ concentration and cytokine concentration

Average ambient 24-hour $PM_{2.5}$ concentrations across various stages of pregnancy and infancy were measured and correlated with plasma/serum cytokine concentrations in infant children (**Table 7.1-1** and **Figure 7.1-1**).

7.1.3.1 Prenatal $PM_{2.5}$ exposure and infant cytokine

Most correlations were observed between the average 24-hour $PM_{2.5}$ concentrations during the first trimester of pregnancy, and cytokine concentrations in infant children at 6–12 months. A significant positive correlation was found with IL-22 (coefficient 0.265, $P = 0.019$) and IL-33 (coefficient 0.368, $P = 0.002$), suggesting that prenatal exposure to $PM_{2.5}$ during early pregnancy may influence pathways involved in epithelial defence and allergic

inflammation. Conversely, an inverse correlation were identified between $PM_{2.5}$ concentrations and proinflammatory cytokines (IL-6 and TNF with coefficient -0.257 and -0.227, P = 0.026 and 0.040, respectively), and epithelial related cytokine (TSLP) with coefficient -0.317, P = 0.006. While in the second trimester, weak significant positive correlations were observed only between the average 24-hour $PM_{2.5}$ exposure and the cytokines IL-2 (coefficient 0.362, P = 0.002).

In the third trimester, several positive correlations were observed between average $PM_{2.5}$ concentrations and cytokine concentrations in infants at 6–12 months. Positive correlations were found with proinflammatory cytokines of IL-6, and IL-8 (coefficient 0.297, 0.275, P = 0.012, 0.016, respectively). In contrast, significant inverse correlations were observed with IL-22 (coefficient -0.267, P = 0.019) and IL-33 (coefficient -0.248, P = 0.027), both cytokines associated with epithelial barrier function and tissue repair.

These findings collectively highlight the complex and dynamic role of $PM_{2.5}$ exposure at different stages of pregnancy in shaping immune development in infants. While the first and second trimesters may influence immune pathways related to inflammation and immune activation, the third trimester appears to represent a critical period where environmental pollutants like $PM_{2.5}$ can significantly impact immune system maturation.

7.1.3.2 Postnatal $PM_{2.5}$ exposure and infant cytokines

Compared to prenatal $PM_{2.5}$ exposure, postnatal $PM_{2.5}$ exposure during the first 6 months of life showed fewer correlations with infant cytokines, though inverse correlations were found

in IL-2, IL-31, IL-33, and TSLP and positive correlations were with average $PM_{2.5}$ exposure during infant children 4-6 months and IL-22 and IL-33.

Furthermore, the significant inverse association between $PM_{2.5}$ exposure and cytokine concentrations persisted over time, with $PM_{2.5}$ exposure during 0-2 months and 2-4 months displaying a significant inverse correlation with IL-2 (coefficient -0.242, and -0.313, $P = 0.030$, and 0.007 for average $PM_{2.5}$ during 0-2 and 2-4 months, respectively). Notably, $PM_{2.5}$ exposure during 4-6 months of age showed a positive correlations with IL-22 (coefficient 0.231, $P = 0.037$) and IL-33 (coefficient 0.422, $P < 0.001$), both of which are involved in epithelial barrier function and tissue repair, while an inverse correlation with IL-31 (coefficient -0.314, $P = 0.007$) and TSLP (coefficient -0.389, $P = 0.001$) was observed.

Overall, these findings highlight the complex and age-dependent nature of the immune response to early life $PM_{2.5}$ exposure. The variation in cytokine correlations depending on the timing of exposure underscores the importance of considering both prenatal and postnatal environmental factors in the development of immune system function in infant children.

7.2 The potential influenced factors of early life and allergic outcomes in infant children

Overall, a univariate logistic regression analysis that examines the association between various variables and the allergic diseases outcome in children showed that gravida, birthweight, maternal body mass index (BMI) before pregnancy, maternal occupation, vitamin intake during 1^{st} pregnancy, exclusive breast feeding ≥ 4 months. The sibling allergy, maternal sensitisation,

and gender were significantly associated with sensitisation in infant children. SES in terms of maternal education, elevated maternal IgE in 1st trimester, birthweight, gender, and birth season factors were associated with elevated infant total IgE concentration (**Table 7.2-1**). However, these factors solely analysis the association with each outcome on a pairwise basis and need to be considered further for interaction with multiple factors, therefore, the multivariable logistic regression was carried out by incorporating these potential factors in the analysis for each outcome.

Table 7.2-1: The univariate logistic regression analysis of potential influential factors during pregnancy and infancy period on allergic outcomes (allergic diseases, sensitisation, and elevated total IgE concentration) in infant children.

Variables	Allergic diseases			Infant Sensitisation			Elevated infant total IgE concentration		
	N	Crude OR (95%CI)	*P*	N	Crud OR (95%CI)	*P*	N	Crude OR (95%CI)	*P*
Site of data collection	86			86			86		
Ramathibodi Hospital		--	--	--	--	--		1	
Rayong Hospital		--	--	--	--	--		0.622 (0.149, 2.594)	0.515
Maternal age at pregnancy ≥ 35 years	86	0.964 (0.233, 3.987)	0.960	86	1.212 (0.230, 6.375)	0.821	86	0.622 (0.149, 2.594)	0.515
Gravida ≥ 3	86	0.191 (0.041, 0.893)	**0.035**	86	0.684 (0.174, 2.692)	0.587	86	0.482 (0.167, 1.392)	0.177
Mean body mass index (BMI) before pregnancy; kg/m^2	83	1.122 (0.998, 1.261)	**0.054**	83	0.981 (0.855, 1.125)	0.783	83	0.916 (0.819, 1.025)	0.126
Family member ≥ 3	86	1.435 (0.557, 3.696)	0.454	86	0.952 (0.312, 2.907)	0.931	86	0.789 (0.331, 1.881)	0.594
Maternal occupation (Pregnant women)	86			86			86		
Unemployed, housewife, student, freelancer		1			1			1	
Medical personal, industry, hairdresser		36.000 (3.124, 414.894)	**0.004**		2.667 (0.417, 17.046)	0.300		1.719 (0.347, 8.508)	0.507
Teacher, officers or employees either in government or private sector		7.412 (0.901, 60.991)	**0.063**		1.067 (0.251, 4.538)	0.930		0.688 (0.231, 2.046)	0.501
Merchant, personal business		7.714 (0.682, 87.252)	**0.099**		0.593 (0.053, 6.572)	0.670		1.375 (0.295, 6.402)	0.685
Family income > 20,000 Thai Baht	82	1.779 (0.525, 6.028)	0.355	82	5.417 (0.663, 44.224)	0.115	82	2.541 (0.826, 7.820)	0.104
Maternal education (P01)	86			86			86		
Below undergraduate		1			1			1	
Undergraduate		1.238 (0.448, 3.419)	0.680		0.444 (0.118, 1.670)	0.230		1.150 (0.446, 2.965)	0.772
Graduate		1.083 (0.235, 4.994)	0.918		2.286 (0.519, 10.057)	0.274		3.354 (0.817, 13.778)	**0.093**
Maternal common allergy (pregnant women)	86	1.040 (0.292, 3.700)	0.952	86	0.756 (0.151, 3.795)	0.756	86	2.359 (0.738, 7.545)	0.148
Paternal common allergy (partner)	86	1.571 (0.415, 5.946)	0.506	86	1.060 (0.205, 5.489)	0.945	86	2.014 (0.562, 7.214)	0.282
Sibling common allergy	86	1.304 (0.113, 15.087)	0.832	86	10.769 (0.909, 127.619)	**0.060**	86	3.187 (0.278, 36.600)	0.352
Maternal sensitisation	86	2.250 (0.688, 7.361)	0.180	86	5.250 (1.477, 18.660)	**0.010**		--	--
Elevated maternal total IgE concentration (≥ 100 KUA/L)									
1st trimester (P01)	86	2.068 (0.586, 7.294)	0.259	86	1.722 (0.406, 7.309)	0.461	86	5.880 (1.461, 23.669)	**0.013**
3rd trimester (P03)	86	3.933 (0.810, 19.106)	**0.089**	86	0.774 (0.086, 6.944)	0.819	86	2.178 (0.456, 10.409)	0.330

Variables	Allergic diseases			Infant Sensitisation			Elevated infant total IgE concentration		
	N	Crude OR (95%CI)	*P*	N	Crud OR (95%CI)	*P*	N	Crude OR (95%CI)	*P*
Infant sensitisation		1.963 (0.613, 6.282)	0.256		--	--		--	--
Elevated infant total IgE concentration		1.435 (0.553, 3.725)	0.458		--	--		--	--
GDM	86	4.286 (0.669, 27.446)	0.125	86	1.196 (0.124, 11.531)	0.877	86	0.364 (0.039, 3.401)	0.375
Folic acid intake during 1st trimester	86	0.964 (0.233, 3.987)	0.960	86	0.436 (0.051, 3.689)	0.446	86	0.857 (0.231, 3.185)	0.818
Vitamin intake during 1st trimester	86	7.895 (1.415, 44.048)	**0.018**	86	0.774 (0.086, 6.944)	0.819	86	1.161 (0.243, 5.546)	0.851
Gestational age (GA ≥ 37 weeks)	83	1.019 (0.184, 5.649)	0.983		--	--	83	1.818 (0.332, 9.971)	0.491
Birth weight (≥ 2,500 gram)	84	0.271 (0.066, 1.116)	**0.071**	84	1.836 (0.212, 15.896)	0.581	84	6.286 (0.748, 52.902)	**0.090**
Small for gestational age (SGA)	83	2.944 (0.842, 10.293)	**0.091**	83	0.892 (0.174, 4.568)	0.891	83	0.430 (0.107, 1.724)	0.234
Mode of delivery	84			84			84		
Vaginal		1			1			1	
Caesarean section		2.179 (0.825, 5.758)	0.116		2.452 (0.758, 7.928)	0.134		1.612 (0.670, 3.877)	0.286
Gender	84			84			84		
Male		1			1			1	
Female		1.773 (0.682, 4.605)	0.240		0.257 (0.067, 0.993)	**0.049**		0.441 (0.178, 1.094)	**0.078**
Birth season	86			86			86		
Winter (November to February)		1			1			1	
Summer (March to May)		0.573 (0.130, 2.521)	0.461		--	--		0.848 (0.212, 3.391)	**0.813**
Rainy (June to October)		0.616 (0.222, 1.708)	0.352		1.219 (0.384, 3.870)	0.737		1.649 (0.635, 4.278)	0.304
Exclusive breastfeeding ≥ 4 months	78	3.571 (1.253, 10.182)	**0.017**	78	0.591 (0.178, 1.960)	0.390	78	0.848 (0.343, 2.101)	0.723
Exclusive breastfeeding ≥ 6 months	80	1.642 (0.357, 7.544)	0.524		--	--	79	0.174 (0.020, 1.490)	0.111

Environmental exposures during pregnancy and their association with the risk of developing allergic diseases in infants were assessed using univariate logistic regression analyses, conducted separately for each trimester of pregnancy (first, second, and third), as shown in **Table 7.2-2**. The environmental factors examined included the season of data collection and various home environmental factors, such as type of residence, ventilation system, visibility of mould, pet exposure, household ETS, proximity to heavy traffic areas, presence of greenery at home, use of common household chemical products, and recent house furnishing or renovations within three months.

Significant associations with allergic diseases ($P \leq 0.1$) were identified for data collection during the summer season in the third trimester, type of residence during the second or third trimester, ventilation system across all trimesters, pet ownership in the second trimester, and recent house furnishing or renovations in the first trimester. These factors were included in the multivariable logistic regression analysis to explore the relationship between multiple environmental exposures and allergic diseases in children.

For the outcome of sensitisation, the significant environmental factors selected for the multivariable logistic regression model included the ventilation system during the second and third trimesters, as well as household ETS during the second and third trimesters. Additionally, for the outcome of elevated infant total IgE concentration, mould visibility at home in the first trimester, pet exposure in the second trimester, and household ETS during the second and third trimesters were identified as important variables ($P \leq 0.1$).

Table 7.2-2: The univariate logistic regression analysis of environmental exposure during pregnancy (*in utero*) and risk for allergic diseases, sensitisation, and elevated total IgE concentration in infant children

Variables	Allergic diseases			Infant sensitisation			Elevated infant total IgE concentration		
	N	**OR (95%CI)**	***P***	**N**	**OR (95%CI)**	***P***	**N**	**OR (95%CI)**	***P***
Season of data collection									
1st trimester	86			86			86		
Winter (November to February)		1			1			1	
Summer (March to May)		2.000 (0.547, 7.312)	0.295		1.647 (0.441, 6.149)	0.458		0.917 (0.298, 2.817)	0.879
Rainy (June to October)		1.692 (0.506, 5.659)	0.393		0.353 (0.076, 1.637)	0.183		0.458 (0.160, 1.316)	0.147
2nd trimester	85			85			85		
Winter (November to February)		1			1			1	
Summer (March to May)		0.778 (0.187, 3.227)	0.729		1.789 (0.290, 11.035)	0.531		1.058 (0.310, 3.613)	0.929
Rainy (June to October)		1.352 (0.406, 4.506)	0.624		2.250 (0.437, 11.588)	0.332		0.815 (0.271, 2.450)	0.715
3rd trimester	84			84			84		
Winter (November to February)		1			1			1	
Summer (March to May)		0.200 (0.038, 1.061)	**0.059**		0.958 (0.143, 6.404)	0.965		2.714 (0.765, 9.634)	0.122
Rainy (June to October)		0.686 (0.242, 1.940)	0.477		2.556 (0.630, 10.362)	0.189		2.221 (0.764, 6.457)	0.143
Type of residence									
1st trimester	86			86			86		
Detached house		1			1			1	
Flat/ condominium/ apartment		0.415 (0.139, 1.246)	0.117		0.966 (0.311, 2.995)	0.952		0.861 (0.344, 2.155)	0.749
Row house		2.077 (0.510, 8.466)	0.308		--	--		0.580 (0.131, 2.575)	0.474
2nd trimester	86			85			85		
Detached house		1			1			1	
Flat/ condominium/ apartment		0.360 (0.122, 1.060)	**0.064**		1.091 (0.337, 3.530)	0.885		1.370 (0.535, 3.506)	0.512
Row house		1.458 (0.366, 5.812)	0.593		0.450 (0.048, 4.218)	0.484		1.000 (0.242, 4.131)	1.000
3rd trimester	86			84			84		
Detached house		1			1			1	
Flat/ condominium/ apartment		0.656 (0.213, 2.019)	0.463		1.394 (0.413, 4.702)	0.593		1.481 (0.559, 3.919)	0.430
Row house		3.000 (0.817, 11.017)	**0.098**		0.343 (0.036, 3.240)	0.350		0.595 (0.151, 2.338)	0.457

Variables	Allergic diseases			Infant sensitisation			Elevated infant total IgE concentration		
	N	OR (95%CI)	*P*	N	OR (95%CI)	*P*	N	OR (95%CI)	*P*
Ventilation system									
1st trimester	86			86			86		
Open windows/ doors		0.228 (0.084, 0.619)	**0.004**		0.513 (0.165, 1.592)	0.248		1.382 (0.546, 3.498)	0.495
Air conditioner (AC)		3.158 (0.962, 10.369)	**0.058**		2.174 (0.560, 8.433)	0.262		1.599 (0.620, 4.128)	0.332
Fan		0.720 (0.235, 2.203)	0.565		0.448 (0.131, 1.534)	0.201		0.581 (0.204, 1.657)	0.310
Ventilation fan		0.855 (0.085, 8.648)	0.894		--	--		1.562 (0.209, 11.656)	0.663
Air purifier		2.652 (0.159, 44.184)	0.497		--	--		1.545 (0.093, 25.572)	0.761
2nd trimester	85			85			85		
Open windows/ doors		0.525 (0.201, 1.369)	0.188		1.833 (0.470, 7.158)	0.383		1.042 (0.427, 2.541)	0.928
Air conditioner (AC)		3.949 (1.057, 14.749)	**0.041**		0.250 (0.073, 0.856)	**0.027**		1.270 (0.484, 3.332)	0.627
Fan		0.836 (0.256, 2.725)	0.766		4.929 (0.291, 83.577)	0.269		0.605 (0.202, 1.806)	0.368
Ventilation fan		2.609 (0.157, 43.468)	0.504		4.929 (0.291, 83.577)	0.269		1.515 (0.092, 25.077)	0.772
Air purifier		2.240 (0.547, 9.179)	0.262		8.250 (1.890, 36.005)	**0.005**		15.385 (1.824, 129.743)	0.012
3rd trimester	84			84			84		
Open windows/ doors		0.350 (0.125, 0.979)	**0.046**		0.223 (0.069, 0.719)	**0.012**		0.758 (0.284, 2.025)	0.580
Air conditioner (AC)		5.500 (1.175, 25.754)	**0.030**		0.971 (0.274, 3.436)	0.963		0.758 (0.284, 2.025)	0.580
Fan		1.571 (0.397, 6.216)	0.519		0.197 (0.055, 0.700)	**0.012**		0.443 (0.139, 1.420)	0.171
Ventilation fan		1.261 (0.109, 14.590)	0.853		2.393 (0.203, 28.249)	0.488		--	--
Air purifier		3.800 (0.782, 18.472)	**0.098**		4.062 (0.805, 20.501)	**0.090**		4.138 (0.753, 22.728)	0.102
Mould visibility at home									
1st trimester	86	0.643 (0.208, 1.986)	0.443	86	0.995 (0.283, 3.506)	0.994	86	2.600 (0.979, 6.905)	**0.055**
2nd trimester	85	1.230 (0.460, 3.291)	0.680	85	0.655 (0.189, 2.272)	0.504	85	1.680 (0.676, 4.176)	0.264
3rd trimester	84	0.778 (0.265, 2.282)	0.647	84	1.316 (0.398, 4.353)	0.653	84	1.361 (0.523, 3.544)	0.528
Pet exposure									
1st trimester	86			86			86		
Any pet exposure		0.692 (0.258, 1.858)	0.465		1.024 (0.328, 3.193)	0.968		0.603 (0.244, 1.488)	0.272
Dog		0.575 (0.171, 1.938)	0.372		1.250 (0.350, 4.463)	0.731		0.778 (0.274, 2.204)	0.636
Cat		0.538 (0.139, 2.088)	0.371		2.727 (0.780, 9.531)	0.116		1.239 (0.413, 3.717)	0.703

Variables	Allergic diseases			Infant sensitisation			Elevated infant total IgE concentration		
	N	**OR (95%CI)**	***P***	**N**	**OR (95%CI)**	***P***	**N**	**OR (95%CI)**	***P***
2nd trimester (P02)	85			85			85		
Any pet exposure		0.288 (0.088, 0.945)	**0.040**		0.655 (0.189, 2.272)	0.504		0.440 (0.167, 1.158)	**0.096**
Dog		0.402 (0.105, 1.531)	0.181		0.844 (0.212, 3.363)	0.810		0.843 (0.294, 2.416)	0.750
Cat		--	--		2.667 (0.585, 12.154)	0.205		0.726 (0.169, 3.124)	0.667
3rd trimester (P03)	84			84			84		
Any pet exposure		0.455 (0.149, 1.388)	0.166		0.727 (0.209, 2.536)	0.617		0.502 (0.189, 1.333)	0.167
Dog		0.516 (0.133, 2.006)	0.340		1.077 (0.265, 4.374)	0.917		0.857 (0.279, 2.631)	0.788
Cat		--	--		2.625 (0.576, 11.968)	0.212		1.200 (0.298, 4.835)	0.798
Household ETS									
1st trimester	86			86			86		
Any household ETS		0.845 (0.296, 2.409)	0.752		1.005 (0.285, 3.540)	0.994		1.318 (0.488, 3.560)	0.587
From partner		0.757 (0.243, 2.360)	0.631		1..719 (0.513, 5.760)	0.380		1.200 (0.442, 3.256)	0.720
From housemate (s)		1.096 (0.341, 3.531)	0.877		1.018 (0.253, 4.102)	0.980		1.980 (0.678, 5.781)	0.211
From neighbour (s)		1.017 (0.384, 2.694)	0.972		0.558 (0.162, 1.929)	0.357		0.706 90.285, 1.7490	0.452
2nd trimester (P02)	85			85			85		
Any household ETS		0.792 (0.276, 2.276)	0.665		0.952 (0.269, 3.368)	0.939		0.575 (0.216, 1.533)	0.269
From partner		0.817 (0.235, 2.837)	0.750		2.682 (0.767, 9.376)	0.122		1.210 (0.403, 3.634)	0.734
From housemate (s)		1.342 (0.406, 4.437)	0.630		1.950 (0.525, 7.2500	0.319		1.394 (0.453, 4.283)	0.562
From neighbour (s)		0.842 (0.324, 2.188)	0.724		0.250 (0.065, 0.9640	**0.004**		0.425 (0.172, 1.051)	**0.064**
3rd trimester (P03)	84			84			84		
Any household ETS		0.739 (0.255, 2.143)	0.578		0.899 (0.253, 3.197)	0.869		0.677 (0.250, 1.8330)	0.443
From partner		0.329 (0.068, 1.584)	0.165		1.187 (0.290, 4.864)	0.811		0.976 (0.312, 3.050)	0.967
From housemate (s)		2.167 (0.661, 7.097)	0.202		0.297 (0.086, 1.0240	**0.055**		3.240 (0.978, 10.731)	**0.054**
From neighbour (s)		0.792 (0.306, 2.045)	0.629		3.333 (0.925, 12.012)	**0.066**		0.364 (0.147, 0.897)	**0.028**
Living near a busy road									
1st trimester	86	0.888 (0.337, 2.342)	0.810	86	1.024 (0.328, 3.193)	0.968	86	1.371 (0.568, 3.3100	0.483
2nd trimester	85	0.737 (0.252, 2.151)	0.576	85	0.848 (0.242, 2.971)	0.797	85	0.788 (0.300, 2.066)	0.627
3rd trimester	84	0.789 (0.251, 2.482)	0.686	84	0.765 (0.193, 3.035)	0.703	84	0.551 (0.188, 1.617)	0.278

Variables	Allergic diseases			Infant sensitisation			Elevated infant total IgE concentration		
	N	OR (95%CI)	*P*	N	OR (95%CI)	*P*	N	OR (95%CI)	*P*
Presence of a green environment at home									
1st trimester	86	0.962 (0.270, 3.421)	0.952	86	0.733 (0.177, 3.031)	0.668	86	0.424 (0.133, 1.356)	0.148
2nd trimester	85	1.224 (0.352, 4.254)	0.750	85	0.569 (0.155, 2.092)	0.396	85	0.827 (0.275, 2.482)	0.734
3rd trimester	84	3.043 (0.631, 14.660)	0.165	84	0.842 (0.206, 3.449)	0.811	84	1.450 (0.448, 1.696)	0.535
Common household chemical product consumption									
1st trimester	86			86			86		
Use any chemical product		1.444 (0.538, 3.877)	0.465		2.012 (0.584, 6.936)	0.268		1.094 (0.451, 2.655)	0.842
Bathroom cleaning liquid		1.318 (0.225, 7.715)	0.759		2.577 (0.427, 15.563)	0.302		1.581 (0.300, 8.332)	0.589
Bleach for washing		0.849 (0.236, 3.070)	0.803		1.192 (0.235, 6.031)	0.832		2.460 (0.624, 9.693)	0.198
Disinfectant		0.500 (0.187, 1.337)	0.167		1.066 (0.349, 3.258)	0.910		0.756 (0.316, 1.810)	0.530
2nd trimester	85			85			85		
Use any chemical product		0.878 (0.340, 2.270)	0.788		0.619 (0.202, 1.897)	0.401		0.645 (0.269, 1.550)	0.327
Bathroom cleaning liquid		0.833 (0.156, 4.499)	0.831		3.250 (0.684, 15.438)	0.138		1.567 (0.364, 6.743)	0.547
Bleach for washing		0.745 (0.225, 2.463)	0.630		0.828 (0.202, 3.388)	0.792		1.000 (0.320, 3.121)	1.000
Disinfectant		0.544 (0.207, 1.430)	0.217		0.667 (0.214, 2.073)	0.484		0.759 (0.318, 1.815)	0.535
3rd trimester	84			84			85		
Use any chemical product		1.111 (0.419, 2.944)	0.832		0.691 (0.224, 2.129)	0.520		0.713 (0.293, 1.735)	0.455
Bathroom cleaning liquid		0.818 (0.153, 4.370)	0.814		3.200 (0.673, 15.205)	0.144		1.533 (0.356, 6.604)	0.566
Bleach for washing		0.882 (0.244, 3.194)	0.849		0.678 (0.162, 2.838)	0.595		0.760 (0.231, 2.497)	0.651
Disinfectant		0.641 (0.243, 1.691)	0.369		0.771 (0.247, 2.401)	0.653		0.619 (0.255, 1.502)	0.289
Recently furnished/renovated house (within 3 months)									
1st trimester	86	3.929 (1.235, 12.496)	**0.020**	86	1.229 (0.300, 5.031)	0.774	86	0.497 (0.144, 1.713)	0.268
2nd trimester	85	1.020 (0.287, 3.631)	0.976	85	0.744 (0.148, 3.732)	0.719	85	1.630 (0.515, 5.157)	0.406
3rd trimester	84	1.647 (0.557, 4.869)	0.367	84	0.828 (0.208, 3.302)	0.789	84	1.091 (0.387, 3.078)	0.869

7.3 The association of early life ETS exposure and infant allergies

No cases of ETS exposure, defined as a cotinine/creatinine ratio (CCR) ≥ 30 ng/mg creatinine, were observed during the first trimester of pregnancy (P01) or at birth (C01) among participants in the allergic disease group. Additionally, there was no statistically significant association between CCR concentrations or a CCR ≥ 30 ng/mg creatinine and allergic disease outcomes. However, significant associations at $P \leq 0.1$, which were included in the multivariable logistic regression model, were identified. These included the CCR in the third trimester of pregnancy for the sensitisation outcome and the CCR in children at birth (C01) for the elevated infant total IgE concentration outcome. The findings are summarised in **Table 7.3-1**.

Table 7.3-1: The univariate analysis for the association between environmental tobacco smoke (ETS) exposure by measuring urine cotinine/creatinine (CCR) during pregnancy (1st and 3rd trimester) and infant children (at birth and 6-12 months) and outcome of allergic diseases, sensitisation, and elevated total IgE concentration in infant children. Allergic diseases were AD and food allergies; sensitisation was categorised by plasma/serum sIgE to PhadiaTop® Infant ≥ 0.35 PAU/L, and elevated infant total IgE concentration cut off at ≥ 10 kU/L. CCR were presented in both continuous data and categorical data (low and high level, cut-off CCR at 30 ng/mg creatinine).

CCR (ng/mg creatinine)	**Allergic diseases**			**Infant sensitisations**			**Elevated infant total IgE concentration**		
	N	**OR (95%CI)**	***P***	N	**OR (95%CI)**	***P***	N	**OR (95%CI)**	***P***
Pregnancy 1st trimester	86	0.553 (0.246, 1.244)	0.152	86	1.000 (1.000, 1000)	0.702	86	1.000 (1.000, 1000)	0.572
≥ 30		--	**--**		--	--		1.545 (0.093, 25.572)	0.761
Pregnancy 3rd trimester	86	0.996 (0.981, 1.010)	0.566	84	1.000 (1.000, 1.000)	**0.087**	84	1.000 (1.000, 1000)	0.153
≥ 30		0.855 (0.085, 8.648)	0.894		17.500 (1.678, 182.501)	**0.017**		--	--
Children at birth	69	0.848 (0.558, 1.288)	0.439	69	1.000 (1.000, 1.001)	0.175	69	1.001 (1.000, 1.001)	**0.053**
≥ 30		--	--		--	--		--	--
Children at 6-12 months	**77**	1.002 (0.991, 1.014)	0.675	**77**	1.000 (1.000, 1.000)	0.336	77	1.000 (1.000, 1000)	0.196
≥ 30		0.877 (0.163, 4.731)	0.879		0.653 (0.074, 5.740)	0.701		1.600 (0.372, 6.883)	0.528

Abbreviations: RR, relative risks, CCR; urine cotinine-creatinine ratio

7.4 The association of prenatal heavy metal exposure and infant allergies

The univariate logistic regression analysis of metal concentrations in first-trimester pregnant women revealed that, among the heavy metals analysed, only Pb demonstrated a statistically significant association with allergic diseases. Notably, Pb was associated with a reduced risk of allergic diseases (OR: 0.870, 95%CI: 0.753, 1.004, P = 0.057). The detailed results are presented in **Table 7.4-1**. Based on this finding, Pb was included in the multivariable logistic regression model to further evaluate the combined effects of multiple environmental factors on allergic diseases in infant children.

For the outcomes of infant sensitisation and elevated infant total IgE concentration, none of the metals (Cr, Mn, As, Cd, Hg, and Pb) demonstrated statistically significant associations at the $P \leq 0.1$.

Table 7.4-1: The univariate logistic regression of metal exposures in the first trimester of pregnant women (P01) and allergic outcomes in infant children (allergic diseases, infant sensitisation, and elevated infant total IgE concentration). Allergic diseases were AD and food allergies; infant sensitisation was categorised by plasma/serum sIgE to PhadiaTop® Infant ≥ 0.35 PAU/L, and elevated infant total IgE concentration cut off at ≥ 10 kU/L.

Metals	**Allergic diseases**			**Infant sensitisation**			**Elevated infant total IgE concentration**		
	N	**Crude OR (95%CI)**	***P***	N	**Crude OR (95%CI)**	***P***	N	**Crude OR (95%CI)**	***P***
As	77	1.803 (0.818, 3.972)	0.144	77	1.100 (0.675, 1.793)	0.701	77	0.951 (0.637, 1.421)	0.806
Cd	77	1.077 (0.941, 1.233)	0.281	77	0.003 (0.000, 178.016)	0.301	77	1.075 (0.059, 19.650)	0.961
Mn	77	0.895 (0.507, 1.582)	0.703	77	0.589 (0.206, 1.682)	0.323	77	0.722 (0.360, 1.448)	0.360
Hg	77	0.002 (0.000, 9.751)	0.149	77	0.896 (0.557, 1.441)	0.649	77	0.895 (0.634, 1.262)	0.526
Mn	77	1.388 (0.898, 2.145)	0.140	77	1.010 (0.903, 1.129)	0.867	77	1.046 (0.958, 1.143)	0.317
Pb	77	0.870 (0.753, 1.004)	**0.057**	77	0.907 (0.789, 1.044)	0.174	77	0.967 (0.900, 1.040)	0.366

Abbreviations: As, arsenic, Cd, cadmium, Cr; chromium, Hg, mercury, Mn; manganese, Pb; lead, RR; relative risks, 95%CI; 95% confidence interval

7.5 $PM_{2.5}$ exposure in early-life and infant allergies

The univariate logistic regression analysis of average 24-hour $PM_{2.5}$ concentrations at each period in pregnancy and infant periods and allergic outcomes showed significant findings for specific early-life exposure periods. $PM_{2.5}$ exposure during the first two months of life was significantly associated with an increased risk of allergic diseases in infants. Additionally, exposure between 2–4 months of age was significantly associated with sensitisation. However, no significant association was observed between $PM_{2.5}$ exposure during any period and elevated total IgE levels in infants. Detailed results for each exposure period during early life are provided in **Table 7.5-1**.

To further explore these associations, a cut-off level of 25 μg/m³ for $PM_{2.5}$ exposure was applied. High $PM_{2.5}$ exposure during the first two months of life was found to be significantly associated with allergic diseases, consistent with the findings from the analysis using continuous $PM_{2.5}$ exposure data for the same period. However, this cut-off approach did not reveal significant associations for other allergic outcomes or exposure periods (**Table 7.5-1**).

Importantly, the study did not identify any significant associations between $PM_{2.5}$ exposure during pregnancy and allergic outcomes in infants.

Table 7.5-1: The univariate logistic regression analysis of $PM_{2.5}$ exposure at each early life period of pregnancy to infant children 6 months and allergic outcomes (allergic diseases, infant sensitisation, and elevated infant total IgE concentration). The average 24-hour $PM_{2.5}$ estimation is presented both in continuous data and categorical data (cut-off of exposure at ≥ 25 μg/m^3 based on the data distribution (50th percentile) in this population of study)

Average 24-hour $PM_{2.5}$ estimation (μg/m^3)	**Allergic diseases**			**Infant sensitisation**			**Elevated infant total IgE level**		
	N	**Crude OR (95%CI)**	***P***	**N**	**OR (95%CI)**	***P***	**N**	**OR (95%CI)**	***P***
Pregnancy 1st trimester	86	0.983 (0.930, 1.040)	0.554	86	1.025 (0.960, 1.094)	0.455	86	1.015 (0.965, 1.068)	0.556
≥ 25		0.469 (0.175, 1.254)	0.131		1.393 (0.456, 4.256)	0.561		1.534 (0.643, 3.660)	0.335
Pregnancy 2nd trimester	86	0.971 (0.907, 1.040)	0.406	86	0.969 (0.893, 1.053)	0.461	86	1.018 (0.958, 1.081)	0.568
≥ 25		0.740 (0.275, 1.989)	0.551		0.768 (0.237, 2.485)	0.659		0.990 (0.407, 2.410)	0.983
Pregnancy 3rd trimester	86	1.016 (0.972, 1.062)	0.483	86	0.821 (0.942, 1.049)	0.821	86	0.980 (0.940, 1.023)	0.356
≥ 25		1.636 (0.634, 4.224)	0.309		0.768 (0.247, 2.385)	0.648		0.505 (0.208, 1.229)	0.132
Infancy at birth - < 2 months	85	1.048 (0.998, 1.102)	**0.060**	85	1.023 (0.967, 1.081)	0.431	85	0.990 (0.948, 1.033)	0.635
≥ 25		2.545 (0.889, 7.291)	**0.082**		2.062 (0.598, 7.116)	0.252		1.131 (0.465, 2.749)	0.786
Infancy at ≥ 2 - < 4 months	85	1.014 (0.971, 1.058)	0.531	85	1.053 (1.001, 1.119)	**0.047**	85	1.029 (0.989, 1.071)	0.155
≥ 25		1.066 (0.413, 2.751)	0.896		2.121 (0.680, 6.612)	0.195		1.744 (0.725, 4.192)	0.214
Infancy at ≥ 4 - 6 months	85	0.988 (0.943, 1.035)	0.607	85	1.022 (0.969, 1.078)	0.416	85	1.030 (0.987, 1.075)	0.181
≥ 25		0.606 (0.233, 1.575)	0.304		0.424			1.739 (0.723, 4.184)	0.217

7.6 Infant cytokines concentration and allergic outcomes

To account for the potential link between environmental factors, immunological alterations, and allergic outcomes, infant cytokines were also considered as variables associated with allergic outcomes. Univariate logistic regression analysis revealed that among the cytokines analysed, only IL-12/IL-23p40 was significantly associated with allergic diseases (**Table 7.6-1**). However, this association was not observed for the other two outcomes: infant sensitisation and elevated infant total IgE concentration.

Table 7.6-1: The univariate logistic regression analysis of cytokine concentrations and allergic outcomes (allergic diseases, infant sensitisation, and elevated infant total IgE concentration

Cytokines	Allergic diseases			Infant sensitisation			Elevated infant total IgE concentration		
	N	Crude OR (95%CI)	P	N	Crude OR (95%CI)	P	N	Crude OR (95%CI)	P
IL-1β	61	0.964 (0.836, 1.111)	0.610	61	0.657 (0.315, 1.371)	0.263	61	1.004 (0.993, 1.016)	0.459
IL-2	61	1.063 (0.774, 1.460)	0.705	61	0.857 (0.524, 1.401)	0.538	61	0.901 (0.645, 1.259)	0.541
IL-4	61	1.210 (0.792, 1.848)	0.378	61	0.828 (0.447, 1.532)	0.548	61	0.774 (0.474, 1.263)	0.305
IL-5	61	1.273 (0.811, 2.000)	0.294	61	0.755 (0.299, 1.907)	0.552	61	0.904 (0.589, 1.389)	0.646
IL-6	61	0.994 (0.977, 1.011)	0.494	58	0.971 (0.893, 1.056)	0.491	58	0.985 (0.941, 1.031)	0.519
IL-8	61	1.000 (0.999, 1.001)	0.483	61	0.991 (0.967, 1.015)	0.461	61	1.000 (1.000, 1.000)	0.613
IL-10	61	0.957 (0.811, 1.131)	0.608	61	0.783 (0.500, 1.225)	0.284	61	1.003 (0.882, 1.141)	0.964
IL-12/IL-23p40	61	0.959 (0.921, 0.998)	**0.039**	61	1.003 (0.970, 1.037)	0.868	61	1.003 (0.975, 1.032)	0.812
IL-13	61	1.005 (0.758, 1.332)	0.974		--	--	61	0.482 (0.032, 7.341)	0.600
IL-17	61	0.907 (0.793, 1.037)	0.153	61	0.938 (0.814, 1.081)	0.377	61	0.928 (0.825, 1.045)	0.218
IL-22	61	1.018 (0.921, 1.125)	0.730	61	1.075 (0.966, 1.196)	0.187	61	1.042 (0.940, 1.155)	0.436
IL-31	61	1.006 (0.996, 1.016)	0.269	61	0.999 (0.992, 1.007)	0.835	61	0.995 (0.985, 1.006)	0.386
IL-33	61	1.001 (0.980, 1.022)	0.936	61	1.018 (0.996, 1.040)	0.101	61	1.013 (0.991, 1.036)	0.248
IFN-γ	61	1.033 (0.901, 1.184)	0.644	61	1.318 (0.897, 1.935)	0.160	61	1.405 (0.886, 2.227)	0.149
TNF	61	1.016 (0.910, 1.135)	0.776	60	0.940 (0.788, 1.122)	0.494	61	0.952 (0.843, 1.075)	0.428
TSLP	61	0.998 (0.949, 1.048)	0.922	61	1.018 (0.968, 1.070)	0.480	61	0.991 (0.946, 1.038)	0.687

7.7 The multivariable logistic regression model results

After the initial univariate logistic regression analysis, several potential factors associated with the outcomes were identified based on a *P*-value of less than 0.1. These factors were then included in a multivariable logistic regression model to conduct a comprehensive analysis of each allergic outcome: allergic diseases, infant sensitisation, and elevated infant total IgE concentration.

The factors identified differed by outcome and by trimester of pregnancy, highlighting variations in the timing and impact of exposures during gestation. For this reason, variables that were statistically significant in each trimester were included in the multivariable logistic regression model as individual variables. This approach aimed to examine the importance and differences in the timing of *in utero* exposures on allergic outcomes.

To further explore the data, the multivariable logistic regression analysis was performed for each allergic disease as a subgroup analysis (group 1: atopic dermatitis vs no other allergic diseases, group 2: food allergy vs no other allergic diseases, and group 3: combined AD and food allergy vs no other allergic diseases). Variables included in the final model for allergic disease outcomes were applied (**Appendix 7**), along with additional variables selected from the univariable logistic regression of each allergic disease (**Appendix 6**), based on the same rationale for allergic outcome assessment. This allowed for the identification of potential differences in significant associations between the two groups. The results of this analysis are presented in the appendix, depending on the observed findings.

7.7.1 Multiple effects of environmental exposure and allergic diseases

The selected variables were summarised in **Table 7.7-1**. Both birthweight ≥ 2,500 gram and SGA yielded P-values < 0.1 for the allergic disease outcome, with birthweight ≥ 2,500 gram showing a slightly stronger association (P = 0.071) compared to SGA (P = 0.091). To address potential collinearity and optimise model parsimony, only the birthweight ≥ 2,500 gram variable was included in the multivariable analysis. This decision was supported by a high correlation between birthweight ≥ 2,500 gram and SGA (Spearman's rho = 0.792, P < 0.001), which could affect model stability if both variables were simultaneously entered. The significant factors associated with allergic diseases in infant children after adjusting for potential confounders and multiple environmental exposures effect are shown in **Table 7.7-2**.

Table 7.7-1: Summary of the selected factors for multivariable logistic regression **Model 1** of infant allergic diseases outcome

Variables	N	OR (95%CI)	*P*
Fixed factors (not timing related factors)			
Maternal factors			
Gravida ≥ 3	86	0.191 (0.041, 0.893)	**0.035**
Mean body mass index (BMI) before pregnancy; kg/m^2	83	1.122 (0.998, 1.261)	**0.054**
Maternal occupation (Pregnant women)	86		
Unemployed, housewife, student, freelancer		1	
Medical personnel, industry, hairdresser		36.000 (3.124, 414.894)	**0.004**
Teacher, officers or employees either in government or private sector		7.412 (0.901, 60.991)	**0.063**
Merchant, personal business		7.714 (0.682, 87.252)	**0.099**
Vitamin intake during 1st trimester	86	7.895 (1.415, 44.048)	**0.018**
Infant factors			
Birth weight (≥ 2,500 gram)	84	0.271 (0.066, 1.116)	**0.071**
Exclusive breastfeeding ≥ 4 months	78	3.571 (1.253, 10.182)	**0.017**
Timing related factors			
1st trimester			
Recently furnished/renovated house (within 3 months)	86	3.929 (1.235, 12.496)	**0.020**
Pb concentration	77	0.870 (0.753, 1.004)	**0.057**
Open windows/ doors		0.228 (0.084, 0.619)	**0.004**
Air conditioner (AC)		3.158 (0.962, 10.369)	**0.058**
2nd trimester			
Any pet exposure	85	0.288 (0.088, 0.945)	**0.040**
Air conditioner (AC)	85	3.949 (1.057, 14.749)	**0.041**
Type of residence	85		
Detached house		1	
Flat/ condominium/ apartment		0.360 (0.122, 1.060)	**0.064**
Row house		1.458 (0.366, 5.812)	0.593
3rd trimester			
Open windows/ doors	84	0.350 (0.125, 0.979)	**0.046**
Air conditioner (AC)	84	5.500 (1.175, 25.754)	**0.030**

Air purifier	84	3.800 (0.782, 18.472)	**0.098**
Type of residence	86		
Detached house		1	
Flat/ condominium/ apartment		0.656 (0.213, 2.019)	0.463
Row house		3.000 (0.817, 11.017)	**0.098**
Season of data collection	86		
Winter (November to February)		1	
Summer (March to May)		0.200 (0.038, 1.061)	**0.059**
Rainy (June to October)		0.686 (0.242, 1.940)	0.477
At birth - < 2 months of infant children			
Average 24-hour $PM_{2.5}$	85	1.048 (0.998, 1.102)	**0.060**
6-12 months of infant children			
IL-12/IL-23p40 concentration	61	0.959 (0.921, 0.998)	**0.039**

In **Model 1**, which included all significant variables from the univariate logistic regression analysis, the following factors remained significant: ventilation practice (open window) during the first trimester, type of residence in the second trimester, and BMI before pregnancy. Open window ventilation during the first trimester emerged as a potential protective factor for allergic diseases, while residing in a row house/attached house and higher BMI before pregnancy were identified as risk factors. For sensitivity analysis, birthweight ≥ 2,500 gram was substituted by SGA in the multivariable model of atopic disease outcome. The results remained unchanged, suggesting that either variable could represent the underlying effect of restricted foetal growth in the model. This reinforces the robustness of the findings, though LBW was retained in the final model for parsimony and clarity.

However, when the analysis was re-conducted using only the retained variables from **Model 1**, and with a larger sample size due to fewer missing data, the only statistically significant factor was the practice of open window ventilation during the first trimester (**Model 2**). This remained significant after adjusting for type of residence in the second trimester and BMI before pregnancy.

The results from **Model 3**, after further adjustment for the site of data collection (hospital) and using sequential Bonferroni correction, showed a consistent trend. The only significant factor was the open window practice in the first trimester that seemed to reduce risk of allergic diseases, with an adjusted OR of 0.216 (95% CI: 0.066, 0.705, $P = 0.012$).

Additionally, a subanalysis of **Model 3** was performed for each allergic disease outcome (AD, food allergy, and combined AD and food allergy), adjusting for factors based on the primary outcome of allergic diseases. The results showed that living in a row house/attached house was significantly associated with an increased risk of AD (adjusted OR: 23.169, 95% CI: 1.417–378.939, $P = 0.028$, N = 66). Prepregnancy BMI was linked to a higher risk of food allergy (adjusted OR: 1.293, 95% CI: 1.009–1.656, $P = 0.042$, N = 66). Additionally, keeping doors/windows open was associated with an increased risk of combined AD and food allergy (adjusted OR: 4.514, 95% CI: 1.088–18.733, $P = 0.038$, N = 71). The detailed results of this subanalysis for each atopic disease are presented in **Appendix 7**.

To ensure the robustness of the subgroup analysis results, univariate logistic regression analysis was conducted to identify potential factors associated with each allergic disease. These factors were then included in the multivariable logistic regression model, following the same procedures as for the main allergic disease outcome (**Appendix 6**). The analysis found that only first-trimester factors were significantly associated with specific allergic diseases. Vitamin intake during the first trimester was significantly associated with an increased risk of atopic dermatitis (AD) (adjusted OR: 24.695, 95% CI: 2.150–283.690, $P = 0.011$) and food allergy (adjusted OR: 15.145, 95% CI: 1.069–214.631, $P = 0.045$). Similarly, living in a recently furnished home during the first trimester was significantly associated with an

increased risk of AD (adjusted OR: 14.135, 95% CI: 2.154–92.744, $P = 0.006$). Additionally, prepregnancy BMI was significantly associated with an increased risk of food allergy (adjusted OR: 1.274, 95% CI: 1.016–1.598, $P = 0.036$). In contrast, keeping doors and windows open was associated with a lower risk of combined AD and food allergy (adjusted OR: 0.176, 95% CI: 0.043–0.713, $P = 0.016$). These findings highlight the potential role of early pregnancy environmental and maternal factors in the development of allergic diseases.

Table 7.7-2: Multivariate logistic regression analysis of the association between early life environmental exposure, lifestyle factors and allergic outcomes (allergic diseases, infant sensitisation, and elevated infant total IgE concentrations) in infant children.

Variables	Model 1			Model 2			Model 3 (Final model)		
	N	adjusted OR (95%CI)	*P*	N	adjusted OR (95%CI)	*P*	N	adjusted OR (95%CI)	*P*
Allergic diseases outcome									
Open Window (1st trimester)	48	0.061 (0.009, 0.439)	**0.005**	83	3.800 (1.272, 11.354)	**0.017**	83	0.216 (0.066, 0.705)	**0.012**
Type of residence (2nd trimester)	48			83			83		
Detached house		1			1			1	
Condominium/ flat		1.076 (0.154, 7.493)	0.941		0.605 (0.183, 2.003)	0.411		0.879 (0.245, 3.151)	0.841
Row house/ attached house		37.565 (2.856, 494.025)	**0.006**		2.849 (0.610, 13.297)	0.183		4.633 (0.839, 25.581)	0.078
BMI before pregnancy	48	1.270 (1.006, 1.602)	**0.044**		1.120 (0.988, 1.270)	0.075		1.122 (0.982, 1.282)	0.089
Infant sensitisation outcome									
Maternal sensitisation [c]	79	8.121 (1.469, 44.907)	**0.016**	82	8.275 (1.495, 45.810)	**0.016**	82	6.132 (1.302, 28.892)	**0.022**
Using air purifier (2nd trimester)	79	20.125 (2.722, 148.796)	**0.003**	82	20.643 (2.789, 152.801)	**0.003**	82	12.111 (2.039, 71.925)	**0.007**
Open window (3rd trimester)	79	0.134 (0.023 (0.797)	**0.027**	82	0.130 (0.022, 0.775)	**0.025**	82	0.247 (0.052, 1.162)	0.076
CCR (3rd trimester)	79	0.034 (1.000, 1.000)	**0.034**	82	1.000 (1.000, 1.000)	**0.033**		--	--
Elevated infant total IgE outcome									
Elevated IgE (1st trimester)	66	9.205 (1.490, 56.874)	**0.017**	68	4.259 (0.589, 30.768)	0.151	66	4.266 (0.573, 31.758)	0.154
Visible mould in the house (1st trimester)	66	4.169 (1.115, 15.595)	**0.034**	68	4.210 (1.188, 14.921)	**0.026**	66	4.198 (1.161, 15.182)	**0.029**
Household ETS exposure from housemate (3rd trimester)	66	6.488 (1.371, 30.697)	**0.018**	68	6.876 (1.539, 30.722)	**0.012**	66	6.745 (1.476, 30.835)	**0.015**
CCR (at birth)	66	1.001 (1.000, 1.002)	**0.036**	68	1.001 (1.000, 1.002)	**0.033**	66	1.001 (1.000, 1.001)	0.034

Model 1: All variables that provided p ≤ 0.1 from univariable logistic regression analysis were included in the analysis with forward, conditional selection method.

Model 2: The retained variables from Model 1 were re-analysis with multivariate logistic regression, enter selection method

Model 3: The retained variables from Model 1 were selected for the multilevel multiple logistic regression analysis considering the cluster effect from site of data collection (hospital) and adjust for multiple comparisons using sequential Bonferroni.

[c] sIgE at 1st trimester ≥ 0.35 kUA/L

7.7.2 Multiple effects of environmental and lifestyle exposure and sensitisation in infant children

Similarly to allergic disease outcomes, early-life *in utero* factors in each trimester of pregnancy and the early infancy period were found to affect sensitisation in infant children in different ways. The results are presented in **Table 7.7-2**. And the summary of selected variables for multivariable logistic regression model was shown in **Table 7.7-3**.

Table 7.7-3: Summary of the selected factors for multivariable logistic regression Model 1 of infant sensitisation outcome

Variables	N	OR (95%CI)	*P*
Fixed factors (not timing related factors)			
Maternal factors			
Maternal sensitisation	86	5.250 (1.477, 18.660)	**0.010**
Infant factors			
Gender: female	84	0.257 (0.067, 0.993)	**0.049**
Sibling common allergy	86	10.769 (0.909, 127.619)	**0.060**
Timing related factors			
1st trimester			
--	--	--	--
2nd trimester			
Air conditioner (AC)Any pet exposure	85	0.250 (0.073, 0.856)	**0.027**
Air purifier	85	8.250 (1.890, 36.005)	**0.005**
Household ETS from neighbour (s)	85	0.250 (0.065, 0.9640	**0.004**
3rd trimester			
Open windows/ doors	84	0.223 (0.069, 0.719)	**0.012**
Fan	84	0.197 (0.055, 0.700)	**0.012**
Air purifier	84	4.062 (0.805, 20.501)	**0.090**
Household ETS from housemate (s)	84	0.297 (0.086, 1.0240	**0.055**
Household ETS from neighbour (s)	84	3.333 (0.925, 12.012)	**0.066**
CCR (ng/mg creatinine)	84	1.000 (1.000, 1.000)	**0.087**
≥ 2 - < 4 months of infant children			
Average 24-hour $PM_{2.5}$	85	1.053 (1.001, 1.119)	**0.047**

In **Model 1**, after adjusting for variables from the univariate logistic regression analysis, the following factors remained significant: maternal sensitisation, using an air purifier during the second trimester, open window ventilation during the third trimester, and CCR concentration during the third trimester. These associations remained consistent in **Model 2**, which was

adjusted for only the retained variables from **Model 1** (maternal sensitisation, using air purifier in second trimester, open window/door in third trimester, and CCR in third trimester).

However, after adjusting for the site of data collection and applying the sequential Bonferroni correction (**Model 3**), CCR concentration was no longer significant. The factors that remained significant were maternal sensitisation and using an air purifier during the second trimester. In the final model (**Model 3**), maternal sensitisation (adjusted OR: 6.132, 95% CI: 0.264, 3.364, $P = 0.022$) and the use of an air purifier during the second trimester (adjusted OR: 12.111, 95% CI: 0.713, 4.276, $P = 0.007$) were identified as risk factors for infant sensitisation. Open window/door for home ventilation remained a potential protective factor for infant sensitisation (adjusted OR: 0.247, 95% CI: -2.948, 0.150, $P = 0.076$), similar to the allergic diseases outcome, although it was not statistically significant.

7.7.3 Multiple effects of environmental and lifestyle exposure and elevated total IgE concentration in infant children

For the final allergic outcome, elevated total IgE concentration in infant children, the univariate logistic regression analysis identified several factors to adjust for in the multivariable logistic regression model. These factors included maternal education, birthweight, infant gender, birth season, maternal elevated maternal total IgE concentration in the third trimester, visible mould in the first trimester, pet ownership in the second trimester, ETS from neighbours (second and third trimester), household ETS from housemates (third trimester), and CCR at birth (**Table 7.7-4**).

Table 7.7-4: Summary of the selected factors for multivariable logistic regression Model 1 of elevated infant total IgE concentration in infant children outcome

Variables	N	OR (95%CI)	*P*
Fixed factors (not timing related factors)			
Maternal factors			
Maternal education	86		
Below undergraduate		1	
Undergraduate		1.150 (0.446, 2.965)	0.772
Graduate		3.354 (0.817, 13.778)	**0.093**
Infant factors			
Birth weight (≥ 2,500 gm)	84	6.286 (0.748, 52.902)	**0.090**
Gender: female	86	0.441 (0.178, 1.094)	**0.078**
Birth season	86		
Winter (November to February)		1	
Summer (March to May)		0.848 (0.212, 3.391)	**0.813**
Rainy (June to October)		1.649 (0.635, 4.278)	0.304
Timing related factors			
1st trimester			
Mould visibility at home	86	2.600 (0.979, 6.905)	**0.055**
2nd trimester			
Any pet exposure	85	0.440 (0.167, 1.158)	**0.096**
Household ETS from neighbour (s)	85	0.425 (0.172, 1.051)	**0.064**
3rd trimester			
Household ETS from housemate (s)	84	3.240 (0.978, 10.731)	**0.054**
Household ETS from neighbour (s)	84	0.364 (0.147, 0.897)	**0.028**
At birth			
CCR (ng/mg creatinine)	85	1.001 (1.000, 1.001)	**0.053**

Similar to the previous two allergic outcomes, stepwise modelling from **Model 1** to **Model 3** was applied, with the results shown in **Table 7.7-2**.

In **Model 1**, the following significant factors were associated with elevated infant total IgE concentrations: elevated maternal total IgE in the first trimester, visible mould in the first trimester, household ETS from housemates in the third trimester, and CCR at birth. These factors were then selected for further analysis in **Model 2**.

After adjusting for maternal elevated maternal total IgE in the first trimester, visible mould in the first trimester, household ETS from housemates in the third trimester, and CCR at birth in **Model 2**, elevated maternal total IgE in the first trimester was no longer significant. In **Model**

3, after adjusting for the site of data collection and applying the sequential Bonferroni correction, visible mould in the house during the first trimester (adjusted OR: 4.198, 95% CI: 1.161, 15.182, $P = 0.029$) and household ETS from housemates in the third trimester (adjusted OR: 6.745, 95% CI: 1.476, 30.835, $P = 0.015$) were identified as risk factors for elevated infant total IgE concentrations.

7.8 Discussion

The discussion in this chapter is divided into 7.8.1 The chemical exposure in early-life and infant cytokines, 7.8.2 Maternal factors and allergic outcomes, and 7.8.3 Early-life environmental factors and allergic outcome in infants.

7.8.1 The chemical exposure in early-life and infant cytokines

7.8.1.1 Prenatal heavy metal exposure and infant cytokines

To facilitate understanding and link cytokines to potential health outcomes, the cytokines were categorised based on their primary roles in the immune system. Briefly, pro-inflammatory cytokines, including IL-1β, IL-6, IL-8, IL-17α, and TNF, are key mediators of inflammation and immune activation. Anti-inflammatory cytokines, such as IL-10, regulate immune responses by counteracting inflammation. Th1 cytokines, including IL-2, IL-12/IL-23p40, and IFN-γ, are associated with cell-mediated immunity, defence against intracellular pathogen and counteract allergic reaction. Th2 cytokines, comprising IL-4, IL-5, IL-13, and IL-31, are involved in humoral immunity, allergic responses, and tissue repair. Th17 cytokines, represented by IL-17α and IL-22, play critical roles in autoimmune diseases, mucosal immunity, and often associated with chronic phase of AD and enhance allergic response through chronic inflammation. Epithelial cell-associated or atopic dermatitis-related cytokines,

IL-33 and TSLP, are implicated in epithelial barrier dysfunction and allergic inflammation. The associations between these cytokine groups and each heavy metal were analysed to understand their potential health implications. The significant association of whole blood heavy metals concentrations in first trimester pregnant women and infant cytokines was summarised in **Table 7.8-1**.

Table 7.8-1: The summary of the significant association of whole blood heavy metals in 1st trimester pregnant women and infant cytokines

Association	As	Cr	Hg	Mn
Positive	IFN- γ, TSLP	IL-10	IL-31, TSLP	IL-5
Negative	-	IL-31, TSLP	IL-6	-
Remarks of representative cytokines function: **Proinflammatory: IL-1β, IL-6, IL-8, IL-17α, and TNF** **Anti-inflammatory: IL-10** **Th1: IL-2, IL-12/IL-23p40, and IFN-γ** **Th2: IL-4, IL-5, IL-13, and IL-31** **Th17: IL-17α and IL-22** **Epithelial (AD-) related: IL-33 and TSLP**				

7.8.1.1.1 Arsenic (As)

Prenatal As exposure is well-documented for its neurotoxicity and has been linked to intellectual deficits, ADHD, immune dysregulation, inflammation, and allergic development via epigenetic mechanisms children [145, 346, 435-441].

In this DPhil study, prenatal As concentration was significantly correlated with increased levels of IFN-γ and TSLP in infants. The concurrent increase in IFN-γ and TSLP—despite their opposing roles in type 2 inflammation, which underlies allergic diseases—suggests a complex immunomodulatory effect of prenatal As exposure, potentially disrupting immune balance in early life. However, no significant associations were found with allergic outcomes in infancy, indicating that additional environmental or genetic factors may be required for the development of clinical allergies.

These findings contrast with results from the Maternal-Infant Research on Environmental Chemicals (MIREC) study in Canada, which found no association between prenatal As exposure and IgE, IL-33, or TSLP. Instead, the MIREC study reported positive associations between Pb and organophosphate metabolites with elevated IL-33 and TSLP levels in cord blood [442]. Similarly, a US birth cohort study observed a positive association between prenatal As exposure and placental IL-1β expression [145], a finding not replicated in this study.

These discrepancies across studies may be attributed to differences in the timing of cytokine measurements and sample types. While the present study measured cytokines in infant plasma/serum at 6–12 months, the US birth cohort study analysed placental tissue, which may reflect distinct immune responses at different developmental stages. The observed positive correlation between prenatal As exposure and IFN-γ and TSLP suggests that early-life immune programming may be influenced by environmental toxicants, highlighting the need for further research on long-term immune consequences and potential disease susceptibility in later childhood.

7.8.1.1.2 Cadmium (Cd)

No significant association was found between maternal Cd concentrations in the first trimester and infant cytokine levels in this study. In contrast, a Chinese study reported positive associations between prenatal Cd exposure (measured by maternal urinary Cd) and IL-4 and IL-6 in preschoolers [443]. The discrepancy may be due to differences in exposure sources, as smoking—a major Cd source—was more prevalent in China (3.8%) than in this study (0%)

[444]. Additionally, the use of blood Cd instead of urinary Cd may have contributed to the differing results.

7.8.1.1.3 Chromium (Cr) and mercury (Hg)

In this study, both Cr and Hg influence the immune system, but their cytokine associations exhibit opposing directions. Cr exposure was associated with an increase in anti-inflammatory cytokines (IL-10) (coefficient: 0.288, $P = 0.016$) and reverse associations with Th2-related (IL-31) and AD-associated (TSLP) cytokines (coefficients: -0.366, $P = 0.003$ and -0.332, $P = 0.007$, respectively).

Hg exposure was positively associated with Th2 cytokines, including IL-5, IL-31, and TSLP (coefficients: 0.191, 0.232, and 0.270, with $P = 0.081$, 0.045, and 0.023, respectively). These findings suggest that Cr and Hg may have opposing effects on immune regulation, with Cr exposure more linked to inflammation suppression, while Hg exposure was associated with enhanced allergic responses through Th2 and epithelial cell-related pathways.

The pro-inflammatory effects of Cr observed in this study align with findings from *in vitro* and animal studies. Cr has been shown to stimulate pro-inflammatory cytokines, including IL-6 and TNF-α, in peripheral blood mononuclear cells (PBMCs) [445] and to increase IgA, IgE, IL-2, IL-6, and TNF-α in rats fed a high-fat diet supplemented with Cr [446].

The Th2-skewing effects of Hg are also supported by experimental studies. Hg enhances basophil and mast cell activation, promoting IgE-mediated histamine release [447, 448].

Additionally, Hg^{2+} exposure induces a proliferation-dependent shift in CD4+ T cells in both allergic and non-allergic individuals, suggesting a role in modulating immune responses [449].

Despite these experimental findings, research on prenatal heavy metal exposure and infant cytokine profiles remains limited. The COCOA study examined umbilical cord blood metals (Pb, Hg, Cr, Cd) and cytokine production (IL-13 and IFN-γ) from stimulated CBMCs. It reported a positive association between IL-13 and Pb/Cr concentrations, with cord blood Cr linked to AD severity at 24 months [450]. However, this study did not assess pro-inflammatory cytokines, and its findings contrast with this DPhil, where no significant changes in IL-13 or other Th2 cytokines were observed. These differences may be due to variations in study design, such as the measurement approach (*in vitro* CBMC stimulation vs *in vivo* infant serum cytokines at 6–12 months) and sample timing (cord blood vs postnatal exposure).

In this study, IL-31, primarily produced by Th2 cells but also by macrophages, dendritic cells, mast cells, basophils, and eosinophils [451], was positively associated with Hg exposure. This aligns with Hg known effects on these immune cells but differs from the COCOA study, which found no association between Hg or Cd and cytokines [450]. These discrepancies highlight the complexity of Hg immunomodulatory effects and the need for further research on its role in early-life immune development.

7.8.1.1.4 Manganese (Mn)

Mn showed a positive association with Th2 cytokines in this study, but positive association with IL-4, IL-5, and IL-13 using the $P \leq 0.1$ (coefficients: 0.207, 0.285, and 0.204 for IL-4, IL-5, and IL-13; $P = 0.065$, 0.018, and 0.067, respectively). Mn is an essential metal involved in

lipid, protein, and carbohydrate metabolism and is critical for various enzymatic functions [452] and host defence mechanisms [435] but exposure to Mn fumes has been shown to impair both cellular and humoral immunity [453].

Evidence linking Mn exposure to Th2 skewing is limited. One study in chickens exposed to high Mn in drinking water demonstrated inflammatory damage and Th1/Th2 imbalance in the nervous system [454]. However, human studies, such as the Japan Environment and Children's Study (JECS), found no association between maternal blood Mn levels during pregnancy and common allergic diseases (AD, food allergy, asthma, or AR) in children [455]. These findings suggest that the observed Th2 cytokine alterations in this study may not directly translate to allergic disease outcomes in children. Larger studies are needed to confirm the immunological effects of Mn exposure and its implications for childhood allergies.

7.8.1.1.5 Lead (Pb)

Prenatal Pb exposure was not associated with altered IL-12/IL-23p40 (coefficient: 0.186, P = 0.087) in correlation analysis but in the univariate logistic regression analysis was associated with a lower risk of allergic diseases (OR: 0.870, 95% CI: 0.753–1.004, P = 0.057, **Table 7.4-1**). IL-12/IL-23p40 concentration was slightly inversely significant associated with allergic diseases (OR: 0.959, 95% CI: 0.921–0.998, P = 0.039, **Table 7.6-1**), suggesting that this cytokine may play a role in mediating the relationship between Pb exposure and reduced allergic risk though this remains a hypothesis because there was no association between Pb levels and those of IL-12/IL-23p40. This could align with emerging role of IL-12/IL-23p40 as a therapeutic target for allergic diseases, such as AD, as previously discussed (**Section 6.4.1,**

Chapter 6). However, further research is needed to clarify the underlying mechanisms and the clinical significance of these associations.

Consistent with this study, prior research reported that maternal Pb exposure was associated with reduced IL-10 and IL-4 levels in 3-year-old children, but not with IL-2, IL-5, IL-6, IFN-γ, or TNF-α [456] but this was not seen in this work. Further research is needed to understand these correlations' underlying mechanisms and implications for health and disease outcomes.

7.8.1.2 Ealy life ETS exposure and infant cytokines

The study result showed complex interactions between ETS, as indicated by urine CCR, and the infant cytokines concentration. Various cytokines exhibited associations with CCR concentration at each time point, as summarised in **Table 7.8-2**. Previous studies showed that tobacco smoke can potentially induce the production of pro-inflammatory cytokines such as IL-1, IL-6, IL-8, granulocyte-macrophage colony-stimulating factor (GM-CSF), and TNF-α. It can also increase the accumulation of immune cells in the airway [457]. However, it should be noted that tobacco smoke can also suppress Th1 and Th17 pathways [458], indicating its complex and multifaceted effects on the immune system. To enhance understanding and link these findings to clinical implications, the functions of cytokines and the timing of CCR measurement are discussed.

Table 7.8-2: The summary of the significant association of CCR in each period and infant cytokines

Association	P01	P03	C01	C05
Positive	IL-1β, IL-4, IL-13, and TSLP	-	IL-2	IL-13 and TSLP
Negative	IL-33	IL-31 and TSLP	TSLP	IL-22
Remarks of representative cytokines function: **Proinflammatory: IL-1β, IL-6, IL-8, IL-17α, and TNF** **Anti-inflammatory: IL-10** **Th1: IL-2, IL-12/IL-23p40, and IFN-γ** **Th2: IL-4, IL-5, IL-13, and IL-31** **Th17: IL-17α and IL-22** **Epithelial (AD-) related: IL-33 and TSLP**				

Pro-inflammatory cytokines exhibited distinct associations with CCR depending on the timing of exposure. During the first trimester, significant positive correlations were observed between CCR and IL-1β, suggesting an early inflammatory response. However, these associations were not evident at later time points, indicating that the pro-inflammatory effects of ETS may be confined to early pregnancy. Some cross-sectional studies also supported the effect of ETS and inflammation in children via increasing IL-1β and TNF-α similar to this DPhil finding [459, 460].

Th2 cytokines, including IL-4, IL-13, and IL-31, showed significant correlations across most evaluation periods except for cord blood at birth. Notably, IL-13 demonstrated consistent significant positive associations with CCR during the first trimester and at 6–12 months of age, highlighting the potential for early and sustained Th2 skewing. However, IL-31 displayed a significant inverse correlation with CCR during the third trimester, suggesting dynamic shifts in Th2 immune responses over time. This finding was support by the evidence the *in vitro* study that ETS in infant children of smoking parents that showed the elevated cytokines production of IL-4, IL-5 and TNF from cultured PBMC, especially cultured PMBC from AD children from parental smoking can exhibited elevated IL-5 earlier at 3 months of age [460].

For infant IL-2, significant positive associations with CCR was observed in cord blood (coefficient 0.251, P = 0.031). Since none of the other Th1 cytokines demonstrated similar associations with CCR, interpreting this as evidence of Th1-driven immune modulation should be approached with caution. IL-2 is a non-specific T cell-activated cytokine, primarily produced by activated T cells (CD4+ and CD8+), and it plays a role in activating various T cell subsets, including Th1, Th2, Th17, and regulatory T cells [461]. Therefore, this finding suggests that ETS exposure during late pregnancy may promote general T cell activation rather than specifically driving a Th1 response.

In contrast, AD-related cytokines, including IL-33 and TSLP, exhibited correlations across multiple time points. Infant IL-33 was significant inverse correlated with CCR in first trimester (coefficient -0.213, P = 0.050), indicating a potential suppressive effect on epithelial-driven immune responses of ETS in early pregnancy. TSLP showed the opposite with a positive correlation with CCR in first trimester pregnant women and children aged 6–12 months (coefficient 0.3376 and 0.309, P = 0.001 and 0.009, respectively), but significant inverse correlated with CCR in third trimester and cord blood (coefficient -0.324 and -0.231, P = 0.006 and 0.044, respectively). It possibly reflecting immune modulation related to ETS exposure depends on timing of exposure. Supporting evidence from animal studies has shown a link between tobacco smoke exposure and increased TSLP and TNF-α receptor expression in mouse lungs, aligning with the observations in this DPhil study [462]. A link between CCR and allergic outcomes was not found from this study. Therefore, replication of these findings in larger studies and the linkage with clinical relevance is needed to validate and better understand these associations.

These findings emphasise the importance of understanding how the timing of ETS exposure interacts with cytokine functions to shape immune system development and this and other similar factors can affect these studies as confounding effect and need to be considered. Clinically, this underscores the need for interventions targeting ETS exposure during critical windows of pregnancy and early infancy to mitigate its potential adverse effects on immune development and allergic disease risks.

7.8.1.3 $PM_{2.5}$ and infant cytokines

Similar to the CCR study, the findings revealed a variable relationship between ambient $PM_{2.5}$ concentration during pregnancy and infancy and infant cytokine concentrations. Although correlations were identified, the direction and significance of these associations varied across different cytokines and exposure periods. The significant associations between 24-hour $PM_{2.5}$ concentrations at each period and infant cytokine concentrations are summarised in **Table 7.8-3**.

Table 7.8-3: The summary of the significant association of average 24-hour $PM_{2.5}$ concentration in each period and infant cytokines

Association	P01	P02	P03	C02	C03	C04
Positive	IL-33, IL-22	IL-2	IL-6 and IL-8	-	-	IL-22 and IL-33
Negative	IL-6, TNF, and TSLP	-	IL-22 and IL-33	IL-2	IL-2	IL-31 and TSLP
Remarks of representative cytokines function: **Proinflammatory: IL-1β, IL-6, IL-8, IL-17α, and TNF** **Anti-inflammatory: IL-10** **Th1: IL-2, IL-12/IL-23p40, and IFN-γ** **Th2: IL-4, IL-5, IL-13, and IL-31** **Th17: IL-17α and IL-22** **Epithelial (AD-) related: IL-33 and TSLP**						

The significant positive correlation between average 24-hour $PM_{2.5}$ concentration during the third trimester of pregnancy and pro-inflammatory cytokines (IL-6 and IL-8) as well as positive correlation but non-significant ($0.05 < P < 0.1$) with other pro-inflammatory cytokines

including IL-1β, , IL-17α, and TNF and the epithelial-related cytokine TSLP aligns with the association observed in the first four months of infancy. Specifically, $PM_{2.5}$ exposure during early infancy (0-2 months) was linked to atopic disease outcomes and sensitisation (2-4 months). This highlights the potential impact of early-life $PM_{2.5}$ exposure, particularly during the third trimester and early infancy, on the development of allergic diseases through inflammatory mechanisms. These findings contrast with the absence of a clear association with Th2 cytokines such as IL-4, IL-5, or IL-13, and inverse correlation observed with IL-31 and $PM_{2.5}$ exposure in children aged 4-6 months.

These results are consistent with the findings of Yseng *et al*., who studied long-term $PM_{2.5}$ exposure in 6-12-year-old children in Taiwan. They reported a significant association between proinflammatory cytokines TNF-α and $PM_{2.5}$ exposure, but no significant associations with other inflammatory cytokines such as IFN-γ, IL-1β, IL-2, IL-6, IL-10, and IL-13 [463]. In contrast, Friedman *et al*. found an association between $PM_{2.5}$ exposure and maternal inflammation cytokines (IL-6 and TNF-α) but not in umbilical cord blood [464].

IL-22 and IL-33, which are involved in inflammation and can contribute to the chronic phase of allergy, associations were inconsistent. There was a significant fluctuation correlation of $PM_{2.5}$ and infant IL-22 and IL-33 that significant positive correlation in $PM_{2.5}$ during first trimester and children 4-6 months, but significant inverse correlation with $PM_{2.5}$ during third trimester of pregnancy, possibly indicating that $PM_{2.5}$ exposure may link to allergic diseases development and to diseases aggravation in early childhood via IL-22 and IL-33 alteration. For epithelial cytokines, TSLP showed the fluctuation correlation with $PM_{2.5}$ exposure like IL-33 but with opposing directions of association (inverse correlations in first trimester pregnant

women and infant 4-6 months). These results inconsistent with *in vitro* studies showing that $PM_{2.5}$ can promote TSLP expression in keratinocytes, possibly contributing to dermatitis risk [465]. Furthermore, a large European cohort study, the MIREC study, found no association between prenatal $PM_{2.5}$ exposure and IL-33 or TSLP, although NO_2 exposure was associated with elevated cord blood concentrations of both cytokines [466].

In the Th1/Th2 cytokine group, IL-2 had a positive correlation with $PM_{2.5}$ exposure during the second trimesters, but inverse association in infants at 0 - 4 months. IL-31, but not for another Th2 cytokines, was inverse correlation with $PM_{2.5}$ exposure during infant 4-6 months. These findings contrast with an animal study that showed $PM_{2.5}$ significantly decreased IFN-γ, but increased IL-4, and IL-13 expressions, along with eosinophil numbers in nasal lavage fluid in a model of AR, suggesting a Th2 skewing effect and AR exacerbation [467].

Overall, while this study's findings on early-life $PM_{2.5}$ exposure and cytokine skewing support the main outcomes related to allergic diseases and sensitisation via proinflammatory and epithelial related cytokines instead of Th2 skewing cytokines, it still provided some inconsistent results when compared to other studies. These inconsistencies may be due to differences in detection methods, cohort sizes, timing of evaluation, and $PM_{2.5}$ exposure concentrations. Harmonisation and consolidation of future studies is needed to clarify the evidence in respect of $PM_{2.5}$ exposure and its long-term health outcomes, because cytokines can play multiple roles in various diseases, and a single disease may be influenced by multiple cytokines.

7.8.2 Maternal factors and allergic diseases

7.8.2.1 Pre-pregnancy body mass index (BMI)

Several birth cohort studies have reported varying results regarding the impact of maternal BMI, both pre-pregnancy and during pregnancy, on childhood allergy outcomes [468-470]. A Swedish birth cohort study, which followed children up to 16 years of age, found a positive association between maternal BMI in early pregnancy and childhood asthma but no significant association with rhinitis, AD, or sensitisation [470]. In contrast, a Finnish birth cohort study identified an association between early pregnancy BMI and allergic diseases in children up to five years old but found that gestational weight gain, rather than BMI alone, increased the risk of AD [468]. Similarly, a nationwide birth cohort study from Japan demonstrated that pre-pregnancy BMI was associated with an increased risk of asthma in three-year-old children but was linked to a decreased risk of food allergy and showed no association with AD [469].

Although this DPhil study had a relatively small sample size, it replicated findings from the JECS study, showing an association between pre-pregnancy BMI and allergic diseases (AD and food allergy) in univariate regression analysis. However, after adjusting for confounding and environmental factors, the association was no longer statistically significant for allergic diseases but still significant increased risk of food allergy in infant children (adjusted OR: 1.274, 95%CI 1.016, 1.598, $P = 0.036$) as shown in **Appendix 6**. These inconsistencies across studies may be attributed to differences in genetic susceptibility, the timing of outcome evaluation, and the influence of other modifying factors, such as environmental exposures.

7.8.2.2 Maternal atopic status

Maternal atopic status, defined by allergic diseases, maternal total IgE, and maternal specific IgE, has been linked to allergy development in offspring, primarily due to genetic susceptibility [433, 471, 472]. However, in this DPhil study, none of the maternal allergy determinants (allergic diseases, elevated maternal total IgE concentration, or maternal sensitisation (sIgE to aeroallergens ≥ 0.35 kU/L) showed a significant association with infant allergic diseases in univariate logistic analysis. Nonetheless, elevated maternal total IgE concentration and maternal sensitisation in first trimester pregnant women were positively associated with infant sensitisation and elevated infant total IgE concentration in univariate logistic regression analysis. This was most pronounced in the association between maternal sensitisation and infant sensitisation, as demonstrated in the multivariable logistic regression analysis adjusted for potential confounders using sequential Bonferroni correction (adjusted OR: 6.132, 95%CI: 1.302, 28.892, *P* 0.022). However, the wide 95%CI suggests that the effect estimate should be interpreted with caution due to small sample size or too many adjustments. Further research with a larger sample size and simplifying the model to reduce unnecessary covariates may be needed to confirm this association. This suggests that maternal atopic status may have a less pronounced impact on infant allergic diseases and elevated infant total IgE concentration compared to another modifying factors such as exclusive breastfeeding or ETS [473, 474]. However, the strong association between maternal and infant sensitisation highlights a significant genetic predisposition for this outcome.

7.8.2.3 Vitamin intake in early pregnancy and allergic outcomes in infants

The univariate logistic regression analysis found that vitamin intake, but not folic acid, was associated with an increased risk of allergic diseases (OR: 7.895, 95% CI: 1.415–44.048, *P* = 0.018). This association did not persist after adjusting for other variables in the final

multivariable logistic regression model with Bonferroni correction. However, the subgroup analysis of each allergic diseases found the significant association of vitamin intake in first trimester with AD or food allergic infant children (adjusted OR: 24.695 and 15.145, 95% CI: 2.150–283.690, 1.069–214.631, P = 0.011 and 0.045 for AD and food allergy, respectively). This finding contradicts previous study, which suggest that certain vitamins intake during pregnancy, such as vitamin D, vitamin E, zinc, magnesium, and multivitamins, may reduce the risk of allergy development [475]. However, a key limitation of this study is the lack of detailed information regarding the specific types, dosages, and duration of vitamin intake, and small sample size which may influence the observed associations. Further research is needed to clarify the role of different vitamin supplements in allergy prevention.

7.8.3 Early-life environmental factors and allergic outcomes in infants

The final model of multivariable logistic regression analysis, adjusted for hospital effects and applying sequential Bonferroni correction, was used to explore the association between multiple environmental exposures in early life and potential confounding factors. These confounders were selected based on clinical significance and further refined through univariate analysis. The analysis examined the relationship with allergic outcomes, including infant allergic diseases, infant sensitisation, and elevated infant total IgE concentrations. The final model revealed that pre-pregnancy BMI, maternal atopic status (maternal sensitisation and elevated maternal total IgE concentration during pregnancy), housing condition including type of residence, home ventilation practices, and mould visibility in the house, and reported household ETS exposure remained significant.

7.8.3.1 Housing conditions and allergy

Housing conditions influence the indoor ventilation system and affect indoor air pollution, possibly linked to allergy development [474]. An indoor air ventilation system is crucial for indoor air quality control since it helps exchange between indoor and outdoor air. Outdoor air is needed to dilute the indoor pollutants. Therefore, poor ventilation can increase indoor air pollutants such as CO, VOCs, and PM accumulation. Ventilation also reduces heat and indoor moisture concentration, decreases mould and bacteria's growth rate, and reduces the concentration of airborne viruses [475]. As a result, it can lead to chronic respiratory infection, sick-building syndrome, and asthma exacerbation [476-480] which was supported by the univariate logistic analysis result in this study that demonstrated a significant association between the housing conditions during the pregnancy period, including residence type, indoor ventilation system, recently furnished, mould visibility in the house, with allergic diseases (AD and food allergy), infant sensitisation, and elevated infant total IgE concentration in a different timing of exposure.

7.8.3.1.1 Indoor air ventilation and temperature-control measures

Ventilation can be achieved by simply opening doors/windows or using a ventilation system with/without an air purifier. Using AC is the temperature-controlled measure that influence indoor air quality. This DPhil study evaluated the common types of indoor ventilation systems and temperature-control measures in Thailand, including keeping doors/windows open, using fans, AC, and air purifiers. The results from univariate logistic regression analysis showed that, using air conditioning (all trimester) seemed to increase risk of allergic diseases, but open door/window for indoor ventilation (first and third trimester) was decreased risk of allergic diseases in infant children. And open door/window (third trimester), using a fan (third trimester) decreased the risk of sensitisation. Interestingly, using air purifiers in second and

third trimester of pregnancy was significantly associated with sensitisation in infants. However, after the multivariable logistic regression with site adjustment and Bonferroni correction was applied, only opening of windows and doors for home ventilation remained as a significant association for decreased risk of allergic diseases in infant children (adjusted OR: 0.216, 95%CI: 0.066, 0.705, P = 0.012), and using air purifier in second trimester increase risk of sensitisation in infant children (adjusted OR: 12.111, 95%CI 2.039, 71.925, P = 0.007). Keeping doors/windows open in 1st trimester also decreased the risk of combined AD and food allergy infant children in the subgroup analysis (adjusted OR: 0.176, 95%CI 0.043, 0.713, P = 0.016). It indicates that, ventilation system especially open doors/ windows seemed to be the strongest protective effect of infant allergies even though is not respiratory allergy and also multiple potential factors were considered including genetic predisposing factor, air pollution, and also other modifying factors. However, the finding that using air purifier in 2nd trimester of pregnancy seemed to contradicts with the benefit of an air purifier, which improves indoor air quality [476] and modifies the negative effect of prenatal exposure to tobacco smoke or incense burning on cord blood IgE [477]. The indirect effect of high indoor air pollution around the participant's residences, high risk of allergy themselves may lead to increase using air purifier and may not reflex the true risk factors of sensitisation in children. The home ventilation practice in each trimester of pregnancy seemed to influence the allergy in different patterns. It implies the window of susceptibility of each stage of *in utero* on allergy development and immunological alterations.

7.8.3.1.2 Residence type

The typical housing in urban areas of Thailand used to be categorised in this study is depicted in **Figure 7.8-1**. Three characteristics were simplified based on the height of the building and attachment to the neighbourhood. Type A flats/condominiums were tall buildings, usually

exceeding four storeys and attached to a neighbourhood (next room). Type B detached house is relatively low-rise and not attached to the neighbourhood. Lastly, Type C. row house/ townhouse/ attached house combines low-rise buildings with neighbourhood attachment. Considering these characteristics, two potential mechanisms may be due to the natural distribution of air pollution, mainly $PM_{2.5}$ related to high buildings, and the effect of natural ventilation. Compared to the ground level, air pollutants at a higher level, such as at a tall building, are lower [478]. And although most high-rise buildings are fully sealed, natural or proper ventilation is usually well-constructed with more attention from the regulatory system. The healthy natural ventilation design also maximises the indoor air quality in tall buildings [479]. Therefore, condominiums still have a lower risk for allergy development than living row houses. In addition, attached houses might have limited natural ventilation, leading to indoor air pollutants accumulation. As a result, increased concentration of indoor air pollution may lead to allergy development [480]. As supported by this study's result that living in row houses/ townhouses/ attached houses (low building + attached to neighbourhood) especially in 2nd and 3rd trimester of pregnancy were the increased risk of developing allergic diseases in infants compared to living in a detached house (low building + detached to neighbourhood). Similarly to this DPhil study, living in apartment also demonstrated the risk of AD development in northern European children [481]. However, the effect was diluted, and no significant association was identified in the multivariate logistic regression analysis with site adjustment and Bonferroni correction. It suggested that other factors such as ventilation practice could modified this risk and could be easier to apply in practice.

Additionally, the effect of ETS on the neighbourhood might be considered since the source of self-reported household ETS exposure from this study was mostly from neighbour (s) (38.6 –

43.2% across all trimesters of pregnancy). Therefore, the attached house may indirectly receive more health effects from neighbourhood ETS exposure than the detached house.

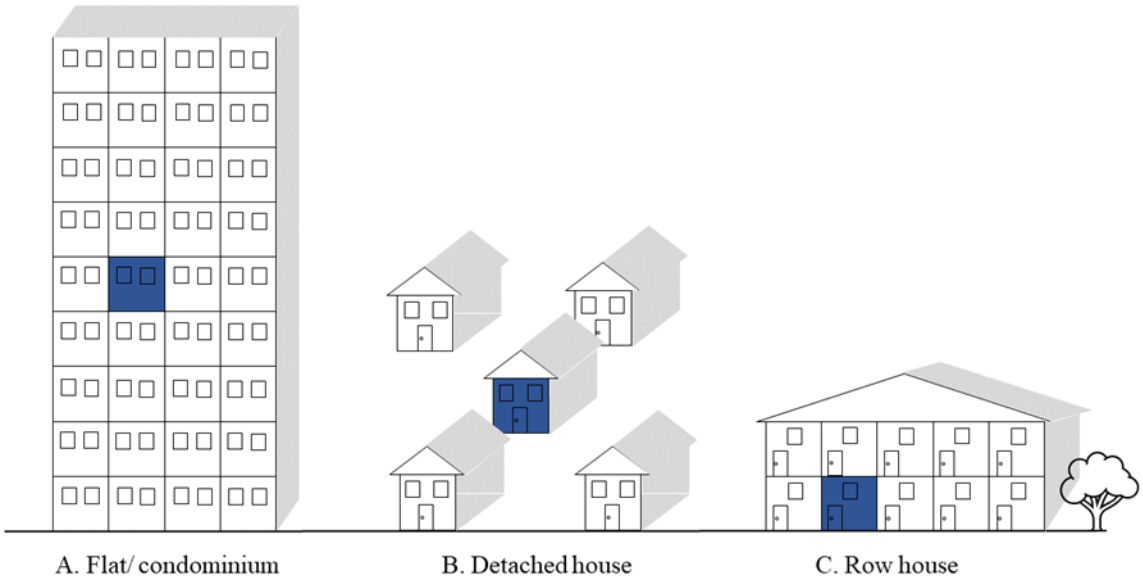


Figure 7.8-1: A schematic of different houses in Thailand, which were included in the self-assessment questionnaires.

7.8.3.1.3 Visible mould at home

The meta-analysis showed that home environments with visible mould and mould spore exposure increase the risk of allergic respiratory (asthma, wheeze, AR) health outcomes in children [482] and the Respiratory Health in Northern Europe (RHINE) study also showed the risk of AD development from visible mould at home [481]. However, no association between mould visibility and allergic outcomes and sensitisation were not found in this DPhil study. The limitation of interpretation may be due to self-reported mould visibility, which depends on participants' attention to thoroughly observe the home environment, which may affect the prevalence of mould growth.

Interestingly, the univariate logistic regression analysis and multivariable logistic regression adjusted with elevated maternal total IgE concentration (first trimester), household ETS from housemate (3rd trimester), CCR (at birth), and site of data collection with Bonferroni correction showed that mould visibility at home increased risk of elevated infant total IgE concentration in children (adjusted OR: 4.198, 95%CI: 1.161, 15.182, P = 0.029) but not for sensitisation outcome. It was supported by previous studies that mould and dampness exposure did not associate with sensitisation to food and/or aeroallergens in children up to 16 years [483] but associated with total IgE level in children 4-7 years [484].

7.8.3.2 ETS exposure and allergy in infant children

ETS can cause numerous adverse health effects in both adults and children. In children, ETS exposure has been linked to sudden infant death syndrome (SIDS), impaired lung development, early onset of cardiovascular diseases, AD, increased susceptibility to respiratory infections, childhood asthma, and asthma exacerbation [485]. Immunological disturbances and allergic sensitisation are also demonstrated [486].

This study found associations between ETS exposure and both infant sensitisation and elevated infant total IgE concentration. However, self-reported household ETS exposure across the three pregnancy trimesters did not significantly differ between infants with and without allergies (**Table 5.1-1**). Univariate logistic analysis of ETS exposure, measured using urinary CCR during pregnancy and infancy, showed no significant association with allergic diseases. However, urinary CCR levels in the third trimester of pregnancy were associated with an increased risk of infant sensitisation, while CCR levels in infants at birth were linked to elevated infant total IgE concentration (**Table 7.3-1**). Despite these initial findings, the

associations did not remain significant in the multivariable logistic regression model, suggesting that other environmental and modifying factors may have a stronger influence on allergic outcomes than ETS, as measured by urinary CCR. Given the potential influence of exposure timing on immune development, future research should consider refined measurements at critical periods to better capture the impact of ETS on allergic outcomes.

7.8.3.3 Recently furnished home during pregnancy

Recently furnished furniture in the home during the first trimester was associated with a significantly increased risk of allergic diseases in the univariate logistic regression analysis. However, in the multivariable logistic regression model with Bonferroni corrections, this significant association persisted only for AD (adjusted OR: 14.135, 95%CI: 2.154, 92.744, $P = 0.006$). Although the wide confidence interval reflects the small sample size, the underlying scientific rationale remains noteworthy. Newly furnished homes can contribute to indoor VOCs exposure, as VOCs are commonly emitted from paints, lacquers, cleaning products, furnishings, copiers, printers, glues, adhesives, and permanent markers [418]. This finding aligns with previous studies reporting an association between house renovation and AD in childhood [487, 488]. Furthermore, exposure to indoor VOCs has been shown to alter cytokine profiles in cord blood and children by promoting Th2-skewed immune responses [489] and increasing proinflammatory cytokines [490] which may underlie this association.

7.9 Conclusion

This chapter examined the associations between early-life factors—including maternal, child, socioeconomic, and environmental factors (from **Chapter 5**)—and immunological and allergic outcomes; allergic diseases, infant sensitisation, and elevated infant total IgE. These findings

highlight the potential role of early environmental exposures in shaping immune responses and influencing allergy development in infancy, contributing to the broader objective of this thesis.

Chapter 8: Discussion

This DPhil research has explored the complex interactions between *in utero* environmental exposures, immunological alterations, and subsequent infant allergic diseases, infant sensitisation, and elevated infant total IgE concentrations. The findings of this DPhil study offer significant contributions to understanding the factors influencing allergic outcomes in early life, with a specific focus on maternal exposures and environmental conditions during pregnancy and infancy. The key findings of the study and their interpretations have been discussed in detail in the previous Chapters. Therefore, this discussion chapter focus on additional insights arising from the findings, including factors that may or may not be directly associated with the main outcomes. Furthermore, the broader implications of these results, study limitations, and potential directions for future research will be explored. A comprehensive synthesis of the study's findings within the broader context of environmental and immunological research related to allergic outcomes in infants are provided.

A unique aspect of this study is its implementation within the context of Thailand, where environmental health issues, particularly related to air pollution are a growing public health concern. Conducting a birth cohort study in Thailand provided valuable insights into the local environmental and cultural factors that influence infant health outcomes, particularly allergic diseases. The research journey in Thailand involved overcoming challenges related to data collection, including the measurement of environmental exposures, recruitment of a diverse cohort, and long-term follow-up. This experience has underscored the importance of context in interpreting research findings and contributes to a more comprehensive understanding of how environmental exposures influence immune development and allergic disease outcomes in Thai infants.

8.1 Lesson learned from conducting multicentre birth cohort study in Thailand

Overall, conducting a birth cohort study with the same objectives as this pilot study, as demonstrated in this DPhil thesis, is achievable. However, certain procedures and protocols need to be refined for improved implementation such as criteria for participants enrolment, following up schedule and methods, data collection procedure, and project management. Beyond the research results, the challenges, problem-solving skills, and valuable insights gained from the multicentre birth cohort study in Thailand have provided critical information about environmental challenges and health-related outcomes. These findings emphasise the need for a robust study design to address the complex questions in this field. Despite various constraints affecting the execution of the research, valuable lessons were learned. These lessons, from the perspective of the DPhil student as the principal investigator, are discussed, and suggestions for improving the feasibility of an extended birth cohort study based on this pilot study are proposed and summarised in the **Table 8.1-1**.

Table 8.1-1: The challenges, lesson learned, and suggestion from conductive birth cohort study in Thailand.

Challenges	Description	Lesson learned	Suggestions
Funding Challenges	Limited funding restricted repeated exposure measurements and scope of the study.	• Be cautious of the challenges associated with fluctuating funding cycles, as support may not be reliable for long-term projects. • Diversify funding sources and explore alternative financial models to ensure sustainability and flexibility should be done.	• Seek diversified funding sources and collaborate with governmental/non-governmental organisations. • Plan budgets for unforeseen costs. • Allocate sufficient budget to ensure that incentives are provided to participants and staff in a timely manner.
Cooperation with Collaborating Hospitals and Staff	Although early communication with collaborating hospitals and staff, along with training and support for the research protocol, was provided before and during the study enrolment, the primary responsibilities of the hospital staff still hindered the research schedule, leading to conflicts in data collection, participant monitoring, and deviations from the research protocol.	Maintaining collaboration with hospitals and medical staff was challenging, especially for ongoing engagement.	• Foster a sense of ownership at the collaborating centres by involving staff and providing adequate incentives. • Employ research assistants to alleviate the workload burden. • Establish early communication channels with hospitals, administrators, and staff, ensuring proper training and continuous support for the research protocol still be important.
Participant enrolment and retention	Difficulty in enrolling participants and retaining them due to socioeconomic barriers and life changes.	• Strict eligibility criteria and relatively low incentives, or the perceived lack of importance by participants, may lead to a low enrolment rate. • Although the inclusion criteria aimed to select participants who intended to follow up until their child reached two years of age, unforeseen circumstances could alter their plans	• Implement regular follow-ups, provide incentives, and allow flexibility in study schedules. • Establish trust and clear communication with participants.
Missing data	Missing data due to non-responses or loss to follow-up,	The mandatory electronic SAQ format can help minimise missing responses for certain questions. However, completely missing data due to non-response for a particular visit remains a	• Develop strategies to manage missing data (e.g., multiple imputation).

Challenges	Description	Lesson learned	Suggestions
	potentially skewing study results.	challenge. It is important to balance this with the potential nuisance or burden of completing multiple questionnaires.	• Regularly remind participants and offer alternative data collection methods.
Patient Movement and Migration	Participants moving out of the study area or changing contact information posed challenges in tracking exposures.	Patient movement was an uncontrollable factor, even though the criteria were set to ensure participants' intention to follow up at the same hospital long term. Implementing flexible data collection methods, such as telemedicine or mobile applications, could help mitigate this challenge.	• Regularly update participant contact details, utilise mobile health technologies for tracking, and offer flexible follow-up systems. • A nationwide birth cohort should be considered to facilitate follow-up at any hospital across the country, and also providing a more representative sample, and generalisability of the result.
Burden of Follow-Up	High burden on participants due to frequent visits, sample collection, and data collection.	Even though the study design aligned with routine ANC, infant health check-ups, and vaccination schedules, the additional burden of completing the SAQ and sample collection still impacted participant follow-up, as required by the research protocol.	Reduce in-person visits, when possible, use remote data collection tools, and offer flexible scheduling for participants.
Control of Research Protocol	Maintaining adherence to the research protocol amidst variations in circumstances and practices.	• The multicentre collaboration and prospective, multi-visit data collection involving multiple researchers and repeated data collection from the same participants posed challenges to maintaining research protocol integrity due to both intra- and inter-personal variability. • The passion, knowledge, and experience of the principal investigator (PI) are crucial in addressing these challenges. A committed and knowledgeable PI plays a key role in ensuring adherence to the research protocol, maintaining high standards, and effectively managing team dynamics. Their expertise helps in navigating complex situations, resolving issues related to data collection, and minimising inconsistencies that may arise due to multiple researchers or diverse participant responses. The PI's involvement and leadership are instrumental in maintaining the integrity and success of the study	• Establish internal monitoring systems, conduct regular protocol review meetings, and provide ongoing training for field staff to ensure consistency and adherence to research standards. • Implement an effective mentoring system to coach less experienced PIs and researchers, fostering skill development and ensuring high-quality research execution.

8.2 Potential confounding factors influencing allergic outcome in infants

8.2.1 Maternal factors

Gravida (the number of pregnancies a woman has experienced) was shown as a significant factor influencing the occurrence of allergic diseases in the univariate logistic regression. Infants born to mothers with three or more pregnancies (≥ G3) exhibited a reduced risk of developing allergic diseases compared to those born to mothers with two or fewer pregnancies (≤ G2). This observation is substantiated by an OR of 0.191 (95%CI: 0.041, 0.893, *P* 0.035), suggesting a protective effect of higher gravidity against allergic diseases. This observation was compatible with many previous studies, as reviewed by Karmaus *et al.* [491].

However, after adjusting for environmental factors in the multivariable logistic regression model, the significant association was no longer observed. This suggests that while these factors may influence the development of allergic diseases in children, the effect may be diluted by the presence of multiple other influencing factors.

8.2.2 Socioeconomic status (SES)

According to the univariate logistic regression analysis, no significant association between family income and allergic outcomes was identified.

8.2.3 Folate uptake

Folate supplementation of 400 μg daily has been recommended during early pregnancy or the pre-conceptual pregnant women to prevent neural tube defect in offspring [492]. According to the baseline characteristics from this study, the percentage of folate uptake prior to or during early pregnancy (first trimester) was relatively low, with only 11.33% of total pregnant

participants meeting this recommendation. This uptake rate shows a slight improvement compared to the findings of Nawapan and Phupong in 2007, where only 9.7% of 401 Thai pregnant women reported taking folate during the periconceptional period [493]. Despite the proven effectiveness of folate consumption during the pre-conceptual and early pregnancy periods in preventing neural tube defects, low uptake remains a significant challenge in many Asian countries [494-496]. The findings of this study emphasise the urgent need for action to improve folate supplementation among Thai women during the pre-conception and early pregnancy periods.

There was though no statistical difference between the non-dyad and dyad groups (10.00% vs. 13.25%, P = 0.472). This contrasts with one previous study that showed low folate intake during pregnancy (<400 μg/day) has been associated with an increased risk of allergic diseases in children, particularly in respiratory allergies [497].

8.2.4 Infant factors

In the analysis of elevated infant total IgE concentration, birth weight emerged as a variable of interest in the univariate regression analysis. Infants with birth weights ≤ 2,500 grams showed a trend towards higher infant total IgE concentrations compared to those with birth weights < 2,500 grams (OR: 6.286, 95%CI: 0.748, 52.902, P = 0.090). These findings contradict previous reports by Liem et al. which found no association between low birth weight and the development of food allergies [498]. This discrepancy warrants further investigation. Additionally, although the mode of delivery, specifically caesarean section, has been associated with an increased risk of allergies in children in other study [499], no such association was observed in this DPhil study. However, a significant association was found between infant

female gender and a decreased risk of both infant sensitisation and elevated infant total IgE concentration but only in the univariate logistic regression analysis. The association between infant gender and IgE levels is supported by other studies in both children and adults; however, the underlying mechanisms remain not fully understood [500-502].

8.2.5 Exclusive breastfeeding (EBF)

In this study, 46.2% of infants were exclusively breastfed at or before 4 months, with a lower percentage in the allergy group (37.5%) compared to the non-allergy group (88.3%). By 6 months, exclusive breastfeeding dropped to 10.1%, below the WHO target of 50% (8.8% in the non-allergy group vs. 13.6% in the allergy group), raising concerns about the need for improved breastfeeding promotion in Thailand. Although univariate analysis showed an increased odds ratio for allergic diseases of 3.571 (95% CI: 1.253, 10.182, $P = 0.017$), this may reflect heightened awareness among mothers in the allergy group, who are more likely to practice exclusive breastfeeding for allergy prevention [408]. However, this association was no longer observed after adjusting for environmental and other modifying factors in the multivariable logistic regression model.

8.2.6 Season of data collection and birth

Since participants were enrolled year-round and the study focused on air pollution ($PM_{2.5}$ and ETS) and home ventilation practices, weather factors could confound the results, and the birth season may affect allergy outcomes [503-505]. Therefore, both the season of data collection and birth season were considered in the analysis. Univariate logistic regression showed that children born during the summer (March to May) had a lower risk of allergic diseases compared to those born in winter (November to February), with an OR of 0.200 (95% CI: 0.038, 1.061, $P = 0.059$). This aligns with the seasonal variation in $PM_{2.5}$ levels, which tend to be higher in

winter in the study provinces. However, the multivariable logistic regression model did not find a statistically significant association, and no significant link between either season or allergic outcomes was identified. These findings need to be confirmed in a larger study.

8.2.7 Green environment

The concept of green environments protecting against allergies, as elucidated by Zednik *et al*., has been acknowledged [506]. Their extensive review emphasises the significance of biodiverse green spaces, encompassing green forests and meadows, in reducing the risk of asthma and allergies. This protection is thought to be mediated through several potential mechanisms, notably cultivating a diverse skin microbiome [507]. This diverse microbiome is fostered by early-life exposure to soil and natural surroundings, especially during childhood, ultimately fortifying the immune system. In alignment with this body of research, this DPhil study also investigated the association between green environment exposure at home during pregnancy and allergic outcomes, encompassing infant allergic diseases, infant sensitisation, and elevated infant total IgE concentration. However, univariate logistic regression analysis could not identify an association between these factors in this study. In terms of objectives the study here was compatible with the large birth cohort study in Finland that also could not find an association between greenness around residences and AD development in children [480].

Additionally, some factors may contribute to these subtle different findings. Firstly, this study's relatively small sample size might have limited its statistical power to detect associations. Secondly, the timing of exposure during pregnancy and its potential influence on immune outcomes may differ from that in early childhood, which is a focus of another research. Lastly, the study only assessed green environment exposure at home and did not account for exposure

in other settings, such as workplaces. This limited scope may not fully capture the comprehensive green environmental exposure experienced by participants in their real-life environments. Further research with larger sample sizes and comprehensive assessments of exposure timing and locations should be considered to refine the understanding of how green spaces influence allergy-related health outcomes.

8.3 Interpretation of findings

8.3.1 Heavy metals exposure and allergy in infant children

The results of the univariable logistic regression analysis suggested that Pb showed a statistically significant association with a decreased risk of allergic diseases among the analysed metals in first-trimester pregnant women (OR: 0.870, 95%CI: 0.753, 1.004, P = 0.057) (**Table 7.4-1**). However, no statistically significant associations were found for the other metals (Cr, Mn, As, Cd, Hg) about infant allergic diseases, infant sensitisation, or elevated infant total IgE concentration at the $P \leq 0.10$ threshold. These findings highlight the complexity of the relationships between metal exposures and allergic health outcomes and suggest that the studied metals might not significantly contribute to the observed allergic conditions in this cohort within the concentrations assessed. The result seemed to contradict several previous studies that indicated the positive association of prenatal Pb exposure to Th2 cytokines (IL-4, IL-10, TNF-α) [456], cord blood IgE [79], and AD severity [450]. However, significant results were no longer observed after conducting a multivariate logistic regression model that included Pb exposure and other potential factors related to allergic outcomes. Further investigation, potentially considering interactions with other environmental factors, is warranted to elucidate the complex relationships between metal exposure and allergic health outcomes.

8.3.2 $PM_{2.5}$ exposure and allergy in infant children

The result in **Chapter 7, Section 7.5** indicated that the reported allergies, sensitisation in infants within the studied population correlate with distinct $PM_{2.5}$ concentration patterns during infancy interval (0-4 months). $PM_{2.5}$ exposure during infancy 0-2 months was a slightly increased risk of allergic diseases (OR: 1.048, 95%CI: 0.998, 1.102, P = 0.060), and $PM_{2.5}$ exposure during infancy 2-4 months slightly increased risk sensitisation (OR: 1.053, 95%CI: 1.001, 1.119, P = 0.047). Therefore, they were not substantial enough to be considered reliable and conclusive, and they could have occurred due to random variation. Further research and analysis with a larger sample size may be needed to determine if there are any meaningful and statistically significant differences in $PM_{2.5}$ exposure between allergic and non-allergic infant children during these specific periods.

However, since the literature suggested the potential effect of $PM_{2.5}$ exposure in early life and allergy, the multivariable logistic regression approach also included $PM_{2.5}$ concentrations where appropriate. It allows for examining potential interactions and confounding effects among $PM_{2.5}$ exposure with other variables, providing a more comprehensive and accurate assessment of their associations with the allergy outcome.

Unfortunately, there was not statistically significant $PM_{2.5}$ exposure for all allergic outcomes. Previous studies majority reported the association between $PM_{2.5}$ and respiratory allergies (wheezing, asthma, and AR), but at a higher concentration of $PM_{2.5}$ exposure (e.g. mean $PM_{2.5}$ concentrations during pregnancy and the first year after birth were 36.2 ± 3.5 $\mu g/m^3$ and 35.6 ± 3.5 $\mu g/m^3$ in Taiwan), longer follow up time (at least four years of age), and larger sample size compared to this study (e.g. 191,330 maternal-child pair in Taiwan) [67, 508, 509], these

may explain the discordance result from this DPhil study. It should be noted that the birth cohort study in Toronto (Toronto Child Health Evaluation Questionnaire (T-CHEQ) also observed no association of early life (birth – 3 years) $PM_{2.5}$ exposure, but O_3 and NO_2 and risk of incidence asthma and AD in children up to 17 years (n = 1,286) [510]. Further research with larger sample sizes, longer follow-ups, and more advanced statistical techniques such as Generalised Estimating Equation (GEE) or Generalised Linear Mixed Model (GLMM) may help comprehensively investigate and understand the potential relationship between longitudinal $PM_{2.5}$ exposure and allergic outcomes in infants during different early life stages.

8.3.3 Multiple effects of environmental exposure and allergic diseases

The multivariable logistic regression analysis examined the relationship between environmental exposures and allergic outcomes in infants, incorporating a comprehensive set of factors. These included maternal characteristics such as gravida, birth weight, and maternal BMI, along with key environmental factors such as residential type, home ventilation practices, recent home furnishing, and exposure to heavy metals (Pb). The analysis identified significant associations between certain environmental factors and the risk of allergic diseases in infants. Notably, keeping doors and windows open for ventilation during the first trimester was linked to a significantly lower risk of allergic diseases (adjusted OR: 0.216, 95% CI: 0.066–0.075, $P = 0.012$).

Additionally, living in a row house or attached house and higher maternal BMI appeared to increase the risk of allergic diseases, although these associations did not reach statistical significance, likely due to the study's limited sample size (as discussed **in Chapter 7, Section 7.7.1**). These findings underscore the importance of the home environment in shaping allergic

outcomes, particularly in settings with relatively low concentrations of other environmental pollutants, such as heavy metals, ETS, and $PM_{2.5}$. Further research is needed to clarify the mechanisms underlying these associations. Meanwhile, promoting optimal home ventilation practices and addressing maternal BMI could be practical strategies for reducing the risk of allergic diseases in children.

8.3.4 Multiple effects of environmental exposure and sensitisation in infant children

The findings in **Chapter 7, Section 7.7.2**, highlight the complex relationship between early-life environmental exposures and the development of sensitisation. Several critical aspects emerged, including the importance of exposure timing, the distinction between protective and risk factors, and the impact of home ventilation practices. Notably, keeping doors and windows open appeared to have a protective effect against sensitisation, similar to its association with atopic diseases. However, this finding did not reach statistical significance, suggesting that ventilation-related environmental factors may influence sensitisation outcomes, but further research is needed to confirm this relationship.

An unexpected finding was that air purifier use during the second trimester was associated with an increased risk of sensitisation. Air purifiers have well-established efficacy in reducing the symptoms of respiratory allergic conditions such as asthma and AR [511-513]. However, their role in primary prevention of allergic diseases or sensitisation remains inconclusive due to limited evidence. For instance, Kim *et al*. reported the presence of mould contamination, an important aeroallergen, in high-efficiency particulate air (HEPA) filters of air purifiers after 6-12 months of use [514]. This raise concerns that, under certain conditions such as improper maintenance, air purifiers could potentially become a source of allergen exposure, particularly

in children with AD who may be more susceptible to sensitisation. Nevertheless, in this DPhil study, allergic sensitisation was assessed primarily through food allergen sensitisation, which may not be directly attributable to airborne mould exposure from air purifiers. Therefore, this mechanism may not fully explain the observed the inversely associations in the study.

Thus, this result warrants cautious interpretation, as potential confounding factors may play a role. One possible explanation is that heightened awareness of air pollution and allergy prevention, particularly among atopic mothers, may lead to increased air purifier use. This aligns with the study's observation that maternal sensitisation was a good predictor of infant sensitisation (adjusted OR: 6.132, 95% CI: 1.302–28.892, P = 0.022) that may reflect the existing allergic conditions. This study was designed as an observational study, not an intervention study. Therefore, any advice or knowledge provided by researchers or healthcare providers was not part of the study. The questionnaire regarding indoor ventilation practices was designed to capture a comprehensive range of available ventilation and temperature control measures in Thailand, including an open-ended question (e.g., "others, please specify"), which was intentionally neutral in tone and did not suggest or imply any specific knowledge. However, since general advice regarding allergy prevention has been widely disseminated to the public and could not be controlled within the study (acting as an uncontrolled confounder), it is plausible that the use of air purifiers may not represent a causal exposure. Instead, it could reflect a response to existing allergic conditions, such as following medical advice or increased awareness of allergy risks. This raises the possibility that air purifier use might act as a proxy for underlying allergic status, potentially representing reverse causality or functioning as an inadvertent confounder. Further research is needed to explore the underlying mechanisms and confirm whether air purifier use itself contributes to the risk or if it is simply a marker of pre-existing susceptibility by clarification details regarding air purifier use, including frequency,

timing, duration, room coverage, type of air purifier, equipment maintenance, and the appropriateness of these factors, as they might influence the effectiveness of the air purifiers. Additionally, factors such as the frequency of window opening and closing and air conditioning use could also play a role. While the DPhil study did not specifically capture these details due to its broader focus on indoor ventilation measures, these aspects were acknowledged as limitations and are suggested for future research, should the initial findings indicate their relevance.

8.3.5 Multiple effects of environmental exposure and elevated infant total IgE concentration

The findings in **Chapter 7, Section 7.7.3** highlight the potential role of genetic susceptibility in influencing allergic outcomes, as demonstrated by the significant variables that remained in the multivariable logistic regression analysis, even though statistical significance was not achieved. This suggests that maternal IgE levels may still be a considerable risk factor for elevated IgE concentration in infants, warranting further investigation. Additionally, the study reinforces the importance of trimester-specific environmental exposures, as home environmental factors continued to show associations with elevated infant total IgE concentration, similar to previous allergic outcomes such as allergic diseases and sensitisation.

In a broader context, these results align with growing evidence that early-life environmental and genetic factors interact to shape immune development and allergy risk. The significant associations observed between elevated IgE concentration and specific exposures—such as visible mould in the home during the second trimester (adjusted OR: 4.198, 95%CI: 1.161, 15.182, P = 0.029) and household ETS exposure from housemates in the third trimester

(adjusted OR: 6.745, 95%CI: 1.476, 30.835, P = 0.015)—underscore the need for targeted public health interventions. Addressing these modifiable environmental risk factors, particularly during critical windows of immune system development, could help reduce the burden of allergic diseases in children. Future research should further explore the complex interplay between genetic predisposition and environmental exposures, incorporating larger, more diverse study populations and mechanistic studies to clarify underlying biological pathways.

8.3.6 Timing of exposures

The timing of environmental exposures plays a crucial role in shaping immune system development and influencing allergic outcomes in infants. Findings from this study suggest that both prenatal and postnatal exposures contribute to immune dysregulation, with trimester-specific effects observed for certain environmental factors. For example, home ventilation practices, such as keeping doors and windows open, appeared to have a protective effect against allergic diseases and sensitisation, particularly during the third trimester. This highlights the importance of adequate air circulation in reducing indoor pollutant concentrations during a critical window of immune development. Conversely, exposure to visible mould in the second trimester and household ETS from housemates in the third trimester were associated with an increased risk of elevated infant total IgE concentrations, suggesting that certain exposures during specific periods of pregnancy may heighten allergic risk.

The observed trimester-specific associations underscore the complexity of immune system maturation and the differential effects of environmental factors at various developmental stages. Early pregnancy exposures may influence immune programming through epigenetic

modifications, while later exposures could have more immediate effects on immune responses at birth and in early infancy. The finding that maternal sensitisation was a strong predictor of infant sensitisation further supports the role of genetic susceptibility in modifying the impact of environmental exposures. However, the unexpected association between air purifier use during the second trimester and increased risk of sensitisation raises questions about potential confounding factors, such as increased allergy awareness among mothers with a family history of atopy.

Recognising these timing-dependent effects has important implications for public health interventions aimed at allergy prevention. Strategies should prioritise minimising harmful exposures during pregnancy and early infancy while promoting protective practices such as optimal home ventilation and reducing ETS exposure. Future research should further explore these associations in larger cohorts to validate these findings and clarify the mechanisms underlying the timing-specific impact of environmental exposures on allergic disease development.

8.3.7 Immunological alterations

Environmental exposures in early life may influence immune function directly or act as mechanisms linking to allergic disease development in children. In this study, Pb exposure emerged as a potential immunomodulatory factor, exhibiting a significant inverse correlation with IL-12/IL-23p40 concentration in infants and a decreased odds ratio for allergic diseases. IL-12/IL-23p40 plays a crucial role in promoting Th1 immune responses, which counterbalance Th2-driven allergic inflammation. A reduction in IL-12/IL-23p40 may indicate an immune shift toward Th2 dominance, theoretically increasing allergy risk. However,

paradoxically, Pb exposure in this study was associated with a lower risk of allergic diseases, which contrasts with findings from previous research suggesting Pb's role in immune dysregulation and increased allergy susceptibility.

Notably, these associations were not statistically significant in the multivariable logistic regression model, where both Pb exposure and IL-12/IL-23p40 levels were included. This suggests that other environmental and genetic factors may confound or modify the relationship between Pb and allergic outcomes. The lack of statistical significance could also be attributed to sample size limitations or the complexity of immune interactions influenced by multiple exposures. Nonetheless, these findings highlight the potential role of heavy metals in shaping immune responses during infancy and raise important questions about their mechanistic links to allergic disease development.

Further studies with larger cohorts and mechanistic investigations are needed to clarify how Pb exposure influences immune signalling pathways and whether its effects on IL-12/IL-23p40 contribute to allergy risk or reflect broader immune dysregulation. Understanding these interactions could help refine risk assessments and public health strategies aimed at reducing heavy metal exposure during pregnancy and early childhood.

8.3.8 Association between environmental factors and allergic outcomes

One of the central challenges for this study was in juxtaposing a small sample size with the multitude of potential influencing factors. A small sample size inherently limits the generalisation of findings to larger populations, mainly when accounting for diverse demographic and geographical variations. Moreover, this diminutive sample can reduce

statistical power, making detecting significant relationships or differences between variables challenging. Higher variability in data may further blur the lines between genuine effects and random fluctuations.

Further the other hand, the vast array of factors that individuals are exposed to in the real world complicates matters. From environmental nuances to lifestyle choices, genetic variations, and socioeconomic influences, the study's task of comprehensively considering these variables can be daunting. Interactions and confounding effects among these myriad factors can muddy the waters, making it difficult to pinpoint the precise impact of $PM_{2.5}$ exposure on health outcomes

Furthermore, was limited it provided further evidence of the involvement of environmental factors of ETS and $PM_{2.5}$ exposure interact with other factors to influence allergic outcomes could provide valuable insights. Resource limits individual studies but collectively the contribute to greater understating. Thus, this study provides a prelude to an extended cohort study with a longer follow-up period, allowing for a comprehensive understanding of the interplay between environmental factors and allergic responses. This expanded scope could shed light on how various environmental elements collectively influence allergic outcomes, contributing to a more holistic view of the subject matter.

8.4 Strengths and limitations

8.4.1 Strengths of the study

This study has several strengths that contribute to its significance in understanding the relationship between early-life environmental exposures and allergic outcomes in children. First, the prospective study design allows for a temporal assessment of exposures during

pregnancy and infancy, reducing recall bias and providing a clearer picture of causality. The longitudinal approach enhances the reliability of findings by capturing dynamic changes in environmental exposures and their potential effects on immune development over time.

Another strength is the detailed exposure assessment, which considers multiple environmental factors, including residential type, home ventilation practices, heavy metal exposure, ETS, and household chemicals. By incorporating trimester-specific exposures, this study provides insights into the critical windows of susceptibility, emphasising the importance of timing in environmental influences on immune function and allergy development. The inclusion of home ventilation practices is particularly relevant, as it highlights modifiable risk factors that can be targeted for intervention.

Additionally, the study integrates both immunological and clinical allergic outcomes, bridging the gap between environmental exposures and immune system alterations. The measurement of cytokines, such as IL-12/IL-23p40, adds a mechanistic dimension to the findings, offering potential pathways through which environmental factors may contribute to allergy risk. This dual approach strengthens the study's contribution to the field of environmental immunology.

Furthermore, the study was conducted in a real-world setting within a Thai population, adding valuable data from an underrepresented region in allergy and environmental health research. The findings can inform local public health policies and interventions tailored to specific environmental challenges, such as air pollution and heavy metal exposure in urban areas.

Finally, the use of multivariable logistic regression analysis accounts for potential confounders and interactions between different environmental, maternal, and infant factors. This robust analytical approach enhances the reliability of the associations identified and allows for a more comprehensive understanding of the complex interplay between early-life exposures and allergic disease development. However, while these strengths contribute to the study's validity, further research with larger sample sizes and additional mechanistic investigations will be essential to confirm and expand upon these findings

8.4.2 Limitation of the study

Despite its strengths, this study also has several limitations. First, the assessment of prenatal heavy metal exposure relied on a single whole blood measurement due to funding constraints. This approach may not fully capture long-term exposure, as heavy metals exhibit different metabolic and excretion patterns. While alternative biomarkers such as hair, nails, or teeth could provide insights into chronic exposure, challenges in standardisation and sample collection remain.

For ETS exposure, self-reported household ETS and urinary CCR were used, but infant self-reported household ETS exposure was not reassessed postnatally. The chosen cutoff for urine CCR (≥ 30 μg/mg Cr) warrants further validation in larger cohorts since relatively low prevalence of ETS exposure due to this cutoff level in the population of this study, given potential genetic variability in nicotine metabolism (e.g., CYP2A6) [515]. Furthermore, pregnant and infant children might metabolise nicotine differently than healthy adults [516-518].

Ambient $PM_{2.5}$ exposure was estimated using 24-hour average concentrations from government monitoring stations. While this provides a general exposure estimate, peak exposure levels and other pollutants (e.g., NO_2, O_3, SO_2) were not assessed. GIS-based interpolation methods, despite adjustments for residential mobility, may introduce exposure misclassification due to uncertainties in assessing individual-level $PM_{2.5}$ exposure (457), particularly in non-industrial residential areas. These uncertainties can arise from various factors, including the regulatory basis for locating monitoring stations primarily in highly polluted areas or hotspots such as heavily trafficked roads and industrial zones. This tendency can potentially lead to overestimations of $PM_{2.5}$ exposure in residential areas [519]. Future studies should refine exposure models by incorporating meteorological and microenvironmental factors such as building types, workplace settings, transportation modes, and the duration of exposure on an hourly basis, while balancing feasibility and accuracy.

The microbiome component and miRNA (epigenetic alteration) were initially planned to explore potential mechanisms linking prenatal exposures to immune outcomes. However, due to resource constraints and challenges in sample processing, this aspect was not fully completed and not included in this thesis.

Regarding immunological outcomes, the study lacked measurements of T-cell populations and responses, limiting insights into cell-mediated immune mechanisms. Additionally, the absence of parasitic screening (e.g., stool or serology tests) means elevated total IgE levels should be interpreted with caution, as soil-transmitted helminths infections in mothers or children were not ruled out [520].

For allergic outcomes, disease severity was not stratified, potentially obscuring associations with early-life exposures. The use of SCORing Atopic Dermatitis (SCORAD) for atopic dermatitis and trans-epidermal water loss (TEWL) measurement could enhance precision. Similarly, while sIgE screening (PhadiaTop®) provided preliminary insights, confirmatory tests such as oral food challenges would offer a more comprehensive allergy assessment.

8.5 Implication for public health and policy

8.5.1 Healthcare provider

Establishing the potential link between urban chemical exposure and immune system alterations in Thai infants from this study will ensure that healthcare professionals working in urban areas with high $PM_{2.5}$ exposure monitor the infant's health, especially the allergy aspect. Early detection and intervention for immune-related issues could mitigate potential health risks.

Pregnant women could be advised to minimise exposure to chemicals during their pregnancies to reduce potential impacts on their infants' immune systems. It might involve recommendations to avoid certain products or environments. Regarding this study's finding that housing, ventilation may affect the allergy, the recommendation of ensuring natural ventilation would be considered.

8.5.2 Policy makers

These findings could encourage collaborations between health professionals, environmental scientists, policymakers, and urban planners. Addressing this issue requires a multi-

disciplinary approach. Health issues from immune system alterations could increase healthcare costs and potential productivity losses. It could motivate stakeholders to invest in pollution control measures.

8.5.3 Public health policies

The findings could lead to more stringent regulations and public health policies to reduce chemical pollutants especially air pollution in urban environments. It could involve stricter emissions controls and increased awareness of potential risks. Along with environmental considering, low folate intake in Thai pregnant population is still needed to tackle.

8.5.4 Environmental protection

The research could underscore the importance of preserving clean and sustainable urban environments. Efforts to reduce air pollution and responsibly use and disposal of chemicals could be crucial in protecting infant health.

8.5.5 Parents and caregivers

Educating parents and caregivers to raise awareness about the potential risks associated with urban chemical exposure is crucial. Increased awareness can lead to better protective measures for infants.

8.5.6 International context

The implications might extend beyond Thailand, as similar patterns of urbanisation and chemical exposure are observed globally. The findings could contribute to a broader understanding of the impact of urban environments on infant health.

In essence, the potential implications underscore the need for comprehensive action to ensure the health and well-being of infants in urban areas. Effective solutions will require collaboration between various sectors and a commitment to safeguarding the environment and public health.

8.6 Future research suggestions

The potential link, such as epigenetic alteration, microbiome disturbance, and epithelial barrier dysfunction, between early life chemical exposures and the development of allergic disease highlights the need for further research to understand causality and mechanisms better. Funding for longitudinal studies that follow infants from birth into childhood could provide more definitive answers. The exposome concept should be considered to thoroughly examine the complexity of environmental factors and health [60, 61, 521]. Harmonisation with international concentration of study will help to robust the equivalent results. The specified suggestions based on the result from this DPhil study are as following.

8.6.1 Study population and geography

The study population's characteristics, such as demographics, socioeconomic status, and lifestyle factors, should always be considered. The geographical residential area also plays a

crucial role in air pollution exposure. Different regions may have varying concentration and sources of air pollution, so the expansion of study to cover more regions in Thailand (multi-centre or national birth cohort study) to ensure that the study population and location are representative of the target population of interest, was suggested.

8.6.2 Exposure assessment

To prioritise exposure assessment based on the data from this pilot birth cohort study, it appears that $PM_{2.5}$ is a more significant concern when compared to heavy metals and ETS in people who live in urban areas. However, several important factors should be considered especially seasonal variation, home ventilation practise, and microenvironment factors, when planning the following study to obtain more representative information. Additionally, utilise advanced exposure assessment methods, such as air quality modelling and personal monitoring devices was suggested, to obtain accurate and detailed data on pollutant concentration at different locations within the study area. It can help identify specific sources of exposure and high-risk areas. In addition, microenvironment details should be considered to robust the finding of environmental effects on health.

8.6.3 Consideration of other air pollutants and composition of $PM_{2.5}$

While $PM_{2.5}$ is a crucial indicator of air quality and can serve as a proxy for air pollution exposure, it is crucial to broaden the scope of the study by considering other air pollutants. It may include assessing concentration of O_3, NO_2), SO2, CO, and VOCs, among others, depending on the specific air quality concerns in the study area. These air pollutants also increase evidence of health-related problems, especially in respiratory allergy and lung development [522, 523].

8.6.4 Long term monitoring

Consider implementing long-term air quality monitoring better to understand the chronic health effects of air pollution exposure. This monitoring can provide insights into seasonal variations, trends over time, and the cumulative effects of exposure. Additionally, extending the follow-up period for disease outcomes is highly recommended to capture the full spectrum of health effects and better assess the role of timing in environmental exposures and disease development.

8.6.5 Outcome assessment

They clearly defined the health outcomes of interest, whether allergies, respiratory conditions, or other health effects are essential. It will guide the selection of exposure metrics and the analysis of potential associations. Additionally, long-term health implications of immune system alterations in infancy need to be studied. There could be effects that extend beyond infancy, potentially leading to a higher risk of immune-related disorders later in life apart from allergic diseases such as autoimmunity, cardiovascular diseases, and neurobehavioral disorders.

8.6.6 The association of environmental factors and allergy

Expanding the research framework to encompass the exposome and adopting omic technologies, advanced statistics, and molecular mechanistic investigations offers a more comprehensive and nuanced understanding of how environmental exposures influence health outcomes. This approach can transform our ability to identify, prevent, and mitigate the impact of environmental factors on disease and health biomarkers.

In addition to examining single exposures and their association with diseases, embracing a more comprehensive approach that considers multiple exposures and the exposome concept is essential [48, 60, 61, 63, 66, 293, 521, 524, 525]. This broader perspective better reflects the complexity of real-life environmental interactions. To achieve this, leveraging advanced omic technologies, including microbiome analysis, epigenome studies, and sophisticated statistical methods, can provide valuable insights [286, 299, 303, 526-529].

By integrating these cutting-edge technologies and analytical approaches, the intricate molecular mechanisms underlying the relationship between environmental exposures and disease outcomes or changes in biomarkers that precede the onset of disease can be explored. This holistic approach identifies potential causal links and underlines the early molecular changes that may serve as pre-disease indicators.

8.7 Conclusion

This DPhil study demonstrates that some early-life environmental exposures particularly home ventilation practices during pregnancy are associated with the development of allergic outcomes in Thai infants. Multiple environmental factors were assessed via maternal questionnaires, including ETS, green environment exposure, pet ownership, household chemical use, and indoor ventilation behaviours and associated with measured allergic outcomes in Thai infants. In respect of ventilation behaviours keeping windows and doors open during the third trimester was consistently linked with a lower risk of infant allergic diseases and allergic sensitisation. This finding alone indicates the importance of factors in the internal air environment in the development to allergies in Thai infants. Further support for this finding was identified by second trimester exposure to visible mould and third trimester ETS exposure

which are found in the indoor environment and positively associated with allergic outcomes in Thai infants. Exposure to both these indoor environmental air contaminants was associated with elevated infant total IgE concentrations, a biomarker of allergy development. Unexpectedly use of an air purifier was also positively associated with allergic outcomes in Thai infants and as discussed in section 8.3.4 this could be due to mould contamination of the air purifier filter.

Heavy metal exposure and $PM_{2.5}$ was exhaustively investigated but was not consistently associated with allergic outcomes. This is a positive finding but needs to be caveated in that the result reflect relatively low exposure levels, a limited sample size, and short follow-up duration.

In terms of biological susceptibilities, maternal sensitisation and infant gender were significant predictors of infant allergic sensitisation and infant total IgE levels, suggesting that biological susceptibility plays a role in early allergic immune development.

In summary the data presented here highlight the importance of allergens in the indoor air environment in the development of allergic outcomes in Thai infants. The public message is that of the importance of modifiable prenatal indoor environmental exposures can provide benefit in the development of allergic outcomes in Thai infants. The work provides evidence to support practical public health interventions, such as promoting natural home ventilation

during pregnancy especially in households there is a higher risk due to biological susceptibilities.

Chapter 9: Conclusions

9.1 Summary

This thesis explores the impact of early-life environmental exposures on the development of allergic diseases and immune responses in Thai infants. Through a birth cohort study conducted in Thailand, several important associations were identified between specific environmental factors and allergic outcomes, such as AD, food allergies, sensitisation, and elevated IgE concentrations in infants. These findings contribute to the growing body of evidence regarding how environmental factors can shape immune development in early life. The summary of key findings from each chapter was shown in **Table 9.1-1**.

Table 9.1-1: The summary of key findings from each chapter of the thesis

Chapter	Key findings	Implications
Chapter 1: Introduction	• Thailand faces a growing burden of NCDs, with childhood allergy being a significant concern. • Gene-environment interactions contribute to allergy development, requiring further exploration. • Urban and industrial environments may be linked to allergy onset. • Early-life exposures (*in utero* and infancy) are critical windows for immune health and allergic disease development. • Exposure assessment is essential but must balance feasibility and precision.	Highlights the importance of understanding environmental influences on allergy development, particularly in Thailand. Emphasises the need for targeted prenatal and early-life interventions.
Chapter 2: Literature Review	• Environmental issues in Thailand (air pollution, heavy metals, pesticides) possibly impact children's health. • Limited research exists on environmental exposures and allergy outcomes in Thai children.	Supports the need for focused research on environmental exposures ($PM_{2.5}$, heavy metals, pesticides) and their effects on allergic outcomes in Thai children.

Chapter	Key findings	Implications
Chapter 3: Study Design	• Describes study design, eligibility, participant selection, and data collection methods. • Ethical considerations and methodological framework established.	• Establishes a methodological foundation, ensuring data quality and ethical research practices
Chapter 4: Birth Cohort Study	• Summary of enrolment and participant characteristics, air quality data.	• Highlights the demographic and environmental factors relevant to the study, providing context for subsequent analysis.
Chapter 5: Exposure Assessment	• Assessed home environment factors, lifestyle hazards, and environmental exposures (ETS, heavy metals, $PM_{2.5}$).	• Identifies key environmental exposures in the study cohort and links them to potential health risks for infant allergy development.
Chapter 6: Outcome Assessment	• Immunological profiles of infants, including cytokine levels and allergic conditions (AD, FA).	• Provides baseline data on infant immune development, crucial for examining associations with early-life environmental exposures.
Chapter 7: Associations of Exposure and Allergies	• Exposure to urban pollutants (e.g., Pb, ETS, $PM_{2.5}$) during pregnancy and infancy disrupted immune cytokine production in infants. • Detailed analysis of the impact of specific cytokines on allergic diseases and sensitisation in infants exposed to urban pollutants. • In-depth analysis of the correlation between environmental exposures (e.g., ETS, heavy metals, $PM_{2.5}$) and allergic outcomes in infants. • Identified association between environment factors (e.g., ventilation, Pb exposure, household cleaning products) and allergic outcomes in infants.	• Provides insight into immunological pathways linking environmental exposure to allergic disease. • Demonstrates the significant role of early-life exposures in shaping allergic disease outcomes, emphasising the need for targeted interventions. • Highlights the need for environmental control in homes to reduce allergy risks. • Emphasises the importance of timing in environmental exposure when assessing allergy risks.

Chapter	Key findings	Implications
Chapter 8: Discussion	• Lessons learned from the birth cohort study in Thailand, general consideration related to the findings including exposure of ETS, heavy metals, $PM_{2.5}$, home environment, immunological, and allergic diseases outcomes.	• Provides an interpretation of findings, offering insights into the complex interactions between environmental exposures and immune health, and discussion in general context link to implication of the study result and included suggestions.
Chapter 9: Conclusions	• Synthesis of findings, implications for policy, healthcare, and future research. Emphasises the importance of addressing urban pollution and environmental risks.	• Calls for enhanced urban planning, stricter environmental regulations, and further research to confirm causality and guide health interventions.

9.2 Conclusions

This thesis provides evidence on the relationship between early-life environmental chemical exposures and allergic outcomes in early childhood. Prenatal exposure to heavy metals, particularly Pb, showed potential immunomodulatory effects, including an inverse association with IL-12/IL-23p40. Household environmental factors, such as ventilation practices and exposure to ETS, were linked to allergic diseases and sensitisation, with trimester-specific effects observed. While ambient $PM_{2.5}$ exposure was considered, its impact on allergic outcomes was not clearly established due to limitations in exposure assessment. The findings emphasise the complex interplay between environmental exposures and immune development, highlighting the need for further research to refine exposure assessments. The findings suggest that improving urban planning, environmental regulation, and health education could help reduce exposure to harmful pollutants. Prevention strategies should focus on prenatal and early childhood interventions to minimise the risk of allergic diseases and reducing the burden of allergies and promoting better health outcomes for future generations.

9.3 Future directions

While this study contributes important knowledge on the relationship between environmental exposures and allergic outcomes, further research is needed to establish definitive causal links. Longitudinal studies with larger, more diverse populations will be essential to validate these findings. Additionally, future research should explore the role of genetic and epigenetic factors, the microbiota, and epithelial dysfunction in modulating the effects of environmental exposures on immune development and allergic diseases.

APPENDIX

Appendix 1 Comparison of ICP-MS analysis for metal elements in Mahidol University and UKHSA

Issues	UKHSA	Ramathibodi Hospital, Mahidol University	Faculty of Science, Mahidol University
Sample preparation Note: 1 ppm = 1 mcg/mL 1 ppb = 0.001 mcg/mL	Microwave digestion: modified digestion buffer 4,800 µL + sample 200 µL (Ultrawave®, 22 position rack, final volume 5 mL each tube: modified acid digestion buffer 4,800 mL + sample 200 µL) Remarks: 100 mL of modified digestion buffer consists of - 75.56 mL DI - 4 mL 30% H_2O_2 (Trace grade, final concentration 1.2%(v/v)) - 20 mL 20% HNO_3 (Trace grade, final concentration 4%(v/v)) - 40 µL Gold standard stock (0.04 mL)(1000 ppm) → 40 mcg, required to reduce intrinsic Hg memory effects - 200 µL Internal standard (10,000 ppb IrBiRh) → 2 mcg of INSD - 200 µL Triton X-100 * Add 250 µL 10% HCl to stabilise Hg in 5 mL digested samples *before transferring the digested sample* **Diluted digested samples to 10 mL by adding 5 mL multielement standard matrix (2%HNO3 + 0.5%HCl) before the ICP-MS analysis	Acid dilution (matrix diluent) 4,800 µL + sample 200 µL Remarks: 100 mL of matrix diluent consists of - Ethylenediaminetetraacetic (H_2EDTA) 0.1 g - Isopropanolol 4 mL - NH_4OH 2 mL - Triton-X-100 100 mL - DI water up to 100 mL - *Able to store at room Temp. for 1 week*	Microwave digestion (ETHOS UP®, Rotor MAXI-44, 44 position rack, 10 mL each tube, sample weight up to 1.5 mL, reagents 8 mL (6 mL 65%HNO_3 + 2 mL 30% H_2O_2 Remarks: 100 mL of reagents consist of - 75 mL 65% HNO_3, final concentration 48.75% (v/v) - 25 mL 30% H_2O_2, final concentration 7.5% (v/v)
Analysis	ICP-MS (Thermo Scientific™ iCAP Q ICP-MS, High-performance quadrupole analyser)	ICP-MS (Agilent 7900 ICP-MS, flexible quadrupole mass spectrometry) Remarks: 800 THB or 20 GBP/samples	ICP-MS (Agilent 7900) ICP-MS, flexible quadrupole mass spectrometry

Issues	UKHSA	Ramathibodi Hospital, Mahidol University	Faculty of Science, Mahidol University
			Remarks: 1,400 THB or 35 GBP/hours
LOD, LLOQ	0.1, 0.3 ppb	0.1, 0.5 ppb	NA

Appendix 2 ICP-MS methods to measure 8 multi-elements in whole blood at Ramathibodi Hospital

1. Scope of measurement

No.	Element	Measurement Isotope	Internal Standard (ITSD)/ Alternative Internal Standard	Reporting Unit (LOQ 0.5, LOD 0.1)
1	Arsenic (As)	^{75}As (mass 75)	**^{72}Ge**/^{45}Sc	µg/L (0.5-1,000)
2	Cadmium (Cd)	^{111}Cd (mass 111)	^{103}Rh/^{113}In	µg/L (0.5-1,000)
3	Chromium (Cr)	^{52}Cr (mass 52)	**^{45}Sc**/^{72}Ge	µg/L (0.5-1,000)
4	Lead (Pb)	^{208}Pb (mass 208)	^{209}Bi/**^{159}Tb**	µg/dL (0.5-1,000)
5	Manganese (Mn)	^{55}Mn (mass 55)	**^{45}Sc**/^{72}Ge	µg/L (0.5-1,000)
6	Mercury (Hg)	^{202}Hg (mass 202)	^{209}Bi/**^{159}Tb**	µg/L (0.5-1,000)
7	Selenium (Se)	^{78}Se (mass 78)	**^{45}Sc**/^{72}Ge	µg/L (0.5-1,000)
8	Thallium (Tl)	^{203}Tl (mass 203)	**^{209}Bi**/^{159}Tb	µg/L (0.5-1,000)

Available ITSD: ^{45}Sc, ^{72}Ge, ^{159}Tb, ^{209}Bi

2. Sample preparation

- Dilution (isopropanol: H2EDTA: ammonium hydroxide: triton+X100 10: 0.1: 1: 0.1 v/w/w/w in DI)
- Sample (whole blood): diluent = 2:48 (25 times)

3. ICP-MS (Agilent 7900)

- Argon gas
- Plasma ionisation source
- Collision-reaction cell process (Octapole reaction system, ORS): Helium collision mode or Hydrogen collision mode for *minimise polyatomic interferences*

4. Method Performance Characteristics

As the table below:

Performance Characteristics:	Results:
Selectivity	No significant interference from the matrix
Sensitivity/ Limit of detection (Estimated LOD)	All elements = 0.1 µg/L
Limitation of quantification (LOQ)	All elements = 0.5 µg/L
The upper limit of quantification (ULOQ)	All elements = 1,000 µg/L
Working range/ Calibration model	All elements = 0.5 – 1,000 µg/L (linear model desired)
Precision	Repeatability/ with-in run: < ±20% CV Reproducibility/ between runs: < ±20% CV
Accuracy	Bias: < ±20% CV
Carryover	No significant carryover at 10,000 mg/L
Dilution Integrity	10x dilution: < ±20% CV 20x dilution: < ±20% CV 50x dilution: < ±20% CV 100x: dilution: < ±20% CV
Processed sample stability	12 hours

5. Specimen requirement

- 1-3 mL whole blood (K_2EDTA-Whole blood)
- ☑ recommend collection medium: BD Vacutainer Plus with K2EDTA Royal Blue Stopper, 6-mL Collection tube (Product number 363381), stored at 4 ± 2 °C

6. Reagents

6.1 > 99.5% Isopropyl alcohol (IPA) (Analytical reagent grade or equivalent)
6.2 65% Nitric acid (Analytical reagent grade or equivalent)
6.3 ≥ 99.95% Ethylenediaminetetraacetic acid (H_2EDTA) (ACS reagent grade or equivalent)
6.4 ≥ 25% Ammonium hydroxide (NH_4OH)
6.5 Triton-X100 (ACS reagent grade or equivalent)
6.6 > 99.99% Multi-element standard mix (Sigma-Aldrich CRM or equivalent)
6.7 ICP-MS tuning solution (^{45}Li, ^{72}Ce, ^{103}Y, ^{113}Co, and ^{159}Tl)

6.8 ICP-MS internal standard mix (^{45}Sc, ^{72}Ge, ^{103}Rh, ^{113}In, ^{159}Tb, and ^{209}Bi)
6.9 Deionised water (≥ 18.2 Mohm)
6.10 High purified argon gas (Ar) ≥ 99.995%
6.11 Ultra-high purify Hydrogen gas ≥ 99.999%
6.12 Ultra-high purify Helium gas ≥ 99.999%

7. Solution preparations

7.1 2% HNO_3
: 2 mL 65% HNO_3 + 80 mL distilled water + DI up to 100 mL? → Storage at RT 1 month, bottle 100 mL

7.2 Internal standard 100 µg/L
: 25 µL ISTD (10 µg/mL) + 25 mL 2% HNO_3 → Storage at 2-8 °C 1 month, bottle 25 mL

7.3 Matrix Dilution Solution (Matrix Diluent)
: 0.1 g H_2EDTA + 50 mL distilled water + 4 mL isopropanol + 2 mL NH_4OH + 100 µL triton X-100 + DI up to 100 mL → Storage at RT 1 week, bottle 100 mL

7.4 Stock Standard 1,000 µg/L and 10 µg/L
Stock Standard 1,000 µg/L: 100 µL multi-element standard (mix) (10 µg/mL) + DI 900 µL in micro-centrifuge tube 1.5 mL, mix by vortex mixer 10 sec
Stock Standard 10 µg/L: 10 µL multi-element standard (10 µg/mL) + DI 990 µL in micro-centrifuge tube 1.5 mL, mix by vortex mixer 10 sec

7.5 QC samples

7.5.1 Diluent Blank (BLK)
: 4,950 mL diluent + 50 µL DI in sample cup, close the sample cup by parafilm the mix by vortex mixer 10 sec

7.5.2 Blank blood/ blood negative control (BNC)
: 1.5 mL BNC, storage at -15 °C for 1 year

7.5.3 Internal Quality Control (IQC)
: Seronorm™ L-1, L-2, L-3 (IQC_L; 3 µg/L, IQC_M; 12 µg/L, IQC_H; 48 µg/L)

7.5.4 Calibration standard as shown in the table below

Level	Concentration	Stock volume (µL)	Stock concentration (µg/L)	Volume of Water (µL)
0	0 µg/L	0	-	1,000
1	0.5 µg/L	50	10	950
2	10 µg/L	10	1,000	990
3	50 µg/L	50	1,000	950
4	100 µg/L	10	10,000	990
5	250 µg/L	25	10,000	975
6	500 µg/L	50	10,000	950
7	1,000 µg/L	100	10,000	900

8. Tools and equipment

8.1 ICP-MS (Agilent 7900)
8.2 Agilent Integrated Autosampler (I-AS) 89 positions
8.3 Vortex mixer
8.4 Micro-centrifuge tube 1.5 mL with cap
8.5 Bottle 10, 50, 100 mL (Pyrex Class A)
8.6 Volumetric Pipette (Pyrex Class A)
8.7 Micropipette 10-100, 20-200, 200-1000, and 500-5000 µL
8.8 ICP sample cup 6 mL
8.9 Sample rack

9. Procedure

9.1 Calibration standard
9.1.1 pipette 4800 µL matrix diluent into sample cup
9.1.2 pipette 200 µL calibration standard into the sample cup, then cover the sample cup with parafilm, vortex 10 sec

9.2 Sample preparation
9.2.1 place the sample blood or QC blood at RT in the hood or sterile incubator 30 min
9.2.2 Mixing sample by flipping the tube 8-10 times or using the rotator
9.2.3 Pipette 4800 µL matrix diluent into sample cup
9.2.4 Pipette 200 µL sample blood/ QC blood into the sample cup

9.2.5 Cover sample cup with parafilm, vortex 10 sec
9.2.6 Analyse by ICP-MS

9.3 ICP-MS RUN Sequence
1) RNB: Reagent Blank with ISTD
2) CAL0
3) CAL1
4) CAL2
5) CAL3
6) CAL4
7) CAL5
8) CAL6
9) CAL7
10) CAL0
11) IQC_L
12) IQC_M
13) IQC_H
14) BLK_I (matrix diluent)
15) BLK_II (matrix diluent)
16) Blood sample (case sample) 1-10
17) IQC at least 1 sample
18) Blood sample (case sample) 11-20
19) IQC sample at least 1 sample

9.4 Quality Control
9.4.1 Correlation of determination (r^2) of calibration curve ≥ 0.99 and concentration calculation ± 20% or ± 30% in the lowest concentration
9.4.2 Internal Quality Control (IQC):
- signal RNB < signal of CAL1,
- signal BLK (Matrix Diluent) < signal of CAL1,
- concentration of IQC_L, IQC_M, IQC_H pre- and post- blood sample location within mean value of control range or ± 20%
- Carryover evaluation: Reagent blank of Matrix Blank ≤ 10% of CAL1
- Duplicate analysis, CV ≤ 20%

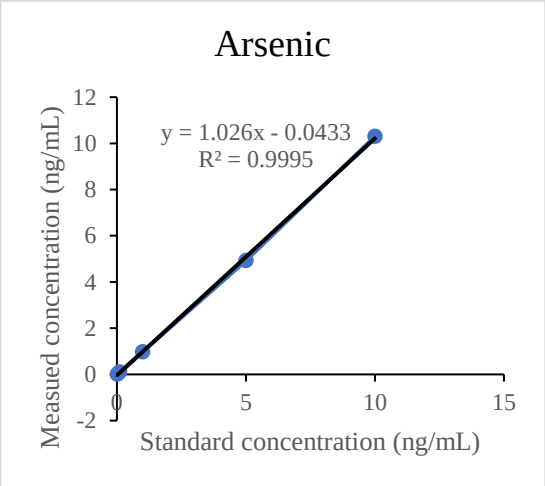
9.5 Reference Value as the table below

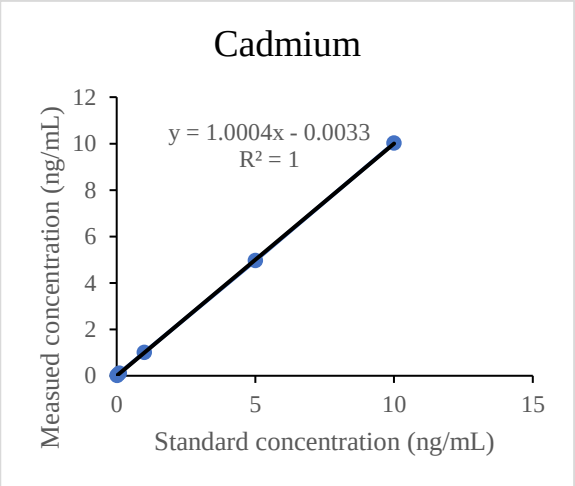
Element in blood	**Reference Values*:**
Total As	< 12 ng/mL
Cd	< 5 µg/L
Cr	< 30 µg/L (< 3 µg/dL)
Pb	< 30 µg/L → adult?
Mn	4.7 – 18.3 µg/L
Hg	< 9 µg/L
Se	146-247 µg/L
Tl	< 0-1 µg/L

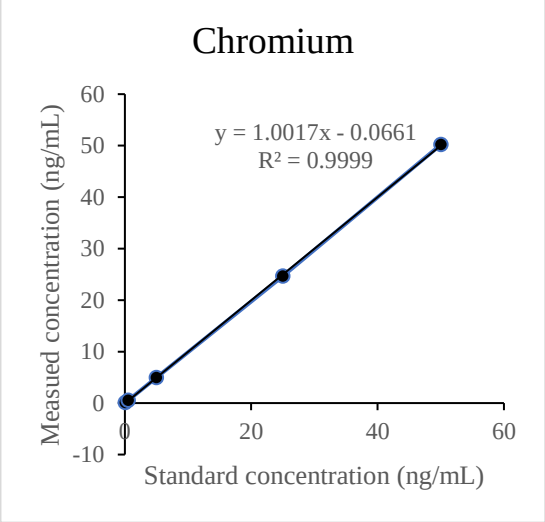
* If > reference value → report as critical value

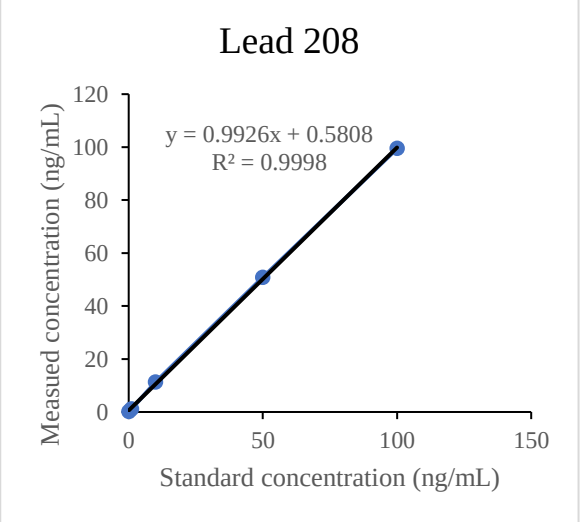
9.6 Reporting Range: 0.5 – 1,000 µg/L
9.7 Possible Variations and Preventions
9.7.1 ICP-MS efficiency → Autotune before analysis
9.7.2 Variation of Internal standard concentration → same ISTD between the preparation of calibration standard, control, and sample
9.7.3 Matrix diluent variation → spate pipette, enough preparation of matrix diluent for calibration standard, IQC, sample
9.7.4 High-quality and certified Argon gas (plasma gas) and collision gas (He, H_2)
9.7.5 Analytical grade, AR grade of reagents, Type I water (> 18.2 Mohm) or background signal CAL1
9.7.6 Tool and equipment: within valid date, clean with distilled water and diluted HNO_3, storage in a safe place to prevent environmental contamination

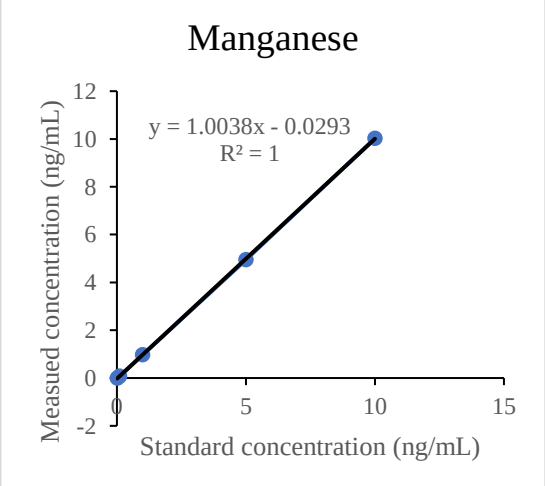
Appendix 3 Calibration curve of each metal measurement by ICP-MS at the PHE for protocol development and LOD/LOQ calculation

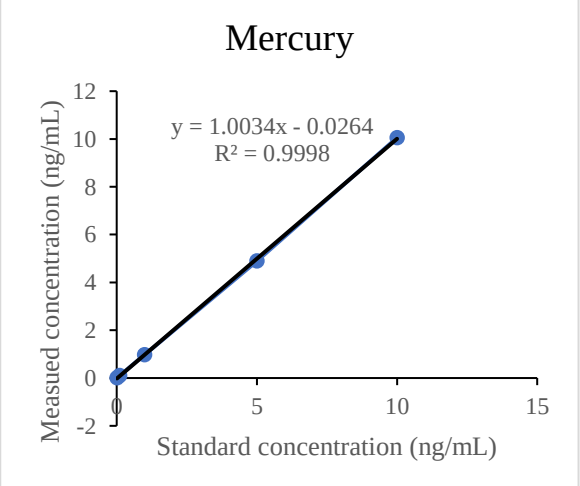






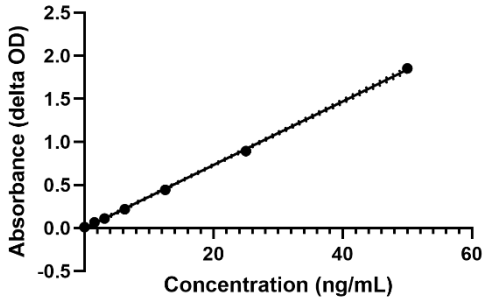




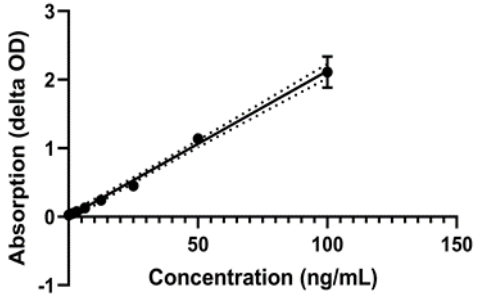


Appendix 4 Standard curve of ELISA for IgA in cord plasma/serum

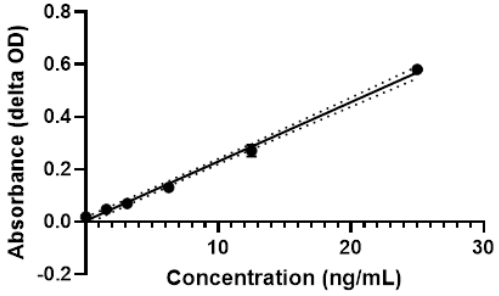
Standard curve of ELISA for IgA, protocol testing dated 28/02/20



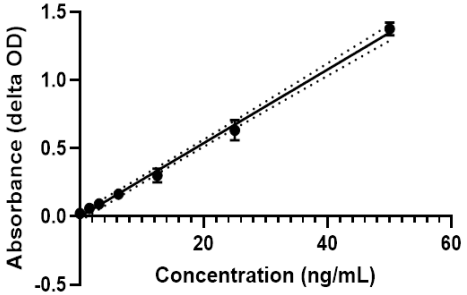
Standard curve of ELISA for IgA, protocol testing dated 03/12/20



Standard curve of ELISA for IgA, dated 05/12/20



Standard curve of ELISA for IgA, dated 07/12/20



Appendix 5 Optimisation of laboratory and GIS interpolation methods

This appendix details the systematic process of refining and enhancing the laboratory techniques used to measure various parameters in the study. Accurately measuring these parameters is essential to ensure the validity and reliability of the research outcomes. The chapter focuses on four specific laboratory methods: 3.1) measurements of heavy metals in whole blood using Inductively Coupled Plasma Mass Spectrometry (ICP-MS), 3.2) measurements of IgE and IgA in plasma/serum using Enzyme-Linked Immunosorbent Assay (ELISA), 3.3) measurements of creatinine in urine using an enzymatic colourimetric technique, and 3.4) Geographic Information System (GIS) analysis of $PM_{2.5}$ exposure at participants' residences.

The initial section introduces the significance of these laboratory measurements in the broader context of the research objectives. Accurately assessing heavy metals, immunoglobulins, creatinine, and $PM_{2.5}$ exposure is critical for understanding various aspects of the study, such as potential health associations, immune responses, and environmental exposures.

It proceeds with a comprehensive explanation of the methodology for optimising each laboratory method. A stepwise approach was employed, systematically exploring different experimental setups, sample preparation techniques, instrument parameters, assay selection, and interpolation methods for GIS analysis. Each parameter was tested and adjusted to ensure the highest possible accuracy and reliability in the laboratory methods while ensuring that the chosen techniques were practical and feasible for the entire project.

Subsequently, the results obtained during the optimisation process are presented, providing a clear overview of the changes made at each stage and their impact on the outcomes. The findings are supported by quantitative data, including root-mean-square error (RMSE) values, recovery rates, calibration curves, and sensitivity analyses.

The discussion section focuses on the rationale behind the final selection of each laboratory method. It extensively explores the various factors and considerations that led to adopting specific techniques over others. Factors such as sensitivity, precision, recovery rate, and suitability for the study's objectives are thoroughly explored and justified.

The conclusion summarises the achievements of the optimisation process, emphasising the importance of employing these refined laboratory methods to achieve reliable and robust results. It underscores the significance of the chapter in enhancing the accuracy and credibility of the subsequent analyses and interpretations presented throughout the thesis.

A5.1 Introduction

A5.1.1 Heavy metal measurement in whole blood: ICP-MS

The ICP-MS method was used to measure the elemental metals in whole blood since it can detect very low concentrations of elemental metals in bio-specimens and simultaneously measure multiple elements in a single analysis. [530] Measurement is based on the mass-charge ratio (m/z) after liquid samples are nebulised into a fine aerosol and passed into a high-temperature argon plasma chamber for atomisation and ionisation samples. The detection limit of ICP-MS is less than 0.001 to 10 µg/dL, the exact value being dependent on the element, the

type of biological matrix, the dilution factor employed during sample preparation, the design of the sample introduction system, instrument operating conditions (including plasma temperature) and background signals (e.g. reagent purity). One of the crucial steps in ICP-MS analysis is the specimen preparation method. The typical methods for sample preparation are 1) dilution with either acid, alkali, or deionised water, or 2) digestion methods with a strong acid(s) or alkali, either at room temperature or heated in a hot water bath, dry heating block or high-pressure microwave to assist digestion microwave. [530, 531] Therefore, ICP-MS was selected for the multi-elemental measurement in this study. However, the digestion methods required evaluation.

A5.1.2 Plasma immunoglobulin: ELISA

The enzyme-linked immunosorbent assay (ELISA) is a gold-standard immunological assay for many immunological measurements. It is commonly used to detect and quantify antibodies, antigens, proteins, glycoproteins and hormones in biological samples. It was selected to measure immunoglobulin concentration in plasma/serum of this study, including IgA, total IgE, and Th/allergy-related cytokines. While the general ELISA process is similar for different biomarkers, protocol validation and verification are required to ensure that results align with their specified performance parameters. Validation demonstrates that an ELISA test consistently produces accurate and reliable results within the specified parameters. Verification refers to confirming the performance characteristics of a commercially available test when it is implemented in a specific laboratory or research setting, e.g., samples from various time points during pregnancy and infancy.

A5.1.3 Urine nicotine, cotinine, and tran-3'-hydroxynicotine

Urine nicotine, cotinine, and tran-3'-hydroxynicotine are biomarkers commonly used to assess nicotine exposure, a major component of tobacco smoke. [532] Nicotine is a chemical compound in tobacco products such as cigarettes and cigars. It is the primary addictive substance in tobacco and is responsible for the psychological and physical dependence associated with smoking. Cotinine is a metabolite of nicotine that forms when the body processes nicotine (**Figure A5.1-1**). It is commonly used as a biomarker of nicotine exposure because it has a longer half-life than nicotine itself. Cotinine concentration in urine reflect recent exposure to nicotine, providing a more accurate measure of tobacco smoke exposure over time. 3-Hydroxynicotine is another metabolite of nicotine formed during the breakdown of nicotine in the body. Like cotinine, it can be measured in urine to assess nicotine exposure.

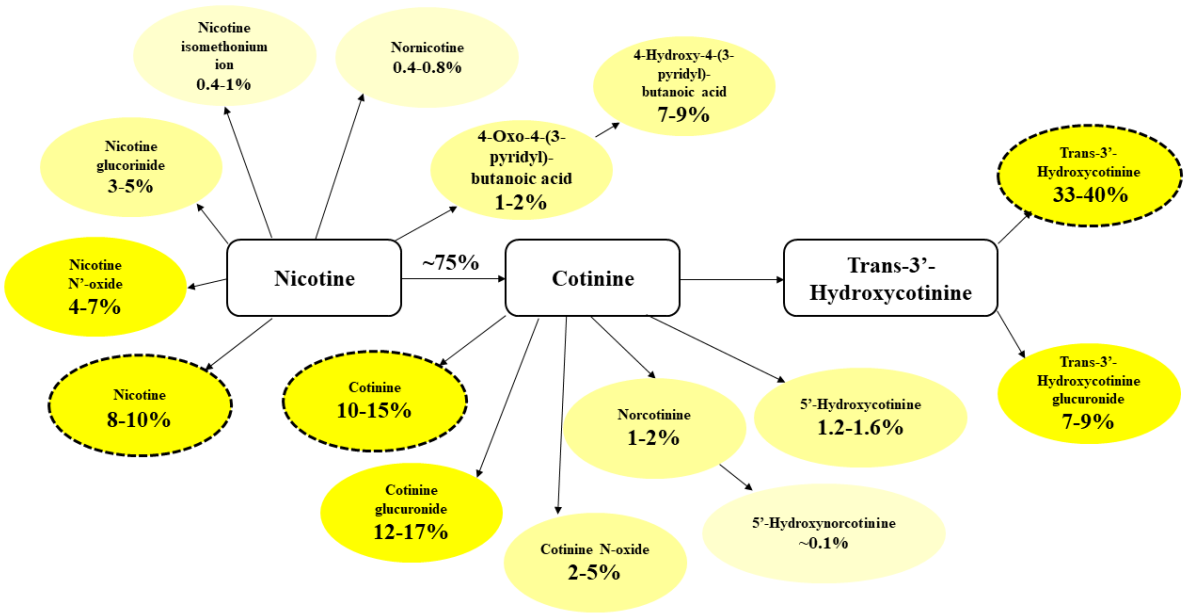


Figure A5.1-1: The quantitative scheme of nicotine metabolism involves the breakdown of nicotine into various metabolites excreted in urine. The percentage estimates the average excretion of nicotine metabolites as a percentage of total urinary nicotine. Adapted from Benowitz et al. [532]

These biomarkers are helpful in research and public health studies to determine exposure to tobacco smoke, including exposure to ETS or second-hand smoke and active smoking. They are measured in urine samples because they are excreted from the body through urine, providing a non-invasive and practical way to assess nicotine exposure.

A5.1.4 Urine creatinine

Physiological changes during pregnancy and infancy may alter urine concentration. [533-535] Therefore, monitoring of biomarkers in urine should be adjusted for urine concentration, particularly in the random urine collection method. [536] Urinary creatinine is acceptable and is widely used for this purpose. [518, 536-539] The measurement techniques of creatinine in urine can be divided by their methods of detection in terms of the substrates (enzyme, molecularly imprinted polymer (MIP), and antibody) or analysis approach (optical, electrochemical, Ion Selective Field-Effect Transistor (ISFET), capacities sensors, and chromatography). [540] The gold standard for creatinine assay in serum and urine is the isotope-dilution mass spectrometry (IDMS) method, which is relatively expensive and burdensome for routine use. [541, 542] The most common method for measuring creatinine concentration in serum and urine is based on Jaffe's reaction, which is spectrophotometric detection of the colour change of creatinine activity? in alkaline conditions. [543] This technique is simple and relatively low-cost. However, its major limitation is interference by ascorbic acid, bilirubin, proteins, ketones, cephalosporins and other drugs. [543, 544] Several methods have been developed for creatinine measurement in various biosamples: blood, urine, saliva, etc. Enzymatic methods can be divided into sarcosine oxidase (SOE), creatinine amide hydrolase, and imine hydrolase. Among these enzymatic methods, SOE has been widely used for research. [545]

This study selected the SOE enzyme reaction with colourimetric assays (Sigma-Aldrich®, Missouri, USA) due to its wide use, simple process, acceptable detection limit and reduced sensitivity to interference from other molecules. [543, 544] In addition, it was used in a similar population to normalise urine nicotine in pregnant women. [415, 546] While the assay was performed according to the company's instructions, sample filtration and dilution were tested to optimise the method.

A5.1.5 GIS analysis of $PM_{2.5}$ exposure at participants' residence

According to the relatively sparse air monitoring station in Thailand that may affect the performance of $PM_{2.5}$ estimation, two common spatial interpolation methods, Inverse Distance Weighting and ordinary kriging, were selected to compare to validate the interpolated $PM_{2.5}$ concentration before applied across the study period. The rationale for selecting the interpolation method is in **Chapter 1, Section 1.2.1**.

A5.2 Methodology

A5.2.1 Heavy metals measuring at the UKHSA

Microwave versus acid digestion methods were compared using reference blood (SeronormTM® Trace Elements Whole Blood L-3 LOT 1509408) and whole blood from a healthy volunteer spiked with increasing concentrations of different metals to optimise detection limits—the analytical value of targeted metals and their analytical uncertainty (95% Confidence Interval) of reference. The reference blood was employed for the recovery assessment to determine the efficacy of each digestion method. It was prepared in different dilutions to generate a calibration curve. This section's method was subdivided into sample

preparation (digestion) and ICP-MS analysis using an ASX-520 autosampler. The validation parameters were LOD, LOQ, linearity and range of calibration curve, accuracy and recovery, repeatability and precision, and inter-precision. [547] The ICP-MS had to pass the tuning procedure before analysis to optimise the instrument sensitivity and verify the mass solution. The reagent blank was used to determine the measurement uncertainty regarding reagents and the associated contribution of preparative, analytical steps. Blanks were measured after each calibration standard and before every ten analysing samples. The limit of detection (LOD) and limit of quantitation (LOQ) for method validation were calculated based on the linear regression of the calibration curve [548], which were expressed as the following equation

$$LOD = 3.3S_a/b,$$

$$LOQ = 10S_a/b,$$

where S_a is the standard deviation of the response and b is the slope of the calibration curve. The standard deviation of either y-residuals or y-intercepts of regression lines can estimate the standard deviation of the response.

A5.2.2 Sample preparation (digestion method)

Reference blood sample (Seronorm L-3®), multielement standard matrix, ten ppm internal standard stock, modified digestion Buffer D (for microwave digestion) and modified digestion buffer E (for acid digestion) were prepared as **Table A5.2-1**.

Table A5.2-1: Method of solution preparation for ICP-MS optimisation of metal measurement in whole blood

Solutions	Specific/detail	Preparation	Storage condition
Reference blood sample	Seronorm L-3®	**Deionised water (DI) 3 ML +** Seronorm L-3 powder, aliquot to 0.8 ml for each tube	-20 °C
Multielement standard matrix	50 ml of 2%HNO3 + 0.5%HCl	20 ml 5%HNO_3 + 2.5 ml 10%HCl + 27.5 ml DI **OR** 5 ml 20%HNO_3 + 2.5 ml 10%HCl + 42.5 ml DI	2-8 °C
10 ppm (10000 ppb) internal standards stock	10 ml 10 ppm of IrBiRh stock. Rh was selected for the internal standardisation of Cd, and Bi was selected for the internal standardisation of Hg and Pb.	0.1 ml 1000 ppm Ir stock + 0.1 ml 1000 ppm Bi + 0.1 ml 1000 ppm Rh + 9.7 ml of multielement standard matrix (2%HNO3 + 10%HCl)	2-8 °C
Modified digestion Buffer D	For microwave digestion	• Adding 1ml 30% H_2O_2 (Trace grade, final concentration 0.6%(v/v)) • 5ml 20% HNO_3 (Trace grade, final concentration 2%(v/v)) • 10µL Gold standard stock (1000ppm) (required when analysing samples for mercury) (final concentration 200 ppb) • 50µL Triton X-100 (trace grade, final concentration 0.1%(v/v)) • 50µL of each internal standard (10000ppb Ir stock) in trace HNO3 (final concentration 10 ppb) • Make up to 50 ml of deionised water (43.9 ml) in 50 ml tube. • Gentle mixing by the rotator for 30 minutes	2-8 °C
Modified digestion Buffer E	• For acid digestion • Prepared 150 ml for 22 samples	• Adding 7.35 ml 34% HCl (Trace grade, final concentration 5%(v/v)) • 14.9ml 67% HNO3 (Trace grade, final concentration 20%(v/v)) • 10µL Gold standard stock (1000ppm) (required when analysing samples for mercury) (final concentration 200 ppb) • 50µL Triton X-100 (trace grade, final concentration 0.1%(v/v)) • Makeup to 50 ml of deionised water (27.64 ml) in a 50 ml tube • Gentle mixing by the rotator for 30 minutes	2-8 °C
Calibration standards	Target elements 52Cr, 75As, 111Cd, 202Hg, 208Pb, 238U, 55Mn, (include 39K as a marker for the presence of blood and potentially for normalisation)	10 ml of mixed element calibration standards (Inorganic Ventures' ESSLAB-1375-REV1A) at 0, 0.01, 0.1, 1, 5, 10 ppb in 15ml falcon tubes, five dilutions as the following • 15 ml Dilution 1 (2%HNO3 + 0.5%HCl), final volume is 9 ml	Fresh prepared on the ICP-MS running day

Solutions	Specific/detail	Preparation	Storage condition
		= 0.75 ml mixed stock (Inorganic Ventures' ESSLAB-1375-REV1A in 10%HNO3) + 0.75 ml 10%HCl + 1.125 ml 20%HNO3 + 12.375 ml H2O • ***Add 15 µL 10 ppm Hg (10%HNO3) into 15 ml Dilution1, final conc. Hg is 10 ppb. • 10 ml Dilution2 (2%HNO3 + 0.5%HCl), the final volume is 10 ml = 5 ml Dilution 1 + 5 ml (2%HNO3 + 0.5%HCl) • 10 ml Dilution 4 (2%HNO3 + 0.5%HCl), final volume is 10 ml = 1 ml Dilution 1 + 9 ml (2%HNO3 + 0.5%HCl) • 10 ml Dilution 6 (2%HNO3 + 0.5%HCl) = 1 ml Dilution 4 + 9 ml (2%HNO3 + 0.5%HCl) • Prepare Zero + Ir = 0 ppb 10 ml 2%HNO3 + 0.5%HCl	

The sample was prepared for overnight acid and microwave digestion using UltraWAVE® digestion.

Acid digestion using digestion buffer E

Samples and digestion buffer E were added into labelled 15ml Falcon tubes as the methods A and B in **Table A 5.2-2**, then mixed using an automatic rotator at 40 rpm speed at room temperature for 18-24 hours. The acid-digested tube was centrifuged at 2000 rpm for 5 minutes. Approximately 1 mL of supernatant was pipetted into 9 mL DI in 15 mL Falcon tubes to dilute the acid-digested samples ten times before ICP MS analysis. Internal standard (50 µL) was added into each diluted acid-digested sample tube.

Table A5.2-2: Optimisation of digestion methods for whole blood ICP-MS

Methods	Labelled tube	Sample	Digestion buffer D (ml)	Digestion buffer E (ml)	Dilution	Digestion time	Note
A*	A1	0.1 (R)	-	4.9	X50	18-24 hrs	Acid digestion
	A1	0.1 (R)	-	4.9	X50		
	A2	0.1 (R)	-	4.9	X50		
	A3	0.1 (R)	-	4.9	X50		
	A4	0.1 (WB)	-	4.9	X50		
	A5	0.1 (WB)	-	4.9	X50		
	A6**	0.1 (WB)	-	4.9	X50		
B*	B1	0.25 (R)	-	4.75	X20	18-24 hrs	Acid digestion
	B2	0.25 (R)	-	4.75	X20		
	B3	0.25 (R)	-	4.75	X20		
C	C1	0.05 (R)	4.95	-	X100	1.5 hrs	Microwave
	C2	0.05 (R)	4.95	-	X100		
	C3	0.05 (R)	4.95	-	X100		
D	D1	H2O	4.9	-	-	1.5 hrs	Microwave
	D1	0.10 (R)	4.9	-	X50		
	D2	0.10 (R)	4.9	-	X50		
	D3	0.10 (R)	4.9	-	X50		
	D4	0.10 (WB)	4.9	-	X50		
	D5	0.10 (WB)	4.9	-	X50		
	D6	0.10 (WB)	4.9	-	X50		
	D2	0.25 (R)	-	4.75	X20		
	D3	0.25 (R)	-	4.75	X20		

***C and D must be diluted 10 times before ICP MS analysis to achieve a similar matrix.**
****According to the incomplete ingestion appearance of acid digestion method after dwelling time of 1.5 hours, 1 ml of H_2O_2 was added to A6 to test the enhancing effect of digestion.**
Abbreviations: R = reference blood (Seronorm™ Trace Elements Whole Blood), WB = whole blood, H_2O = deionised water

Microwave digestion using UltraWAVE®

Samples and digestion buffer D were added into labelled, pre-washed (10-20% HNO_3 soaked at least 24 h) microwave digestion tubes (with 22 positions rack) as the methods C and D in **Table A5.2-2**. A Base load (130mL H2O + 5mL 30% H2O2 + 2mL H2SO4) was added to the main Titan Microwave digestion (TFM) vessel and placed into the single reaction rack. The rack was then placed into the UltraWAVE® microwave digestion chamber within the instrument and run as **Table A5.2-3**.

Table A5.2-3: Blood method setting in the UltraWAVE® program

Step	Time	T1	T2	P	Power
1	00:10:00	200°C	60°C	160 bar	1500 W
2	00:15:00	200°C	60°C	160 bar	1500 W

(Pressure release rate to 4 bar/min when analysing for mercury and other 'volatile' elements to prevent loss by evaporation)

Following microwaved digestion, the machine was left to cool down completely. The reaction rack was removed from the chamber and gently rinsed with DI water before adding 250 µL 10% HCl to stabilise Hg in 5 mL digested samples and transferring the digested sample into a new 15 mL falcon tube. The sample was diluted to 10 mL before the ICP-MS analysis by adding 5 mL multielement standard matrix (2%HNO_3 + 0.5%HCl).

Calibration standards were prepared as **Table A5.2-1.** Spike-in internal standards (Ir, Bi, Rh) were added to each calibration standard dilution, zero + Ir, and digested to make a final concentration of 10 ppb internal standards. Positioning standard calibration, zero + Ir, blank (matrix) and digested samples in the rack of ASX-520 workstation.

A5.2.3 ICP-MS Analysis using ASX-520 autosampler

ICP-MS was set up by running the performance report and autotuning before analysis. Measurement parameters were set at three main runs, as shown in **Table A5.2-4.** Results were exported into an Excel file for further statistical analysis and data interpretation.

Table A5.2-4: Measurement parameters of iCAP Q ICP-MS

Isotope	Mode	Dwell time (s)	# of Channels	Spacing	Resolution
27Al	KED	0.01	1	0.1	Normal
39K	KED	0.01	1	0.1	Normal
51V	KED	0.01	1	0.1	Normal
52Cr	KED	0.01	1	0.1	Normal
55Mn	KED	0.1	1	0.1	Normal
59Co	KED	0.2	1	0.1	Normal
60Ni	KED	0.01	1	0.1	Normal
63Cu	KED	0.01	1	0.1	Normal
75As	KED	0.8	1	0.1	Normal
95Mo	KED	0.1	1	0.1	Normal
111Cd	KED	0.8	1	0.1	Normal
140Ce	KED	0.01	1	0.1	Normal
193Ir	KED	0.05	1	0.1	Normal
202Hg	KED	0.2	1	0.1	Normal
206Pb	KED	0.005	1	0.1	Normal
207Pb	KED	0.005	1	0.1	Normal
208Pb	KED	0.005	1	0.1	Normal
209Bi	KED	0.05	1	0.1	Normal
103Rh	KED	0.05	1	0.1	Normal
238U	KED	0.005	1	0.1	Normal

2.03s /sweep, one main run = 2.03 d /sweep x 15 sweeps = 30.45s
3 main runs x 30.42s = ca. 91.5 s
KED, kinetic energy discrimination

A5.2.4 Heavy metals measuring at the Faculty of Medicine Ramathibodi Hospital, Mahidol University

The ICP-MS method with dilution method at the Faculty of Medicine, Ramathibodi Hospital, Mahidol University, has been recently opened for clinical service in 2021. The target elements of measurement were As, Cd, Cr, Pb, Mn, and Hg. The differences between UKHSA and Ramathibodi Hospital laboratories regarding substances and instruments, mainly related to the internal standard solution, method validation (sensitivity, selectivity, accuracy, precision), calibration, reference blood, and carryover (machine setting parameters), are detailed in **Appendix 1**. And technical method is in **Appendix 2**.

These differences may affect the outcome interpretation. Therefore, some whole blood samples were re-analysed to compare heavy metal concentration between the UKHSA and Ramathibodi

Hospital laboratories for inter-precision assessment (degree of reproducibility). Paired whole blood samples of first-trimester pregnant women were randomly selected for re-analyses. The method of sample preparation and ICP-MS measurement used the dilution technique instead of microwave digestion. Approximately 200 μL of whole blood was diluted with 4,800 μL of matrix diluent (Ethylenediaminetetraacetic; H_2EDTA, isopropanolol, NH_4OH, triton-X100 and deionised water) and vortex mixed. The calibration standard was prepared by adding 200 μL of each calibration standard concentration (0, 0.5, 10, 50, 100, 250, 500, and 1,000 μg/L) with 4,800 μL of matrix diluent and vortex mixed. Diluted whole blood samples and calibration standards were directly injected into the ICP-MS by an integrated autosampler (I-AS)—the ICP-MS and I-AS settings, as shown in **Table A5.2-5** and **Table A5.2-6.** The clotted samples were excluded from the analysis due to the uncertainty of the result and equipment limitations. Samples that gave uncertain values (did not fit on the calibration curve) were re-analysed to confirm measurement. ICP-MS was daily autotuning before analysis. Reagent blank with internal standard (ISTD) and quality-controlled blood (Seronorm^{TM} Trace Elements Whole Blood) of low (3.0 μg/L), medium (12.0 μg/L), and high (48.0 μg/L) concentrations were measured before and after every ten samples for quality control. Internal standard (45Sc, 72Ge, 103Rh, 113In, 159Tb, and 209Bi) was automatically injected by the ICP-MS machine.

The concentration of six heavy metals (As, Cd, Cr, Pb (208), Hg, and Mn) were expressed as mean (SD), median (IQR), minimum, and maximum. Differences between measured metal concentration at UKHSA and Ramathibodi Hospital laboratories were compared using a Two-sided p, Paired Sample T-Test (parametric data) or Wilcoxon Signed Ranks Test (nonparametric data). The correlation of each metal concentration between the two laboratory settings was done by Pearson’s or Spearman's rho coefficients, depending on the data

distribution type. The significant statistical level (*P*) was 0.05. Statistical analysis was performed in SPSS version 28.0.

Table A5.2-5: The ICP-MS (Agilent 7900) setting of H2 and He mode at the Faculty of Medicine Ramathibodi Hospital, Mahidol University

Parameters	Values	Units
RF power	1550	W
RF matching	1.8	V
Sample depth	8.0	mm
Carrier gas	1.05	L/min
Nebuliser pump	0.10	rps
S/C Temp.	2	ⁱC
Gas switch	Makeup Gas	

Table A5.2-6. Sample acquisition of ICP-MS (Agilent 7900) at the Faculty of Medicine Ramathibodi Hospital Mahidol University

Parameters	Time (sec)	Speed (rps) nebulizer pump
Stabilise	20	Tune Parameter
Speed	--	Tune Parameter
Post Run		
Probe rinse (sample)	20	0.3
Probe rinse (Std)	20	0.3
Rinse 1	30	0.3
Rinse 2	30	0.3
Preemptive Rinse: start rinse before acquisition and complete 30 sec		

LOD and LOQ were calculated using 3SD blank and 5SD blank methods, respectively. The samples that measured a concentration < LOD were substituted by LOD/$\sqrt{2}$

A5.2.5 Plasma immunoglobulin: ELISA

Optimisation parameters

Per the manufacturer's instructions, several parameters were optimised for the ELISA assay. These included the antigen concentration, antibody dilutions, incubation times, blocking agents, washing steps, and detection system components. Each parameter was systematically

adjusted and fine-tuned for the highest assay sensitivity and specificity. The antigen concentration was optimised to ensure the optimal binding of the target molecule with the immobilised capture antibodies, leading to reliable and accurate measurements. Similarly, the antibody dilutions were optimised to achieve the appropriate signal-to-noise ratio, allowing for precise and consistent detection of the antigen-antibody complex. Incubation times were adjusted to balance maximising the binding interactions while preventing non-specific binding or background noise. Appropriate blocking agents were chosen to prevent non-specific binding and reduce background interference, enhancing the assay's specificity. The washing steps were optimised to ensure the thorough removal of unbound molecules and contaminants while retaining the specific antigen-antibody complexes immobilised on the solid surface. The detection system components were selected and adjusted to provide the best sensitivity for signal detection, allowing accurate quantification of the target analytes. Sample dilution was crucial to ensure that the sample concentration fell within the assay's linear range, avoiding signal saturation or dilution artefacts. Additionally, sample suitability was assessed to ensure that the samples met the assay's requirements and were compatible with the optimised parameters.

Total IgE

Stored cord serum from representative samples was thawed overnight at 2-8 °C and thoroughly mixed before analysis. The project involves analysing plasma samples from pregnant women and infant children at different times to study the variation in IgE concentration, considering that age might influence this concentration. Carefully chosen representative plasma samples from pregnant women and infants were selected for testing to assess the method's effectiveness in detecting IgE. Reagents, including coating buffer, capture antibody, blocking buffer, assay

buffer, human IgE standard, and diluted detection antibody, were prepared as per the manufacturer's protocol (IgE Human Uncoated ELISA Kit, Invitrogen®, Thermo Fisher Scientific, catalogue number 88-50610-88). The 96-well plate was coated with capture antibody in coating buffer (100 µL/well), sealed, and stored overnight at 2-8 °C. The coated plate was washed twice by soaking with 400 µL/well of washing solution for one minute, aspirated and blotted on clean napkin paper. Blocking buffer was added into the well (250 µL/well), sealed, and incubated for two hours at room temperature. Two-fold serial dilutions of the human IgE standard were performed with duplicate wells to create a standard curve of seven concentrations. In triplicate, the assay buffer solution was added into each blank well (100 µL) and sample well (90 µL). 10 µL/well of the sample was added into the appropriate wells, sealed, and then incubated at room temperature for 1 hour on a microplate shaker (300 rpm). The primary antibody (HRP-conjugated anti-human IgE monoclonal antibody) diluted 1: 250 in assay buffer A was added to all wells (100 µL/well) and then sealed and incubated at room temperature for 1 hour on a microplate shaker at 300 rpm. After incubation, all wells were washed with the wash buffer (eBioscience™ Wash Buffer (20X), catalogue number BMS408.0500) four times, 100 µL/well of substrate solution was added to each well and incubated at room temperature for 15-30 minutes. Subsequently, 100 µL/well of the stop solution was meticulously added to all wells, and the resulting absorbance or fluorescence measurements were obtained at wavelengths of 450 nm and 570 nm using a Biotek Synergy™ HT Microplate Reader. The calculation of delta OD involved subtracting the values obtained at 570 nm from those at 450 nm, and this data was then subjected to detailed analysis. Sample values were obtained using a pre-established standard curve, which underwent processing through GraphPad Prism version 9.3.1. This software was employed to interpolate the IgE concentration of unknown samples. Then, analyse the resulting data using GraphPad Prism software version 9.3.1. The final calculated values were then presented as the average,

represented as mean ± standard deviation (SD). The conversion from the unit of ng/ml to the standard unit of IU/ml was performed using the conversion factor of 1 IU/ml = 2.4 ng/ml.

Cord plasma/serum IgA

Stored cord plasma/serum of representative samples were thawed overnight at 2-8 °C and mixed well before analysis. Undilated samples and sample dilutions of 1:10, 1:100, 1:1,000, 1:5,000, 1:10,000, 1:20,000, and 1:100,000 in Assay Buffer A were prepared to optimise sample concentration for the IgA assay. Measurement was performed per the manufacturer's instructions (IgA Human Uncoated ELISA Kit, Invitrogen®, Thermo Fisher Scientific, catalogue number 88-50600-88). Briefly, duplicates of six dilutions of standards, blanks, and the five sample dilutions were added into 96 flat bottom-well plates (Corning™ Costar™ plates). The plate was read using a Biotek SynergyTM HT Microplate Reader at a wavelength of 450 nm and at 570 nm. Afterwards, delta OD was calculated by subtracting the values obtained at 570 nm from the measurements taken at 450 nm. The calibration curve was constructed based on the delta OD in GraphadPrism software version 9.3.1 and used to interpolate the IgA concentration of unknown samples. The final IgA concentration was calculated by multiplying it with the dilution factor, and the resulting values were expressed as the mean ± standard deviation (SD). Additionally, the unit of ng/mL was converted to mg/dL by multiplying with 0.0001.

A5.2.6 Urine cotinine, nicotine, and trans-3'-hydroxycotinine

Frozen urine samples (-80 ^{i}C) were thawed at room temperature and extracted for the cotinine, nicotine, and trans-3'-hydroxycotinine according to the company protocol (Biotage®, Uppsala, Sweden). The internal working standard solution was made up of nicotine-d4 (100 µg/ml in

MeOH, stored in -20°C freezer), cotinine-d3 (100 µg/ml), stored in -20°C freezer), and 3-hydroxycotinine -d3 (100 µg/ml in MeOH, stored in -20°C freezer). The solid-liquid extraction was done by making an extraction matrix comprising of 120 µL urine sample, 10 µL internal standard working solution (50 µl nicotine -d4, 50 µL cotinine-d3, 50 µL 3-hydroxycotinine-d3 plus 2350 µL deionised water), 230 µL 0.25% ammonia solution, and vortex mixing. Then, 100µL Methanolic 200mM HCl was added to a fresh 96-well deep-well collection plate (Biotage®), and the SLE extraction plate on top of this collection plate. Add 150 µL (50 µL sample) extraction matrix to a well on the SLE extraction plate. After loading all samples, tap the plate to ensure the liquid is evenly loaded. Put SLE-plate/collection plate assembly onto the SLE extraction system. Briefly, pulse N_2 flow to load extraction matrix onto SLE material and leave for 5 minutes. Eluted sample with 1ml dichloromethane: isopropanol (95:5 v/v) into deep-well plate containing 100 µL methanolic 200 mM HCl using SLE extraction system. They were evaporated to dryness in Genevac® centrifugal evaporation system (approximately 90 minutes). Re-suspend with deionised water (200 µL), cover, and mix for 10 minutes on a flat-bed shaker. Liquid chromatography and mass spectrometry (Q Exactive™ Plus Hybrid Quadrupole-Orbitrap™ Mass Spectrometer) were conducted to measure cotinine, nicotine, and 3-hydroxycotinine with the mobile phase solution, which was A. 5mM Ammonium Formate + 0.1% Formic acid (FA) and B. Methanol + 0.1% FA. The calibration standards were prepared as a serial dilution at 0, 10, 25, 50, 100, and 1,000 ng/ml mixed standard (nicotine, cotinine, and trans-3'-hydroxycotinine). Each standard solution was spiked with an internal standard. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the response and slope of the calibration curve as below.

$$LOD = 3.3\sigma / S$$

$$LOQ = 10\sigma / S$$

Where σ is the standard deviation of the response and S is the slope of the calibration curve.

A5.2.7 Urine creatinine

Preparation of urine sample

Frozen urine samples (-80°C) were thawed at room temperature and mixed well before analysis. Ten µL of 5 representative samples from each time point of collection in the pregnancy and infancy period (first trimester; P01, third trimester; P03, at birth; C01, and at 6-12 months; C05) with similar (uncorrected) concentrations were selected and assayed as shown in **Figure A5.2-2**. Briefly, samples were divided into two groups; 1) unfiltered (assayed directly) versus 2) filtered (deproteinised) with a 10kDa MWCO spin filter (Amicon® Ultra 0.5 mL, Merk, Darmstadt, Germany) at 14,000 rpm, room temperature for 10 minutes. The supernatant of the filtered sample was collected for measurement. Pooled samples from unfiltered and unfiltered tubes were diluted 1:10, 1:100, and 1:1000 with the assay buffer.

For the deproteinised process, approximately 500 µL of thawed urine samples were deproteinised at 14000 rpm for ten minutes, and the supernatant was collected for further assay. Deproteinised urine samples were diluted at 1: 100 for initial analysis. However, the samples whose values exceeded the standard curve were re-measured by 1:200 and 1:500 where appropriate.

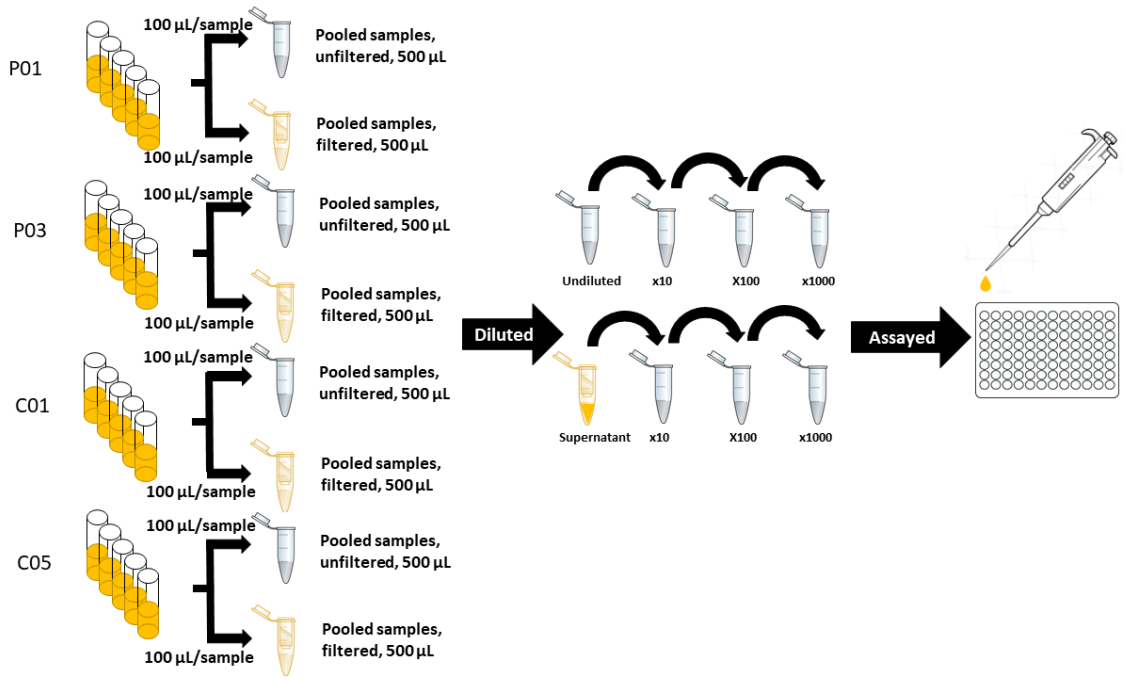


Figure A5.2-2: Sample preparation to optimise the measurement of creatinine in urine. P01, P03, C01, and C05 represented urine sample of first trimester pregnant women, third trimester pregnant women, newborn baby at birth (within 72 hours after birth), and infant children at 6-12 months of age, respectively.

Creatinine assay

The urine samples were assayed as per the manufacturer's instructions. Standards and samples were measured in duplicate. Standards were prepared at 0 (blank), 2, 4, 6, 8, and 10 nmol/well. The result was read with a microplate reader at a wavelength of 570 nm. Urine samples 50 µL/well were triplicate added. The reaction mixed solutions, 50 µL/well, were added into appropriate wells (standard and samples wells: 42 µL of Creatinine Assay Buffer, 2 µL of Creatinase, 2 µL of Creatininase, 2 µL of Creatinine Enzyme Mix and blank sample wells: 44 µL of Creatinine Assay Buffer, 2 µL of Creatinase, 2 µL of Creatinine Enzyme Mix). Plates were covered and incubated at room temperature with a horizontal plate shaker for 60 minutes. The absorbance at 570 nm was measured for colourimetric mode. The 0 (blank) creatinine standard was subtracted from all readings to correct the background. If the blank sample value was more than 0, it was subtracted from the sample reading to correct the measurement. The

corrected values were used to calculate the amount of creatinine by determining from the standard curve, using GraphPad Prism version 8.3.0 for Windows (GraphPad Software, San Diego, California USA, www.graphpad.com).

Calculation of creatinine concentrations

The creatinine amount was calculated per the manufacturer's instructions as the equation below and multiple with dilution factor (x100, x200, or x500).

$$Sa/Sv = C$$

Where Sa = Amount of Creatinine in the unknown sample (nmol) from the standard curve

Sv = Sample volume (μL) added into the wells

C = Concentration of Creatinine in sample

In short, background values (creatinine standard 0 blank wells) were subtracted from all readings. Values obtained from the appropriate Creatinine standards were plotted on a standard curve. Results and standard curve were calculated in GraphPad Prism version 8.3.0 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com. Final concentrations were obtained by multiplication with the dilution, and final values were expressed as mean ± SD. The creatinine concentration in urine samples was converted from nmol/μL to ng/mL by multiplying with Creatinine molecular weight (113.12 g/mole). The original result unit of ng/μL was subsequently converted to mg/ml by multiplying it by 0.001 to facilitate comparison with other studies.

A5.2.8 GIS analysis of $PM_{2.5}$ exposure at participants' residence

$PM_{2.5}$ information was selected for interpolation in January 2018, 2019, and 2020. Before applying IDW and OK to the entire dataset, a validation process was conducted to compare the performance of both interpolation methods. The validation procedure was carried out using the QGIS.

Through this validation, the accuracy and reliability of IDW and OK were assessed by comparing their interpolation results against known reference points or ground monitoring station data. The goal was to determine which method provided the most accurate representation of the underlying spatial distribution of the variable of interest, in this case, $PM_{2.5}$ exposure.

By employing QGIS, the validation process allowed for spatial analysis and comparison of the interpolation outputs. This analysis facilitated the identification of any differences or discrepancies between the predicted values from IDW and OK and the actual observed values at the ground monitoring stations.

A5.3 Result

A5.3.1 Digestion methods for heavy metals measurement at UKHSA

According to the visual observation after sample digestion, the precipitated protein was observed only in acid digestion tubes (**Table A5.3-1**). Almost all samples (reference blood and human whole blood) were detected at the level within the calibration curve except for mercury measurement, especially with the acid digestion method at x50 dilution (A1-A6)

(**Table A5.3-2**). The x50 and x100 dilution reference blood samples were detected in the calibration curve of all six metals with microwave digestion but only in the x20 dilution of acid digestion.

Table A5.3-1: Visual observation after sample digestion

Tubes	Supernatant	Precipitated protein
A1-3	Clear, straw colour	Yes, dark brown colour
A4-5	Clear, straw colour	Yes, dark brown colour
A6	Clear, straw colour, a large number of gases and bubbles	Yes, whitish colour
B1-3	Clear, straw colour	Yes, dark brown colour
C1-3	Clear, straw colour	No
D1-3	Clear, straw colour	No
D4-6	Clear, straw colour	No

Table A5.3-2: The result of the method development of heavy metal concentration in whole blood by ICP-MS at the PHE (before multiple by dilution factor).

Digestion methods	Sample	Label	As (ppb)	Cd (ppb)	Cr (ppb)	Hg (ppb)	Mn (ppb)	Pb206 (ppb)	Pb207 (ppb)	Pb208 (ppb)
Acid digestion A	Reference blood	A1	0.061	0.020	0.028	0.050	0.093	1.291	0.785	1.143
		A2	0.055	0.017	0.014	0.062	0.082	1.161	0.679	0.998
		A3	0.058	0.019	0.026	0.059	0.096	1.222	0.738	1.083
	Average		0.058	0.058	0.019	0.057	0.090	1.225	0.734	1.074
	%RSD		0.044	0.044	0.060	0.088	0.065	0.043	0.059	0.056
	SD		0.003	0.003	0.001	0.006	0.005	0.006	0.053	0.060
	Whole blood	A4	0.008	-0.002	-0.048	-0.111	0.027	-0.004	0.004	0.000
		A5	0.007	-0.002	-0.042	-0.112	0.040	-0.004	0.004	0.000
		A6	0.002	-0.002	-0.051	-0.091	0.018	0.000	-0.002	-0.001
	Average		0.006	0.006	0.006	-0.002	-0.047	-0.105	0.029	-0.003
	%RSD		0.456	0.456	0.456	-0.020	-0.078	-0.093	0.319	-0.707
	SD		0.003	0.003	0.003	0.000	0.004	0.010	0.009	0.002
Acid digestion B	Reference blood	B1	0.126	0.046	0.105	0.003	0.185	2.816	1.639	2.425
		B2	0.155	0.056	0.136	0.024	0.203	2.816	1.639	2.425
		B3	0.127	0.047	0.096	0.000	0.196	2.780	1.668	2.461
	Average		B6	0.136	0.050	0.112	0.009	0.195	2.804	1.648
	%RSD		0.099	0.099	0.092	0.151	1.216	0.037	0.006	0.008
	SD		0.014	0.014	0.005	0.017	0.011	0.007	0.017	0.014
Microwave C	Reference blood	C1	0.264	0.098	0.544	0.294	0.548	5.742	3.499	5.062
		C2	0.246	0.090	0.656	0.193	0.327	5.317	3.141	4.610
		C3	0.266	0.104	1.428	0.209	-0.030	6.166	3.659	5.330
	Average		0.259	0.259	0.097	0.876	0.232	0.282	5.742	3.433
	%RSD		0.035	0.035	0.058	0.449	0.192	0.845	0.060	0.063
	SD		0.009	0.009	0.006	0.393	0.045	0.238	0.347	0.216
Microwave D	Reference blood	D1	0.502	0.183	1.085	0.417	0.271	10.567	6.379	9.327
		D2	0.496	0.174	0.645	0.377	0.670	10.089	6.156	8.890
		D3	0.506	0.181	1.074	0.381	0.772	11.218	6.721	9.847
	Average		0.501	0.501	0.179	0.935	0.391	0.571	10.625	6.419
	%RSD		0.008	0.008	0.021	0.219	0.046	0.378	0.044	0.036
	SD		0.004	0.004	0.004	0.205	0.018	0.216	0.463	0.232
	Whole blood	D4	0.023	0.001	0.540	-0.068	0.571	0.479	0.331	0.468
		D5	0.023	0.001	0.404	-0.077	0.331	1.089	0.705	1.019
		D6	0.022	0.003	0.461	-0.081	0.329	1.080	0.727	1.027

Digestion methods	Sample Label	As (ppb)	Cd (ppb)	Cr (ppb)	Hg (ppb)	Mn (ppb)	Pb206 (ppb)	Pb207 (ppb)	Pb208 (ppb)
	Average	0.0227	0.023	0.002	-0.076	0.410	0.883	0.588	0.838
	%RSD	0.0194	0.019	0.374	-0.071	0.277	0.323	0.309	0.312
	SD	0.0004	0.000	0.001	0.056	0.005	0.114	0.285	0.262
The analytical value of reference blood (Seronorm™)		**27.3**	**9.9**	**7.1**	**22.4**	**33.3**	**na**	**na**	**362**

Abbreviations: RSD; relative standard deviation (means/SD), SD; standard deviation

According to the protocol, the sample in the final tube in the Autosampler was 50 times diluted (originally from 200 **µ**L of a whole blood sample). Therefore, the final concentration of each element in the original whole blood sample is multiplied by 50.

Comparing the recovery percentage (% recovery) of reference blood between the two digestion methods, microwave digestion methods provided higher concentration of % recovery than acid digestion methods (85.71% - 146.75% vs 3.15% – 16.91% for x50 dilution of microwave digestion vs acid digestion) (**Table A5.3-3**). The standard curves of each metal are in **Appendix 3**.

Table A5.3-3: The recovery analyses and calculations were based on reference blood digestion at UKHSA.

Methods	As (µg/L)	Cd (µg/L)	Cr (µg/L)	Hg (µg/L)	Mn (µg/L)	Pb (µg/L)
Reference blood (Seronorm™)	**27.3**	**9.9**	**35.5**	**22.4**	**33.3**	**362**
Microwave A, mean (SD)	2.89	0.94	1.12	< LOD	4.52	
Microwave B, mean (SD)	2.72	0.99		0.174	3.89	
Microwave C, mean (SD)	25.86	9.72	87.58	23.22	28.18	574.2
Microwave D, mean (SD)	25.06	8.96	46.73	19.57	28.54	531.24
% Recovery						
Acid digestion A	10.59	9.49	3.15	< LOD	13.56	16.91
Acid digestion B	9.96	10.00	6.34	0.78	11.69	15.49
Microwave C	94.73	98.18	246.70	103.66	84.62	158.62
Microwave D	91.78	90.45	131.63	87.37	85.71	146.75

A5.3.2 Heavy metals measuring at the Faculty of Medicine Ramathibodi Hospital, Mahidol University

Sixty whole blood samples were available for re-analysis. All the mean metal concentrations were significantly different between the two laboratories, except Cd (**Table A5.3-4**). The metal concentrations, including arsenic, chromium, manganese, mercury, and lead, measured at UKHSA were higher than at Ramathibodi Hospital. However, there was a strong correlation between the As, Mn, Hg, and Pb concentration measured at the two laboratories ($r > 0.5$ and $P < 0.05$) (**Table A5.3-5**).

Table A5.3-4: The comparison of metal concentrations in whole blood, measured by ICP-MS between PHE and Ramathibodi Hospital (N = 60)

Metals	PHE	Ramathibodi	*P*	
Arsenic				
Median (IQR)	2.35 (1.49)	1.62 (1.09)	<0.001	*
Min	1.01	0.11		
Max	7.75	5.9		
Cadmium				
Median (IQR)	0.45 (0.34)	0.42 (0)	0.584	*
Min	0.26	0.42		
Max	1.05	0.98		
Chromium				
Median (IQR)	5.40 (4.15)	0.95 (1.32)	<0.001	*
Min	2.04	0.01		
Max	33.75	4.23		
Manganese				
Median (IQR)	15.43 (7.53)	10.68 (5.25)	<0.001	*
Min	4.65	0.03		
Max	38.15	29.12		
Mercury				
Median (IQR)	2.45 (1.90)	1.4 (1.59)	<0.001	*
Min	0.58	0.03		
Max	11.3	11.22		
Lead				
Median (IQR)	21.03 (8.46)	10.09 (5.71)	<0.001	**
Min	6.84	0.4		
Max	41.3	27.22		

***Wilcoxon Signed Ranks Test**
****Paired Samples T-Test**

Table A5.3-5: The correlation of heavy metal measurement between UKHSA and Ramathibodi Hospital by ICP-MS (N = 60)

Metals	UKHSA		Ramathibodi		Correlation	*P*		95% Confidence Intervals (2-tailed)	
	Mean (µg/L)	SD	Mean (µg/L)	SD				Lower	Upper
Arsenic	2.78	1.43	1.93	1.17	0.740	<0.001	**	0.593	0.839
Cadmium	0.49	0.23	0.45	0.10	0.363	0.004	**	0.113	0.570
Chromium	6.65	4.90	1.12	0.99	0.307	0.017	*	0.050	0.526
Manganese	16.95	6.82	11.20	5.03	0.645	<0.001	**	0.462	0.776
Mercury	3.07	2.06	1.86	1.78	0.769	<0.001	**	0.636	0.858
Lead	21.58	7.43	10.71	4.94	0.538	<0.001	***	0.325	0.694

*** Spearman's rho, significant at the 0.01 level (2-tailed)**
**** Spearman's rho, significant at the 0.01 level (2-tailed)**
***** Pearson Correlation, significant at the 0.01 level (2-tailed)**

A5.3.3 Plasma immunoglobulin

Plasma total IgE

Initially, the incubation time of the last step was 15 minutes, and the signals seemed very weak (**Table A5.3-6**).

Table A5.3-6: Protocol testing of IgE result for the first plate (incubation 15 minutes of the last step)

Sample	450 nM			570 nM			Delta OD		
	1	2	3	1	2	3	1	2	3
STD	0.297	0.171	--	0.033	0.032	--	0.264	0.139	--
STD 2x	0.265	0.17	--	0.032	0.033	--	0.233	0.137	--
STD 4x	0.253	0.173	--	0.033	0.032	--	0.22	0.141	--
STD 8x	0.155	0.117	--	0.028	0.029	--	0.127	0.088	--
STD 16x	0.103	0.085	--	0.033	0.026	--	0.07	0.059	--
STD 32x	0.108	0.083	--	0.058	0.031	--	0.05	0.052	--
STD 64x	0.069	0.053	--	0.038	0.027	--	0.031	0.026	--
BLANK	0.039	0.045	--	0.023	0.028	--	0.016	0.017	--
Positive control	0.116	0.13	0.141	0.032	0.028	0.028	0.084	0.102	0.113
Negative control	0.044	0.045	0.045	0.032	0.032	0.032	0.012	0.013	0.013
P1	0.046	0.043	0.048	0.030	0.029	0.029	0.016	0.014	0.019
P2	0.155	0.106	0.15	0.028	0.029	0.029	0.127	0.077	0.121
P3	0.08	0.059	0.064	0.026	0.025	0.025	0.054	0.034	0.039
P4	0.044	0.043	0.044	0.027	0.027	0.027	0.017	0.016	0.017
P5	0.043	0.043	0.042	0.023	0.023	0.023	0.02	0.02	0.019
P6	0.039	0.045	0.039	0.02	0.021	0.021	0.019	0.024	0.018

The protocol was reviewed, and the ELISA kit manufacturer's representative advised addressing the low signal issue by increasing the incubation time of the substrate solution from 15 minutes to 30 minutes. Following this recommendation, the signal detection improved (**Table A5.3-7**). This improvement aligns with the manufacturer's sensitivity information of 7.8 ng/ml.

Table A5.3-7: Protocol testing of IgE result for the second plate (incubation 30 minutes of the last step)

Sample	450 nM			570 nM			Delta OD		
	1	2	3	1	2	3	1	2	3
STD	2.238	2.204	--	0.055	0.061	--	2.183	2.143	--
STD 2x	1.69	1.651	--	0.057	0.049	--	1.633	1.602	--
STD 4x	0.916	0.964	--	0.051	0.042	--	0.865	0.922	--
STD 8x	0.497	0.496	--	0.03	0.037	--	0.467	0.459	--
STD 16x	0.27	0.287	--	0.029	0.039	--	0.241	0.248	--

STD 32x	0.168	0.167	--	0.031	0.032	--	0.137	0.135	--
STD 64x	0.107	0.114	--	0.024	0.03	--	0.083	0.084	--
BLANK	0.058	0.081	--	0.024	0.049	--	0.034	0.032	--
Positive control	0.493	0.511	0.407	0.028	0.029	0.025	0.465	0.482	0.382
Negative control	0.037	0.040	0.030	0.020	0.023	0.014	0.017	0.017	0.016
P1	0.203	0.203	0.201	0.053	0.033	0.033	0.15	0.17	0.168
P2	1.558	1.401	1.54	0.099	0.038	0.036	1.459	1.363	1.504
P3	0.706	0.7	0.733	0.029	0.033	0.029	0.677	0.667	0.704
P4	0.07	0.092	0.065	0.048	0.072	0.04	0.022	0.02	0.025
P5	0.094	0.103	0.11	0.028	0.031	0.033	0.066	0.072	0.077
P6	0.097	0.098	0.104	0.028	0.026	0.034	0.069	0.072	0.07

After the protocol was validated for the incubation period, plasma total IgE concentration of pregnant women and the control samples (adult healthy and allergic individuals) were successfully measured using undiluted samples—(**Table A5.3-7** and **Table A5.3-8**). Standard curves consistently demonstrated a linear range between 0 and 300 ng/ml (**Figure A5.3-1**). However, when the same protocol was used to test cord plasma/serum from infants at 6-12 months, the signal was low and undetectable, even with undiluted samples (data not shown).

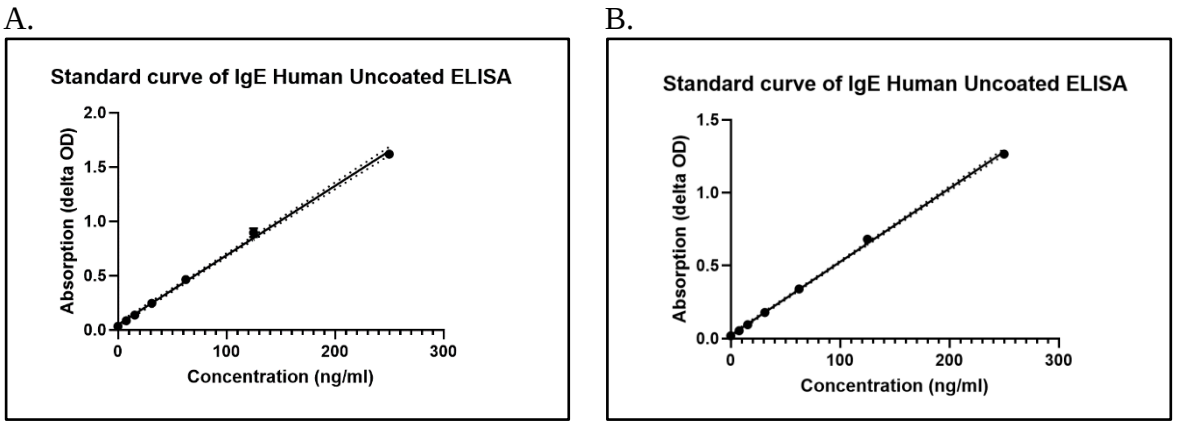


Figure A5.3-1: The standard curves of plasma total IgE during the protocol optimisation. A. was done on 02/12/2019, B. was done on 11/02/2020

Table A5.3-8: Plasma total IgE concentration of pregnant women and the control samples (adult healthy and allergic individuals for negative and positive control, respectively)

Sample	Plasma IgE (ng/ml)	Plasma IgE (IU/ml)
P26	182.91	76.21
P27	< LOD	< LOD
P29	40.83	17.01
P30	2180.35	908.48
P33	994.79	414.50
P34	38.75	16.14
P35	< LOD	< LOD

Sample	Plasma IgE (ng/ml)	Plasma IgE (IU/ml)
P36	483.72	201.55
P37	< LOD	< LOD
P39	217.26	90.52
P40	55.40	23.08
P41	82.46	34.36
P42	< LOD	< LOD
P43	300.01	125.00
P44	162.61	67.75
P45	76.74	31.97
P46	593.54	247.31
P47	198.52	82.72
P48	< LOD	< LOD
P49	591.45	246.44
P50	< LOD	< LOD
P51	5.44	2.27
P52	< LOD	< LOD
P53	96.00	40.00
Positive control	620.60	258.58
Negative control	< LOD	< LOD
LOD = 7.8 ng/ml or 3.25 IU/ml)		

Cord plasma/serum IgA

The optical density (OD) signal of diluted plasma samples, 1:10, 1:100 and 1:1,000 diluted cord plasmas/serums exceeded the assay upper limit of quantitation (ULOQ) (data not shown). The result of further dilution of 1:5,000 to 1:100,000 is shown in **Table A5.3-9**. The OD of 1:100,000 diluted cord plasma/serums of all six samples were lower than the assay lower limit of quantification (LLOQ). One (sample C6) of eight diluted plasma/serums (1:10,000 and 1:20,000) were within the assay linear quantification range (standard curve). An example of the standard curve is shown in **Figure A5.3-2**. The assay range was 1.6-100 ng/mL (0.00016 - 0.01 mg/dL), and the analytical sensitivity was 1.6 ng/mL.

Table A5.3-9: Cord plasma/serum IgA concentration of infant children.

Sample	Dilution	IgA conc. interpolation	Final IgA conc (IgA conc. Interpolation x dilutions)	
			(ng/mL)	(mg/dL)
C1	5000 x	1.322	33040.887	3.304
C2		1.157	28924.664	2.892
C3		1.133	28336.632	2.834
C4		1.133	28336.632	2.834
C5		1.181	29512.695	2.951
C6		> ULOQ	> ULOQ	> ULOQ
C7		1.157	28924.664	2.892
C8		1.228	30688.759	3.069
C1	10000 x	1.204	60201.455	6.020
C2		1.133	56673.263	5.667
C3		1.204	60201.455	6.020
C4		1.110	55497.200	5.550
C5		1.133	56673.263	5.667
C6		59.654	2982719.763	298.272
C7		1.133	56673.263	5.667
C8		1.157	57849.327	5.785
C1	20000 x	1.251	125107.164	12.511
C2		1.157	115698.654	11.570
C3		1.322	132163.546	13.216
C4		1.204	120402.909	12.040
C5		1.181	118050.782	11.805
C6		4.944	494391.167	494.391
C7		1.157	115698.654	11.570
C8		1.181	118050.782	11.805
C1	100000 x	1.157	578493.272	57.849
C2		1.157	578493.272	57.849
C3		1.204	602014.546	60.201
C4		1.204	602014.546	60.201
C5		1.181	590253.909	59.025
C6		1.181	590253.909	59.025
C7		1.157	578493.272	57.849
C8		1.181	590253.909	59.025

Abbreviation: conc, concentration

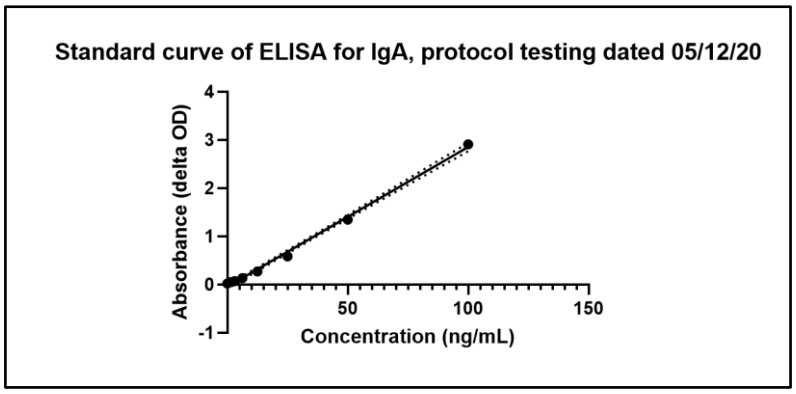


Figure A5.3-2: The standard curves of plasma total IgA during the protocol optimisation on 5/12/2020

A5.3.4 Urine creatinine

An example of a standard curve is shown in **Figure A5.3-3**. All twenty-eight blank samples detected OD values less than the background. The detailed sample results are shown in **Table A5.3-10**. The undiluted and 10x diluted samples of pregnant women (first and third trimester) exceeded the highest standard value. In children's urine samples, undiluted samples at birth and 6-12 months and 1000x at birth were out of the standard range. The 100x dilution enabled the measurement of all samples.

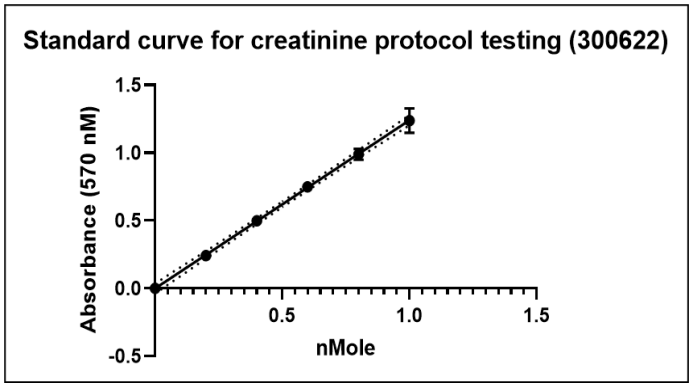


Figure A5.3-3: The standard curves of urine creatine during the protocol optimisation on 30/06/2022

Table A5.3-10: Result of creatinine measurement in urine for protocol optimisation

Visit	Dilution	Filtered samples (ng/µL)	Unfiltered samples (ng/µL)	Suitable dilution
P01	Undiluted	na	Exceeding highest standard	No
P01	10x	Exceeding highest standard	Exceeding highest standard	No
P01	100x	126.21	103.68	Yes
P01	1000x	74.78	86.63	Yes
P03	Undiluted	na	Exceeding highest standard	No
P03	10x	Exceeding highest standard	Exceeding highest standard	No
P03	100x	79.52	72.59	Yes
P03	1000x	54.71	26.44	No
C01	Undiluted	na	Exceeding highest standard	No
C01	10x	11.17	10.93	Yes
C01	100x	9.39	7.57	Yes
C01	1000x	Under lowest standard	Under lowest standard	No
C05	Undiluted	na	Exceeding highest standard	No
C05	10x	21.79	21.11	Yes
C05	100x	25.62	22.61	Yes
C05	1000x	6.38	3.65	No

Abbreviations:
P01, first-trimester pregnancy; P03, third-trimester pregnancy; C01, newborn infant within 72 hours after birth; C05, infant children 6-12 months.

When comparing filtered versus unfiltered samples, the creatinine concentration was higher in almost all filtered samples (**Table A5.3-11**), indicating the potential benefits of sample filtration for accurate measurements. However, there was one exception to this trend. The x1000 dilution of the first-trimester sample showed the opposite result of increased creatinine concentration in the unfiltered vs filtered sample (2-tailed $P = 0.039$, Sign Test).

Table A5.3-11: The sign test result of creatinine measurement comparison (filtered vs unfiltered urine samples)

Frequencies		
		N
Unfilter - Filter	Negative Differences[a]	8
	Positive Differences[b]	1
	Ties[c]	0
	Total	9

a. Unfilter < Filter
b. Unfilter > Filter
c. Unfilter = Filter

A5.3.5 GIS analysis of $PM_{2.5}$ exposure at participants' residence

The number of available air monitoring stations for $PM_{2.5}$ concentration gradually increased from a minimum of 24 stations (February 2018) to 139 stations (November 2020), as shown in **Figure A5.3-4**. There were 25, 75, and 101 available stations in January 2018, 2019, and 2020, respectively.

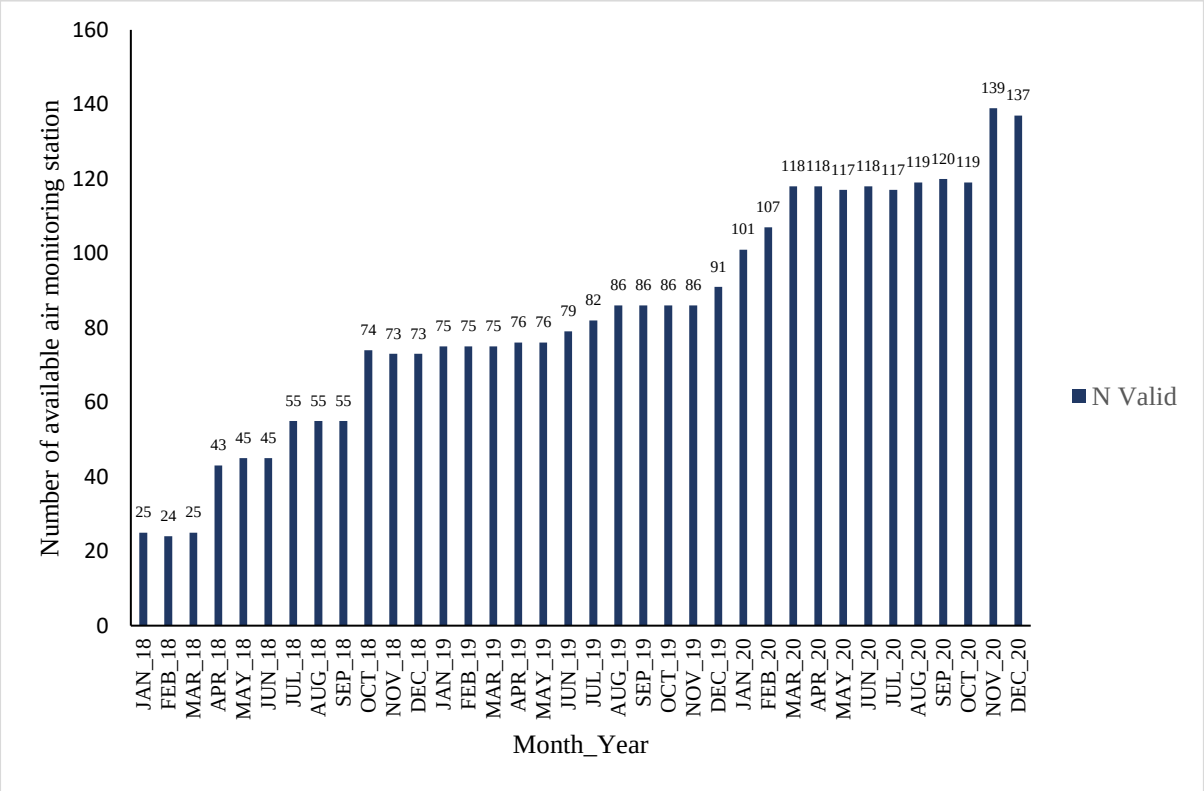


Figure A5.3-4: Number of available air monitoring stations for $PM_{2.5}$ estimation across the study period (Jan 2018 - Dec 2020)

The air monitoring stations for $PM_{2.5}$ interpolation in January 2018, 2019, and 2020 were 25, 75, and 101, respectively. Comparing the $PM_{2.5}$ estimation results, IDW provided minimum, maximum, and mean $PM_{2.5}$ interpolation closer to the observed values at the ground monitoring station than OK for all three months (**Table A5.3-12**).

Table A5.3-12: Cross-validation results of two interpolation methods for $PM_{2.5}$ estimation (inverse distance weighting (IDW) vs Ordinary Kriging (OK))

	Observed values (Ground monitoring station)	inverse distance weighting (IDW)	Ordinary Kriging (OK)
Min (mg/m^3)			
Jan 2018	11.6	11.6048	13.6456
Jan 2019	12.9354	12.9388	10.6967
Jan 2020	12.2903	12.2962	13.8230
Max (mg/m^3)			
Jan 2018	54.7419	54.6635	44.7795
Jan 2019	72.5357	72.3036	63.4893
Jan 2020	176	123.5642	65.4784
Mean (mg/m^3)			
Jan 2018	32.9351	31.4421	27.3296
Jan 2019	40.2388	35.5508	31.3156
Jan 2020	41.0068	37.8415	35.0730
Root-mean-square error (RMSE)	--	17.5872	37.3480

A5.4 Discussion and conclusion

A5.4.1 Digestion methods for heavy metals measurement at UKHSA

The microwave digestion method at x50 dilution demonstrated greater efficacy by providing more yield of heavy metal detection. It also added the benefit of relatively simple preparation requiring reduced time consumption. The intricate acid digestion method may potentially increase the risk of metal contamination originating from the laboratory environment. Therefore, the microwave digestion method was initially selected for blood and urinary elemental metal analysis in this study.

Due to the COVID-19 pandemic, the measurement of heavy metals in whole blood was severely interrupted. Eventually, it was moved from UKHSA to the Faculty of Science,

Mahidol University and Faculty of Medicine Ramathibodi Hospital, Mahidol University. The availability of microwave digestion and ICP-MS equipment, internal standard solution, laboratory accessibility, and solutions were significantly different (**Appendix 1**). Therefore, samples prepared in this study were re-optimised.

A5.4.2 Heavy metals measuring at the Faculty of Medicine Ramathibodi Hospital, Mahidol University

Although the mean concentration of all metals, except Cd, exhibited statistical differences between the two laboratories, a significant correlation was still observed for most targeted metals, namely As, Mn, Hg, and Pb.

Considering this correlation and the need to ensure consistent and reliable analysis, the decision was made to proceed with the analysis of the remaining samples at the Faculty of Medicine, Ramathibodi Hospital, Mahidol University.

Utilising the facilities at Ramathibodi Hospital allowed for a more comprehensive and robust evaluation of the targeted metals in the samples while ensuring that any potential biases arising from differences between the two laboratories would be minimised. This approach provided a solid foundation for the continued investigation, ensuring the accuracy and integrity of the data obtained for the rest of the samples.

Ultimately, the collaboration between the two institutions and the utilisation of the analytical resources available at Ramathibodi Hospital significantly contributed to the successful

completion of this study, providing reliable and meaningful insights into the concentrations of targeted metals in the samples under investigation.

In conclusion, the measurement of heavy metals for the entire project was conducted using the ICP-MS available at Ramathibodi Hospital, which is routinely utilised for clinical purposes.

A5.4.3 Plasma immunoglobulin

Plasma total IgE

In the initial ELISA experiments, plasma total IgE concentration were detected within the standard range, indicating the assay was functioning correctly. Moreover, increasing the incubation time of the substrate solution from 15 minutes to 30 minutes can improve signal detection.

Despite the improved signal detection in the adult and pregnant samples, the cord plasma/serum from infants and children at 6-12 months remained undetectable, even with undiluted samples. The potential reasons for the lack of signal in the cord and infant samples might be age-related differences in total IgE concentration that could affect the assay's sensitivity. The study of Hansen et al. demonstrated that cord serum IgE can be detected with the average at 0.5 kU/l (geometric mean was 0.13 kU/l and 0.12 kU/l) equal to 1.2 ng/ml. [549] and less than 1.6 ng/ml LOD for this ELISA method. The solid phase immunoassays for the routine diagnosis of specific IgE, which provided higher sensitivity to detect IgE at 0.1 kU/l, should be considered in future. [550] Additionally, factors specific to cord and infant samples may interfere with the ELISA assay, resulting in a lack of detectable signals.

Cord plasma/serum IgA

The results of IgA measurement revealed a wide range of concentrations in diluted cord serum/plasma, spanning from 1:10,000 to 1:20,000. This variation could be attributed to individual differences in IgA concentration and specific conditions affecting IgA production or metabolism.

The choice of the 1:10,000 dilution factor for the rest of the samples was supported by practical considerations. The straightforward sample preparation process at this dilution allowed for efficient and consistent analysis of IgA concentrations. Furthermore, this decision was aligned with the recommendations provided in the manufacturer's user guide. The study aimed to maintain standardisation and comparability in IgA measurements across all samples by adhering to the recommended dilution factor.

The measurement of IgA in cord serum/plasma is of significant interest in the context of this study to determine maternal blood contamination that may cause false positives in the IgE concentration in cord blood, leading to misinterpretation of the result. The selected dilution factor 1:10,000 efficiently detected the IgA concentrations to indicate maternal contamination.

In conclusion, after successfully validating and verifying the IgA and IgE ELISA tests, the results demonstrated that both assays performed as expected, meeting the intended objectives. This outcome instils confidence in the reliability and accuracy of these tests, ensuring their suitability for further use in the project.

However, it was noted that the measurement of total IgE in cord plasma/serum and infant plasma/serum using the ELISA kit was constrained by its low sensitivity (the analyte level in samples was lower than the test's detection limit). As a result, an alternative method – ImmunoCap® - capable of detecting lower concentration of total IgE in cord plasma/serum and infant children was employed for more accurate measurements in this project.

A5.4.4 Urine creatinine

The creatinine measurement by the enzymatic method validated the protocol and optimised the appropriate sample preparation for dilution and filtration. The 1:100 dilution was detected in the standard range for all representative samples, either filtrated or unfiltered urine samples**.** This concentration was similar to the report from Allegaert *et al*., which used 1:50 dilutions to measure urine creatinine in newborn. [551] The dilution x100 was selected for the remaining samples.

Regarding urine sample filtration, creatinine concentration was statistically different between filtered and unfiltered urine samples (paired measurement), with the filtered urine generally providing a greater level of creatinine. The filtered process functions as deproteinisation, which helps remove and thus minimise interference from other proteins, such as bilirubin (309), which are known to interfere with creatinine detection by enzymatic methods [552]. Therefore, it was decided that the remaining samples should be filtered prior to the creatinine detection assay.

Additionally, all twenty-eight sample blanks were detected below the background. Therefore, two representative sample blanks per assay were applied instead of per sample. Moreover, triplicate samples were assayed to maximise the precision of the result.

A5.4.5 GIS analysis of $PM_{2.5}$ exposure at participants' residence

The comparison of the two interpolation methods revealed that IDW outperformed OK in all three months. IDW consistently showed lower root-mean-square error (RMSE) values, indicating that its estimated $PM_{2.5}$ values were closer to the observed values at the ground monitoring stations. The smaller RMSE values suggested that IDW offered better accuracy and precision in estimating $PM_{2.5}$ concentrations than OK.

One possible reason for the superior performance of IDW over OK could be the nature of the dataset. IDW is effective when the data's underlying spatial distribution is relatively simple and exhibits a strong spatial autocorrelation. [553] In contrast, OK assumes that the data follows a stationary Gaussian process, which may not hold for certain types of environmental data, such as $PM_{2.5}$ concentrations that complex local factors can influence. [126]

Another factor that could contribute to the differences in performance is the choice of the semivariogram model used in OK. The semivariogram model plays a critical role in OK interpolation, and selecting an inappropriate model may lead to less accurate predictions. On the other hand, IDW does not require the estimation of a semivariogram, which may make it more straightforward to implement and less sensitive to model selection. [127]

In conclusion, based on the cross-validation results, it can be concluded that IDW) is a more suitable method for $PM_{2.5}$ interpolation compared to the studied dataset in January 2018, 2019, and 2020. IDW consistently provided $PM_{2.5}$ estimates closer to the observed values at the ground monitoring stations, as evidenced by the lower root-mean-square error (RMSE) values and will be used for this study.

Appendix 6 The univariate logistic regression analysis of potential variables and each allergic disease (AD, FA, and combined AD and FA)

Table A6-1: The univariate logistic regression analysis between maternal and infant factors and each allergic disease in infant children (AD, FA, and combined AD + FA).

Variables	Allergic dermatitis (AD)			Food allergy (FA)			Combined AD and FA		
	N	Crude OR (95%CI)	*P*	N	Crud OR (95%CI)	*P*	N	Crude OR (95%CI)	*P*
Site of data collection	69			68			73		
Ramathibodi Hospital		--	--	--	--	--		--	--
Rayong Hospital		--	--	--	--	--		--	--
Maternal age at pregnancy ≥ 35 years	69	1.310 (0.137, 12.529)	0.815	68	3.929 (0.605, 25.509)	0.152	73	--	--
Gravida ≥ 3	69	--	--	68	0.420 (0.046, 3.837)	0.442	73	0.210 (0.025, 1.756)	0.150
Mean body mass index (BMI) before pregnancy; kg/m^2	66	1.020 (0.826, 1.259)	0.856	66	1.337 (1.051, 1.702)	**0.018**	71	1.101 (0.941, 1.288)	0.230
Family member ≥ 3	69	1.619 (0.334, 7.847)	0.550	68	1.214 (0.227, 6.494)	0.820	73	1.457 (0.402, 5.282)	0.567
Maternal occupation (Pregnant women)	69			68			73		
Unemployed, housewife, student, freelancer		1			1			1	
Medical personal, industry, hairdresser		--	--		--	--		18.000 (1.375, 235.686)	**0.028**
Teacher, officers or employees either in government or private sector		--	--		--	--		3.176 (0.355, 28.460)	0.302
Merchant, personal business		--	--		--	--		2.571 (0.141, 47.017)	0.524
Family income > 20,000 Thai Baht	66	0.791 (0.132, 4.726)	0.797	65	--	--	71	1.779 (0.348, 9.097)	0.489
Maternal education (P01)	69			68			73		
Below undergraduate		1			1			1	
Undergraduate		--	--		0.232 (0.024, 2.214)	0.204		0.929 (0.241, 3.581)	0.914
Graduate		--	--		0.812 (0.079, 8.352)	0.861		0.650 (0.066, 6.410)	0.712
Maternal common allergy (pregnant women)	69	0.867 (0.094, 8.000)	0.900	68	1.040 (0.109, 9.879)	0.973	73	1.156 (0.216, 6.169)	0.866
Paternal common allergy (partner)	69	3.143 (0.510, 19.372)	0.217	68	3.929 (0.605, 25.509)	0.152	73	--	--
Sibling common allergy	69	--	--	68	6.000 (0.460, 78.236)	0.171	73	--	--

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Variables	Allergic dermatitis (AD)			Food allergy (FA)			Combined AD and FA		
	N	Crude OR (95%CI)	*P*	N	Crud OR (95%CI)	*P*	N	Crude OR (95%CI)	*P*
Maternal sensitisation	69	--	--	68	1.350 (0.139, 13.091)	0.796	73	5.625 (1.387, 22.809)	**0.016**
Elevated maternal total IgE concentration (≥ 100 KUA/L)									
1st trimester (P01)	69	--	--	68	--	--	73	6.548 (1.577, 27.191)	**0.010**
3rd trimester (P03)	69	--	--	68	--	--	73	11.238 (2.074, 60.880)	**0.005**
Infant sensitisation at birth	69	--	--	68	--	--	73	--	--
Infant sensitisation at 6-12 months	69	0.981 (0.105, 9.144)	0.987	68	1.178 (0.123, 11.290)	0.887	73	3.365 (0.816, 13.883)	**0.093**
Elevated infant total IgE concentration									
At birth	69	--	--	68	--	--	73	--	--
At 6-12 months	69	0.283 (0.032, 2.497)	0.256	68	0.848 (0.144, 4.997)	0.855	73	4.522 (1.089, 18.771)	**0.038**
GDM	69	5.000 (0.393, 63.584)	0.215	68	6.000 (0.460, 78.236)	0.171	73	3.000 (0.248, 36.258)	0.388
Folic acid intake during 1st trimester	69	2.700 (0.446, 16.336)	0.279	68	1.350 (0.139, 13.091)	0.796	73	--	--
Vitamin intake during 1st trimester	69	12.000 (1.381, 104.251)	**0.024**	68	15.000 (1.652, 136.172)	**0.016**	73	3.000 (0.248, 36.258)	0.388
Gestational age (GA ≥ 37 weeks)	66	--	--	65	0.463 (0.045, 4.779)	0.518	70	0.926 (0.098, 8.790)	0.947
Birth weight (≥ 2,500 gram)	67	0.095 (0.016, 0.581)	**0.011**	66	0.357 (0.033, 3.838)	0.395	71	0.714 (0.072, 7.070)	0.774
Small for gestational age (SGA)	66	0.151 (0.027, 0.842)	**0.031**	65	0.566 (0.056, 5.686)	0.629	70	0.509 (0.089, 2.930)	0.450
Mode of delivery	67			66			71		
Vaginal		1			1			1	
Caesarean section		1.744 (0.359, 8.479)	0.491		6.528 (0.719, 59.419)	**0.095**		1.569 (0.431, 5.712)	0.494
Gender	67			66			71		
Male		1			1			1	
Female		2.000 (0.410, 9.744)	0.391		3.000 (0.509, 17.687)	0.225		1.250 (0.343, 4.561)	0.735
Birth season	69			68			73		
Winter (November to February)		1			1			1	
Summer (March to May)		2.100 (0.257, 17.143)	0.489		--	--		0.350 (0.037, 3.311)	0.360
Rainy (June to October)		1.016 (0.156, 6.613)	0.987		0.677 (0.125, 3.684)	0.652		0.452 (0.113, 1.797)	0.259
Exclusive breastfeeding ≥ 4 months	63	2.222 (0.452, 10.916)	0.325	62	3.333 (0.561, 19.796)	0.185	65	5.833 (1.107, 30.740)	**0.038**
Exclusive breastfeeding ≥ 6 months	64	1.733 (0.173, 17.416)	0.640	63	2.080 (0.201, 21.484)	0.539	66	1.300 (0.134, 12.613)	0.821

Table A6-2: The univariate logistic regression analysis between home environment factors and each allergic disease in infant children (AD, FA, and combined AD + FA).

Variables	Allergic dermatitis (AD)			Food allergy (FA)			Combined AD and FA		
	N	Crude OR (95%CI)	*P*	N	Crude OR (95%CI)	*P*	N	Crude OR (95%CI)	*P*
Season of data collection									
1st trimester	69			68			73		
Winter (November to February)		1			1			1	
Summer (March to May)		1.250 (0.158, 9.879)	0.832		3.750 (0.355, 39.586)	0.272		1.875 (0.279, 12.613)	0.518
Rainy (June to October)		1.154 (0.176, 7.575)	0.882		1.538 (0.130, 18.192)	0.733		2.308 (0.420, 12.672)	0.336
2nd trimester	68			67			72		
Winter (November to February)		1			1			1	
Summer (March to May)		--	--		--	--		2.333 (0.218, 24.923)	0.483
Rainy (June to October)		0.362 (0.071, 1.843)	0.221		--	--		3.379 (0.378, 30.202)	0.276
3rd trimester	67			66			71		
Winter (November to February)		1			1			1	
Summer (March to May)		0.333 (0.031, 3.555)	0.363		--	--		0.200 (0.021, 1.909)	0.162
Rainy (June to October)		0.571 (0.103, 3.172)	0.522		1.143 (0.188, 6.949)	0.885		0.571 (0.143, 2.279)	0.428
Type of residence									
1st trimester	69			68			73		
Detached house		1			1			1	
Flat/ condominium/ apartment		0.600 (0.093, 3.867)	0.591		0.600 (0.093, 3.867)	0.591		0.257 (0.049, 1.346)	0.108
Row house		3.600 (0.474, 27.349)	0.216		1.800 (0.154, 20.987)	0.639		1.543 (0.245, 9.700)	0.644
2nd trimester	68			67			85		
Detached house		1			1			1	
Flat/ condominium/ apartment		0.618 (0.081, 4.722)	0.642		0.154 (0.016, 1.477)	0.105		1.370 (0.535, 3.506)	0.512
Row house		5.250 (0.706, 39.029)	0.105		0. 875 (0.082, 9.376)	0.912		1.000 (0.242, 4.131)	1.000
3rd trimester	67			66			84		
Detached house		1			1			1	
Flat/ condominium/ apartment		1.312 (0.112, 15.405)	0.829		0.438 (0.067, 2.844)	0.387		1.481 (0.559, 3.919)	0.430
Row house		12.000 (1.142, 126.122)	**0.098**		1.000 (0.089, 11.239)	1.000		0.595 (0.151, 2.338)	0.457

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Variables	Allergic dermatitis (AD)			Food allergy (FA)			Combined AD and FA		
	N	Crude OR (95%CI)	*P*	N	Crude OR (95%CI)	*P*	N	Crude OR (95%CI)	*P*
Ventilation system									
1st trimester	69			68			73		
Open windows/ doors		0.239 (0.048, 1.192)	**0.081**		0.319 (0.058, 1.752)	0.189		0.182 (0.047, 0.710)	**0.014**
Air conditioner (AC)		3.789 (0.429, 33.449)	0.231		--	--		1.684 (0.406, 6.981)	0.472
Fan		0.600 (0.104, 3.476)	0.569		0.240 (0.043, 1.340)	0.104		2.400 (0.280, 20.604)	0.425
Ventilation fan		--	--		3.933 (0.343, 45.143)	0.271		--	--
Air purifier		10.167 (0.562, 184.011)	0.117		--	--		--	--
2nd trimester	68			67			72		
Open windows/ doors		1.312 (0.234, 7.350)	0.757		0.105 (0.012, 0.958)	**0.046**		0.630 (0.172, 2.309)	0.486
Air conditioner (AC)		3.385 (0.382, 29.958)	0.273		--	--		2.538 (0.503, 12.812)	0.259
Fan		--	--		0.440 (0.071, 2.712)	0.376		0.587 (0.134, 2.574)	0.480
Ventilation fan		--	--		12.000 (0.648, 222.070)	**0.095**		--	--
Air purifier		4.480 (0.685, 29.293)	0.118		2.240 (0.217, 23.110)	0.498		1.120 (0.118, 10.626)	0.921
3rd trimester	67			66			71		
Open windows/ doors		1.500 (0.165, 13.667)	0.719		0.250 (0.045, 1.397)	0.114		0.208 (0.054, 0.800)	**0.022**
Air conditioner (AC)		--	--		--	--		2.250 (0.444, 11.409)	0.328
Fan		--	--		1.122 (0.119, 10.590)	0.920		1.010 (0.191, 5.344)	0.990
Ventilation fan		--	--		5.800 (0.445, 75.661)	0.180		--	--
Air purifier		7.600 (1.019, 56.676)	**0.048**		3.800 (0.331, 43.634)	0.284		1.900 (0.179, 20.139)	0.594
Mould visibility at home									
1st trimester	69			86	0.995 (0.283, 3.506)	0.994	86	2.600 (0.979, 6.905)	**0.055**
2nd trimester	68			85	0.655 (0.189, 2.272)	0.504	85	1.680 (0.676, 4.176)	0.264
3rd trimester	67			84	1.316 (0.398, 4.353)	0.653	84	1.361 (0.523, 3.544)	0.528
Pet exposure									
1st trimester	69			68			73		
Any pet exposure		0.576 (0.103, 3.208)	0.529		1.385 (0.259, 7.414)	0.704		0.308 (0.061, 1.544)	0.152
Dog		1.150 (0.203, 6.524)	0.875		0.575 (0.062, 5.300)	0.625		0.288 (0.034, 2.426)	0.252
Cat		1.508 (0.262, 8.677)	0.646		--	--		0.377 (0.044, 3.219)	0.373

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Variables	Allergic dermatitis (AD)			Food allergy (FA)			Combined AD and FA		
	N	Crude OR (95%CI)	*P*	N	Crude OR (95%CI)	*P*	N	Crude OR (95%CI)	*P*
2nd trimester (P02)	68			67			72		
Any pet exposure		0.691 (0.123, 3.865)	0.674		0.720 (0.122, 4.237)	0.716		--	--
Dog		1.125 (0.198, 6.385)	0.894		0.563 (0.061, 5.187)	0.612		--	--
Cat		--	--		--	--		--	--
3rd trimester (P03)	67			66			71		
Any pet exposure		0.464 (0.094, 2.300)	0.464		1.727 (0.321, 9.307)	0.525		--	--
Dog		1.446 (0.251, 8.331)	0.680		0.723 (0.078, 6.746)	0.776		--	--
Cat		--	--		--	--		--	--
Household ETS									
1st trimester	69			68			73		
Any household ETS		0.464 (0.094, 2.300)	0.347		0.174 (0.029, 1.042)	**0.055**		--	--
From partner		--	--		0.575 (0.062, 5.300)	0.625		1.643 (0.424, 6.361)	0.472
From housemate (s)		0.694 (0.076, 6.323)	0.746		--	--		2.381 (0.599, 9.470)	0.218
From neighbour (s)		1.272 (0.261, 6.194)	0.766		0.848 (0.144, 4.997)	0.855		0.969 (0.256, 3.672)	0.963
2nd trimester (P02)	68			67			72		
Any household ETS		0.435 (0.087, 2.167)	0.310		0.652 (0.108, 3.924)	0.641		1.467 (0.285, 7.559)	0.647
From partner		--	--		--	--		2.333 (0.586, 9.286)	0.229
From housemate (s)		0.850 (0.092, 7.849)	0.886		--	--		2.914 (0.716, 11.854)	0.135
From neighbour (s)		0.884 (0.182, 4.289)	0.878		2.357 (0.401, 13.845)	0.343		0.442 (0.107, 1.827)	0.256
3rd trimester (P03)	67			66			71		
Any household ETS		0.406 (0.081, 2.034)	0.273		0.609 (0.101, 3.681)	0.589		1.370 (0.264, 7.095)	0.708
From partner		--	--		--	--		0.803 (0.154, 4.186)	0.795
From housemate (s)		1.083 (0.115, 10.217)	0.944		--	--		5.417 (1.334, 21.986)	**0.018**
From neighbour (s)		1.247 (0.257, 6.057)	0.784		1.871 (0.318, 10.998)	0.488		0.352 (0.085, 1.542)	0.148
Living near a busy road									
1st trimester	69	0.247 (0.028, 2.175)	0.208	68	2.960 (0.503, 17.406)	0.230	73	0.846 (0.224, 3.195)	0.805
2nd trimester	69	--	--	67	2.211 (0.408, 11.975)	0.357	72	0.829 (0.198, 3.475)	0.798
3rd trimester	69	0.500 (0.056, 4.495)	0.536	84	0.600 (0.065, 5.553)	0.653	71	1.125 (0.264, 4.795)	0.873

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Variables	Allergic dermatitis (AD)			Food allergy (FA)			Combined AD and FA		
	N	Crude OR (95%CI)	*P*	N	Crude OR (95%CI)	*P*	N	Crude OR (95%CI)	*P*
Presence of a green environment at home									
1st trimester	69	0.481 (0.082, 2.834)	0.418	68	--	--	73	0.865 (0.162, 4.620)	0.866
2nd trimester	69	1.469 (0.161, 13.383)	0.733	67	--	--	72	0.653 (0.150, 2.839)	0.570
3rd trimester	69	--	--	84	1.383 (0.148, 12.902)	0.776	71	2.766 (0.324, 23.637)	0.353
Common household chemical product consumption									
1st trimester	69			68			73		
Use any chemical product		1.806 (0.325, 10.039)	0.500		0.722 (0.135, 3.867)	0.704		1.926 (0.466, 7.964)	0.366
Bathroom cleaning liquid		2.417 (0.231, 25.264)	0.461		2.577 (0.427, 15.563)	0.302		1.450 (0.147, 14.344)	0.751
Bleach for washing		1.019 (0.109, 9.493)	0.987		--	--		0.453 (0.101, 2.036)	0.302
Disinfectant		0.167 (0.019, 1.467)	0.106		0.500 (0.085, 2.932)	0.442		0.833 (0.230, 3.018)	0.781
2nd trimester	68			67			72		
Use any chemical product		0.557 (0.115, 2.707)	0.468		0.371 (0.063, 2.184)	0.273		1.981 (0.479, 8.201)	0.346
Bathroom cleaning liquid		--	--		3.250 (0.684, 15.438)	0.138		2.037 (0.354, 11.706)	0.425
Bleach for washing		--	--		0.980 (0.103, 9.316)	0.986		0.343 (0.084, 1.396)	0.135
Disinfectant		--	--		1.812 (0.309, 10.643)	0.510		0.755 (0.208, 2.740)	0.669
3rd trimester	67			66			71		
Use any chemical product		0.889 (0.182, 4.331)	0.884		0.667 (0.124, 3.583)	0.637		1.778 (0.428, 7.383)	0.428
Bathroom cleaning liquid		--	--		3.200 (0.673, 15.205)	0.144		0.900 (0.098, 8.303)	0.926
Bleach for washing		--	--		0.882 (0.092, 8.464)	0.914		0.471 (0.105, 2.118)	0.326
Disinfectant		--	--		1.069 (0.200, 5.727)	0.938		1.283 (0.353, 4.661)	0.705
Recently furnished/renovated house (within 3 months)									
1st trimester	69	10.476 (1.931, 56.832)	**0.006**	68	3.929 (0.605, 25.509)	0.152	73	1.746 (0.312, 9.773)	0.526
2nd trimester	68	2.040 (0.346, 12.029)	0.431	67	1.020 (0.107, 9.692)	0.986	72	0.510 (0.059, 4.442)	0.542
3rd trimester	67	3.000 (0.591, 15.237)	0.185	66	0.800 (0.085, 7.502)	0.845	71	1.500 (0.345, 6.524)	0.589

Table A6-3: The univariate logistic regression analysis between infant cytokines and each allergic disease in infant children (AD, FA, and combined AD + FA).

Cytokines	Allergic dermatitis (AD)			Food allergy (FA)			Combined AD and FA		
	N	Crude OR (95%CI)	P	N	Crude OR (95%CI)	P	N	Crude OR (95%CI)	P
IL-1β	48	0.919 (0.572, 1.476)	0.727	46	0.851 (0.411, 1.762)	0.664	51	0.979 (0.876, 1.095)	0.715
IL-2	47	1.309 (0.917, 1.868)	0.138	46	1.123 (0.672, 1.876)	0.658	51	0.117 (0.009, 1.592)	0.107
IL-4	47	1.482 (0.914, 2.402)	0.110	46	1.054 (0.438, 2.536)	0.548	51	0.926 (0.434, 1.979)	0.843
IL-5	48	1.163 (0.407, 3.325)	0.778	46	1.694 (0.946, 3.030)	**0.076**	51	0.318 (0.009, 11.477)	0.531
IL-6	45	0.994 (0.962, 1.026)	0.698	43	0.992 (0.944, 1.043)	0.764	48	0.995 (0.977, 1.014)	0.623
IL-8	48	0.985 (0.931, 1.043)	0.608	46	0.996 (0.978, 1.015)	0.708	51	1.000 (0.999, 1.001)	0.670
IL-10	48	1.040 (0.884, 1.224)	0.638	46	0.910 (0.560, 1.479)	0.704	51	0.674 (0.331, 1.375)	0.279
IL-12/IL-23p40	48	0.943 (0.875, 1.016)	**0.039**	46	0.993 (0.938, 1.052)	0.815	51	0.952 (0.900, 1.007)	**0.088**
IL-13	48	1.120 (0.843, 1.489)	0.434		--	--	51	--	--
IL-17	48	0.854 (0.680, 1.072)	0.173	46	0.807 (0.603, 1.080)	0.150	51	0.988 (0.847, 1.153)	0.881
IL-22	48	0.979 (0.811, 1.182)	0.730	46	0.897 (0.593, 1.359)	0.609	51	1.055 (0.945, 1.177)	0.339
IL-31	48	0.998 (0.972, 1.025)	0.899	46	1.028 (0.995, 1.063)	0.101	51	0.998 (0.978, 1.019)	0.882
IL-33	48	0.940 (0.839, 1.053)	0.285	46	0.981 (0.913, 1.055)	0.609	51	1.012 (0.990, 1.035)	0.280
IFN-γ	48	0.968 (0.701, 1.336)	0.842	46	0.990 (0.720, 1.363)	0.953	51	1.061 (0.920, 1.224)	0.418
TNF	47	1.083 (0.958, 1.224)	0.203	45	1.015 (0.836, 1.233)	0.879	50	0.767 (0.391, 1.503)	0.439
TSLP	48	0.990 (0.911, 1.076)	0.817	46	1.016 (0.939, 1.110)	0.695	51	0.993 (0.931, 1.060)	0.833

Table A6-4: The univariate logistic regression analysis between maternal heavy metal concentrations and each allergic disease in infant children (AD, FA, and combined AD + FA).

Metals	Allergic dermatitis (AD)			Food allergy (FA)			Combined AD and FA		
	N	Crude OR (95%CI)	*P*	N	Crude OR (95%CI)	*P*	N	Crude OR (95%CI)	*P*
As	62	0.680 (0.276, 1.675)	0.144	61	1.681 (0.938, 3.011)	**0.081**	64	0.283 (0.082, 0.975)	**0.046**
Cd	62	0.068 (0.000, 201.738)	0.511	61	--	--	64	0.008 (0.000, 377.744)	0.379
Cr	62	2.523 (1.000, 6.366)	**0.050**	61	0.513 (0.081, 3.237)	0.477	64	1.748 (0.722, 4.233)	0.216
Hg	62	1.272 (0.807, 2.005)	0.300	61	1.212 (0.705, 2.083)	0.487	64	0.515 (0.197, 1.345)	0.176
Mn	62	1.041 (0.917, 1.181)	0.140	61	0.976 (0.826, 1.154)	0.779	64	0.925 (0.784, 1.092)	0.359
Pb	62	0.871 (0.716, 1.058)	0.164	61	0.976 (0.862, 1.105)	0.699	64	0.796 (0.640, 0.990)	**0.041**

Abbreviations: As, arsenic, Cd, cadmium, Cr; chromium, Hg, mercury, Mn; manganese, Pb; lead, RR; relative risks, 95%CI; 95% confidence interval

Table A6-5: The univariate logistic regression analysis between urine cotinine/creatinine (CCR) ratio and each allergic disease in infant children (AD, FA, and combined AD + FA).

CCR (ng/mg creatinine)	Allergic dermatitis (AD)			Food allergy (FA)			Combined AD and FA		
	N	OR (95%CI)	*P*	N	OR (95%CI)	*P*	N	OR (95%CI)	*P*
Pregnancy 1st trimester	69	1.000 (0.999, 1.001)	0.494	68	1.000 (0.999, 1001)	0.702	73	0.999 (0.996, 1.001)	0.325
≥ 30		--	--		--	--		--	--
Pregnancy 3rd trimester	68	1.000 (1.000, 1.000)	0.697	67	0.995 (0.989, 1.002)	0.175	71	1.000 (1.000, 1000)	0.832
≥ 30		--	--		--	--		1.967 (0.186, 20.836)	0.574
Children at birth	64	1.000 (0.999, 1.001)	0.621	53	1.000 (0.999, 1.001)	0.823	56	1.000 (0.999, 1.000)	0.537
≥ 30		--	--		--	--		--	--
Children at 6-12 months	**62**	1.000 (1.000, 1.000)	0.900	61	1.000 (1.000, 1.000)	0.117	66	--	--
≥ 30		1.556 (0.159, 15.182)	0.704		1.867 (0.186, 18.734)	0.596		1.600 (0.372, 6.883)	0.528

Abbreviations: RR, relative risks, CCR; urine cotinine-creatinine ratio

Table A6-6: The univariate logistic regression analysis between $PM_{2.5}$ exposure and each allergic disease in infant children (AD, FA, and combined AD + FA).

Average 24-hour $PM_{2.5}$ estimation ($\mu g/m^3$)	Allergic dermatitis (AD)			Food allergy (FA)			Combined AD and FA		
	N	Crude OR (95%CI)	*P*	N	OR (95%CI)	*P*	N	OR (95%CI)	*P*
Pregnancy 1st trimester	69	0.979 (0.894, 1.073)	0.656	68	1.002 (0.911, 1.101)	0.975	73	0.977 (0.907, 1.053)	0.548
≥ 25		0.703 (0.145, 3.406)	0.662		0.469 (0.080, 2.749)	0.401		0.352 (0.085, 1.451)	0.148
Pregnancy 2nd trimester	69	1.049 (0.948, 1.161)	0.356	68	0.924 (0.799, 1.068)	0.286	73	0.930 (0.833, 1.038)	0.197
≥ 25		1.973 (0.406, 9.586)	0.399		0.740 (0.126, 4.351)	0.739		0.329 (0.065, 1.652)	0.177
Pregnancy 3rd trimester	69	1.022 (0.949, 1.100)	0.561	68	0.993 (0.914, 1.079)	0.868	73	1.023 (0.965, 1.085)	0.437
≥ 25		1.846 (0.380, 8.960)	0.447		1.385 (0.259, 7.414)	0.704		1.662 (0.458, 6.033)	0.440
Infancy at birth - < 2 months	68	1.017 (0.943, 1.096)	0.667	67	1.069 (0.980, 1.165)	0.131	72	1.057 (0.986, 1.133)	0.116
≥ 25		0.636 (0.131, 3.088)	0.575		4.242 (0.468, 38.491)	0.199		8.385 (1.022, 70.435)	**0.048**
Infancy at ≥ 2 - < 4 months	68	0.991 (0.924, 1.064)	0.812	67	1.032 (0.961, 1.108)	0.392	72	1.015 (0.960, 1.074)	0.591
≥ 25		0.504 (0.091, 2.801)	0.433		2.519 (0.429, 14.800)	0.307		1.049 (0.289, 3.812)	0.942
Infancy at ≥ 4 - 6 months	68	0.968 (0.890, 1.052)	0.444	67	1.001 (0.923, 1.085)	0.989	72	0.992 (0.933, 1.056)	0.810
≥ 25		0.636 (0.131, 3.088)	0.575		0.848 (0.159, 4.542)	0.848		0.485 (0.129, 1.829)	0.285
Accumulative PM2.5 exposure									
1st trimester	69	1.000 (0.999, 1.001)	0.656	68	1.000 (0.999, 1.001)	0.975	73	1.000 (0.999, 1.001)	0.548
2nd trimester	69	1.000 (0.999, 1.002)	0.356	68	0.999 (0.998, 1.001)	0.286	73	0.999 (0.998, 1.000)	0.197
3rd trimester	69	1.000 (0.999, 1.001)	0.477	68	1.000 (0.999, 1.001)	0.541	73	1.001 (1.000, 1.001)	0.120
Infant 0-2 months	68	1.000 (0.999, 1.002)	0.667	67	1.001 (1.000, 1.003)	0.131	72	1.001 (1.000, 1.002)	0.116
Infant 2-4 months	68	1.000 (0.999, 1.001)	0.812	67	1.001 (0.999, 1.002)	0.392	72	1.000 (0.999, 1.001)	0.591
Infant 4-6 months	68	0.999 (0.998, 1.001)	0.444	67	1.000 (0.999, 1.001)	0.989	72	1.000 (0.999, 1.001)	0.810

Table A6-7: The multivariable logistic regression analysis between early life exposure and each allergic disease in infant children (AD, FA, and combined AD + FA).

Variables	Model 1			Model 2			Model 3 (Final model)		
	N	adjusted OR (95%CI)	*P*	N	adjusted OR (95%CI)	*P*	N	adjusted OR (95%CI)	*P*
Allergic dermatitis (AD)									
Vitamin intake (1st trimester)	41	64.000 (2.831, 1446.883)	**0.009**	69	53.000 (3.272, 858.463)	**0.005**	69	24.695 (2.150, 283.690)	**0.011**
Recently furbished (1st trimester)	41	48.000 (3.303, 697.517)	**0.005**	69	30.286 (2.950, 310.886)	**0.004**		14.135 (2.154, 92.744)	**0.006**
Food allergy (FA)									
Vitamin intake (1st trimester)	64	--	--	66	--	--	66	15.145 (1.069, 214.631)	**0.045**
Prepregnancy BMI	64	1.707 (1.027, 2.835)	**0.039**	66	1.469 (1.024, 2.108)	**0.037**	66	1.274 (1.016, 1.598)	**0.036**
Open window (1st trimester)	64	--	--	66	--	--	66	0.713 (0.107, 4.748)	0.722
Combined AD + FA									
Open doors/ windows (1st trimester)	40	0.052 (0.005, 0.548)	**0.014**		--	--	73	0.176 (0.043, 0.713)	**0.016**

Model 1: All variables that provided p ≤ 0.1 from univariable logistic regression analysis were included in the analysis with forward, conditional selection method.
Model 2: The retained variables from Model 1 were re-analysis with multivariate logistic regression, enter selection method
Model 3: The retained variables from Model 1 were selected for the multilevel multiple logistic regression analysis considering the cluster effect from site of data collection (hospital) and adjust for multiple comparisons using sequential Bonferroni.
[c] sIgE at 1st trimester ≥ 0.35 kUA/L

Appendix 7 Subanalysis of allergic diseases outcome (atopic dermatitis, food allergy, and combined atopic dermatitis and food allergy) of final Model.

The multivariable logistic regression analysis by SPSS, and the result from SPSS was shown below for each outcome of allergic diseases (infant children diagnosed AD, FA, or combined AD and FA). The adjusted variables were based on the final model (**Model 3**) of allergic diseases.

Table A7-1: The subgroup analysis multivariable logistic regression model between early life exposure and each allergic diseases outcome (AD, FA, and combined AD + FA), adjusted factors based on the final model (Model 3) of allergic diseases outcome in **Chapter 7**.

Variables	**Atopic dermatitis (AD)**			**Food allergy (FA)**			**Combined AD and FA**		
	N	**Adjusted OR (95%CI)**	***P***	**N**	**Adjusted OR (95%CI)**	***P***	**N**	**Adjusted OR (95%CI)**	***P***
Open Window (1st trimester)	66	0.210 (0.024, 1.834)	0.155	66	2.514 (0.374, 16.882)	0.343	71	**4.514 (1.088, 18.733)**	**0.038**
Type of residence (2nd trimester)	66			66			71		
Detached house		1			1			1	
Condominium/ flat		2.954 (0.184, 47.513))	0.439		0.289 (0.026, 3.187)	0.311		0.706 (0.154, 3.233)	0.653
Row house/ attached house		**23.269 (1.417, 378.939)**	**0.028**		1.710 (0.124, 23.551)	0.688		0.915 (0.078, 10.759)	0.944
BMI before pregnancy	**66**	1.021 (0.793, 1.316)	0.868	**66**	**1.293 (1.009, 1.656)**	**0.042**	71	1.070 (0.909, 1.261)	0.416

Model 1: All variables that provided p ≤ 0.1 from univariable logistic regression analysis were included in the analysis with forward, conditional selection method.

Model 2: The retained variables from Model 1 were re-analysis with multivariate logistic regression, enter selection method

Model 3: The retained variables from Model 1 were selected for the multilevel multiple logistic regression analysis considering the cluster effect from site of data collection (hospital) and adjust for multiple comparisons using sequential Bonferroni.

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