This is to certify that the
dissertation entitled

**ALLERGIC DISORDERS
AND
SEX HORMONE DISRUPTERS**

presented by

KEVIN ROYD BROOKS

has been accepted towards fulfillment
of the requirements for the

Doctoral degree in Epidemiology

Major Professor's Signature

January , 2007

Date

ALLERGIC DISORDERS AND SEX HORMONE DISRUPTERS

By

Kevin Royd Brooks

A DISSERTATION

Submitted to
Michigan State University
In partial fulfillment of the requirements
for the degree of

DOCTORAL DEGREE

DEPARTMENT OF EPIDEMIOLOGY

2007

ABSTRACT

ALLERGIC DISORDERS AND SEX HORMONE DISRUPTERS

By

Kevin Royd Brooks

Allergic disorders are among the most disruptive of childhood. Further, the prevalence and severity of these disorders are increasing.

Great strides have been made toward disentangling the etiology of allergies; however a clear understanding is still elusive. Recently, the role of sex hormone disrupters (SHD) in triggering childhood allergies have gained increased attention. Specifically, halogenated organochlorine compounds (HOCs) such as dichlorodiphenyl dichloroethene (DDE) and polychlorinated biphenyls (PCBs) in addition to synthetic hormones such as estrogen and progesterone in the form of contraception have been implicated.

We investigated the role of SHD in the etiology of allergic diseases. First we investigated the association between placental p,p'-DDE and cord blood immune markers. p,p'-DDE concentration and cord plasma interleukin (IL)-13, IL-4 and INF-γ were determined in 19 neonates from the ongoing Pregnancy Environment and Child Health (PEACH) study. Results showed increased placental p,p'-DDE to be associated with a statistically significant increase in cord plasma interleukin (IL)-13. Furthermore, both cord plasma IL-4/INF-γ and IL-13/INF-γ ratios were significantly positively associated with placental p,p'-DDE concentration.

Second, we estimated associations between maternal oral contraceptive (OCp) use and humoral immune markers in offspring. We hypothesized that maternal OC use increases humoral immune markers of allergy in offspring. Toward this end, data from a cross-sectional investigation including 334 mother child (aged 7–10 years) pairs from Hesse, Germany were used. Results showed that female offspring of mothers who used OC had significantly lower ($p<0.05$): IgA (123.43 mg/dL vs. 150.52 mg/dL), and IgE (22.96 kU/L vs. 50.83 kU/L) levels as well as basophilic surface IgE counts (783 vs. 842), compared to those of mothers who did not use OC. For male offspring, statistical significance was only seen in an increased number of basophilic surface IgE (911 vs. 876).

Third, using data from a 1986–1987 survey of 11-12 years old Jamaican children, an investigation of the association between maternal OCp use before pregnancy and childhood allergic phenotypes suggest that offspring's asthma or wheezing may be related to OCp use (aOR: 1.81; 95% CI (1.25- 2.61)).
Fourth, the relationship between childhood DDE and immune markers was quantified using data from the above-mentioned Germany cohort.
Higher DDE concentration was significantly related to increased serum IgE levels, IgE count on basophils, and the reduced eosinophilic granula.

These findings suggest that SHD may be important in the in etiology of childhood allergy. Of note, SHD may play a role in the prenatal priming of childhood allergy. Exploring the specific immune mechanism behind the possible effect of SHD on allergy may be worthwhile.

DEDICATIONS

This work is especially dedicated first to my wife Valrie, daughter Nessia, son Kevin and mother Patricia. I also dedicate this monograph to my very promising nephew Jevon O. Brooks.

ACKNOWLEDGEMENTS

The completion of this work is the result of a team of enthusiastic and devoted individuals. Sincere gratitude to my advisor, Dr. Wilfried Karmaus, for his prudence, and moral support in the process of completing this work.

I also thank the other members of my committee, Drs. Joseph Gardiner, David Todem and Venugopal Gangur for taking time from their busy schedule to provide unswerving intellectual support.

For their many years of encouragement, example and technical support, I thank Drs. Maureen Samms-Vaughn and Rainford Wilks. It would be remiss of me not to acknowledge my collegues Kathy, Ali and Azfar for their empathy and friendship.

This work would not be possible without the contribution of all study participants. I thank you all for your time and patience.

TABLE OF CONTENTS

LIST OF TABLES

COMPONENT ONE
In utero exposure to SHD affects perinatal biomarkers of allergy

COMPONENT TWO
In utero SHD affects postnatal biomarkers of allergy

COMPONENT THREE
In utero exposure to SHD affects allergic outcomes in infancy

LIST OF TABLES (CONTINUED)

COMPONENT FOUR
Postnatal exposure to SHD affects biomarkers of allergy in infancy

LIST OF FIGURES

OVERVIEW

Childhood allergies are among the most vexing diseases: purported to be responsible for enormous personal and public health burden. In spite of numerous efforts, the etiology of these diseases continues to evade researchers. The purpose of this monograph is to investigate the role of sex hormone disrupters (SHD) in the etiology of allergic diseases. Specifically, this work explores the prenatal origins of childhood allergies (*in utero* programming) by probing the possible role of SHD in this process. Next, the association between exposure to SHD and biomarkers of allergic susceptibility in infancy is assessed.

The quest for appropriate pieces of the SHD-allergic disorders puzzle started with the development of a conceptual model (**Figure 1;** more details provided in the "Components of the conceptual model" section). This model depicts areas that speak to the purpose of epidemiology, which is to provide clues to the cause of a health related problem. An inventory of suitable data was then undertaken and the following four components developed.

1. *In utero* exposure to SHD affects **perinatal** biomarkers of allergy.
2. *In utero* SHD affects **postnatal** biomarkers of allergy.
3. *In utero* exposure to SHD affects allergic outcomes in infancy.
4. Postnatal exposure to SHD affects biomarkers of allergy in infancy.

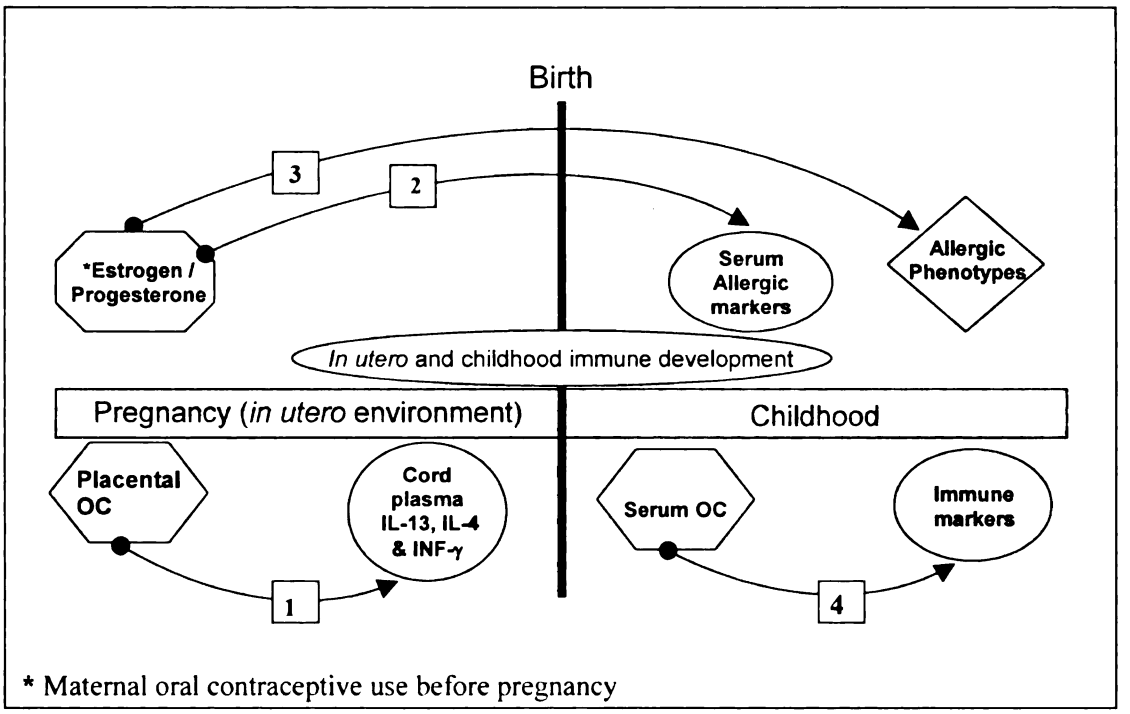

Figure 1 Sex Hormone Disrupters and Childhood Allergy - Conceptual model

Data from three cohorts were used to address the above components. These cohorts are: the ongoing Pregnancy Environment and Child Health (PEACH) study from which information on placental dichlorodiphenyl dichloroethene (DDE) concentration and cord plasma interleukin (IL)-13 were derived (component 1). Data on maternal oral contraceptive use and on childhood biomarkers of allergic susceptibility (Immunoglobulins (Igs), white blood cell, eosinophils, and basophilic surface IgE) were obtained from the Child Health and Environment Cohort Study conducted in 1995 (component 2). This study also provided data on childhood organochlorine exposure and the above mentioned childhood biomarkers of allergic susceptibility (component 4). A geographic sub-cohort from the Jamaican Perinatal Morbidity, Mortality Survey conducted in 1986-1987 provided data on maternal oral contraceptive use before pregnancy and

childhood biomarkers of effect such as asthma, wheezing and eczema (component 3). Detailed description for each respective study is presented in Chapter 2.

Ethical approval for this work was obtained from the Institutional Review Board of the Michigan State University.

This first chapter presents the definition and immunology of asthma, its prevalence and burden, risk factors and the possible effects of various SHD (sex hormone disrupters) on childhood allergy. Next, the importance of time windows of exposures and the main concepts that may explain the increase in the prevalence of asthma are introduced. Then the conceptual model with its four components (see above) followed by their respective backgrounds are presented. Chapter 2 - 'Methods' - describes different aspects of the materials and methods not discussed comprehensively in the manuscripts to be found in Chapter 3 - 'Results'. Additional details on materials and methods can be found in the respective manuscripts in Chapter 3. Furthermore, Chapter 3 showcases four manuscripts; two published, one submitted and one in preparation for publication. These manuscripts provide findings that address the following research questions:

1. Is there evidence for the immunemodulation of cord immune markers of allergy by *in utero* exposure to sex hormone disrupters?

2. Is maternal oral contraceptive use (a proxy for *in utero* estrogen/progesterone exposure) associated with immune markers of allergy in the offspring?

3. Is maternal oral contraceptive use a risk factor for allergic diseases in offspring?

4. Does serum organochlorine alters immune markers in children?

The fourth and last chapter of this monograph, 'Discussion and Conclusion', discusses and present concluding remarks of the results found in chapter 3. Practical public health issues are here presented along with future scientific steps toward a better understanding and early life prevention of childhood asthma.

CHAPTER I

ALLERGIC DISORDERS

AND

SEX HORMONE DISRUPTERS

1.1 ALLERGY

Allergy is a hypersensitivity reaction initiated by immunologic mechanisms[2].

Allergy can be antibody- or cell-mediated. In most patients, the antibody typically responsible for an allergic reaction belongs to the IgE isotype and these patients may be said to suffer from IgE-mediated allergy. In cell-mediated allergy, as in allergic contact dermatitis, immunologically sensitized lymphocytes play a major role [2]. Allergic disease represents a dysregulation of the intact immune system.

Box 1 Definitions

ALLERGY is a hypersensitivity reaction initiated by immunologic mechanisms [2]. The term allergy is also used to denote allergic disorders such as asthma and allergic rhinitis [3].

ASTHMA is a chronic inflammatory disorder of the lungs that is characterized by a reversible obstruction of the airways [4 5]

ATOPY is a personal and/or familial tendency to become sensitized and produce IgE antibodies in response to ordinary exposures to allergens. As a consequence, asthma, rhinoconjunctivitis, or eczema may occur [2].

WHEEZE is a high pitched sound made when breathing, which probably results from turbulence through narrowed tubes [6].

SENSITIZATION is the state where, following exposure to allergens, the immune system is primed to produce IgE antibodies specific to that allergen.

Patients usually develop a hypersensitive immune response to environmental antigens, resulting in local tissue damage and inflammation [7]. Diseases such as allergic rhinitis, dermatitis, and asthma represent a significant personal and public health burden. Approximately 25% of all children experience at least one allergic manifestation [8]. Asthma however, has received the most attention seeing

that it is the most common chronic disease of childhood and is considered the most debilitating [9].

1.2 Asthma

Definition and immunology

Definition

A universally accepted definition of asthma has been frustrated by a lack of understanding of the mechanisms involved in the disease. The American Thoracic Society defines asthma as "a disease characterized by an increased responsiveness of the airways (bronchial hyperresponsiveness) to various stimuli and manifested by slowing of forced expiration (due to narrowing of the airways) which changes in severity either spontaneously or as a result of therapy". This narrowing of the airways ultimately produces "wheeze", which is considered to be the necessary clinical criterion for asthma. Of note; not all that wheezes is asthma [10].

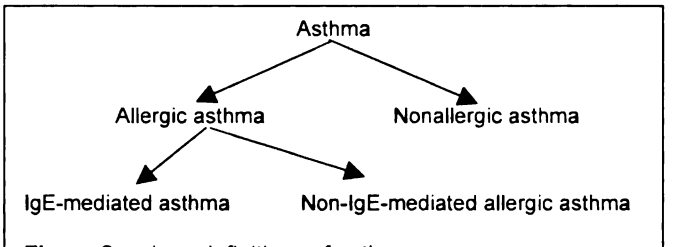

Figure 2 various definitions of asthma

Recently, the Nomenclature Review Committee of the World Allergy Organization [2] proposed the use of the term allergic asthma as the basic term for asthma mediated by immunologic mechanisms (Figure 2). The organization further suggests that when there are indications of IgE-mediated mechanisms, the term should be IgE-mediated

asthma [2]. In addition to IgE, several other immunologic factors such as IL-4 and IL-13 are involved in the etiology of asthma.

Immunology

For the purpose of this work, the immunology of asthma is here discussed within the framework of the type 1 and 2 helper T cells (Th1/Th2) model. This model/paradigm was born out of a series of experiments developed by Tom Wegmann [11] as he perused the placenta in search of clues that migt explain spontaneous abortion. To date, mechanistic explanations for allergy have often invoked the Th1/Th2 paradigm [12,13 14,15].

Theoretically, the immune system 'idles' in a Th0 or naïve state until it is required to change response by some external stimuli (Figure 3). These stimuli may be in the form of environmental antigens in which case non-allergic Th1 response is initiated, leading to secretion of IgG antibodies, removal of the allergen, and the suppression of IgE synthesis [16,17]. In addition

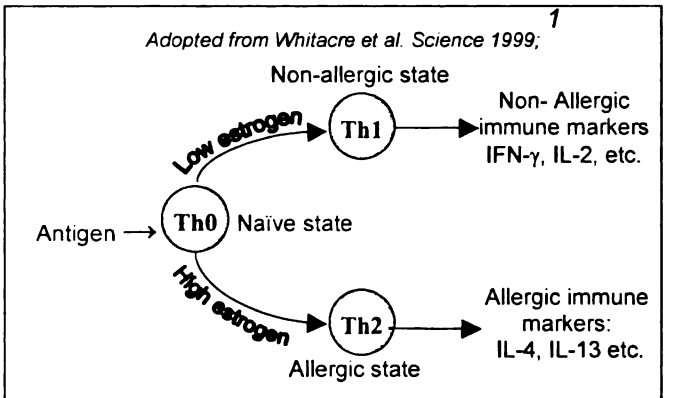

Figure 3 Theoretical immune responses

to antigens, hormones may cause a shift in immune response. However, it is believed that sex hormones such as progesterone and high concentration of estrogen may result in an allergic – Th2 type response [1] (Figure 3).

There is a general consencious in the literature that a shift to a Th2 polarize state occurs during dramatic changes in immune responses associated with pregnancy. To prevent termination of the fetus (removal of the allergen), the feto-maternal immune system shifts to a Th2 direction [18,19]. This Th2 shift begins with the secretion of human chorionic gonadotropin, which in turn stimulates progesterone production. Progesterone then promotes the secretion of Th2 cytokines over the secretion of phagocytic [20] Th1 cytokines. In other words, progesterone is believed to prime the immature fetal immune system towards an allergic direction which may be manifed as allergic diseases (such as asthma) during infancy.

Fundamentally, the underlying problem in asthma appears to be immunologic with children in the early stages of asthma showing signs of excessive imflamation in their airways. While chemokines (*a subset of cytokines that chemically attracts immune cells*) are important in the recruitment of imflamatory cells to the airway, the lymphocytes – particularly those producing Th2 cytokines – are responsible for

Box 2 Select markers of immune response

Th1 response:

IgG - immunoglobulin G
IFN-γ - interferon gamma
IL-2 – interleukin 2
IgA - immunoglobulin A
IgM - immunoglobulin M

Th2 response:

IgE – immunoglobulin E
IL-4 - interleukin 4
IL-13 - interleukin 13

directing and maintaining this inflammatory process [21]. Therefore, the cardinal phenotypic features of allergy are associated with the production of the Th2 cytokines IL-4 and IL-13 which are central to the synthesis of IgE; the forerunner of allergic manifestations (Box 2). Of note, recent reports indicate that cord blood mononuclear cells (CBMC) produce IL-13 and that cord blood IL-13 is associated with allergy in childhood [22,23]. Further evidence suggests that IL-13 can participate in allergies independent of IL-4 [24].

As mentioned earlier, another important marker of a Th2 response is IgE, which is central to the etiology of allergic diseases such as allergic asthma. In allergic individuals, specific IgE is bound to high affinity receptors on mast cells and is cross-linked by antigen; mast cell activation/degranulation occurs and sets in motion a cascade of events resulting in the clinical manifestations of allergic disease [25]. The interaction of IgE and antigen results in an immediate hypersensitivity reaction may be responsible for the classic asthma symptoms exhibited during acute exacerbation, for example, mucosal edema, mucus production, and smooth muscle constriction. Eventually, these and other reaction cascades can induce the production of cells responsible for airway inflammation that underlies allergic asthma [26]. Nonallergic-asthma, conversely, manifests all the clinical feature of allergic asthma without the IgE mediated immunologic aspect. At a population level, it is estimated that 50% of all asthma cases have

an allergic etiology [8,27]. Regarding Non-IgE-mediated asthma (Figure 2) this subtype may be characterized by the following four criteria:

1) clinical phenotype of IgE mediated asthma;
2) absence of other allergic diseases such as allergic dermatitis and rhinoconjunctivitis;
3) negative sensitization (Box 1) to common inhalant and food allergens ; and
4) normal total serum IgE levels.

In contrast to a Th2 response, a Th1 response leads to secretion of IgG antibodies, removal of the allergen, and the suppression of IgE synthesis [28-30]. Other immune markers are associated with a Th1 type response (Box 2). In general, Th1-responses are highly protective against infections mounted by the majority of microbes.

It is widely accepted that changes leading to allergic disorders such as allergic asthma begin in childhood, thus it is important to study the natural history of childhood asthma in an effort to identify risk factors associated with its development.

Asthma in children

Asthma may develop during the first few months of life, but it is often difficult to make a definite diagnosis until the child is older [4]. Approximately 30 % of asthmatic patients are symptomatic by one year of age, 80-90 % have their first symptoms before four to five year of age. The majority of affected children have

only occasional attacks of slight to moderate severity, which are managed with relative ease. A minority experience severe, intractable asthma, usually perennial rather than seasonal. The relationship of age of onset to prognosis is uncertain. Most severely affected children have an onset of wheezing during the first year of life and a family history of asthma and other allergic diseases (particularly atopic dermatitis) [31].

Prevalence and burden of asthma

Asthma remains an enormous personal and public health concern [32]. Asthma symptoms in children vary in prevalence from 0% to 35% in different populations [33]. The prevalence of asthma symptoms is on average 14% among children aged 6-7 years and 18% among those aged 13-14 years [33]. Overall, the prevalence and severity of asthma and other allergic diseases is increasing [4,34].

Globally, approximately 300 million people currently have asthma and it is estimated that there may be an additional 100 million more asthmatics by 2025 [9]. Furthermore, asthma accounts for about one in 250 deaths worldwide.

In the United States (US), asthma affects more than 17 million individuals [35] and results in approximately 10 million visits to physicians, 2 million emergency room visits and approximately 7,000 deaths annually [36]. The annual cost of caring for

asthmatics exceeds six billion dollars per year in the US, and the worldwide market for asthma medication is currently valued at 5.5 billion dollars each year [37]. It is noteworthy that the cost of asthma should be divided into direct and indirect cost. Direct costs include the costs of medication, medical bills, and documented episodes of health service utilization such as clinic visits and hospital admissions. Indirect costs include the adverse economic impact of the disease on an individual, family, and society. This includes the "cost" of premature mortality and productivity loss. In the US, the cost of medical treatments for asthma is on average 10% of total family income [38 39].

The economic cost of asthma is alarming both in terms of direct medical costs (drugs, hospitalization) and indirect medical costs (time lost from work and premature death). The number of disability-adjusted life years (DALYs) lost due to asthma worldwide is currently estimated about 15 million/year, and asthma accounts for around 1% of all DALYs lost, reflecting the high prevalence and severity of asthma [9]. Asthma in general is incapacitating and interferes with school attendance, play activity, and day-to-day functioning. In a 1988 National Health Interview Survey, children with asthma missed 10.1 million days from school (2 times the number of days missed by children without asthma), and had 12.9 million contacts with medical doctors and 200,000 hospitalizations [40]. In the same survey, it was determined that almost 30% of children with asthma

experienced some limitation of physical activity, compared to only 5% of children without asthma [41].

Risk factors for asthma

Risk factors for asthma may be classified as **host factors** that predispose individuals to developing asthma, and **environmental factors** that influence the susceptibility to the development of asthma in predisposed individuals, precipitate asthma exacerbations, and/or cause symptoms to persist [4].

Host factors

These include sex and race along with the genetic predisposition to the development of either asthma or allergic sensitization.

Sex

Sex biases in the susceptibility to, and severity of, autoimmune and allergic diseases are well-recognized [7]. Preadolescent boys have about 1.5 times the asthma prevalence of girls [42-44]. However, after adolescence, the sex difference is reversed [45]. Some authors suggest that the increased risk for males in childhood is probably related to narrower airways, increased airway tone [46-48], and possibly higher IgE [44] in boys. These factors, it is believed, predispose boys to enhanced airflow limitation in response to a variety of insults. More recently, other studies have suggested that the observed difference may be due to endocrine influences [1,7]. In an effort to explain this difference, some authors [49-51] postulate that the acquired immune system of females differs from that of males, because estrogens stimulate immunologic processes driven by CD4 + TH2 cells and B cells, whereas androgens enhance CD4 + TH1 and CD8 + cell

activity. Androgens are also known to promote production of IL-2 by TH1 cells. Interleukin 2 reduces TH2 activity and stimulates CD8 + T cells [52]. Further discussion about the possible role of estrogen as an immune modulator is presented in section **'Estrogen and progesterone.'**

Race

Most experts agree that race as a social construct represents a continuum rather than a term with clear-cut boundaries, a point that has been illustrated by large-scale genotyping among diverse biogeographic populations [53].

Asthma morbidity and mortality is disproportionately high and continues to increase among African Americans [54]. As documented by national surveys, there are higher rates of asthma in black than White children, a disparity that has widened since 1980 [55]. Simon and co-workers also found that among children with asthma, Blacks were more likely than Whites to report asthma-related limitations in physical activity and need for urgent medical services [56].

Furthermore, at the molecular level, authors have detected increased expression of the CD80/CD86 lymphocytes in African American adults following mitogen and antigen stimulation [57]. CD80/86 are crucial molecules in T-cell activation [58]. This finding was supported by that of Willwerth et al. [58] which showed a more generalized increased T-cell responsiveness that included responsiveness to the mitogen phytohemagglutinin and to the allergens in children of black ethnicity.

These reports give credence to the idea that differential neonatal immune responses may underlie the higher incidence of asthma in blacks.

Genetic predisposition

There is evidence that asthma has genetic components [4]. A number of studies have shown an increased prevalence of asthma phenotypes among the offspring of subjects with asthma compared to the offspring of subjects without [59] [60]. Family studies have convincingly indicated that atopy (as measured by allergen skin tests, total IgE, and/or specific IgE), airway hyper-responsiveness, and asthma as diagnosed by questionnaire are at least partly under genetic control [59] [61].

Numerous studies of twins have demonstrated that concordance rates for asthma phenotypes are all substantially higher for monozygotic than for dizygotic twins, suggesting a strong genetic contribution. In population-based studies of twins, the estimated effect of genetic factors is about 35 to 70 %, depending on the population and the design of the study [59,60].

Environmental factors

Early attention to environmental factors that may influence the susceptibility to the development of asthma in predisposed individuals focused on the effect of allergens, air pollution and tobacco smoke. Recently, the role of environmental SHD (synthetic hormones: estrogen and progesterone as well as toxicants: halogenated organochlorine compounds (HOCs) such as dichlorodiphenyl

dichloroethene (DDE) and polychlorinated biphenyls (PCBs)) in the etiology of allergy has gained increased attention [62-69].

Exposure to tobacco smoke

Tobacco burning, a ubiquitous source of irritants, produces a large and complex mixture of gases, vapors, and particulate matter. More than 4,500 compounds and contaminants have been identified in tobacco smoke, among them respirable particles, polycyclic hydrocarbons, carbon monoxide, carbon dioxide, nitric oxide, nitrogen oxides, nicotine, and acrolein [4].

There is evidence that both maternal smoking during pregnancy and passive smoke (environmental tobacco smoke (ETS)) exposure after pregnancy affect asthma later in offspring [70-74]. Actually, ETS was reported to increase both prevalence and severity of asthma, as judged by increases in the frequency of attacks, the number of emergency room visits and the risk of intubations [75]. Using data from a large cohort study (n~60,000), Jaakkola et al. demonstrated that maternal smoking in pregnancy increases the childhood risk of asthma [76].

A large study of 20,000 children (aged 6 to 12 years) from nine countries in Europe and North America, reported that smoking during pregnancy decreases lung function parameters [77]. The effects of past and current passive exposure to tobacco smoke were smaller than the effect of smoking during pregnancy. The aforementioned study strongly suggests that maternal smoking during pregnancy

subsequently affects the lung function and intuitively asthma in children. However, in a cohort of New Zealand children, Sherill et al., [78] found no significant detrimental effects for absolute forced expiratory volume (FEV), or vital capacity related to ETS (including *in utero*) exposure. Likewise, Cunningham et al., [79] compared lung function, measured by FEV1, and were unable to find significant reduction.

Breast-feeding

Ever since Grulee and Sanford in 1936 [80] first reported that breastfeeding protects against an infantile allergic response, researchers have been exploring this topic. Some authors reported that breastfeeding appears to protect against atopic manifestations [81-86] while others found no such association [87-92]. To-date, despite numerous studies, the search for consensus continues [81,82,93].

Breast milk, as a functional food, is thought to contain its own complex immune system [94]. The selective colonization of the mammary gland during lactation by a population of T lymphocytes which displays the phenotype and functional characteristics of memory T cells may be one of the mechanisms whereby the suckling infant benefits from its mother's immunological history [95]. Cytokines and chemokines derived from milk cells and mammary gland epithelium may

contribute to the activation of intestinal T lymphocytes to enhance immunity during the early neonatal period [96].

Findings from a population-based case-contral study (cases=2,315 and control =21,513) of 6–15 year old Japanese children showed a significantly higher prevalence of asthma among children who had been breastfed (adjusted OR = 1.2; 95% CI: 1.05, 1.36). Conversely, a systematic review of prospective studies evaluating the association between exclusive breast-feeding during the first 3-months after birth and asthma, reported opposite findings. The aforementioned study found summary odds ratio (OR) for the protective effect of breast-feeding to be 0.70 (95% CI 0.60, 0.81) thus concluding that exclusive breast feeding during the first months after birth is associated with lower asthma rates during childhood [81].

Maternal allergy

Children born to parents with allergic diseases present an increased risk of developing similar diseases [75,97]. The proportion of children of two allergic parents can be as high as 58 %, when one parent is allergic it is about 29% and a child with neither parents having history of allergy still has about 13 % risk of developing allergic disease sometime during his life [98]. However, maternal atopy is a stronger predictor of childhood allergy that paternal. Mother's atopic history was found to be significantly associated with cord-blood IgE [99]. Likewise, maternal history of atopy was associated with an elevated IgE among newborns.

For maternal asthma, this association was only evident in infant girls [100].

Recently, Kuiper et al. found that maternal asthma was associated with elevated total IgE at all total IgE cut-off levels studied except 0.5 IU/mL [101]. This finding was supported by Shah et al. who report a significant association between maternal history of allergic disease and elevated cord serum IgE among newborns [98].

Environmental sex hormone disrupters

A balanced endocrine system is essential for optimum health. Sex hormones are considered crucial in maintaining this balance. Therefore any disruption/alteration of these hormones could intuitively bring about adverse health effects such as allergic diseases [7] or even death. A more extensive discussion on sex hormone disrupters and their possible role in the etiology of diseases is presented on the next page.

Summary

The prevalence and severity of allergic diseases continue to increase. Sex is an important risk factor with evidence showing that asthma is more prevalent in boys throughout childhood, but in adolescence a gender reversal occurs. African-Americans are disproportionately affected while the idea of a genetic predisposition to allergy continues to intrigue researchers. There is virtually universal agreement that tobacco smoke represents a significant risk factor for allergies. Conversely, the 'verdict' on breast-feeding is inconclusive. Mothers

with allergies tend to have children with allergies: clinical and/or immunologic (increased IgE). Sex hormone disrupters have been implicated in the etiology of allergic disorders. Recently, a plethora of studies have focused on this area in an effort to explain the rising prevalence of allergies.

1.3 Sex hormone disrupters

One difficulty experienced when considering endocrine disruption is the lack of consensus as to an appropriate definition for the term. Various definitions have been suggested including those of the US EPA (Kavlock et al., 1996) and a European workshop (EC, 1997). For this dissertation, the approach taken by the European workshop was adopted and modified to apply specifically to chemical disruption of the endocrine system: "A sex hormone disrupter is an exogenous substance that causes adverse health effects of an intact organism or its progeny, consequent to changes in endocrine function."

Sex hormones are synthesized mainly by endocrine glands such as the gonads (testis and ovary), the adrenals and (during gestation) by the fetoplacental unit, and are then released into the blood circulation.

The biological hormonal properties of these substances have earned them a great deal of attention in the past decade as possible etiologic agents of certain diseases. Some of these chemicals display estrogen like activity, hence their importance in endocrine disruption and subsequent immune modification is closely scrutinize. Sources of these xenobiotics includes life style practices such

as diet (e.g., phytoestrogens coumestrol and genestein), oral contraceptive use or from the environment (e.g., dichlorodiphenyl dichloroethene (DDE), and polychlorinated biphenyls (PCBs). Sex hormones or similar substances from the above-mentioned sources may be classified as endocrine disrupters (EDs). These endocrine disrupters alter normal hormonal regulation and may be naturally occurring or environmental contaminants[103].

There are two classes of substances that may cause disruption: 1) Synthetic hormones; mainly estrogen and progesterone as in the case of oral contraceptives (Ocp), and 2) toxicants including the halogenated organochlorine compounds (HOCs): DDE & PCBs. It has been broadly suggested that these substances bring about adverse effects in at least two ways: by mimicking the action of a naturally-produced hormone and thereby setting off similar bio-chemical reactions and/or by affecting the synthesis, transport, metabolism and excretion of hormones, thus altering the concentrations of natural hormones[104]. However, there is yet to be a clear mechanistic understanding of how these proposed sex hormones disrupt the immune system. Recent attention has focused on the role of the 'promiscuous' estrogen receptors (ERs) through which sex hormone disrupters may communicate with cells prior to altering immune response.

Estrogen and progesterone

There is mounting evidence that the immune system is regulated in part by sex steroid hormones such as estrogen and progesterone. It is known that proper immune function is critical for the delivery of a healthy baby a process that is also believed to be of importance in the etiology of immune diseases. The involvement of estrogen is hinged on the fact that estrogen receptors are found on T lymphocytes: Families of specialized white blood cells that help orchestrate the body's immune responses. Generally, estrogens are seen as enhancers at least of the humoral immunity [49]. Furthermore, many of the allergic family of cytokines are under the control of estrogen [105]. Estrogen and progesterone appears to work in tandem. In order for progesterone to work on the immune system, progestin receptors must be synthesized on the reticuloendothelial cells (cells which trap and consume foreign agents, except leukocytes circulating in the bloodstream) of the thymus [106]. Such receptors are usually the result of estrogen stimulation.

Under normal circumstances, increased estrogen leads to an increase in INF-γ concentration, presumably as a mechanism of feedback inhibition to prevent unopposed stimulation of TH2 cells [107]. Estrogen also stimulates secretion of IL-4, -5, -6, and -10 by TH2 lymphocytes. These cytokines are potent stimulators of B-cell proliferation, maturation into plasma cells, and synthesis of antibody.

Ha

To

foc

pro

Interleukins 4, 5, 6, and 10 are expressed in greater quantity in an estrogen-dominant hormonal milieu [50,52,108,109].

Progesterone seems to promote the preferential development of Th2 cells and to induce IL-4 production [110-113]. Th2 type cytokines are considered vital for the maintenance of pregnancy by controlling the immune and endocrine systems [19,114]. Simultaneously, it is this ability of progestin to invoke this Th1/Th2 shift that makes it important in the etiology of allergic diseases. However, the tight interdependence between estrogen and progestin secretions during the ovarian cycle and concomitant sex hormone hypersecretion during pregnancy pose substantial difficulties for the assessment of the individual contribution of physiological changes in progestin levels to immune regulation.

Epidemiologically, Michel and colleagues reported that progestin administered during pregnancy increased the levels of cord blood IgE in 136 neonates: when mothers received progesterone, 53% of the newborns had detectable IgE (<0.5 IU/ml) versus only 24% of the newborns in mothers without progesterone administration [97].

Halogenated organochlorine compounds (HOCs)

To date studies assessing the role of HOCs in altered immune response have focused mainly on their estrogen-like effects. However, some authors have proposed that the estrogenic activity of environmental toxicants is very low,

relative to estradiol, and thus unlikely to make significant contribution to the etiology of diseases [115,116]. This view has been challenged by reports of low dose effects of estrogenic toxicants [117,118] while others have been unable to confirm these effects [119,120]. Nonetheless, environmental toxicants continue to be regarded as important contributing factors in the pathogenesis of allergic diseases.

Dichlorodiphenyldichloroethene (DDE)

Dichlorodiphenyltrichloroethane (DDT) was originally prepared in 1873, but it was not until 1939 that Paul Muller of Geigy Pharmaceutical in Switzerland discovered the effectiveness of DDT as an insecticide [121]. The use of DDT increased enormously worldwide after World War II, primarily because of its effectiveness against the mosquito and lice. The World Health Organization estimates that during the period of its use approximately 25 million lives were saved predominantly from malaria and typhus. However, many species of insects developed resistance to DDT; it proved to have a high toxicity toward fish; and it was responsible for the near extinction of several bird species because of its interference with the formation of egg shells. For these reasons and because of its environmental persistence, the use of DDT was banned in the United States in 1972. However, DDT is still in use in many countries (including some South American, African countries) as an insecticide [122].

Humans are exposed to DDT mainly through foods, and infants through the placenta and breast-feeding [123]. DDE, a metabolite of DDT, is persistent and is stored in fat tissue. The long half-life of DDE accounts for its ubiquity in the general population [124,125]. Though exposure is of relatively low toxicity, DDE does have troubling effects as it is known to have weak estrogenic [126] and considerable anti-androgenic activity resulting in its endocrine disrupting capabilities [127-131]. A more detailed description of DDE possible effect on the etiology of allergic diseases is presented in the 'Literature Review of Components Comprising the Conceptual model' section.

Polychlorinated biphenyls (PCBs)

PCBs are mixtures of up to 209 individual chlorinated compounds (known as congeners). There are no known natural sources of PCBs. PCBs are either oily liquids or solids that are colorless to light yellow. Some PCBs can exist as a vapor in air. PCBs have no known smell or taste. Many commercial PCB mixtures are known in the U.S. by the trade name Aroclor.

PCBs have been used as coolants and lubricants in transformers, capacitors, and other electrical equipment because they do not burn easily and are good insulators. The manufacture of PCBs was stopped in the U.S. in 1977 because of evidence they build up in the environment and can cause harmful health effects. Products made before 1977 that may contain PCBs include old fluorescent

lighting fixtures and electrical devices containing PCB capacitors, and old microscope and hydraulic oils [132].

Residues have been detected in foods and in human adipose tissue, milk, and serum fat [133]. Reports of PCBs having estrogenic effects [129] and altering immune responses have been recorded.

Reichrtova et al. [65] reported that higher levels of the congener PCB118 and DDE in human placentas are associated with increased cord blood IgE levels. Specific to PCBs and various congeners: A study using pregnant minks documented that serum progesterone concentrations were significantly increased by experimental exposure to PCB-153 [134]. Similarly, an in vitro study documented increased secretion of progesterone from ovaries after exposure to PCB-153 [135,136]. In seals, progesterone metabolism was significantly decreased with increasing liver PCB concentrations [137]. In polar bear dams, the plasma concentration of total PCBs is associated with an increase in placenta progesterone and accounts for 27% of the variance in the progesterone concentration [138].

Sex hormones and immunophysiology - Conclusion

There is an abundance of evidence suggesting that the sex hormone disrupters DDE, PCBs, and synthetic progesterone, and estrogen may bring about alteration of normal endocrine functions resulting in allergic manifestations.

However, no clear unifying hypothesis exists to explain how sex hormone disrupters affect the development and function of the immune system.

1.4 Time-window of exposure

In an attempt to sort out the complex interrelationships between sex steroid hormones and allergic diseases, this work will focus on two important time-windows: the *in utero* and perinatal.

The *in utero* environment sets the stage for prenatal priming of allergic diseases. This concept (prenatal priming) considers fetal life as the critical period of exposure and suggests that *in utero* exposure to environmental factors such as sex steroid hormones are important for the rise in the prevalence of asthma and allergy [139-141].

The prenatal priming concept

'Priming or programming' describes the process whereby a factor at a critical period of development has lasting effects [142]. As an example, dramatic changes in maternal immune responses occur during pregnancy. To avoid aborting the fetus, the feto-maternal immune system is shifted in an allergic/Th2 direction [18,19]. This Th2 reaction is characterized by the secretion of cytokines which then promote IgE production, resulting finally in allergies later in life. Hormones are believed to be responsible for controlling this shift [105].

In assessing the in utero impact of sex steroid hormones on allergies, this dissertation investigates associations between: placental organochlorine and cord blood markers of allergy; maternal oral contraceptive use and offspring's serum allergic markers followed by an investigation of maternal oral contraceptive use and allergic phenotypes.

The postnatal priming concept

Seeing that hormones may play an important in the prenatal priming of allergies, it is plausible to postulate that postnatal exposure may also have a role in the etiology of allergies. Using serum OC, this work further determined the importance of postnatal exposure to sex steroid hormones in altering allergic markers and henceforth the development of asthma.

A theoretical construct outlining the components of both processes (pre- and postnatal), are presented in the forthcoming section.

1.5 Components of the conceptual model

The etiology of asthma, for example, is the result of a complex conflation of events. This section presents a model for assessing one of these complex processes: the involvement of sex hormone disrupters. The components of this model span the pre – and postnatal periods while using a combination of qualitative and quantitative measures of exposure and outcome. Specific pieces of evidence provided are: **First,** evidence that prenatal SHD exposure can disrupt allergic markers measured *in utero.* For this piece of evidence, placental *p,p'* DDE was used as measure of exposure while cord plasma IL-4, IL-13 and

INF-γ (cytokines typical of the atopic response) as measures outcome. This will show the effect of SHD on allergic markers that cannot be attributed to postnatal influences. Note, both exposure (placental *p,p'* DDE) and outcomes (IL-4, IL-13 and INF-γ) are quantitative measures. The hypotheses behind this piece of evidence are: Increased placental p,p'-DDE concentration is associated with an increase in cord plasma IL-4 and IL-13, and that increased placental p,p'-DDE concentration will lead to a reduction in cord plasma INF-γ. **Second,** existence of an association between proxy measures of *in utero* exposure to sex hormone disrupters and postnatal markers of allergic susceptibility. This bit of evidence suggests that the possible *in utero* immunomodulating effect of sex hormone disrupters may persist into child hood. **Third,** as an extension of the second condition above, evidence is required linking proxy measures of *in utero* exposure to sex hormone disrupters (maternal oral contraceptive use) with markers of effect: clinical manifestations of allergy such as asthma-like symptoms and atopic eczema in childhood. This along with evidence from the second condition will provide further support for the idea that sex steroid hormones may be associated with allergic disorders by modulating the immune status, through endocrine disruption. **Fourth,** serum organochlorines stimulate an allergic response. Results from the serum DDE / markers of allergic susceptibility association add a new level of support to the sex hormone disrupters / immune modulating idea in that both exposure and outcome are measured postnatally. This suggests that the effects of sex hormone disrupters do not only occur during the early stages of immune development (*in utero*) when

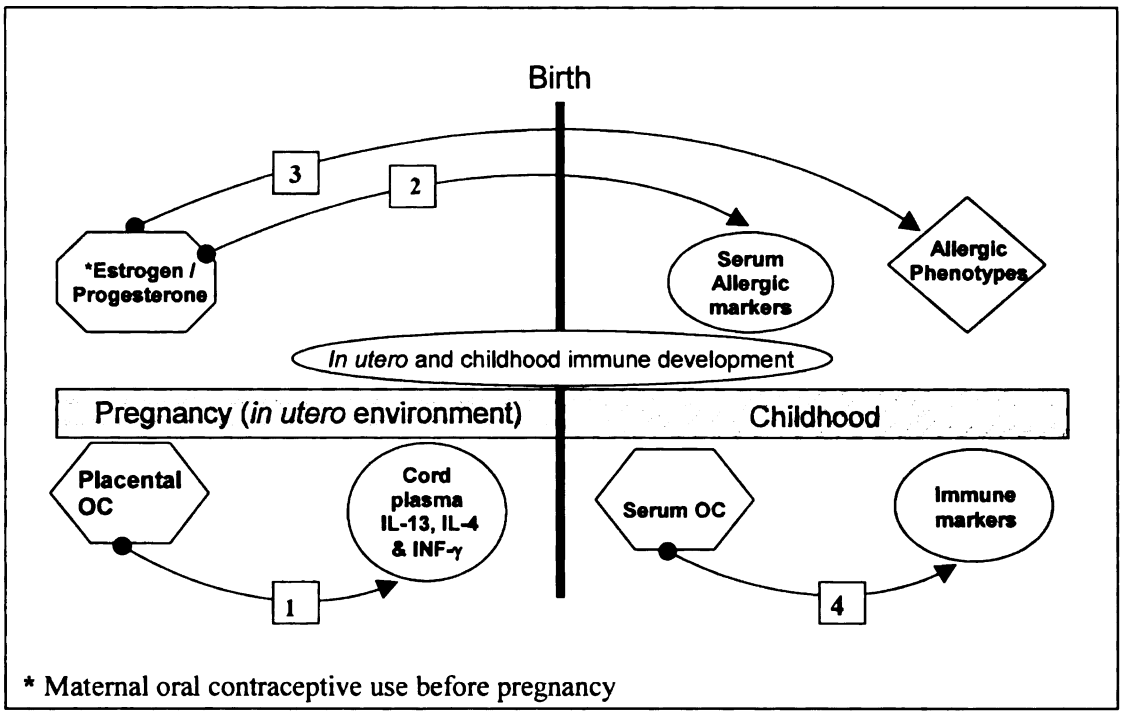

Figure 1 Sex Hormone Disrupters and Childhood Allergy - Conceptual model

the cells are more susceptible to change but may also produce change in immune development postnatally. A schematic representation of a proposed model that encapsulates the overarching theme of SHD and childhood allergy is presented in Figure 1 and discussed in the following background section.

1.6 Literature review of components comprising the conceptual model.

The effect of placental p,p' DDE on cord plasma IL-4, IL-13 & INF-γ.

There is evidence that the cytokine IL-13 is both necessary and sufficient to induce all features of allergic asthma [143]. In humans, this was supported by consistent associations between tissue IL-13 levels and genetic variants in the IL-13 gene with asthma and related traits [143]. Cellular response (including IL-4, IL-13, and IFN-γ,) to allergens have been reported at week 23 gestation [144].

Recent findings suggest that in the etiology of allergic diseases, the Th2/Th1 ratio may be of more importance that individual cytokines [145].

DDE is known to cross the placenta [146] potentially interfering with fetal immune development. This interference is thought to be via its ability to reduce the binding of progesterone to its receptor [147].

Prenatally, Noakes and co-workers suggest a possible immunomodulating effect of cord blood DDE on phytohaemagglutinin mitogen stimulated cord blood IL-13 and IFN-γ [148]. However, findings on the effect of placental *p,p'* DDE on cord plasma cytokines and their ratios (Th1/Th2) are yet to be reported.

In utero exposure to sex hormone disrupters is related to immune markers of allergic susceptibility in childhood.

To date, Frye et al. is the only team to report on the association between maternal OCp use and immune markers (specific immunoglobulin E against common inhalant allergens) in offspring. For atopic sensitization, they reported similar effects for maternal OCp use before and after birth. This finding raises doubts in the previously reported association [93,105,149] between maternal hormone level and allergic diseases.

In an effort to allay these doubts, this dissertation investigates the association between maternal oral contraceptive use and humoral immune markers (such as serum immunoglobulin (Ig)-A, IgE and basophilic surface IgE counts) in offspring.

In utero exposure to sex steroid hormones and their relation to childhood markers of allergic effect.

Studies assessing the association between *in utero* exposure to sex hormone disrupters (using maternal oral contraceptive use as proxy) and allergic phenotypes have reported inconsistent findings.

In 1987, Peters and Golding, presented findings suggestive of an increased risk of childhood eczema for children of mothers who used OC in the 18 months prior to pregnancy in a cohort of 10,900 5-year-old children [93].

Approximately ten years later, using data from an aggregative (ecological) study, Wjst et al. found a geographic trend between asthma prevalence in children and maternal OCp use. This similarity was most notable during 1970 when a sharp decrease in OCp use was followed by a similar decrease in rate of hospital discharge with a diagnosis of asthma. They concluded that mother's OCp use fits well into the geographic and temporal background of this increase in asthma prevalence [105].

In addition, findings from a geographically defined cohort of 5,188 subjects showed that the prevalence of atopy at age 31 years was lower in children whose mothers reached menarche at a later age, especially after age 15 [149]. Early age of menarche is associated with higher levels of estrogen in adulthood [150-152].

Contrary to the above findings, Xu et al. [153] found no differences in the mean concentrations of maternal sex steroid hormones according to the presence of allergic rhinitis or atopic eczema among offspring. In addition, while Frye et al. found maternal OC use before birth to be risk factor for asthma, allergic rhinitis, and atopic eczema; they also found similar effects for OC use after birth [69].

To date, these associations have only been tested using data from predominantly Caucasian cohorts. Assessing this association in a cohort with a different ethnic profile may provide cogency to the controversy. It is in this spirit that we investigated the *in utero* sex hormone exposure / allergic phenotypes association using data form an African-Caribbean cohort.

Immunologic consequence of childhood exposure to organochlorine.

Previously, in this monograph, we proposed the assessment of prenatal exposure to DDE and its association with allergic markers in childhood. This, as mentioned, provided evidence in favor of the prenatal priming concept; purporting that DDE is able to alter immune status during its early stages of development. In this section we extend this idea by determining whether DDE is also able to impose a similar effect postnatally.

There are reports of DDE being associated with changes in cellular and humoral immunity [154,155], in particular cytokines related to allergy, such as interleukin-4 [156,157]. In addition, using data from the Child Health and Environment Cohort

Study - Germany, Karmaus et al. showed serum DDE to be strongly related with increases in total IgE [66]. All the above-mentioned studies focused on adult populations, with the exception of that of Karmaus et al. However, this study in children only reported on one allergic marker – total IgE. Here, using the same study of Germany school children, we investigated whether serum DDE concentrations is associated with several immune markers including IgE, IgG, and IgA levels, IgE count on basophils along with eosinophilic granula, and white blood cell count.

References

1. Whitacre CC, Reingold SC, O_Looney PA. A gender gap in autoimmunity. Science 1999;283(5406):1277-8.

2. Johansson SG, Bieber T, Dahl R, Friedmann PS, Lanier BQ, Lockey RF, Motala C, Ortega Martell JA, Platts-Mills TA, Ring J, Thien F, Van Cauwenberge P, Williams HC. Revised nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. J Allergy Clin Immunol 2004;113(5):832-836.

3. Galli SJ. Allergy. Curr Biol 2000;10(3):R93-5.
4. National institutes of Health, LaBI. NH. GLOBAL STRATEGY FOR ASTHMA MANAGEMENT AND PREVENTION., 2005.

5. MERCKMedicus. Updated 2006. Available at: http://www.merckmedicus.com

6. Elphick HE, Sherlock P, Foxall G, Simpson EJ, Shiell NA, Primhak RA, Everard ML. Survey of respiratory sounds in infants. Arch Dis Child 2001;84(1):35-39.

7. Shames RS. Gender differences in the development and function of the immune system. Journal of Adolescent Health 2002;30(4):59-70.

8. Kuehr J, Frischer T, Karmaus W, Meinert R, Barth R, Urbanek R. Clinical atopy and associated factors in primary-school pupils. Allergy 1992;47(6):650-5.

9. Humbert M. Asthma, a priority for the allergist. Allergy 2006;61(5):515-7.

10. Murray DM, Lawler PG. All that wheezes is not asthma. Paradoxical vocal cord movement presenting as severe acute asthma requiring ventilatory support. Anaesthesia 1998;53(10):1006-11.

11. Chaouat G, Ledee-Bataille N, Dubanchet S, Zourbas S, Sandra O, Martal J. TH1/TH2 paradigm in pregnancy: paradigm lost? Cytokines in pregnancy/early abortion: reexamining the TH1/TH2 paradigm. Int Arch Allergy Immunol 2004;134(2):93-119.

12. Maggi E. The TH1/TH2 paradigm in allergy. Immunotechnology 1998;3(4):233-44.

13. Wills-Karp M, Santeliz J, Karp CL. The germless theory of allergic disease: revisiting the hygiene hypothesis. Nat Rev Immunol 2001;1(1):69-75.

14. Robinson DS, Larche M, Durham SR. Tregs and allergic disease. J Clin Invest 2004;114(10):1389-97.

15. Romagnani S. Immunologic influences on allergy and the TH1/TH2 balance. J Allergy Clin Immunol 2004;113(3):395-400.

16. Beeh KM, Ksoll M, Buhl R. Elevation of total serum immunoglobulin E is associated with asthma in nonallergic individuals. Eur Respir J 2000;16(4):609-14.

17. Baena-Cagnani CE, Teijeiro A. Role of food allergy in asthma in childhood. Curr Opin Allergy Clin Immunol 2001;1(2):145-9.

18. Gaunt G, Ramin K. Immunological tolerance of the human fetus. Am J Perinatol 2001;18(6):299-312.

19. Thellin O, Coumans B, Zorzi W, Igout A, Heinen E. Tolerance to the foeto-placental 'graft': ten ways to support a child for nine months. Curr Opin Immunol 2000;12(6):731-7.

20. Romagnani S. The role of lymphocytes in allergic disease. journal of allergy and clinical immunology 2000;105(3):399-408.

21. Monaco C, Andreakos E, Kiriakidis S, Feldmann M, Paleolog E. T-cell-mediated signalling in immune, inflammatory and angiogenic processes: the cascade of events leading to inflammatory diseases. Curr Drug Targets Inflamm Allergy 2004;3(1):35-42.

22. Ohshima Y, Yasutomi M, Omata N, Yamada A, Fujisawa K, Kasuga K, Hiraoka M, Mayumi M. Dysregulation of IL-13 production by cord blood CD4+ T cells is associated with the subsequent development of atopic disease in infants. Pediatr Res 2002;51(2):195-200.

23. Williams TJ, Jones CA, Miles EA, Warner JO, Warner JA. Fetal and neonatal IL-13 production during pregnancy and at birth and subsequent development of atopic symptoms. J Allergy Clin Immunol 2000;105(5):951-9.

24. Wynn TA. IL-13 effector functions. Annu Rev Immunol 2003;21:425-56.

25. Siraganian RP. Allergy: principles and practice. . 5th ed. ed. St.Louis: C V Mosby, Inc, 1998.

26. Oettgen HC, Geha RS. IgE in asthma and atopy: cellular and molecular connections. J Clin Invest 1999;104(7):829-35.

27. Ponsonby AL, Gatenby P, Glasgow N, Mullins R, McDonald T, Hurwitz M. Which clinical subgroups within the spectrum of child asthma are attributable to atopy? Chest 2002;121(1):135-42.

28. Renz H. The central role of T-cells in allergic sensitization and IgE regulation. Exp Dermatol 1995;4(4 Pt 1):173-82.

29. Daser A, Meissner N, Herz U, Renz H. Role and modulation of T-cell cytokines in allergy. Curr Opin Immunol 1995;7(6):762-70.

30. Erb KJ. Atopic disorders: a default pathway in the absence of infection? Immunol Today 1999;20(7):317-22.

31. Behrman RE, Kiliegman, R. M., Jenson, H. B. Nelson Textbook of Pediatrics. 17 ed W.B. Saunders Company, 2004.

32. Guilbert T, Krawiec M. Natural history of asthma. Pediatric Clinics of North America 2003;50(3):523-38.

33. Johnson CC, Ownby DR, Zoratti EM, Alford SH, Williams LK, Joseph CLM. Environmental epidemiology of pediatric asthma and allergy. Epidemiologic Reviews 2002;24(2):154-175.

34. Beasley R, Crane J, Lai CK, Pearce N. Prevalence and etiology of asthma. The Journal of Allergy and Clinical Immunology 2000;105(2 Pt 2):S466-72.

35. MMWR Morb Mortal Wkly Rep. Forecasted state-specific estimates of self-reported asthma prevalence-United States. MMWR Morb Mortal Wkly Rep, 1998;47:1022–5.

36. Mannino DM, Homa DM, Akinbami LJ, Moorman JE, Gwynn C, Redd SC. Surveillance for asthma--United States, 1980-1999. MMWR Surveill Summ 2002;51(1):1-13.

37. Palmer LJ, Cookson W. Genomic approaches to understanding asthma. Genome Research 2000;10(9):1280-1287.

38. Marion RJ, Creer TL, Reynolds RVC. Direct and Indirect Costs Associated with the Management of Childhood Asthma. Annals of Allergy 1985;54(1):31-34.

39. Vance VJ, Taylor WF. The financial cost of chronic childhood asthma. Ann Allergy 1971;29(9):455-60.

40. Department of Health and Human Services. Action against asthma: A strategic plan for the Department of Health and Human Services. 2000.

41. Taylor WR, Newacheck PW. Impact of childhood asthma on health. Pediatrics 1992;90(5):657-62.

42. Taussig LM, Wright AL, Holberg CJ, Halonen M, Morgan WJ, Martinez FD. Tucson Children's Respiratory Study: 1980 to present. J Allergy Clin Immunol 2003;111(4):661-75; quiz 676.

43. Kuehr J, Frischer T, Karmaus W, Meinert R, Barth R, Herrmann-Kunz E, Forster J, Urbanek R. Early childhood risk factors for sensitization at school age. Journal Of Allergy And Clinical Immunology 1992;90(3 Pt 1):358-63.

44. Sears MR, Burrows B, Flannery EM, Herbison GP, Holdaway MD. Atopy in childhood. I. Gender and allergen related risks for development of hay fever and asthma [see comments]. Clinical and Experimental Allergy 1993;23(11):941-8.

45. Camargo CA, Jr., Schatz M. The relationship of gender to asthma prevalence, healthcare utilization, and medications in a large managed care organization. Acad Emerg Med 2003;10(5):508.

46. Gissler M, Jarvelin MR, Louhiala P, E. H. Boys have more health problems in childhood than girls: follow-up of the 1987 Finnish birth cohort. Acta Paediatr 1999;88:310-4.

47. LeSouef PN. Expression of predisposing factors in early life. Asthma: physiology, immunopharmacology and treatment. . London: Academic Press 1993.

48. Smith JM, Harding LK, G. C. The changing prevalence of asthma in school children. . Clin Allergy 1971;1:57-61.

49. Cutolo M, Sulli A, Capellino S, Villaggio B, Montagna P, Seriolo B, Straub RH. Sex steroid hormones influence on the immune system: basic and clinical aspects in autoimmunity. Lupus 2004;13(9):635-638.

50. Beagley KW, Gockel CM. Regulation of innate and adaptive immunity by the female sex steroid hormones oestradiol and progesterone. FEMS Immunol Med Microbiol 2003;38(1):13-22.

51. Seli E, Arici A. Sex steroids and the immune system. Immunology and Allergy Clinics of North America 2002;22(3):407-433.

52. Parham P. The Immune System. NY: Elsevier Science Inc, 2000.

53. Barnes KC. Genetic epidemiology of health disparities in allergy and clinical immunology. Journal of Allergy and Clinical Immunology 2006;117(2):243-254.

54. Joseph CLM, Williams LK, Ownby DR, Saltzgaber J, Johnson CC. Applying epidemiologic concepts of primary, secondary, and tertiary prevention to the elimination of racial disparities in asthma. Journal of Allergy and Clinical Immunology 2006;117(2):233-240.

55. Centers for Disease Control and Prevention. Measuring childhood asthma prevalence before and after the 1997 redesign of the National Health Interview Survey - United States (Reprinted from MMWR, vol 49, pg 908-911, 2000). Jama-Journal of the American Medical Association 2000;284(18):2312-2313.

56. Simon PA, Zeng ZW, Wold CM, Haddock W, Fielding JE. Prevalence of childhood asthma and associated morbidity in Los Angeles County: Impacts of race/ethnicity and income. Journal of Asthma 2003;40(5):535-543.

57. Hutchings A, Purcell WM, Benfield MR. Peripheral blood antigen-presenting cells from African-Americans exhibit increased CD80 and CD86 expression. Clinical and Experimental Immunology 1999;118(2):247-252.

58. Willwerth BM, Schaub B, Tantisira KG, Gold DR, Palmer LJ, Litonjua AA, Perkins DL, Schroeter C, Gibbons FK, Gillman MW, Weiss ST, Finn PW. Prenatal, perinatal, and heritable influences on cord blood immune responses. Annals of Allergy Asthma & Immunology 2006;96(3):445-453.

59. Holloway JW, Beghe B, Holgate ST. The genetic basis of atopic asthma. Clin Exp Allergy 1999;29(8):1023-32.

60. Wiesch DG, Meyers DA, Bleecker ER. Genetics of asthma. J Allergy Clin Immunol 1999;104(5):895-901.

61. Holgate ST. Genetic and environmental interaction in allergy and asthma. J Allergy Clin Immunol 1999;104(6):1139-46.

62. Weisglas_Kuperus N, Sas TC, Koopman_Esseboom C, van_der_Zwan CW, De_Ridder MA, Beishuizen A, Hooijkaas H, Sauer PJ. Immunologic

effects of background prenatal and postnatal exposure to dioxins and polychlorinated biphenyls in Dutch infants. Pediatric Research 1995;38(3):404-10.

63. Dewailly E, Ayotte P, Bruneau S, Gingras S, Belles_Isles M, Roy R. Susceptibility to infections and immune status in Inuit infants exposed to organochlorines. Environmental Health Perspectives 2000;108(3):205-11.

64. Belles_Isles M, Ayotte P, Dewailly E, Weber JP, Roy R. Cord blood lymphocyte functions in newborns from a remote maritime population exposed to organochlorines and methylmercury. 2002;65(2):165-82.

65. Reichrtova E, Ciznar P, Prachar V, Palkovicova L, Veningerova M. Cord serum immunoglobulin E related to the environmental contamination of human placentas with organochlorine compounds. Environ Health Perspect 1999;107(11):895-9.

66. Karmaus W, Kuehr J, Kruse H. Infections and atopic disorders in childhood and organochlorine exposure. Arch Environ Health 2001;56(6):485-92.

67. Karmaus W, Brooks KR, Nebe T, Witten J, Obi-Osius N, H. K. Immune function biomarkers in children exposed to lead and organochlorine compounds: a cross-sectional study. Environ Health. 2005 Apr 14;4(1):5. . Environ Health. 2005;I4(1):5.

68. Brooks K, Samms-Vaughan M, Karmaus W. Are oral contraceptive use and pregnancy complications risk factors for atopic disorders among offspring? Pediatric Allergy and Immunology 2004;15(6):487-496.

69. Frye C, Mueller JE, Niedermeier K, Wjst M, Heinrich J. Maternal oral contraceptive use and atopic diseases in the offspring. Allergy 2003;58(3):229-32.

70. Gold DR, Burge HA, Carey V, Milton DK, Platts-Mills T, ST. W. Predictors of repeated wheeze in the first year of life: the relative roles of cockroach, birth weight, acute lower respiratory illness, and maternal smoking. . Am J Respir Crit Care Med 1999;160:227-36.

71. Nafstad P, Kongerud J, Botten G, Hagen JA J, JJ. a. The role of passive smoking in the development of bronchial obstruction during the first 2 years of life. . Epidemiology 1997;8:293-7.

72. Jaakkola JJ, MS. J. Effects of environmental tobacco smoke on the respiratory health of children. . Scand J Work Environ Health 2002;28(Suppl 2):71-83.

73. London SJ, James Gauderman W, Avol E, Rappaport EB, JM. P. Family history and the risk of early-onset persistent, early-onset transient, and late-onset asthma. . Epidemiology 2001;12:577-83.

74. Gilliland FD, Li YF, JM. P. Effects of maternal smoking during pregnancy and environmental tobacco smoke on asthma and wheezing in children. Am J Respir Crit Care Med 2001;163:429-36.

75. Arruda LK, Sole D, Baena-Cagnani CE, Naspitz CK. Risk factors for asthma and atopy. Curr Opin Allergy Clin Immunol 2005;5(2):153-9.

76. Jaakkola JJ, Gissler M. Maternal smoking in pregnancy, fetal development, and childhood asthma. . Am J Public Health 2004;94:136-40.

77. Moshammer H, Hoek G, Luttmann-Gibson H, al. e. Parental Smoking and Lung Function in Children: an International Study. . Am J Respir Crit Care Med 2006.

78. Sherrill DL, Martinez FD, Lebowitz MD, Holdaway MD, Flannery EM, Herbison GP, Stanton WR, Silva PA, Sears MR. Longitudinal effects of passive smoking on pulmonary function in New Zealand children. Am Rev Respir Dis 1992;145(5):1136-41.

79. Cunningham J, Dockery D, Speizer FE. Maternal Smoking during Pregnancy as a Predictor of Lung Function in Children American Journal of Epidemiology 1994;139(12):1139-1152

80. Takemura Y, Sakurai Y, Honjo S, Kusakari A, Hara T, Gibo M, Tokimatsu A, Kugai N. Relation between breastfeeding and the prevalence of asthma : the Tokorozawa Childhood Asthma and Pollinosis Study. American Journal of Epidemiology 2001;154(2):115-9.

81. Gdalevich M, Mimouni D, David M, Mimouni M. Breast-feeding and the onset of atopic dermatitis in childhood: A systematic review and meta-analysis of prospective studies. Journal of the American Academy of Dermatology 2001;45(4):520-7.

82. Gdalevich M, Mimouni D, Mimouni M. Breast-feeding and the risk of bronchial asthma in childhood: a systematic review with meta-analysis of prospective studies. Journal of Pediatrics 2001;139(2):261-6.

83. Saarinen UM, Kajosaari M. Breastfeeding as prophylaxis against atopic disease: prospective follow-up study until 17 years old. Lancet 1995;346(8982):1065-9.

84. Peat JK. Prevention of asthma. Eur Respir J 1996;9(7):1545-55.

85. Oddy WH, Holt PG, Sly PD, Read AW, Landau LI, Stanley FJ, Kendall GE, Burton PR. Association between breast feeding and asthma in 6 year old children: findings of a prospective birth cohort study. Bmj 1999;319(7213):815-9.

86. Wafula EM, Limbe MS, Onyango FE, Nduati R. Effects of passive smoking and breastfeeding on childhood bronchial asthma. East Afr Med J 1999;76(11):606-9.

87. Taylor B, Wadsworth J, Golding J, Butler N. Breast feeding, eczema, asthma, and hayfever. Journal Of Epidemiology And Community Health 1983;37(2):95-9.

88. Midwinter RE, Morris AF, Colley JR. Infant feeding and atopy. Arch Dis Child 1987;62(9):965-7.

89. Pratt WR. Allergic diseases in pregnancy and breast feeding. Ann Allergy 1981;47(5 Pt 1):355-60.

90. Pratt HF. Breastfeeding and eczema. Early Hum Dev 1984;9(3):283-90.

91. Wjst M, Dold S, Reitmeier P, Wulff A, Nicolai T, von Mutius E. [Does breast feeding prevent asthma and allergies? Results of the Munich asthma and allergy study]. Monatsschr Kinderheilkd 1992;140(10):769-74.

92. Rusconi F, Galassi C, Corbo GM, Forastiere F, Biggeri A, Ciccone G, Renzoni E. Risk factors for early, persistent, and late-onset wheezing in young children. SIDRIA Collaborative Group. Am J Respir Crit Care Med 1999;160(5 Pt 1):1617-22.

93. Peters TJ, Golding J. The epidemiology of childhood eczema: II. Statistical analyses to identify independent early predictors. Paediatric and Perinatal Epidemiology 1987;1(1):80-94.

94. Hanson LA, Korotkova M, Haversen L, Mattsby-Baltzer I, Hahn-Zoric M, Silfverdal SA, Strandvik B, Telemo E. Breast-feeding, a complex support system for the offspring. Pediatr Int 2002;44(4):347-52.

95. Bertotto A, Gerli R, Fabietti G, Crupi S, Arcangeli C, Scalise F, Vaccaro R. Human breast milk T lymphocytes display the phenotype and functional characteristics of memory T cells. Eur J Immunol 1990;20(8):1877-80.

96. Takahata Y, Takada H, Nomura A, Nakayama H, Ohshima K, Hara T. Detection of interferon-gamma-inducible chemokines in human milk. Acta Paediatr 2003;92(6):659-65.

97. Michel FB, Bousquet J, Greillier P, Robinet_Levy M, Coulomb Y. Comparison of cord blood immunoglobulin E concentrations and maternal allergy for the prediction of atopic diseases in infancy. The Journal of Allergy and Clinical Immunology 1980;65(6):422-30.

98. Shah S, MM. B. Parental history of allergy, maternal serum IgE & cord serum IgE. Indian J Med Sci. 2006 60(1):13-8.

99. Bergmann RL, Schulz J, Günther S, Dudenhausen JW, Bergmann KE, Bauer CP, Dorsch W, Schmidt E, Luck W, Lau S, al e. Determinants of cord-blood IgE concentrations in 6401 German neonates [see comments]. Allergy 1995;50(1):65-71.

100. Johnson CC, Ownby DR, Peterson EL. Parental history of atopic disease and concentration of cord blood IgE. Clin Exp Allergy 1996;26(6):624-9.

101. Kuiper S, Muris JWM, Dompeling E, van Schayck CP, Schonberger HJAM, Wesseling G, Knottnerus JA. Association between first-degree familial predisposition of asthma and atopy (total IgE) in newborns. Clinical & Experimental Allergy 2006;36(5):594-601.

102. Steimer T. Reproductive health. Ares-Serono Symposia Series - Frontiers in Endocrinology. Vol. 2. Rome: Ares Serono Symposia Publications, 1993.

103. Waring RH, Harris RM. Endocrine disrupters: a human risk? Mol Cell Endocrinol 2005;244(1-2):2-9.

104. Choi SM, Yoo SD, Lee BM. Toxicological characteristics of endocrine-disrupting chemicals: Developmental toxicity, carcinogenicity, and mutagenicity. Journal of Toxicology and Environmental Health-Part B-Critical Reviews 2004;7(1):1-32.

105. Wjst M, Dold S. Is asthma an endocrine disease? Pediatric Allergy and Immunology 1997;8(4):200-4.

106. Druckmann R. Review: female sex steroid hormones, autoimmune diseases and immune response. Gynecol Endocrinol 2001;15 Suppl 6:69-76.

107. Ackerman LS. Sex steroid hormones and the genesis of autoimmunity. Arch Dermatol 2006;142(3):371-6.

108. Verthelyi D. Sex steroid hormones as immunomodulators in health and disease. INTERNATIONAL IMMUNOPHARMACOLOGY 2001;1(6):983-993.

109. Wilder RL, Elenkov IJ. Hormonal regulation of tumor necrosis factor-alpha, interleukin-12 and interleukin-10 production by activated macrophages. A disease-modifying mechanism in rheumatoid arthritis and systemic lupus erythematosus? Ann N Y Acad Sci 1999;876:14-31.

110. Szekeres-Bartho J, Wegmann TG. A progesterone-dependent immunomodulatory protein alters the Th1/Th2 balance. J Reprod Immunol 1996;31(1-2):81-95.

111. Piccinni MP, Giudizi MG, Biagiotti R, Beloni L, Giannarini L, Sampognaro S, Parronchi P, Manetti R, Annunziato F, Livi C. Progesterone favors the development of human T helper cells producing Th2-type cytokines and promotes both IL-4 production and membrane CD30 expression in established Th1 cell clones. Journal of Immunology (Baltimore, Md. : 1950) 1995;155(1):128-33.

112. Szekeres-Bartho J, Par G, Szereday L, Smart CY, Achatz I. Progesterone and non-specific immunologic mechanisms in pregnancy. American Journal Of Reproductive Immunology 1997;38(3):176-82.

113. Hamano N, Terada N, Maesako K, Hohki G, Ito T, Yamashita T, Konno A. Effect of female hormones on the production of IL-4 and IL-13 from peripheral blood mononuclear cells. Acta Otolaryngol Suppl 1998;537:27-31.

114. Piccinni MP, Maggi E, Romagnani S. Role of hormone-controlled T-cell cytokines in the maintenance of pregnancy. Biochem Soc Trans 2000;28(2):212-5.

115. Safe SH, Zacharewski T. Organochlorine exposure and risk for breast cancer. Progress In Clinical And Biological Research 1997;396:133-45.

116. Safe SH. Xenoestrogens and breast cancer. N Engl J Med 1997;337(18):1303-4.

117. vom Saal FS, Timms BG, Montano MM, Palanza P, Thayer KA, Nagel SC, Dhar MD, Ganjam VK, Parmigiani S, Welshons WV. Prostate enlargement in mice due to fetal exposure to low doses of estradiol or diethylstilbestrol and opposite effects at high doses. Proc Natl Acad Sci U S A 1997;94(5):2056-61.

118. Welshons WV, Nagel SC, Thayer KA, Judy BM, Vom Saal FS. Low-dose bioactivity of xenoestrogens in animals: fetal exposure to low doses of methoxychlor and other xenoestrogens increases adult prostate size in mice. Toxicol Ind Health 1999;15(1-2):12-25.

119. Ashby J, Tinwell H, Haseman J. Lack of effects for low dose levels of bisphenol A and diethylstilbestrol on the prostate gland of CF1 mice exposed in utero. Regul Toxicol Pharmacol 1999;30(2 Pt 1):156-66.

120. Odum J, Ashby J. Neonatal exposure of male rats to nonylphenol has no effect on the reproductive tract. Toxicol Sci 2000;56(2):400-4.

121. Africa). ACfEAaGC. Approaches to Effective Malaria Control that Avoid DDT in Kenya: Use of Bacillus thuringiensis israelensis (BTi). 2006.

122. ATSDR. Toxicological Profile for DDT/DDD/DDE. Update. (Draft for Public Comment). In: Registry AfTSaD, ed. Atlanta, GA, 2000;433 pp.

123. Sunyer J, Torrent M, Munoz-Ortiz L, Ribas-Fito N, Carrizo D, Grimalt J, Anto JM, Cullinan P. Prenatal dichlorodiphenyldichloroethylene (DDE) and asthma in children. Environ Health Perspect 2005;113(12):1787-90.

124. Woodruff T, Wolff MS, Davis DL, Hayward D. Organochlorine exposure estimation in the study of cancer etiology. Environ Res 1994;65(1):132-44.

125. Longnecker MP, Rogan WJ, Lucier G. The human health effects of DDT (dichlorodiphenyltrichloroethane) and PCBS (polychlorinated biphenyls) and an overview of organochlorines in public health. Ann Rev Public Health 1997;18:211-44.

126. Chedrese PJ, Feyles F. The diverse mechanism of action of dichlorodiphenyldichloroethylene (DDE) and methoxychlor in ovarian cells in vitro. Reprod Toxicol 2001;15(6):693-8.

127. Kelce WR, Wilson EM, Lambright CR, Gray LE, Jr., Roberts KP, Stone CR, Laws SC, Gray LE, Kemppainen JA.. J Mol Med 1997;75(3):198-207.

128. Kelce WR, Lambright CR, Gray LE, Jr., Roberts KP, Stone CR, Laws SC, Gray LE, Kemppainen JA, Wilson EM. Toxicol Appl Pharmacol 1997;142(1):192-200.

129. Hansen LG. Stepping backward to improve assessment of PCB congener toxicities. Environmental Health Perspectives 1998;106 Suppl 1:171-89.

130. Ulrich EM, Caperell-Grant A, Jung SH, Hites RA, Bigsby RM. Environmentally relevant xenoestrogen tissue concentrations correlated to

biological responses in mice. Environ Health Perspect 2000;108(10):973-7.

131. Burow ME, Tang Y, Collins-Burow BM, Krajewski S, Reed JC, McLachlan JA, Beckman BS. Effects of environmental estrogens on tumor necrosis factor alpha- mediated apoptosis in MCF-7 cells. Carcinogenesis 1999;20(11):2057-61.

132. ATSDR. ToxFAQs™ for Polychlorinated Biphenyls (PCBs)

133. Safe S. Polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). Crit Rev Toxicol 1990;21(1):51-88.

134. Patnode KA, Curtis LR. 2,2',4,4',5,5'- and 3,3',4,4',5,5'-hexachlorobiphenyl alteration of uterine progesterone and estrogen receptors coincides with embryotoxicity in mink (Mustela vision). Toxicol Appl Pharmacol 1994;127(1):9-18.

135. Wojtowicz AK, Gregoraszczuk EL, Lyche JL, Ropstad E. Time dependent and cell-specific action of polychlorinated biphenyls (PCB 153 and PCB 126) on steroid secretion by porcine theca and granulosa cells in mono- and co-culture. J Physiol Pharmacol 2000;51(3):555-68.

136. Wojtowicz A, Ropstad E, Gregoraszczuk E. Estrous cycle dependent changes in steroid secretion by pig ovarian cells in vitro to polychlorinated biphenyl (PCB 153). Endocr Regul 2001;35(4):223-8.

137. Troisi GM, Mason CF. PCB-associated alteration of hepatic steroid metabolism in harbor seals (Phoca vitulina). J Toxicol Environ Health A 2000;61(8):649-55.

138. Haave M, Ropstad E, Derocher AE, Lie E, Dahl E, Wiig O, Skaare JU, Jenssen BM. Polychlorinated biphenyls and reproductive hormones in female polar bears at Svalbard. Environ Health Perspect 2003;111(4):431-6.

139. Holt PG, Macaubas C. Development of long-term tolerance versus sensitisation to environmental allergens during the perinatal period. Curr Opin Immunol 1997;9(6):782-7.

140. Strachan DP. Is allergic disease programmed in early life? Clinical And Experimental Allergy 1994;24(7):603-5.

141. Bjorksten B, Kjellman NI. Perinatal environmental factors influencing the development of allergy. Clin Exp Allergy 1990;20 Suppl 3:3-8.

142. Barker DJ. In utero programming of chronic disease. Clin Sci (Lond) 1998;95(2):115-28.

143. Wills-Karp M, Chiaramonte M. Interleukin-13 in asthma. Curr Opin Pulm Med 2003;9(1):21-7.

144. Evans PC, Lambert N, Maloney S, Furst DE, Moore JM, Nelson JL. Long-term fetal microchimerism in peripheral blood mononuclear cell subsets in healthy women and women with scleroderma. Blood 1999;93(6):2033-7.

145. Gabrielsson S, Soderlund A, Nilsson C, Lilja G, Nordlund M, Troye-Blomberg M. Influence of atopic heredity on IL-4-, IL-12- and IFN-gamma-producing cells in in vitro activated cord blood mononuclear cells. Clinical and Experimental Immunology 2001;126(3):390-396.

146. Cohn B. DDT and DDE: Second Generation Time to Pregnancy Effects.

147. Lundholm CE. The effects of DDE, PCB and chlordane on the binding of progesterone to its cytoplasmic receptor in the eggshell gland mucosa of birds and the endometrium of mammalian uterus. Comp Biochem Physiol C 1988;89(2):361-8.

148. Noakes PS, Taylor P, Wilkinson S, Prescott SL. The relationship between persistent organic pollutants in maternal and neonatal tissues and immune responses to allergens: A novel exploratory study. Chemosphere 2006;63(8):1304-1311.

149. Xu B, Jarvelin MR, Hartikainen AL, Pekkanen J. Maternal age at menarche and atopy among offspring at the age of 31 years. Thorax 2000;55(8):691-3.

150. Vihko R, Apter D. Endogenous steroids in the pathophysiology of breast cancer. Critical Reviews in Oncology/Hematology 1989;9(1):1-16.

151. Apter D, Reinila M, Vihko R. Some endocrine characteristics of early menarche, a risk factor for breast cancer, are preserved into adulthood. International Journal of Cancer. Journal International Du Cancer 1989;44(5):783-7.

152. Moore JW, Key TJ, Wang DY, Bulbrook RD, Hayward JL, Takatani O. Blood concentrations of estradiol and sex hormone - binding globulin in relation to age at menarche in premenopausal British and Japanese women. Breast Cancer Research and Treatment 1991;18 Suppl 1:S47-50.

153. Xu B, Pekkanen J, Husman T, Keski-Nisula L, Koskela P. Maternal sex steroid hormones in early pregnancy and asthma among offspring: a case-control study. The Journal of Allergy and Clinical Immunology 2003;112(6):1101-4.

154. Cooper GS, Martin SA, Longnecker MP, Sandler DP, Germolec DR. Associations between plasma DDE levels and immunologic measures in African-American farmers in North Carolina. Environ Health Perspect 2004;112(10):1080-4.

155. Vine MF, Stein L, Weigle K, Schroeder J, Degnan D, Tse CK, Backer L. Plasma 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE) levels and immune response. Am J Epidemiol 2001;153(1):53-63.

156. Bilrha H, Roy R, Moreau B, Belles-Isles M, Dewailly E, Ayotte P. In vitro activation of cord blood mononuclear cells and cytokine production in a remote coastal population exposed to organochlorines and methyl mercury. Environ Health Perspect 2003;111(16):1952-7.

157. Daniel V, Huber W, Bauer K, Suesal C, Conradt C, Opelz G. Associations of dichlorodiphenyltrichloroethane (DDT) 4.4 and dichlorodiphenyldichloroethylene (DDE) 4.4 blood levels with plasma IL-4. Arch Environ Health 2002;57(6):541-7.

158. Kavlock RJ, Daston GP, Derosa C, et al., Research needs for the risk assessment of health and environmental effects of endocrine disruptors Environ Health Perspect 1996;104 (suppl 4), 715-740.

159. EC (1997) European Workshop on the Impact of Endocrine Disrupters on Human Health and Wildlife: Report of the Proceedings (EUR 17549), Weybridge, UK, European Commission

CHAPTER 2

BACKGROUND AND METHODS

FOR THE

RESPECTIVE STUDIES

The four research questions posed in this monograph utilizes data from three cohorts: namely the Pregnancy Environment and Child Health (PEACH) study, the Jamaican Perinatal Morbidity, Mortality Survey and the Child Health and Environment Cohort Study-Germany. This chapter describes the background and methods used the respective studies. Methodologic details not covered in this chapter are presented in the four respective manuscripts found in the 'Results' section.

2.1 The PEACH study – Background

PEACH is a multi-center longitudinal study of mother-infant pairs, which had its inception in Berrien County Michigan (Figure 4) at the Benton Harbor and St. Joseph study sites.

Situated along the South East shore of Lake Michigan in Berrien County, Benton Harbor and St. Joseph are two adjoining but very different cities. According to City-Data.com [1], in 2004, Benton Harbor had over 10, 000 residence (92.4% are African-American) with a median household income 0f approximately $17,500.

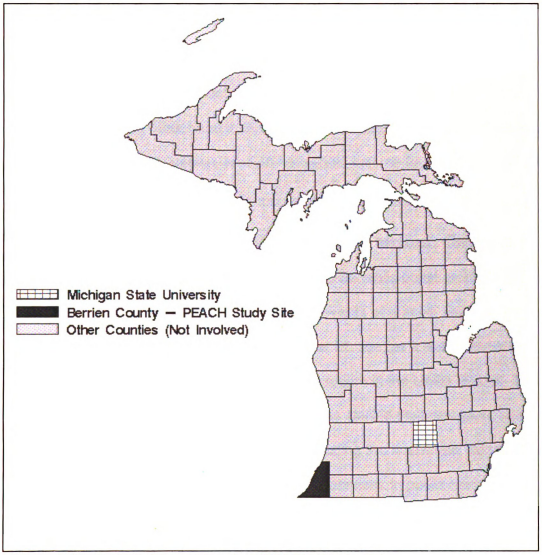

Figure 4. Map of Michigan showing PEACH Study Site

In 1894, the Baushke family, prominent citizens in Benton Harbor, built America's first car, with 4-wheels and seating for 5 people. This was the inception of Benton Harbor's industries which now includes the headquarters for the world renowned Whirlpool Corporation formally known as the Upton Machine Co. As in any other towns or cities, the introduction of industries is good news for the economy; however their possible impact on public health is of grave concern.
St. Joseph on the other hand in 2004 had an estimated population of 9,000 of which 89.6% are Caucasian. Median household income for the year 2000 was $37,032 [1]. Its ideal location at the mouth of the St. Joseph River (linking it to Chicago) earned the city the title 'The Riviera of the Midwest'. This location encourages much commerce and tourism. Fertile lands boast orchards full of apples, peaches and cherries along with vineyards of grapes, fields of corn, rows of strawberries and blueberries. Little wonder St. Joe (as it is affectionately called) is the largest non-citrus fruit-growing region in the United States. There are also several industries (In 1911 the Uptons started their company, now Whirlpool – later moved to Benton Harbor) dotting the landscape, hence as is the case of its 'sister' city Benton Harbor, a possible risks to public health looms.

The cities of Benton Harbor and St. Joseph are ideal sites for studying the human health effects of exposure to environmental sex steroid hormones for the following reasons. First, both cities are adjacent to each other; second, they are located along the South East shore of Lake Michigan, a known 'reservoir' for

environmental toxicants and; third, the discrete ethnic and socio-economic differences between cities.

It is in this setting that the PEACH study had its 'genesis' in May of 2003. The target population was women in their first trimester of pregnancy living in Benton Harbor or St. Joseph. The study population was women from target population who met the inclusion criteria of being primiparous (expecting first live birth), having no previous stillbirth, no multiple births, no diabetes, thyroid or adrenal disorders, and age 18-34 years.

The PEACH study – Methods

Questionnaires

At the time of enrollment, a brief face-to-face interview was conducted to collect data on the mother's lifestyle and mediation usage. A more detailed telephone interview was then conducted two weeks after to collect data on participants' general and reproductive health. Approximately two weeks after delivery, another telephone interview was conducted that focuses on the mother's pregnancy experience such as duration of pregnancy and delivery, and complications during pregnancy. Information on the mother's general health along with that of her newborn was also ascertained.

Cord blood and placenta tissue sampling

Following delivery of the placenta, the external surface of the cord was washed to avoid contamination with maternal cells, and then approximately 10mL cord blood was collected by cordocentesis in heparinized tubes. The tube with blood

is then gently shaken to allow proper mixing of heparin and cord blood. The mixture was then refrigerated and prepared for mononuclear cell separation within 24 hours of collection.

Within an hour of delivery, approximately one quarter of placental tissue (100g) was cut and stored in a glass container for the determination of DDE and other organochlorine compounds. The sample was then stored at -20°C until analyzed.

Laboratory analyses of placental samples

All organochlorine analyses were done at the Analytical Chemistry Section (ACS), Michigan Department of Community Health. Compounds were extracted, fractionated and quantified according to procedures described by Najam et al. [2], with modifications.

Laboratory analyses of cord plasma samples

Fresh cord blood was diluted with equal volume of sterile saline, carefully layered onto Histopaque-1077 (Sigma Chemical Company, St. Louis, MO) and centrifuged 30 minutes (at 600 G) at room temperature. The cells harvested from the interface were washed in serum free saline, then re-suspended into 2-3 ml of RPMI 10 (Gibco BRL, Life Technologies Inc., New York, USA) and the number of viable cells determined using trypan blue exclusion. CBMC staining will also be performed with Turk's stain to exclude nucleated erythrocytes from cell counts and determine the degree of contamination. PHA-P (Sigma Chemical Company),

as a polyclonal activator at 5 ug/mL in culture, and dust mite allergen (Greer Labs), at 100 and 500 ug/mL, were used in cell cultures. Cells were cultured in triplicates using 96-well flat bottom plates in a final volume of 200 µl/well at a final concentration of 1 x 106 leukocytes/mL.

Cytokine assays: Cytokine protein levels were measured using cytokine-specific, sandwich enzyme-linked immunoassays (ELISAs).
IL-4 and IL-13: Recombinant human IL-4 and IL-13 standards of specific activity equivalent to that of WHO standard 88/656 (21) were included in each ELISA. The sensitivity of this assay was 0.9 to 1.9 pg/ml and the linear range of detection between 0.9 to 250 pg/mL.

IFN-γ: Similar procedure to that used for IL-13 was used to quantify IFN-γ. However, the lower limit of detection for the assay was typically 0.3 U/ml and the linear range for quantitation was between 0.3 to 25 U/mL. This assay was calibrated using human IFN-γ reference reagent Gg23–901–530 (specific activity 7 x 105 U/mg, 1 NIH unit = 115 pg, provided by Dr. C. Laughlin, NIAID, Bethesda, Md.).

The Institutional Review Board of the Michigan State University approved the study in addition; all participants gave written, informed consent.

Statistical analysis

Adjusted betas (β) and standard errors (SE) for the associations between exposure and outcomes were determined using linear regression models. Statistical tests are 2-sided and declared significant if *p* value < 0.05. The statistical software package SAS (version 9.1; SAS Institute Inc, Cary, NC) was used for all analyses [3].

Linear regression

In its simplest form a linear regression analysis involves finding the best straight line relationship that explains how the variation in an outcome (or dependent) variable, Y, depends on the variation in a predictor (or independent) variable, X, and is expressed by the following equation:

$Y = b_0 + b_1X$

where b_1 is called the slope of the equation and b_0 the intercept.

From a sample $\{(Y_i, X_i) : I = 1, \ldots, n\}$, b_1, b_0 are estimated by

$$\hat{b}_1 = \frac{\Sigma X_i Y_i - (\Sigma X_i)(\Sigma Y_i)}{\Sigma X_i^2 - (\Sigma X_i)^2}$$

b_0 is called the intercept and estimated by the following formula;

$$\hat{b}_0 = \frac{\Sigma Y - \hat{b}_1 \Sigma X}{n}$$

This method is called the process of Least Squares that calls for minimization with respect to (b_0, b_1) of the error sum of squares; $\sum_{i=1}^{n} e_i^2 = \sum_{i=1}^{n} (Y_i - b_0 - b_1 x_i)^2$

which leads to the expression for $\hat{b}_0, \hat{b}_1$. Since most outcomes are dependent on more than one factor (variable) it is necessary to explain the outcome in terms of the combined effect of these multiple independent factors or variables. This is done by multiple regression which is an extension of simple linear regression in which the dependent variable is predicted by a linear combination of the explanatory variables. For example, if an outcome Y depends on two independent variables X1 and X2, the regression equation will be $Y = b_0 + b_1X_1 + b_2X_2$ where the b_0 is the intercept, and b_1 and b_2 are slopes of X_1 and X_2 respectively.

The amount of variation in the dependent variable that is accounted for by variation in the predictor variables is measured by the value of the coefficient of determination, or R^2 (R squared). The R^2 from multiple regression models is called Adjusted R^2 when penalized for the number of variables in the model. This Adjusted R^2 is the total percent variation in the dependent variable that is explained by the predictor variables together.

2.2 Child Health and Environment Cohort Study – Germany.

The Child Health and Environment Cohort Study was conducted in 1994, targeting children residing in 18 townships in Hesse, Germany.

Hesse is one of Germany's sixteen federal states and has an area of 21,110 km² and just over six million inhabitants. Most inhabitants live in the southernmost part of Hesse between the rivers Main and Rhine. The latter is one of the longest and most important rivers in Europe and borders Hesse on the southwest without running through the state. The valley formed by the Rhine (Rhine Valley) in Hesse is where two of the regions targeted in this study are situated [4]. Figure 5 shows the location of study sites and the toxic waste incinerator in the Rhine Valley. Two regions are situated in the Rhine Valley with low mountains on both sides.

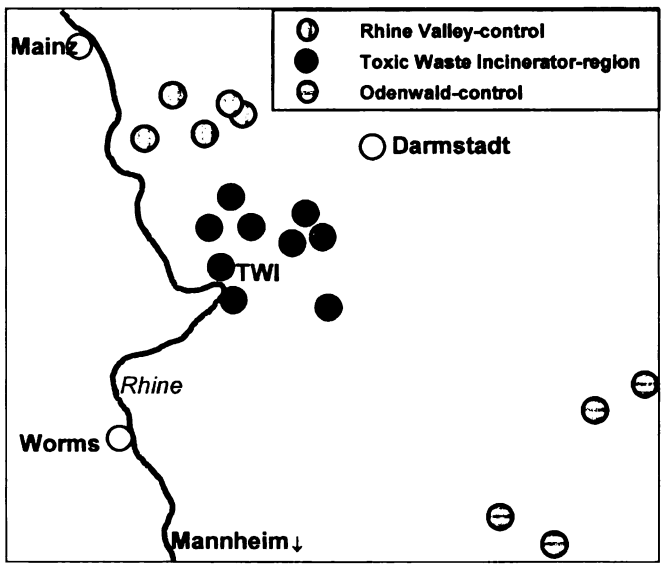

Figure 5: The Rhine Valley

For details regarding the **Questionnaires and Laboratory procedures** used in this study, please see the manuscript entitled *'Immune function biomarkers in children exposed to lead and organochlorine compounds: a cross-sectional study'* in the 'Results' section.

Statistical analysis

The statistical software package SAS (version 9.1; SAS Institute Inc, Cary, NC) was used for all analyses [3]. The linear regression method is described in pages 39 and 40. Statistical tests are 2-sided and decleared important if p value < 0.05.

2.3 The Jamaican Perinatal Morbidity, Mortality Survey – Background

The Jamaican Perinatal Morbidity, Mortality Survey targeted all women who had a live birth, or stillbirth of 500 g or more in Jamaica during the period September 1, 1986 to October 31, 1986.

Jamaica is an island nation 240 kilometres (150 miles) in length and 85 kilometres (50 miles) in width, which is slightly smaller than Connecticut-USA. The island is situated in the Caribbean Sea, approximately 804 kilometres (500 miles) south south east of the southern tip of Florida (Figure 5). Jamaica, with its mostly mountains interior and narrow discontinuous coastal plains, enjoys a mainly tropical climate with a temperate interior (average minimum temperature 22.0°C (71.6°F) and average maximum of 30.3°C (86.5°F)). The population (N=2,758,124) is mainly African-Caribbean (90.9%) of West African descent with a labor force (approximately 1.2 million) made up of workers in services (64.1%; mainly tourism), agriculture (19.3%) and industry (16.6%; mainly bauxite/alumina).

The Jamaican Perinatal Morbidity, Mortality Survey was motivated by results of the Child Mortality Study of 1972 that clearly demonstrated the extent of under-reporting of early infant deaths. Shortly after the island wide survey (Figure 6) was designed using the First British Perinatal Mortality Survey [5] as a blue print.

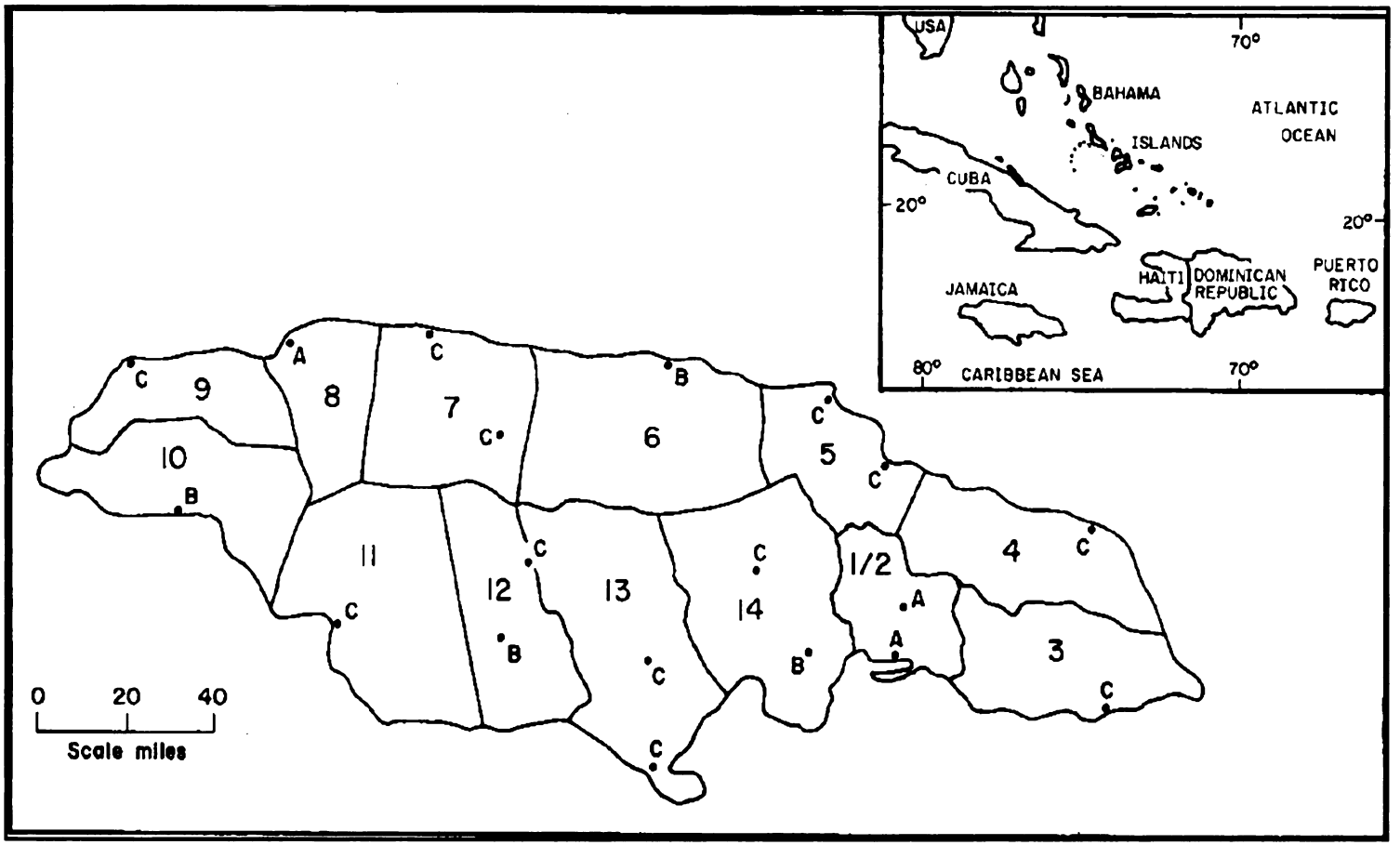

Figure 6. Jamaica: parishes, regional administrative areas and distribution of hospitals. West: 10=Westmoreland; 9=Hanover; 8=St James; 7=Trelawny. South: 11=St Elizabeth; 12=Manchester; 13=Clarendon. North East: 6=St. Ann; 5=St Mary; 4=Portland. South East: 14=St Catherine; 1 / 2=Kingston and St Andrew; 3=St Thomas.

The overall aim of the study was to provide information to help improve maternal and childcare services and reduce perinatal morbidity and mortality in Jamaica.

The Jamaican Perinatal Morbidity, Mortality Survey – Methods

At enrollment, a face-to-face interview was conducted with each prticipant and their babies examined, usually within the first 48 hours after delivery. A standardized questionnaire was used to get at data during the antenatal, labor and delivery, and perinatal periods. Data was also abstracted from the newborn nursery charts and mother's medical record to supplement interview data where necessary. Overall, 10,310 (94%) of the births in the two-month period were identified and included in the study (main cohort study) [5].

The first follow-up occurred six weeks after delivery when another face-to-face interview was conducted aimed at information about breastfeeding practices and the infant's health status.

The study included in this monograph utilizes data from a geographic sub-sample (n= 1,720) of eleven to twelve year old children, representing those born in Kingston and St. Andrew. In a cross-sectional survey of these children, data on health outcomes were collected from the guardian or parent; who was often time the mother.

The Research Ethics Committees of the University of the West Indies Jamaica and Michigan State University approved the assembly of the cohort and analysis of data for the included manuscript, respectively.

Statistical analysis

The statistical software package SAS (version 9.1; SAS Institute Inc, Cary, NC) was used for all analyses [3]. The method of statistical analysis used was logistic regression with 2-sided statistical tests declaring associations significant if p value < 0.05.

Logistic regression

Logistic regression is an adaptation to dichotomous outcomes of the classical linear regression model for continuous outcomes. It is primarily used to describe the relationship to the outcome of a primary exposure after adjusting for the effects of other (independent) variables that may influence both the outcome and exposure.

Formally, in formulating a logistic model let Y (0 or 1) denoting the dichotomous outcome of interest with Y =1 labeling the presence of the condition under study. Then the logistic model for $P[Y = 1 \mid X_1, X_2 \cdots X_k]$ is written simply as $P(\underline{X})$ where X is a shorthand for X_1 through X_k. The model formula is given by

$P(\underline{X}) = \frac{1}{1 + e^{-(\alpha + \Sigma \beta_i X_i)}}$ in which α and β_i are unknown parameters to be estimated based on the observed exposure variables X_1 through X_k and the outcome variable Y. The coefficients β_1 through β_k can be interpreted as adjusted log-odds ratios. For example, if X_1 is the primary exposure, coded X_1 = 1 for "exposed" and X_1 =0 for "not exposed", then assuming the variables X_2 through X_k do not include X_1, the adjusted odds ratio is given by $\exp(\beta_1)$.

References

1. City-Data.com. www.City-Data.com.

2. Najam AR, Kover MP, Williams CC, Burse VW, Needham LL. Analysis of a Mixture of Polychlorinated Biphenyls and Chlorinated Pesticides in Human Serum by Column Fractionation and Dual-Column Gas Chromatography with Electron Capture Detection. Journal of the AOAC International 1999;82:177-185.

3. SAS Institute. Statistical Analysis System, Version 9.1. In: Institute S, ed. Gary, NC, 2002-2003.

4. Wikipedia. Hesse. 2006.

5. Ashley D, McCaw-Binns A, Golding J, Keeling J, Escoffery C, Coard K, Foster-Williams K. Perinatal mortality survey in Jamaica: aims and methodology. Paediatr Perinat Epidemiol 1994;8 Suppl 1:6-16.

CHAPTER 3

RESULTS

In this chapter, four manuscripts are presented. Their titles and order are:

1. Placental p, p'-dichlorodiphenyldichloroethylene and cord blood immune markers **(forthcoming)**.
2. Maternal oral contraceptive use and immune markers in the offspring **(In progress)**.
3. Are oral contraceptive use and pregnancy complications risk factors for atopic disorders among offspring? **(Published)**
4. Immune function biomarkers in children exposed to lead and organochlorine compounds: a cross-sectional study **(Published).**

These manuscripts correspond to the four research questions:

1. Is there evidence for the immunemodulation of cord immune markers of allergy by *in utero* sex hormone exposure?
2. Is maternal oral contraceptive use (a proxy for *in utero* estrogen/progesterone exposure) associated with immune markers of allergy in the offspring?
3. Is maternal oral contraceptive use (a proxy for *in utero* estrogen/progesterone exposure) a risk factor for markers of allergic susceptibility in offspring?
4. Does serum organochlorine alters immune markers in children?

COMPONENT ONE

In utero exposure to SHD affects perinatal biomarkers of allergy

Placental *p, p'*-dichlorodiphenyldichloroethylene and cord blood immune markers

Kevin Brooks[1], Hanem Hasan[2], Sridhar Samineni[2], Venu Gangur[2] and Wilfried Karmaus[1]

[1] Department of Epidemiology and Biostatistics,
Norman J. Arnold School of Public Health,
University of South Carolina
800 Sumter Street, Columbia, SC., 29208-0001

[2] Food Allergy & Immunology Laboratory, Food Science & Human Nutrition; National food Safety & Toxicology Center, Michigan State University, East Lansing, MI 48824, USA

Abstract

Placental *p, p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE) concentration and cord blood atopic markers were determined in 19 neonates. Increased placental *p,p'*-DDE was associated with a statistically significant increase in cord plasma interleukin (IL)-13.

Furthermore, both cord plasma IL-4/INF-γ and IL-13/INF-γ ratios were significantly positively associated with placental *p,p'*-DDE concentration.

Key words:

DDE, placenta, cytokines, cord, allergy

The possible immune modulating role of 'hormone-like substances' is under investigation [1,2]. Previously, in a cohort of German school children, we demonstrated significant associations between serum DDE and biomarkers of allergic susceptibility; including serum immunoglobulin E [2]. In addition, for a group of 26 mother-infant pairs, Noakes et al. reported on the ability of maternal DDE and other 'hormone-like' substances to alter the fetal immune response [1]. More recently, Sunyer and co-workers showed that *in utero* DDE exposure is related to increased incidence of asthma in children [3]. It is therefore very timely to present the yet-to-be-reported relation between placental *p,p'*-DDE and cord plasma levels of prototypic type-1 and type-2 cytokines.

Our objective was to test the hypothesis whether increased placental *p,p'*-DDE concentration is associated with an increase in cord plasma IL-4 and IL-13. In addition, since type-1 and type-2 cytokines often antagonize each others activity, we hypothesize that increased placental *p,p'*-DDE concentration is associated with a reduction in cord plasma INF-γ. The Th2/Th1 cytokine ratio has been used as a measure of relative atopic reactivity [4]. We therefore further hypothesize that increased placental *p,p'*-DDE concentration is associated with an increase in the ratio of cord plasma IL-4/INF-γ as well as the ratio of IL-13/INF-γ.

Methods and results

Primiparous women in their first trimester were enrolled in the ongoing Environment and Child Health (PEACH) study. Ethical approval was granted by the Michigan State University Committee on Research Involving Human Subjects.

Telephone interview data on passive smoking (ETS), mother's age, and allergic status (defined as mother ever had one or any combination of: asthma, hay fever, itchy rash, or wheezing) were collected. Cord blood samples were drawn from the umbilical vein and transported to the lab within 24 hours. Cytokine protein levels were measured using ultrasensitive Elisa based assays optimized using paired monoclonal antibodies (PharMingen). Typical assay sensitivities; IL-4: 0.05pg/ml; IL-13:1.5 pg/ml; and INF-γ. 0.5pg/ml).

Placental *p,p'*-DDE was extracted into diethyl ether-hexane (1:1 v/v), passed over a Florisil column and quantified by high-resolution gas chromatography with electron capture detection. *p,p'*-DDE values below the detection limit (DL = 0.50 pg/g) were replaced with half the DL and all concentrations were lipid adjusted.

We used linear regression models (PROC GLM) with cytokines as dependent and *p,p'*-DDE as independent variables; while controlling for mother's age, atopic status, and ETS. In order to obtain a multivariate normal distribution we log-transformed cytokine levels.

Complete data (interview, cytokine and DDE concentration) are available for 19 mother-infant pairs. Mothers were on average 29 years of age while 2 of 19 were exposed to passive cigarette smoke in their homes during pregnancy (Table 1). Median value for placental *p,p'*-DDE was 58.3 pg/g. For IL-4 and IL-13 their median values were 4.2 pg/mL and 61.4 pg/mL respectively (Table 1). Adjusting for maternal age, allergy and exposure to tobacco smoke, we found that placental *p,p'*-DDE statistically significantly increase the level of cord plasma IL-13 (β = 0.007 pg/g; p=0.03, Table 2). Placental *p,p'*-DDE was also found to increase IL-4 levels while reducing INF-γ; however these associations were not statistically significant. Nevertheless, both cord plasma IL-4/INF-γ and IL-13/INF-γ ratios were significantly increased with increasing placental *p,p'*-DDE concentration (β = 0.03 pg/g; p=0.007 and β = 0.17 pg/g; p=0.02 respectively; Table 2)

Discussion

Using placental and cord blood samples of 19 mother-infant pairs we demonstrated, a statistically significant association between placental *p,p'*-DDE and cord plasma IL-13. The results support our hypotheses that placental DDE is related to increased cord plasma IL-13 and may be associated with increased IL-4 and reduced INF-γ. Considering the aforementioned results for IL-4, IL-13 and INF-γ, we expected placental DDE to be associated with the ratio of IL-4/INF-γ along with that of IL-13/INF-γ. The associations (increased DDE and increased Th2/Th1 ratios) were found to be statistically significant.

DDE is known to cross the placenta. However, in their Western Australian samples, Noakes et al. were unable to detect placental *p,p'*-DDE and used maternal levels instead. This was not the case in our North American samples. The fact that we determined *p,p'*-DDE in placental samples may be explained by different exposure in the southern and northern hemisphere, resulting from a long-range atmospheric transport of organochlorines enhanced by the meridional flow [5].

Whereas both IL-4 and IL-13 play critical roles in the initiation as well as maintenance of allergic inflammation and asthma [6], INF-γ dominated responses are associated with a 'non-allergic' response [7]. Notably, IL-4 and IL-13 share many but not all proallergenic activities. Recent studies suggest that IL-13 is a key player in asthma independent of IL-4 [6]. It is suggested that decreased IFN-γ in cord blood mononuclear cells is the hallmark of newborns from high-risk population [8]. We are uncertain whether the relationships observed in this study between cytokines and placental *p,p'*-DDE are direct or indirect. Maternal and fetal immune responses during pregnancy are under endocrine influence [9]. Thus, it is possible that an explanation for our finding may be that DDE introduce an endocrine disrupting effect [10], which in turn might alter the fetal T-cell cytokine profile.

We are not aware of studies implicating direct effect of DDE on Th2/Th1 cell development or cytokine responses. Our findings suggest that the assessment of DDE exposure on Th2/Th1 ratios may be of more importance than the investigation of individual cytokines.

Noakes et al. reported a significant inverse correlation ($r = 0.406$, $p = 0.049$) between maternal adipose tissue levels of OC *p,p'*-DDE and maternal INF-γ reaction to mitogens [1]. The authors also found increased phytohaemagglutinin mitogen stimulated IL-13 and decreased IFN-γ with increasing cord blood *p,p'*-DDE levels. Though these findings, from their small sample (n=26), were not statistically significant; they were in the same direction as those we presented using a similar size cohort (n=19). Since these small studies reported comparable findings, the effects are likely to gain statistical significance in a study with a larger sample size and improved statistical power.

Our findings suggest that DDE may play a role in the prenatal priming of allergic diseases via promoting IL-13 dominance. This priming – to a greater extent – may also be through the overall dominance of the ratio of Type-2 cytokines (both IL-4 and IL-13) over Type-1 (IFN-γ) cytokine. Larger epidemiologic studies are needed to explicate possible mechanistic explanations for our findings.

Acknowledgement

This work was supported by United States Environment Protection Agency STAR grant number R830825-01-0.

Table 1. Descriptive characteristics of the cohort

		Study cohort (n=19)
Maternal		
Ever had:		
Asthma	Yes	1/19
Hay fever	Yes	3/19
Itchy rash	Yes	2/19
Itchy eyes or stuffy nose	Yes	7/19
Wheezing	Yes	3/19
Allergy	Yes	10/19
Exposure to passive smoke in home	Yes	2/19
Ethnicity	Caucasian	15/19
Age (mean, range, years)		29.3 (21-42)
Placental *p,p'*-DDE (median, 5-95% value, pg/g)		58.3 (40.2 – 388.4)
Cord plasma (median, 5-95% value, pg/mL):		
IL-4		4.2 (0.7 – 29.8)
IL-13		61.4 (9.2 – 963)
INF-γ		119.8 (0.35 – 1398.1)
IL-4/ INF-γ ratio		0.05 (0.003 – 15.41)
IL-13/ INF-γ ratio		0.72 (0.04 – 614.14)

Table 2. Association (betas (β) and standard errors (SE)) between *p,p'*-DDE concentrations and IL-4, IL-13, INF-γ, and the ratio of IL-4/ INF-γ, and IL-13/ INF-γ (n=19)

	IL-4 (pg/mL)	IL-13 (pg/mL)	INF-γ (pg/Ml)	IL-4/ INF-γ ratio	IL-13/ INF-γ ratio
	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)
p,p'-DDE (µg/g)					
Crude	0.003 (0.003)	0.006 (0.003)	-0.007(0.006)	0.027 (0.010)	0.014 (0.006)
Adjusted §	0.002(0.003)	0.007* (0.003)	-0.010(0.006)	0.033* (0.010)	0.017* (0.006)

§ Adjusted for mother's age, allergy and exposure to passive smoke in home

* $p < 0.05$ based on a t-test

References

1. Noakes PS, Taylor P, Wilkinson S, Prescott SL. The relationship between persistent organic pollutants in maternal and neonatal tissues and immune responses to allergens: A novel exploratory study. Chemosphere 2006: 63: 1304-1311.

2. Karmaus W, Brooks KR, Nebe T, Witten J, Obi-Osius N, Kruse H. Immune function biomarkers in children exposed to lead and organochlorine compounds: a cross-sectional study. Environ Health 2005: 4: 5.

3. Sunyer J, Torrent M, Garcia-Esteban R, et al. Early exposure to DDE, breastfeeding and asthma at age six (in print). Clin Exp Allergy 2006.

4. Tanaka T, Kouda K, Kotani M, et al. Vegetarian diet ameliorates symptoms of atopic dermatitis through reduction of the number of peripheral eosinophils and of PGE2 synthesis by monocytes. J Physiol Anthropol Appl Human Sci 2001: 20: 353-361.

5. Tanabe S, Tatsukawa R, Kawano M, Hidaka H. Global distribution and atmospheric transport of chlorinated hydrocarbons: HCH (BHC) isomers and DDT compounds in the Western Pacific, Eastern Indian and Antarctic Oceans. Journal of Oceanography 1982: 38: 137-148.

6. Wills-Karp M. Interleukin-13 in asthma pathogenesis. Immunol Rev 2004: 202: 175-190.

7. Romagnani S. Immunologic influences on allergy and the TH1/TH2 balance. J Allergy Clin Immunol 2004:113: 395-400.

8. Kondo N, Kobayashi Y, Shinoda S, et al. Reduced interferon gamma production by antigen-stimulated cord blood mononuclear cells is a risk factor of allergic disorders--6-year follow- up study. Clin Exp Allergy 1998: 28: 1340-1344.

9. Piccinni MP, Maggi E, Romagnani S. Role of hormone-controlled T-cell cytokines in the maintenance of pregnancy. Biochem Soc Trans 2000: 28: 212-215.

10. Sonnenschein C, Soto AM. An updated review of environmental estrogen and androgen mimics and antagonists. J Steroid Biochem Mol Biol 1998: 65: 143-150.

COMPONENT TWO

In utero SHD affects postnatal biomarkers of allergy

Maternal oral contraceptive use and immune markers in the offspring.

BACKGROUND: Maternal oral contraceptive (OC) use is shown to be associated with clinical manifestations of allergic diseases in offspring. The aim of this study was to assess the, yet to be reported, association between OC use and humoral immune markers in offspring. We hypothesized that maternal OC use increases humoral immune markers of allergy in offspring.

METHODS: A cross-sectional investigation included 334 mother child (aged 7–10 years) pairs from Hesse, Germany. In 1995, detailed self-administered questionnaires were used to collect information on maternal OC use and atopy, passive smoking, breastfeeding, offspring's gender and number of infections in the previous 12 months. Immunoglobulins (Igs) along with white blood cell, eosinophils, and basophilic surface IgE were also quantified. The data was analyzed using linear regression, while controlling for confounders. Since allergic reactions are different for boys and girls, we stratified by sex.

RESULTS: In children ages 7 to 10, female offspring of mothers who used OC had significantly lower ($p<0.05$): IgA (123.43 mg/dL vs. 150.52 mg/dL), and IgE (22.96 kU/L vs. 50.83 kU/L) levels as well as basophilic surface IgE counts (783 vs. 842), compared to those of mothers who did not use OC. For male offspring, statistical significance was only seen in an increased number of basophilic surface IgE (911 vs. 876).

CONCLUSIONS: This study suggests that maternal OC use may result in sex-related differences of the immunomodulating effects in offspring: a finding that could play a role in the etiology of allergic diseases.

Allergic/atopic diseases are among the most vexing disorders of childhood. The increasing prevalence and severity of asthma and other allergic diseases [1,2] render them of enormous personal and public health concern [3]. Early efforts to elucidate the etiology of these conditions focused on factors such as genetic predisposition, air pollution and tobacco smoke.

The role of synthetic hormones (estrogen and progesterone) as risk factors for allergic diseases has gained increased traction [4-6]. Specifically, the importance of prenatal (*in utero*) exposure to these hormones has been presented [1,7].

Xu and co-workers assessed the effect of maternal age at menarche on the occurrence of atopy among offspring. In a follow-up study of 5188 subjects, they collected data during pregnancy and a follow up examination was completed at 31 years of age. Atopy was determined by skin prick tests while maternal age at menarche was obtained from perinatal data. The authors reported that the prevalence of atopy was lower in children whose mothers reached menarche at a later age [7]: a finding that may be explained by the fact that early age of menarche is associated with higher levels of estrogen in adult women [8-10].

Further support for the possible prenatal endocrine disruption-allergic diseases idea comes from the work of Frye et al. They reported that maternal oral contraceptive (OC) use before birth was associated with a higher risk of atopic

diseases in the offspring compared with children of mothers who had never taken OC [4]. Moreover, in a recent study, we demonstrated significant associations for asthma or wheezing, and cough between 11-12 year old offspring of OC users and non-users [5]. These studies show associations between OC use and clinical manifestations of allergic diseases in offspring; however, a relationship between OC use and humoral immune markers in offspring is yet to be presented.

Using data from a cross-sectional investigation conducted in Hesse, Germany, we assess the relationship between maternal OC use and immune markers in offspring. We hypothesized that maternal OC use significantly increases humoral allergic markers in offspring.

Methods

Subjects and materials

In 1995, parents of 1091 second-grade school children in 18 townships of Hesse, Germany were invited to participate in the study. Children were allowed to participate only if passive smoking in the private household did not exceeded 10 cigarettes per day during the previous 12 months. The study was approved by the Data Protection Agency of the State of Hamburg, Germany, the Ministry of Cultural Affairs of Hesse, Germany, and the participating local school committees.

Questionnaires

Four self-administered parental questionnaires were used in the survey: one regarding the living condition and nutrition of the family, one for each parent, and one regarding information on the child. Maternal oral contraceptive use was determined in an interview on reproductive history. The question posed was "In the 12 months before conception of the index child, did you use oral contraceptives at any time (yes\no)?" Duration of breastfeeding was recorded in weeks of total and in weeks of exclusive nursing. Environmental tobacco smoke (ETS) was graded as smoking in the child's home in the previous 12 months (no cigarettes, 1-20 cigarettes, 11-20 cigarettes, 21-30 cigarettes, more than 30 cigarettes per day). We recorded age, gender, and the number of infections, defined as cold, coughing, and sore throat with or without fever in the last 12 months (none, less than 5 infections, 5-10 infections, more than 10 infections).

Immunologic assays

For phlebotomy, we used the 'Vacutainer System' (Becton, Dickinson & Company, San José, California,). Approximately 25 mL were drawn and separated into different aliquots. Immunoglobulin (Ig) E in serum was quantified at the Medical, Alimentary and Veterinary Institute for Research Middle Hesse, Division of Human Medicine, Dillenburg, Germany, using a florescence-immunoassay (CAP, Pharmacia, Uppsala, Sweden). We also measured IgA, G, and M by laser immunonephelometry (Dade Behring, Liederbach, Germany). The results for IgA, G and M were provided in mg/dL and for IgE in kU/L serum.

For leukocyte subsets, we collected 8 mL of blood in tubes containing EDTA and mixed them to prevent clotting. This aliquot was transported to the Central Laboratory of the University Clinic of Mannheim and analyzed on the same day. We used 200 μL of blood for the automated differential (laser-based hematology analyzer CD3500, Abbott Diagnostics, Santa Clara, California), and 100 μL for each of the nine three-color test tubes analyzed by flow cytometry (FACScan, Becton, Dickinson, & Company, San José, California, equipped with a 488 nm air-cooled argon ion laser).

Eosinophils were determined according to their specific depolarisation characteristics and their eosinophilic granula content by the intensity of light scatter by flow cytometry. IgE on basophils were identified by their high density on the basophil surface using immunofluorescence with a Phycoerythrin labeled anti-IgE antibody.

Statistical methods

Since data for leukocytes (WBC) and their subsets (eosinophils), immunoglobulins were not normally distributed, the geometric mean, 5-, 95-percentiles are provided. In order to obtain a multivariate normal distribution, we log-transformed the number of cells and the immunoglobulins before testing associations with possible predictors.

All statistical analyses were performed using SAS software versions 9.1 [11]. We used multiple linear regression models (PROC GLM) stratified by sex with

immune markers of allergic diseases as dependent variables. The potential confounders; maternal atopy (positive if mother reported a history of asthma, Eczema, or hay fever), age of offspring, ETS, number of infections during the last 12 months, and breastfeeding were controlled for in our models. Age of the child was divided into three groups: 7, 8 and 9-10 years of age. Our main exposure of interest, OC use in the 12 months before conception of index child, was coded 'yes/no'. Information on passive smoking (ETS) in the child's home in the previous 12 months was divided into three categories (no cigarettes, 1-20 cigarettes (a pack), 20 cigarettes per day and more). For the number of infections we considered three categories (none, 1-5, more than 5). Duration of breastfeeding was considered as either: 0 weeks, 1-5 weeks or more than 5 weeks.

From the results of the regression analyses, we calculated adjusted geometric means of immune markers for the categories within each covariate. T-tests were used to compare the statistical effect of maternal OC use with no maternal OC use (reference).

Results

Of 1091 subjects invited, 671 (61.5%) agreed to participate. Of these, only 'natural' mother-child pairs (n=663) were selected for this paper. Phlebotomy was performed on 350 of the 663 children; 340 of whom had immunoglobulins quantified. Complete information (questionnaires and immune markers) was available for 334 children.

A comparison of potential confounders' characteristics between the sub-cohort of 'natural' mother-child pairs and that with complete information is presented in Table 1. Approximately 40 % of mothers who gave birth to boys used OC before pregnancy compared to about a third of those who gave birth to girls. Of the maternal atopy 'family' of variables, hay fever was the most prevalent at 15%. Ninety six percent of the children were between 7 and 8 years of age and approximately 74 % experienced 1 to 5 infections during the last 12 months (Table 1). Due to the eligibility criterion of passive smoking of less than 10 cigarettes in the child's home for being included in blood sampling, the prevalence of passive smoking was also lower in the group with phlebotomy than in the total group (Table 1). Nevertheless, the fact that parents were separated or divorced and shared cohabitation for their child, resulted in a re-assessment of the passive smoking status after phlebotomy. Eligibility was determined on the information provided by one parent (mother or father) for their household. In the case of separate dwellings, we re-assessed the exposure by taking the average number of cigarettes smoked in both homes. As a consequence, 24 (7.5%) children who were exposed to more than 10 cigarettes per day at home had a phlebotomy and were included in the analyses.

Tables 2 reports that for girls whose mothers used OC before pregnancy, their level of IgE was half that of girls whose mothers did not used OC before pregnancy. The level of IgA was also decreased in girls of OC users compared to girls of none-OC users. This decreased level however was less dramatic than that reported for IgE. IgG, IgM and IgE showed an increase with increasing age

in boys while only IgM showed this pattern in girls. IgE showed an increase with age in girls but not in a dose dependent fashion.

Regarding IgE count on basophils; a higher number was evident for boys of mother who use OC compared to boys of mother who were none-OC users. The opposite was true for girls: higher in girls of none-OC-users compared to OC users. In addition, IgE count on basophils was found to increase with increasing age and, conversely, decrease with increasing number of infections (Table 3).

Results from adjusted regression models showed significantly decreased IgA ($p=0.04$; 123.43 mg/dL vs. 150.52 mg/dL) and IgE ($p=0.04$; 22.96 kU/L vs. 50.83 kU/L) in girls of mothers who used OC before pregnancy (Table 5). In boys of OC users, IgE count on basophils was significantly increased ($p=0.04$; 911 vs. 876); concomitantly, they were significantly decreased in girls ($p<0.01$; 783 vs. 842; Table 5).

Discussion

The data for children 7-10 years of age suggest, for the first time, sex-related differences in the association between maternal OC use before pregnancy and serum immune markers in offspring. Decreased serum IgA, and IgE levels as well as basophilic surface IgE counts were observed in female offspring of OC users compared to their female counterpart. In addition, increased basophilic surface IgE counts was present for boys if their mother used OC before pregnancy.

The cross-sectional design of the study is a limitation as it brings into focus the possibility of recall bias. However, mothers did not know the result from their child's serum immunologic analyses therefore the possibility of recall bias in this instance was minimized. Furthermore there is no major public interest about OC use and atopic diseases [4].

Contrary to our *a priori* hypothesis, the data did not reveal an overall significant association between maternal OC use and humoral immune markers in offspring. Similarly, Xu and co-workers failed to find significant associations between maternal sex hormone concentrations (in serum) during early pregnancy and onset of allergic disease in early childhood [12]. Furthermore, a study by Frye et al. found no significant difference for atopic manifestations between offspring of mothers who used OC before and those of mothers who used OC after pregnancy [4]. Nonetheless, our significant sex-related findings are intriguing and consistent with the established hypothesis that there are sex-related differences in immune response [13-16],suggesting the involvement of sex steroid hormones [16,17].

OCp have two major components: progesterone, which is known to stimulate the development of TH2 (allergic type) cells [18], and estrogen that is suppose to increase the production of TH1 (non-allergic type) cells [19,20]. To date, there is no clear explanation of how sex steroid hormones affect the development and

function of the immune system. Nonetheless, it is know that some cells have estrogen and progesterone receptors; creating binding sites for these hormones and ultimately altering immune responses [14,15].

Regarding IgA, Seli and Arici suggest that estrogen may increase or decrease IgA levels in an organ dependent manner [15]. We found that maternal OC use may decrease serum IgA levels in girls. IgA was also decreased in boys of OC users though not statistically significant.

For IgE, our finding of decreased levels in girls of mothers who used OC suggests that hormones may be influencing IgE levels. Since IgE is a strong determinant of asthma [34], this finding supports the existing hypothesis that hormonal factors may be involved in the pathogenesis of asthma.

Concerning basophil-bound IgE, studies have shown increased counts in atopic children [29,30] and deduced that they can serve as an indicator of allergic sensitization [31]. Our finding of decrease counts in girls of mothers who used OC is in concordance with our findings for IgE. Similar explanation may apply. On the other hand, boys of OC users had an increased number of basophil-bound IgE. An increase was also seen for IgE in boys of OC users, however this was not statistically significant. These findings may be due to the estrogen/androgen ratio in boys, which is known to modulate the activities of all cells involve in an

immune response [32]. The pattern of androgenic and estrogenic stimulation during early development (*in utero*) [33] may also be of importance. Further studies are needed to examine these hypotheses.

In conclusion, our study supports the notion that there are sex-related differences in the susceptibility to allergic diseases. Furthermore, the study adds evidence to the idea that maternal OC (oral contraceptive) use may have immunomodulatory effects in offspring.

There are yet unanswered questions. Studies are needed to elucidate the mechanisms by which sex steroid hormones alter immune function. In addition, clarity is needed on how the levels of sex steroids correlate with disease severity. An idyllic setting to begin to answer these questions is that of a follow-up study where women are enrolled early in pregnancy and hormone levels determined from samples taken at different time-points (at least 3) during pregnancy. Cord immune marker of allergy should then be quantified and the association between hormone levels and these markers assessed. In addition to testing for an overall effect, analyses should be stratified by sex to get at possible sex-related differences.

References

1. Beasley R, Crane J, Lai CK, Pearce N: Prevalence and etiology of asthma. The Journal of Allergy and Clinical Immunology 2000;105:S466-472.

2. National institutes of Health, LaBI. NH: GLOBAL STRATEGY FOR ASTHMA MANAGEMENT AND PREVENTION. In, 2005.

3. Guilbert T, Krawiec M: Natural history of asthma. Pediatric Clinics of North America 2003;50:523-538.

4. Frye C, Mueller JE, Niedermeier K, Wjst M, Heinrich J: Maternal oral contraceptive use and atopic diseases in the offspring. Allergy 2003;58:229-232.

5. Brooks K, Samms-Vaughan M, Karmaus W: Are oral contraceptive use and pregnancy complications risk factors for atopic disorders among offspring? Pediatric Allergy and Immunology 2004;15:487-496.

6. Wjst M, Dold S: Is asthma an endocrine disease? Pediatric Allergy and Immunology 1997;8:200-204.

7. Xu B, Jarvelin MR, Hartikainen AL, Pekkanen J: Maternal age at menarche and atopy among offspring at the age of 31 years. Thorax 2000;55:691-693.

8. Vihko R, Apter D: Endogenous steroids in the pathophysiology of breast cancer. Critical Reviews in Oncology/Hematology 1989;9:1-16.

9. Apter D, Reinila M, Vihko R: Some endocrine characteristics of early menarche, a risk factor for breast cancer, are preserved into adulthood. International Journal of Cancer. Journal International Du Cancer 1989;44:783-787.

10. Moore JW, Key TJ, Wang DY, Bulbrook RD, Hayward JL, Takatani O: Blood concentrations of estradiol and sex hormone -binding globulin in relation to age at menarche in premenopausal British and Japanese women. Breast Cancer Research and Treatment 1991;18 Suppl 1:S47-50.

11. SAS Institute: Statistical Analysis System, Version 8. In Institute S (ed):Gary, NC, 2002-2003.

12. Xu B, Pekkanen J, Husman T, Keski-Nisula L, Koskela P: Maternal sex steroid hormones in early pregnancy and asthma among offspring: a case-control study. The Journal of Allergy and Clinical Immunology 2003;112:1101-1104.

13. Inman RD: Immunologic sex differences and the female predominance in systemic lupus erythematosus. Arthritis Rheum 1978;21:849-852.

14. Bouman A, Heineman MJ, Faas MM: Sex steroid hormones and the immune response in humans. Hum Reprod Update 2005;11:411-423.

15. Seli E, Arici A: Sex steroids and the immune system. Immunology and Allergy Clinics of North America 2002;22:407-433.

16. Shames RS: Gender differences in the development and function of the immune system. Journal of Adolescent Health 2002;30:59-70.

17. Whitacre CC, Reingold SC, O_Looney PA: A gender gap in autoimmunity. Science 1999;283:1277-1278.

18. Piccinni MP, Giudizi MG, Biagiotti R, Beloni L, Giannarini L, Sampognaro S, Parronchi P, Manetti R, Annunziato F, Livi C: Progesterone favors the development of human T helper cells producing Th2-type cytokines and promotes both IL-4 production and membrane CD30 expression in established Th1 cell clones. J Immunol 1995;155:128-133.

19. Fox HS, Bond BL, Parslow TG: Estrogen regulates the IFN-gamma promoter. J Immunol 1991;146:4362-4367.

20. Whitacre CC: Sex differences in autoimmune disease. Nature Immunology 2001;2:777-780.

21. Klein KO, Baron J, Colli MJ, McDonnell DP, Cutler GB, Jr.: Estrogen levels in childhood determined by an ultrasensitive recombinant cell bioassay. J Clin Invest 1994;94:2475-2480.

22. Garnett SP, Hogler W, Blades B, Baur LA, Peat J, Lee J, Cowell CT: Relation between hormones and body composition, including bone, in prepubertal children. American Journal of Clinical Nutrition 2004;80:966-972.

23. Sears MR, Burrows B, Flannery EM, Herbison GP, Holdaway MD: Atopy in childhood. I. Gender and allergen related risks for development of hay fever and asthma [see comments]. Clinical and Experimental Allergy 1993;23:941-948.

24. Maziak W, Behrens T, Brasky TM, Duhme H, Rzehak P, Weiland SK, Keil U: Are asthma and allergies in children and adolescents increasing? Results from ISAAC phase I and phase III surveys in Munster, Germany. Allergy 2003;58:572-579.
25. Obendorf M, Patchev VK: Interactions of sex steroids with mechanisms of inflammation. Curr Drug Targets Inflamm Allergy 2004;3:425-433.

26. Akdis M, Blaser K, Akdis CA: T regulatory cells in allergy: novel concepts in the pathogenesis, prevention, and treatment of allergic diseases. J Allergy Clin Immunol 2005;116:961-968; quiz 969.

27. Stock P, Akbari O, DeKruyff RH, Umetsu DT: Respiratory tolerance is inhibited by the administration of corticosteroids. Journal of Immunology 2005;175:7380-7387.

28. Jansson L, Holmdahl R: Estrogen-mediated immunosuppression in autoimmune diseases. Inflamm Res 1998;47:290-301.

29. Stallman PJ, Aalberse RC: Quantitation of basophil-bound IgE in atopic and nonatopic subjects. Int Arch Allergy Appl Immunol 1977;54:114-120.

30. Wada T, Toma T, Shimura S, Kudo M, Kasahara Y, Koizumi S, Ra C, Seki H, Yachie A: Age-dependent increase of IgE-binding and FcepsilonRI expression on circulating basophils in children. Pediatr Res 1999;46:603-607.

31. Lander F, Meyer HW, Norn S: Serum IgE specific to indoor moulds, measured by basophil histamine release, is associated with building-related symptoms in damp buildings. Inflamm Res 2001;50:227-231.

32. Cutolo M, Seriolo B, Villaggio B, Pizzorni C, Craviotto C, Sulli A: Androgens and estrogens modulate the immune and inflammatory responses in rheumatoid arthritis. Ann N Y Acad Sci 2002;966:131-142.

33. Martin JT: Sexual dimorphism in immune function: the role of prenatal exposure to androgens and estrogens. Eur J Pharmacol 2000;405:251-261.

34. Jaakkola MS, Ieromnimon A, Jaakkola JJ.K: Are atopy and specific IgE to mites and molds important for adult asthma? J Allergy Clin Immunol 2006; 117:642-648

Table 1. Descriptive characteristics of the children's cohort

		Group of natural mother-child pairs	Subgroup with maternal oral contraceptive and immune markers data
		(N=663)	(n=334)
		%	%
Sex			
	Boy	53.2	57.4
Maternal oral contraceptive use before pregnancy			
Boy	Yes	38.0	42.4
	Missing	4.5	2.6
Girl	Yes	37.4	33.8
	Missing	4.8	2.8
Maternal			
Asthma	Yes	3.8	3.9
Hay fever	Yes	15.8	15.3
Eczema	Yes	9.8	11.1
Atopy	Yes	23.8	23.7
Age-groups			
	7 years	23.5	46.5
	8 years	25.0	49.5
	9-10 years	2.1	3.9

Table 1 (continued). Descriptive characteristics of the children's cohort

	Group of natural mother-child pairs	Subgroup with maternal oral contraceptive and immune markers data
	(N=663)	(n=334)
	%	%
Passive smoking in the child's home during the last 12 months (cigarettes per day)		
None	51.6	66.7
1-20	37.1	29.7
More than 20	10.1	2.4
Missing	1.2	1.2
Number of infections during the last 12 months		
None	6.0	6.0
1- 5	74.5	74.8
>5	19.3	18.9
Duration of total breastfeeding (weeks)		
0	18.8	14.1
1-5	18.7	15.9
More than 5	57.5	65.5
Missing	5.0	4.5

Table 2: Gender specific geometric mean and 5-, 95 values for immunoglobulins by covriates.

Covariate	IgG (mg/dL)	IgA (mg/dL)	IgM (mg/dL)	IgE (kU/l)
Age-groups				
7 years (155)	1104.4 (739-1560)	130.3 (65-277)	121.6 (59-267)	31.6 (1.6-396.1)
8 years (165)	1121.1 (787-1620)	136.9 (69-279)	123.6 (60-265)	30.7 (2.6-395.6)
9-10 years (14)	1104.0 (739-1560)	146.7 (76-328)	160.5 (59-267)	61.7 (3.8-460.3)
Passive smoking in the child's home during the last 12 months (cigarettes per day)				
None (223)	1111.6 (755 – 1604)	130.4 (65 – 274)	123.7 (60 – 267)	30.3 (2.6 – 416.4)
1-20 (99)	1133.5 (763 – 1560)	148.3 (75 – 294)	121.4 (59 – 219)	35.7 (1.5 – 346.3)
More than 20 (8)	1255.2 (908 – 1880)	100 (21 – 186)	164.8 (63 – 383)	36.9 (7.2 – 183.8)
Number of infections during the last 12 months				
None (20)	1059.1 (797 – 1489.5)	152.8 (75 – 268)	105.6 (66–283.5)	28.1 (1.8 – 476.8)
1- 5 (250)	1126.3 (755 – 1595)	132.5 (65 – 280)	126.6 (59 – 268)	31.2 (2.6 – 379.2)
>5 (63)	1112.5 (824 – 1604)	135.5 (72 – 240)	120.1 (67 – 233)	38.2 (2.1 – 416.4)
Duration of total breastfeeding (weeks)				
0 (47)	1106.9 (762 – 1850)	132.9 (60 – 279)	121.1 (60 – 279)	22.9 (0.84–770.8)
1-5 (53)	1103.7 (763 – 1550)	135.5 (64 – 280)	117.2 (55 – 255)	24.9 (2.1 – 334.1)
More than 5 (219)	1131.7 (755 – 1620)	133.7 (65 – 281)	126 (65 – 267)	36.1 (2.7 – 396.1)

Table 3: Gender specific geometric mean and 5-, 95 values for white blood cell, eosinophilic characteristics, and basophilic surface IgE by covriates.

Covariate		White blood cells, $x10^3/\mu L$	Eosinophil cell count, $x10^3/\mu L$	Eosinophilic granula	IgE count on basophils
Sex					
	Boy (190)	8304.7 (5900 – 12200)	205.2 (68 – 888)	900.3 (804 – 984)	849.5 (655 – 964)
	Girl (142)	8433.3 (6200 – 11900)	149.5 (51 – 649)	898.4 (799 – 972)	822.5 (629 – 952)
Maternal oral contraceptive use					
Boy	Yes (81)	8255.5 (5900 – 12500)	203.2 (66 – 820)	899.1 (806 – 984)	863.2 (656 – 964)
	No (104)	8317.0 (5920 – 12100)	201.7 (68 – 798)	900.6 (794 – 982)	836.3 (647 – 961)
Girl	Yes (49)	8757.0 (6280 – 12300)	132.1 (50 – 509)	891 (780 – 979)	784.3 (626 – 924)
	No (89)	8323.7 (6200 – 11300)	161.1 (51 – 746)	902.3 (828 – 962)	843.3 (660 – 959)
Maternal atopy					
	Yes (81)	8422.4 (6100 – 11500)	189.6 (45 – 780)	898.7 (794 – 981)	842.2 (638.8 – 962)
	No (251)	8339.3 (5920 – 12200)	175.8 (62 – 793)	899.7 (809 – 980)	836.7 (638.8 – 962)
Age-groups					
	7 years (155)	8547.1 (5900 – 12000)	196.8 (45 – 75)	899.2 (796 – 979)	834.7 (656 – 957)
	8 years (164)	8174.3 (6000 – 12200)	161.4 (51 – 726)	899 (806 – 985)	839.3 (630 – 961)
	9-10 years (13)	8511.4 (6360 – 12500)	213.9 (65 – 1575)	910.3 (839 – 973)	861 (688 – 973)
Passive smoking in the child's home during the last 12 months (cigarettes per day)					
	None (220)	8365.6 (6135 – 12050)	186 (59 – 795.5)	897 (796 – 981)	835.9 (632 – 961)
	1-20 (100)	8416.8 (5700 – 12400)	197 (62 – 924)	906.2 (821 – 981.5)	841.4 (658 – 963)
	More than 20 (8)	7812.8 (5160 – 10400)	22.6 (0.01 – 447)	877.7 (633 – 957)	826 (625 – 959)

Table 3 (continued): Gender specific geometric mean and 5-, 95 values for white blood cell, eosinophilic characteristics, and basophilic surface IgE by covriates.

Covariate	White blood cells, $x10^3/\mu L$	Eosinophil cell count, $x10^3/\mu L$	Eosinophilic granula	IgE count on basophils
Number of infections the last 12 months during				
None (20)	8408.3 (6100 – 11600)	198.4 (77.5 – 574.5)	902 (843 – 984)	837.3 (656 – 989)
1- 5 (247)	8498.7 (5960 – 11800)	166.1 (54 – 785)	897.6 (805 – 979)	835.6 (638.4– 952)
>5 (64)	8285 (6000 – 12500)	231.7 (85 – 924)	906.5 (786 – 982)	849.5 (630 – 965)
Duration of total breastfeeding (weeks)				
0 (47)	8322.7 (5580 – 12500)	157.2 (59 – 1260)	893.5 (806 – 981)	826 (623.1 – 964)
1-5 (54)	8277.1 (6200 – 12500)	147.5 (44 – 726)	899.1 (780 – 995)	805.5 (614 – 957)
More than 5 (217)	8403.2 (6000 – 12100)	191.2 (68 – 785)	900.4 (805 – 980)	847.7 (660 – 954)

Table 4: Maternal oral contraceptive use by immunoglobulins

Outcome		IgG (mg/dL)		IgA (mg/dL)		IgM (mg/dL)		IgE (kU/l)	
		Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls
N									
Maternal oral contraceptive use									
Crude	Yes	1096	1129	134	114	110	138	42	14
	No	1093	1153	139	139	119	139	39	29
Adjusted §	Yes	1156	1257	138	123*	126	151	75	23*
	No	1144	1291	144	151	133	155	69	51

§ Adjusted for maternal atopy, child's age, passive smoking in the child's home during the last 12 months, number of infections during the last 12 months, and duration of total breastfeeding.

* $p<0.05$ based on a t-test compared to no maternal oral contraceptive use as the reference.

Table 5: Maternal oral contraceptive use by white blood cell, eosinophilic characteristics, and basophilic surface IgE

Outcome		White blood cells, $x10^3/\mu L$		Eosinophil cell count, $x10^3/\mu L$		Eosinophilic granula		IgE count on basophils	
		Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls
N									
Maternal oral contraceptive use									
Crude	Yes	8255	8757	203	152	899	891	863	784
	No	8317	8324	202	161	901	902	836	843
Adjusted §	Yes	8112	9084	281	127	903	887	911*	783*
	No	8085	8680	270	120	905	900	876	842

§ Adjusted for maternal atopy, child's age, passive smoking in the child's home during the last 12 months, number of infections during the last 12 months, and duration of total breastfeeding.

* $p<0.05$ based on a t-test compared to no maternal oral contraceptive use as the reference.

COMPONENT THREE

In utero exposure to SHD affects allergic outcomes in infancy

Are oral contraceptive use and pregnancy complications risk factors for atopic disorders among offspring?

Kevin Brooks [1], Maureen Samms-Vaughan [2], Wilfried Karmaus [1]

[1] Department of Epidemiology, School of Human Medicine, Michigan State University, East Lansing, United States;

[2] Department of Obstetrics, Gynecology and Child Health, University of the West Indies, Mona campus, Jamaica.

Abstract

In utero programming of atopic manifestations has been suggested. We investigated the association between oral contraceptive (OC) use before, and complications during pregnancy (CDP) and asthma, along with other atopic manifestations. The study is based on neonates from Kingston and St. Andrew, a geographic sub-cohort from the Jamaican Perinatal Morbidity, Mortality Survey conducted in 1986-1987. Information on OC use and CDP was extracted from maternal interviews and medical records. In a follow-up in 1997-1998, via interviews with mothers, trained nurses collected information on asthma/wheezing, coughing, eczema, and hay fever. Data, specific to this paper, from birth and 11 – 12 years of age was available for a total of 1040 of the 1720 members of the geographic sub-cohort. Using logistic regression, controlling for confounders, we estimated adjusted odds ratio (aOR) and corresponding 95 % confidence intervals (CI). For asthma or wheezing, and coughing, aOR for OC use were 1.81 (95% CI 1.25- 2.61), and 2.72 (95% CI 1.41-5.24), respectively. CDP was only shown to be a significant risk factor for hay fever. Additionally, a higher number of older siblings were protective for hay fever. The results suggest that asthma in childhood may be programmed *in utero*.

Key words: asthma; eczema; hay fever; atopy; oral contraceptives; pregnancy complications

The prevalence of allergies is increasing in many parts of the world, and asthma has become the most common chronic disease of childhood. The etiology of asthma and allergic disease remains poorly understood, despite considerable research [1]. Recent work has expanded to include the study of novel factors such as *in utero* and perinatal exposures that may program initial susceptibility [2]. Some authors have reported that maternal estrogen levels and maternal health complications during pregnancy are associated with asthma and other atopic manifestations later in life [3-6].

The most appropriate design to test an association between perinatal conditions and asthma in offspring is a longitudinal study commencing with mothers in the antenatal period and following their offspring through childhood. The Jamaican Perinatal Mortality and Morbidity Survey (JPMMS) [7], and the subsequent follow-up studies offered a unique opportunity for testing these hypotheses.

The JPMMS, originally designed to ascertain causes of maternal and neonatal mortality and morbidity, obtained detailed information about maternal lifestyle before and during pregnancy, the labor and delivery process. The subsequent follow-up studies of the children were designed to identify factors promoting and preventing optimum childhood outcomes, including child health.

Women who participated in the JPMMS in 1986 were contacted at six weeks postpartum and again eleven to twelve years later. We investigated whether *in utero* and perinatal factors contributed to the development of asthma and other atopic manifestations in children 11 to 12 years of age. Specifically, we tested the association between two risk factors:

(1) Maternal use of oral contraceptives (OC) before pregnancy

(2) Maternal complications during pregnancy (CDP)

and the prevalence of four atopic manifestations:

(a) Asthma/wheezing,

(b) frequent nighttime/early morning cough,

(c) eczema, and

(d) hay fever/sinus problems/other allergy

Methods

Study population

The Jamaican Perinatal Morbidity and Mortality Survey included all pregnant women who had a live or stillbirth during the two-month period from September 1st to October 31st, 1986. Women were interviewed and their babies examined, usually within the first 48 hours after delivery. Data were gathered during this phase using the main questionnaire (MQ). A total of 10392 babies or 94 % of the births in the two-month period were identified and included in the study (main cohort study) [8]. The first follow-up study of the children occurred at six weeks of age when 8,800 (84.7 %) mother-child pair from the main cohort were

interviewed. Information on breastfeeding and the infant's health was obtained using the first follow-up questionnaire (FFUQ). These 8800 mother-child pairs were used as the baseline cohort (entire island) for the analyses.

Follow-up

A geographic sub-sample of 1,720 eleven to twelve-year-old children, representing those residing in Kingston or St. Andrew, the two most urban of Jamaica's fourteen parishes, was selected for a second follow-up study. In a cross-sectional survey of these children, data on health outcomes were collected from participating parents or guardians (n=1,163), mostly the mothers, and recorded using a second follow-up questionnaire (SFUQ). We linked the three datasets using a unique identification number assigned at birth and dates of birth for both mother and child.

Questionnaires and examinations

During the JPMMS, trained nurse midwives administered face-to-face interviews with mothers and conducted a brief examination of the babies shortly after birth. Babies were also weighed and measured. Hospital delivery notes and maternal health records were consulted to verify and supplement the data obtained by interview. The MQ provided data on past obstetric history and complications during pregnancy (CDP), labor and delivery.

Data on the current status of the baby (alive/healthy, alive/ill, dead or don't know) and what the baby was currently being fed (breast, breast and formula or formula only) was extracted from the FFUQ. Information on the presence of asthma or

wheezing, coughs, eczema and hay fever/ sinus problem/ other allergy in the children at 11 to 12 years of age was collected from the general health section of the SFUQ. Trained nurse interviewers administered the questionnaire to either the parents or guardians.

Definition of variables

Maternal age at birth was calculated from the mother's date of birth and child's date of delivery. Based on questions from the MQ, a yes, no, or don't know response was used to define the following:

- Did you smoke tobacco regularly at any time during this pregnancy?
- Have you ever used contraception? If yes, which methods have you used? (Contraceptive pill was among a list of contraception.)
- Were you admitted to hospital or rural maternity center during this pregnancy but before going into labor? (Used to determine maternal hospital admissions.)
- Are any of the following services available within 1-2 miles walking distance of where you live?' (Health center was among a list of services. – Used to determine access to health care.)

Mother's education level was grouped into three categories for the purpose of this study (less than secondary, secondary, and more than secondary education).

Maternal CDP was defined as a positive response to any of the following questions:

1) Did you have vaginal bleeding in the *first* 28 weeks of pregnancy?
2) Did you have vaginal bleeding *after* 28 weeks (7 months)?

3) After starting antenatal care, were you referred for treatment during pregnancy for any reason?

4) Were you referred during pregnancy for any reason?

5) Was the mother diagnosed as having hypertension, pre-eclampsia or eclampsia during this pregnancy?

6) Did the mother have any of the following during this pregnancy; epilepsy, eclamptic fits or heart disease and lastly,

7) Did the mother have any other complications, disorder or serious illness during this pregnancy?

Responses to the first five questions above were obtained by interview, while the remainder was obtained from health records.

Maternal infection during pregnancy was defined as a positive answer to any of the following question based on information from medical records:

1) Did the mother have a vaginal discharge or infection during this pregnancy?

2) Did the mother have urinary tract infection, tuberculosis, rubella (German measles), gonorrhea, syphilis, a positive Venereal Disease Research Laboratory test or genital sores or blisters during pregnancy?

The following variables were used to define maternal health complications during labor or delivery (positive if indicated in the medical records):

1) Did the mother have eclamptic fits?

2) Did she hemorrhage?

3) Was labor obstructed?

4) Did the uterus rupture?

5) Was mother transferred during labor?

6) Did the cord prolapse?

7) Was there meconium in the liquor?

8) Were there any other complications?

The number of preceding live births determined birth order of the index child. The child's birth weight was also recorded during this period (MQ) and grouped into three categories for the purpose of our analyses (<2.5kg, 2.5-4.0 kg and >4.0 kg).

The variable child's illness or health problems in first week of life was defined as a positive response to any of the following questions from the FFUQ:

1) Was the baby referred to a medical officer?

2) Was the baby admitted to hospital or special care baby unit?

3) Were any abnormal symptoms noted in the baby?

Outcomes of interest at 11 to 12 years of age were ascertained from the SFUQ. The original question to the parents was, 'Has your child ever had...' This question was followed by a list of conditions; including asthma or wheezing, frequent nighttime or early morning cough, eczema, and hay fever sinus problem or other allergy.

Statistical analysis

We tabulated the prevalence of the four atopic manifestations for the different ante- and perinatal exposures. Differences in proportions between the different sub-samples were assessed. Adjusted odds ratios (aOR) and their 95 %

confidence intervals (95% CI) were estimated by logistic regression analysis. Statistically, we controlled for maternal infection, hospital admissions and smoking during pregnancy, as well as maternal complications during labor/delivery, induced labor, anesthetic/analgesic during labor, gender, birth weight, breastfeeding, child's illness in the first week of life, access to health care and mother's level of education. These potential confounders were included based on previous publications and after satisfactorily, checking for collinearity between all predictors (risk factors and all confounders) [9]. We estimated aOR for all four outcome variables independently.

Further steps were taken to determine whether having both, asthma or wheezing and frequent nighttime or early morning cough, was associated with OC use and complications during pregnancy. All calculations were carried out using SAS for Microsoft Windows (release 8.2) [10].

Results

From the three data sets, we successfully linked 1040 (60.4 %) of the 1720 available mother-child pairs from the Kingston and St. Andrew sub-cohort. Compared characteristics between exposure variables from the baseline cohort (entire island) and the linked data set (urban population) are presented in Table 1. Approximately 50 % of mothers used OC, and approximately, 50 % had CDP. In the baseline sample, maternal education was lower than in our sub-sample (Table 1: less than secondary level of education – baseline: 35.6%, subsample:

25.4%). The proportion of children born with low birth weight (<2.5 kg) was higher in the more urban setting of Kingston and St. Andrew (12.2 %), where the second follow-up cohort originated. A significant rural-urban difference was also indicated by the difference in breastfeeding categories.

When comparing the follow-up group for whom health information was available, (*n*=1163 of 1720) with the linked sample (*n*=1040) there was no substantial difference (asthma or wheezing: 17.0 % vs. 16.8 %, frequent nighttime or early morning cough: 4.8 % vs. 5.3 %, eczema: 6.5 % vs. 6.3 %, hay fever or sinus problem: 19.6 % vs. 20.1 %).

Table 2 presents the extent to which atopic manifestations occur with each other. The proportion of atopic disorders in relation to *in utero* risk factors is presented in Table 3. Children of mothers, who used, compared to those who did not use OC before pregnancy, had a higher prevalence of asthma (20 % *vs.* 13.4 %), frequent nighttime or early morning cough (7.4 % *vs.* 3.6 %) and eczema (7.4 % *vs.* 5.5 %). Asthma and wheezing, as well as hay fever or sinus problems were more common in children whose mothers had complications during pregnancy (CDP) than in those who did not.

Of the perinatal risk factors, induced labor was related to a higher prevalence of eczema (11.4 % *vs.* 6 %, Table 4). Children of mothers who had complications during labor or delivery had a greater proportion of frequent nighttime or early

morning cough (8.1 % *vs.* 4.8 %). In children who had illness or health problems in their first week of life, eczema (10.5 % *vs.* 5.3 %) and hay fever sinus problem or some other allergy (27.4 % *vs.* 18.3 %) were more frequently reported at 11-12 years of age.

Information on breastfeeding was available for only 763 children (Table 4). For this reason, breastfeeding was not considered when testing the hypotheses with a full model. A possible confounding effect of breastfeeding was investigated in additional analyses.

Adjusted odds ratios for asthma or wheeze, and frequent nighttime or early morning cough are reported in Table 5 while those for eczema and hay fever, sinus problems or other allergy are reported in Table 6. Maternal OC use before pregnancy was significantly associated with both asthma (aOR = 1.81) and frequent nighttime or early morning cough (aOR = 2.72) in the offspring. The association between maternal OC use before pregnancy and asthma was stronger for girls (aOR = 2.02, 95% CI 1.21-3.37) than for boys (aOR = 1.60, 95% CI 0.92- 2.76). Regarding eczema, hay fever, and sinus problem or other allergy at age 11-12 years of age, the confidence limits of the aOR for maternal OC use included the null-value (Table 6).

Additionally, it is well established that 'All that wheezes is not asthma' [11], therefore we conducted further analysis with a combined variable, defined as the

conditions asthma or wheezing and frequent nighttime or early morning cough occurring concurrently. Of children whose mothers used OC, 5.1 % had asthma or wheezing, and coughing compared to 1.9 % of children of mothers who did not. This association was then assessed controlling for confounders in the full model. When compared to the two separate effects, the association of the combined outcome was stronger (aOR=3.32, 95% CI 1.33-8.32).

Complications during pregnancy gained statistical importance only for hay fever or sinus problems (Table 6). When type of feeding was introduced into the models, analyses were then based on 763 children. For CDP, this resulted in an increased odds ratio for hay fever (aOR=1.72, 95% CI 1.13-2.63) (data not shown). However, the significant odds ratio for oral contraceptive use did not change.

A higher number of older siblings (birth order) showed a significant protective effect for hay fever (Table 6). For asthma or wheezing and for coughing, similar association was found though not statistically significant (Table 5).

Discussion

The findings support the hypothesis that children whose mothers used OC (oral contraceptive) before pregnancy, are at a higher risk for developing asthma or wheezing, and frequent nighttime or early morning cough at 11 to 12 year of age. The data also supported the hypothesis that a maternal complication during pregnancy is a risk factor for atopic manifestations in offspring.

The association between OC use and asthma or wheezing and coughing is in concordance with a suggestion of Wjst et al. who, based on aggregative data on OC sales and hospital discharge data, were the first to hypothesize that maternal use of OC is a risk factor for asthma in the offspring [3]. To the best of our knowledge, the present study is the first to prospectively assess the association between, maternal OC use and asthma or wheeze in offspring, using individual data.

The most frequently used OC in Jamaica during the study period was the Pearl ®, in which the active components were ethinyloestradiol (30 μg) and levonorgestrel (150 μg), an estrogen and progesterone respectively. It has been suggested that women who use OC have higher estrogens levels after discontinuing their use [12], however, the evidence is not consistent [13].

The OC-atopy association may be explained by endocrine effects on T-helper 2 (Th_2) cells. Th_2 cells produce interleukin (IL)-4, IL-5, and IL-13, which promote the production of IgE and eosinophil infiltration in the airways and ultimately orchestrate the development of allergy and asthma [14-16]. Wjst and co-workers suggest that estrogen might enhance the activity of Th_2 type cytokines [3], predisposing the fetus to atopic disorders later in life. Furthermore, in vitro studies suggest that progesterone promotes the preferential development of Th_2-type cells and to function as a potent inducer of the production of the Th_2 type

cytokines [17-19]. Michel et al. reported that IgE levels in cord blood were significantly increased among neonates whose mothers had taken progesterone during pregnancy [20].

We suggest two possible scenarios for the endocrine mechanism, which may explain our OC-atopy association. Either maternal exposure to estrogen via OC use may trigger an increase Th_2 activity continuing into pregnancy (prolonged Th_2 effect), consequently predisposing the fetus to a higher risk of developing atopy later in life, a Th_2-mediated disorder [15,21,22]. Alternately, women who used OC continuously, discontinuing before becoming pregnant, may have created an environment of higher estrogen or progesterone levels (prolonged endocrine effect), resulting in an increased Th_2 activity impacting the fetus transplacentally.

Evidence for the prolonged Th_2 effect is supported by findings that women exposed *in utero* to the estrogen diethylstilbestrol, were found to have alterations in their T-cell-mediated immunity [23]. In addition, children of mothers who used OC in the 18 months prior to being pregnant were found to be more likely to have eczema at age five [24]. This finding was later corroborated by Xu et al. [25].

Evidence in support of an prolonged endocrine effect: Xu et al. reported that atopy in adults was more common among those whose mothers experienced an early age of menarche [4]. A likely explanation for the atopy – age at menarche

association is that early age of menarche is associated with higher levels of estrogen in adult women [13, 26-28]. Hence, the fetus of women with an early age at menarche may experience higher exposure to estrogens. This could then lead to the programming of the immune system of the fetus affecting atopic status later in life [29].

In a cross-sectional study, Frye et al. [30] provided data to support the prevailing OC – atopy association. They however downplayed the significance of this finding on the basis that similar results were found in the offspring of women who used OC exclusively after they delivered. It is noteworthy to accentuate the fact that Frye and co-workers used a cross-sectional design wherein OC use was assessed retrospectively. This design will no doubt be plagued with biases. These results may be an artifact of 'reverse causation' introduced by behavior modification of the parents. An example of this is women in the 'exclusive OC use after birth' group may have chosen to resume the use of OC after the index child developed atopic eczema. The reason is that a child with eczema will require more attention at nights. Therefore, a mother with a demanding child may decide to delay having another by using OC.

There is a paucity of studies on the relationship between maternal complications during pregnancy and the risk of having asthma or any atopic disorders. Nafstad et al. in a population-based, four-year, cohort study involving 2531 children. Asthma was assessed at age four [5]. The authors reported that uterus-related

complication (antepartum hemorrhage, preterm contractions, insufficient placenta, and restricted growth of the uterus) increased the risk of having asthma (OR = 3.0, 95 % CI 1.8-5.4) and allergic rhinitis (OR = 2.9, 95 % CI 1.6-5.2). Annesi-Maesano et al. also found maternal complications during pregnancy to be a risk factor of asthma (OR = 2.01, 95 % CI 1.52-2.67) in the offspring using a large British birth cohort (4065 natural children of 2583 mothers) [6]. More recently, two studies have supported these findings [31,32], one by using data on Norwegian live births (1967-1993, n=1,548,429) [31]. Our study found maternal complications during pregnancy (CDP) to be a significant risk factor for hay fever and sinus problems (aOR = 1.5, 95 % CI 1.05-2.16, p=0.03).

Further analyses for items included in the predictor complication during pregnancy (CDP) indicated that only the variable 'have there been any other complications, disorder or serious illness during this pregnancy?' conferred a significantly increased risk for asthma in the child ($p< 0.001$), but none of the specific items. This finding is in agreement with a recent report by Nafstad et al. who also found that pregnancy complications in general might present risk factors of stress for the developing fetus [31]. It might be that CDP (complications during pregnancy) is reflective of factors such as a threatened pregnancy, found to be associated with frequent wheezing [33], which will alter the Th_1 / Th_2 balance [34,35]. This hypothesis warrants further investigations with adequate perinatal and breastfeeding information.

Our study adds to the body of evidence, which suggest that the number of siblings, in particular older siblings, is important in the etiology of atopic disorders [28]. We found number of older siblings to be protective for hay fever or sinus problems (Table 6). Number of older siblings and oral contraceptive use were statistically significantly correlated ($r_{Spearman}$ = 0.3, p=0.0001, n=1020).

The lack of association between low birth weight and asthma is in contrast to some previous studies [6,36-38] and in agreement with others [39-42]. If is worth mentioning that the finding from our Afro-Caribbean cohort is similar to that of an African-American cohort [40].

The strengths of the current investigation are in its prospective design, being derived from a large population based cohort, with all the exposure information collected during pregnancy, at birth and shortly after. This design presents a clear time-order, as outcomes were not assessed until approximately 11 years after. The impact of recall bias is also reduced. Additionally, controlling for a broad range of confounders minimize the possibility of these findings occurring purely by chance.

A limitation of this study is the follow-up proportion of 60.4 %. This is largely explained by parental participation in the second follow-up study. Though

parental consent was obtained for the 1720 children evaluated using the child's completed reports, only 1163 parents provided interview and questionnaire data. The significant differences found between the characteristics of some of the variables from the baseline cohort when compared with those of the linked dataset were expected. This, we infer, is due to the rural-urban difference between these groups.

The difference between reported occurrence of CDP in the baseline cohort (38.4%) and our sub-sample from Kingston/St. Andrew (49.2%) is a possible source of selection bias. The baseline cohort has both rural and urban populations, whereas the sub-sample if predominantly urban. Different proportions of maternal complications in these samples can be explained by two factors, namely maternal education level and access to health care. For instance, of the women in our sub-sample who reported having complications during pregnancy, 3.6% were characterize as having low level education (less than secondary) and less access to health care compared to 8.9% of those with similar characteristics from the baseline cohort. Recently, McCaw-Binns et al reported that access to health care in Jamaica differs from rural to urban areas [43]. Since these two factors (maternal education level and access to health care) seem to affect the reported proportion of maternal complications, and as the prevalence of asthma, eczema, and sinus problems/allergies increase with level of education, they were included as confounders in our models to minimize the impact of a selection effect (Tables 5 and 6).

Another limitation is the absence of information on how long before pregnancy OC was used and if it was the last method of birth control used. This limitation introduces a non-differential misclassification, which tends to underestimate the association between OC and asthma or wheezing, and coughing. To evaluate, whether the OC hypothesis holds true, we investigated data of children from pregnancies that occurred despite birth control (*n*=90), 42 with and 35 without using oral contraceptives. Asthma was more frequently reported at 11-12 years of age in children whose mothers used OC, 23.6 %, compare to 17.1 % in children whose mothers did not use OC when they conceived.

We did not collect information on parental atopy and thus could not adjust for it in our model. While there is data showing parental history of atopy to be a risk factor for atopic disorders in the offspring, data supporting an association between parental history of atopy and oral contraceptive use is lacking and the plausibility of such an association is questionable. Hence, it is highly unlikely that parental history of atopy would have confounded the association between oral contraceptive and atopy, since confounding requires both, association with exposure and outcome [44].

The present study was not originally designed to address our hypotheses. However, an advantage of the cohort study design is that data collected can be used to provide answers to newly emerging research questions. We therefore

took advantage of the utility and efficiency of this study to test our respective hypotheses. Additionally, the unique nature of this cohort makes this the first study to test perinatal risk factors for asthma in an Afro-Caribbean population.

Regarding coughing, we asked for 'frequent night time or early morning coughing', but did not include the adjective 'dry'. Nevertheless, the validity of 'early morning coughing' as a marker for asthma has been found to be sufficient [45,46]. On the other hand, it has been shown that bronchial hyperreactivity was not related to dry cough or to nocturnal cough [47]. According to Chang, coughing and bronchoconstriction may be caused by different mechanisms. Hence, different asthma-like symptoms may indicate different features [48].

The definition or measurement of asthma or wheezing may also be of concern as wheezing is a heterogeneous disorder [49] and not all that wheezes is asthma [11]. The impact this misclassification may have, was assessed by combining the variables asthma or wheezing and frequent nighttime or early morning cough to create a compound outcome variable. The combined condition was less prevalent and represented a more specific state of bronchial reactivity. The association between the combined variable and maternal OC use before pregnancy was assessed and results showed a stronger association (aOR = 3.15, 95 % CI 1.29- 7.71). Additionally, we investigated whether the association with asthma was more likely due to an atopic response. For this purpose, we

combined two variables: having both, asthma and allergies. The association between maternal OC and the new combined variable was stronger (aOR 2.26, 95% CI 1.18-4.35) compared to the association with only asthma (aOR 1.81, 95% CI 1.25-2.61). This strengthening suggests that the association is likely to have been the result of an atopic mechanism.

In summary, this data contributes to existing literature suggesting that maternal oral contraceptive use may be important in the development of asthma in offspring. In particular, the data helps to address the ongoing question as to whether it is 'hormones or hygiene' [50] that is responsible for atopy. There are only a few existing studies that allow for the investigation of maternal OC use and the child's risk of atopic manifestation. The hypothesis that oral contraceptive use may contribute to an increase of asthma in children as put forward by Wjst [3] is very thought provoking. We suggest this hypothesis be tested in future studies with more precise exposure assessment.

Acknowledgements

Funding for the Jamaican Perinatal Morbidity, Mortality Survey was provided by the International Development Research Center of Canada while the Jamaica Cohort study was supported by the Policy Development Unit of the Planning Institute of Jamaica.

References

1. ASHER MI, KEIL U, ANDERSON HR, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. The European Respiratory Journal : Official Journal of the European Society For Clinical Respiratory Physiology 1995;8:483-91.

2. BEASLEY R, CRANE J, LAI CK, PEARCE N. Prevalence and etiology of asthma. The Journal of Allergy and Clinical Immunology 2000;105:S466-72.

3. WJST M, DOLD S. Is asthma an endocrine disease? Pediatric Allergy and Immunology 1997;8:200-4.

4. XU B, JARVELIN MR, HARTIKAINEN AL, PEKKANEN J. Maternal age at menarche and atopy among offspring at the age of 31 years. Thorax 2000;55:691-3.

5. NAFSTAD P, MAGNUS P, JAAKKOLA JJ. Risk of childhood asthma and allergic rhinitis in relation to pregnancy complications. J Allergy Clin Immunol. 2000;106:867-73.

6. ANNESI-MAESANO I, MOREAU D, STRACHAN D. In utero and perinatal complications preceding asthma. Allergy 2001;56:491-7.

7. ASHLEY D, MCCAW-BINNS A, FOSTER-WILLIAMS K. The perinatal morbidity and mortality survey of Jamaica 1986-1987. Paediatr Perinat Epidemiol 1988;2:138-47.

8. ASHLEY D, MCCAW-BINNS A, GOLDING J, et al. Perinatal mortality survey in Jamaica: aims and methodology. Paediatr Perinat Epidemiol 1994;8 Suppl 1:6-16.

9. KLEINBAUM K, MULLER. Applied Regresion Analysis and Other Multivariable Methods, 1988.

10. SAS INSTITUTE. Statistical Analysis System, Version 8. Gary, NC, 2000.

11. MURRAY DM, LAWLER PG. All that wheezes is not asthma. Paradoxical vocal cord movement presenting as severe acute asthma requiring ventilatory support. Anaesthesia 1998;53:1006-11.

12. BARBIERI RL, GAO X, XU H, CRAMER DW. Effects of previous use of oral contraceptives on early follicular phase follicle-stimulating hormone. Fertility and Sterility 1995;64:689-92.

13. MOORE JW, KEY TJ, WANG DY, BULBROOK RD, HAYWARD JL, TAKATANI O. Blood concentrations of estradiol and sex hormone -binding globulin in relation to age at menarche in premenopausal British and Japanese women. Breast Cancer Research and Treatment 1991;18 Suppl 1:S47-50.

14. MOVERARE R, ELFMAN L, STALENHEIM G, BJORNSSON E. Study of the Th1/Th2 balance, including IL-10 production, in cultures of peripheral blood mononuclear cells from birch-pollen-allergic patients. Allergy 2000;55:171-5.

15. MAZZARELLA G, BIANCO A, CATENA E, DE PALMA R, ABBATE GF. Th1/Th2 lymphocyte polarization in asthma. Allergy 2000;55:6-9.

16. KUO ML, HUANG JL, YEH KW, LI PS, HSIEH KH. Evaluation of Th1/Th2 ratio and cytokine production profile during acute exacerbation and convalescence in asthmatic children. Ann Allergy Asthma Immunol 2001;86:272-6.

17. PICCINNI MP, GIUDIZI MG, BIAGIOTTI R, et al. Progesterone favors the development of human T helper cells producing Th2-type cytokines and promotes both IL-4 production and membrane CD30 expression in established Th1 cell clones. Journal of Immunology (Baltimore, Md. : 1950) 1995;155:128-33.

18. SZEKERES-BARTHO J, WEGMANN TG. A progesterone-dependent immunomodulatory protein alters the Th1/Th2 balance. J Reprod Immunol 1996;31:81-95.

19. HAMANO N, TERADA N, MAESAKO K, et al. Effect of female hormones on the production of IL-4 and IL-13 from peripheral blood mononuclear cells. Acta Otolaryngol Suppl 1998;537:27-31.

20. MICHEL FB, BOUSQUET J, GREILLIER P, ROBINET_LEVY M, COULOMB Y. Comparison of cord blood immunoglobulin E concentrations and maternal allergy for the prediction of atopic diseases in infancy. The Journal of Allergy and Clinical Immunology 1980;65:422-30.

21. WARNER JA, JONES CA, WILLIAMS TJ, WARNER JO. Maternal programming in asthma and allergy. Clin Exp Allergy 1998;28 Suppl 5:35-8; discussion 50-1.

22. JONES CA, HOLLOWAY JA, WARNER JO. Does atopic disease start in foetal life? Allergy 2000;55:2-10.

23. BURKE L, SEGALL-BLANK M, LORENZO C, DYNESIUS-TRENTHAM R, TRENTHAM D, MORTOLA JF. Altered immune response in adult women exposed to diethylstilbestrol in utero. Am J Obstet Gynecol 2001;185:78-81.

24. PETERS TJ, GOLDING J. The epidemiology of childhood eczema: II. Statistical analyses to identify independent early predictors. Paediatric and Perinatal Epidemiology 1987;1:80-94.

25. XU B, JARVELIN MR, PEKKANEN J. Prenatal factors and occurrence of rhinitis and eczema among offspring. Allergy 1999;54:829-36.

26. VIHKO R, APTER D. Endogenous steroids in the pathophysiology of breast cancer. Critical Reviews in Oncology/Hematology 1989;9:1-16.

27. APTER D, REINILA M, VIHKO R. Some endocrine characteristics of early menarche, a risk factor for breast cancer, are preserved into adulthood. International Journal of Cancer. Journal International Du Cancer 1989;44:783-7.

28. KIRCHENGAST S, HARTMANN B. Association between maternal age at menarche and newborn size. Soc Biol 2000;47:114-26.

29. WESTERGAARD T, BEGTRUP K, ROSTGAARD K, KRAUSE TG, BENN CS, MELBYE M. Reproductive history and allergic rhinitis among 31145 Danish women. Clin Exp Allergy 2003;33:301-5.

30. FRYE C, MUELLER JE, NIEDERMEIER K, WJST M, HEINRICH J. Maternal oral contraceptive use and atopic diseases in the offspring. Allergy 2003;58:229-32.

31. NAFSTAD P, SAMUELSEN SO, IRGENS LM, BJERKEDAL T. Pregnancy complications and the risk of asthma among Norwegians born between 1967 and 1993. Eur J Epidemiol 2003;18:755-61.

32. CALVANI M, ALESSANDRI C, SOPO SM, et al. Infectious and uterus related complications during pregnancy and development of atopic and nonatopic asthma in children. Allergy 2004;59:99-106.

33. STAZI MA, SAMPOGNA F, MONTAGANO G, GRANDOLFO ME, COUILLIOT MF, ANNESI-MAESANO I. Early life factors related to clinical manifestations of atopic disease but not to skin-prick test positivity in young children. Pediatr Allergy Immunol 2002;13:105-12.

34. NILSSON L, KJELLMAN NI, LOFMAN O, BJORKSTEN B. Parity among atopic and non-atopic mothers. Pediatr Allergy Immunol 1997;8:134-6.

35. PICCINNI MP, MAGGI E, ROMAGNANI S. Role of hormone-controlled T-cell cytokines in the maintenance of pregnancy. Biochem Soc Trans 2000;28:212-5.

36. SCHWARTZ J, GOLD D, DOCKERY DW, WEISS ST, SPEIZER FE. Predictors of asthma and persistent wheeze in a national sample of children in the United States. Association with social class, perinatal events, and race. Am Rev Respir Dis 1990;142:555-62.

37. SVANES C, OMENAAS E, HEUCH JM, IRGENS LM, GULSVIK A. Birth characteristics and asthma symptoms in young adults: results from a population-based cohort study in Norway. Eur Respir J 1998;12:1366-70.

38. STEFFENSEN FH, SORENSEN HT, GILLMAN MW, et al. Low birth weight and preterm delivery as risk factors for asthma and atopic dermatitis in young adult males. Epidemiology 2000;11:185-8.

39. GREGORY A, DOULL I, PEARCE N, et al. The relationship between anthropometric measurements at birth: asthma and atopy in childhood. Clin Exp Allergy 1999;29:330-3.

40. OLIVETI JF, KERCSMAR CM, REDLINE S. Pre- and perinatal risk factors for asthma in inner city African-American children. American Journal of Epidemiology 1996;143:570-7.

41. SEARS MR, HOLDAWAY MD, FLANNERY EM, HERBISON GP, SILVA PA. Parental and neonatal risk factors for atopy, airway hyper-responsiveness, and asthma. Arch Dis Child 1996;75:392-8.

42. KATZ KA, POCOCK SJ, STRACHAN DP. Neonatal head circumference, neonatal weight, and risk of hayfever, asthma and eczema in a large cohort of adolescents from Sheffield, England. Clin Exp Allergy 2003;33:737-45.

43. MCCAW_BINNS A, STANDARD_GOLDSON A, ASHLEY D, WALKER G, MACGILLIVRAY I. Access to care and maternal mortality in Jamaican hospitals: 1993-1995. International Journal of Epidemiology 2001;30:796-801.

44. ROTHMAN KJ. Modern epidemiology, 1986.

45. KUEHR J, FRISCHER T, KARMAUS W, MEINERT R, BARTH R, URBANEK R. Clinical atopy and associated factors in primary-school pupils. Allergy 1992;47:650-5.

46. FERRIS BG. Epidemiology Standardization Project (American Thoracic Society). Am Rev Respir Dis 1978;118:1-120.

47. STRAUCH E, NEUPERT T, IHORST G, et al. Bronchial hyperresponsiveness to 4.5% hypertonic saline indicates a past history of asthma-like symptoms in children. Pediatr Pulmonol 2001;31:44-50.

48. CHANG AB. Cough, cough receptors, and asthma in children. Pediatr Pulmonol 1999;28:59-70.

49. DELL S, TERESA , T. Breastfeeding and asthma in young children. Arch of Pediatric Adolescence medicine 2001;155:1261-1265.

50. RANGARAJ S, DOULL I. Hormones not hygeine? Birth order and atopy. Clin Exp Allergy 2003;33:277-8.

Table 1. Comparison characteristics of exposure variables in baseline and linked datasets

Factors	Baseline Cohort (%) (Perinatal and six-week follow-up) (*N*=8800)	Linked Data (%) from the sub-sample in Kingston and St. Andrew (*n*=1040)
Maternal oral contraceptive use	45.7	49.9
Maternal complications during pregnancy	38.4	49.2
Maternal infections during pregnancy	22.5	25.7
Maternal hospital admission during pregnancy	9.6	12.3
Induced labor	4.3	6.7
Analgesic or anesthetic given during labor	12.0	22.6
Maternal health complications during labor or delivery	11.6	14.5
Maternal education - Less than secondary	35.6	25.4
Higher than secondary	7.9	15.3
Live within 1-2 miles of Health centre	78.3	86.1
Feeding – Breast only	46.2	34.9
Breast and formulae	49.3	59.0
Formulae only	4.5	6.4
Birth weight - <2.5 kg	10.7	12.2
2.5 kg - 4.0 kg	85.1	83.4
>4.0 kg	4.2	4.4
Child's illness or health problems in first week of life	10.9	20.3

Table 2. Proportions of simultaneous occurrence of the different atopic manifestations

	Eczema[†]		Asthma or Wheezing		Frequent nighttime or early morning cough		Total group
	Yes *(n=65)*	No *(n=962)*	Yes *(n=173)*	No *(n=857)*	Yes *(n=53)*	No *(n=944)*	*(n=1040)*
Asthma or Wheezing	36.9	15.4					16.6
Frequent nighttime or early morning cough	13.8	4.6	16.1	3.1			5.1
Hay fever or sinus problem or some other allergy	50.0	18.0	34.9	17.1	47.0	18.6	19.8

[†]Total proportions = 6.3.

Table 3. Proportion of atopic disorders in relation to their *in utero* risk factors

	Gender		Maternal oral contraceptive use		Maternal complications during pregnancy		Maternal infections during pregnancy		Maternal hospital admissions during pregnancy		Smoking during pregnancy	
Outcome %	Male (*n*=488)	Female (*n*=547)	Yes (*n*=511)	No (*n*=509)	Yes (*n*=528)	No (*n*=512)	Yes (*n*=267)	No (*n*=773)	Yes (*n*=127)	No (*n*=907)	Yes (*n*=78)	No (*n*=962)
Asthma or Wheezing	16.7	16.9	20.0	13.4	18.9	14.8	18.9	16.1	18.1	16.7	11.5	17.2
Frequent nighttime or early morning cough	4.5	6.0	7.4	3.6	5.5	5.2	5.9	5.1	7.1	5.1	5.3	5.3
Eczema	6.0	6.7	7.4	5.5	7.3	5.4	5.7	6.5	10.2	5.8	6.4	6.3
Hay fever sinus problem or some other allergy	19.1	21.1	20.3	20.0	22.7	17.7	21.0	19.8	15.9	20.8	11.7	20.8

Table 4. Proportion of atopic disorders in relation to their perinatal potential risk factors

	Induced labor		Analgesic or anesthetic given during labor		Maternal complications during labor or delivery	
Outcome %	Yes (*n*=70)	No (*n*=970)	Yes (*n*=232)	No (*n*=794)	Yes (*n*=522)	No (*n*=518)
Asthma or Wheezing	18.6	16.7	18.3	16.6	18.0	16.6
Frequent night time or early morning cough	4.4	5.4	5.0	6.6	8.1	4.8
Eczema	11.4	6.0	12.2	4.7	7.3	6.2
Hay fever sinus problem or some other allergy	20.3	20.1	28.8	17.2	21.5	19.9

Table 4. (continued) Proportion of atopic disorders in relation to their perinatal potential risk factors

	Feeding			Birth weight			Child's illness or health problems in first 4 weeks of life	
Outcome %	Breast only (*n*=266)	Breast & Formulae (*n*=449)	Formulae only (*n*=48)	< 2.5 kg (*n*=125)	2.5 kg 4.0 kg (*n*=852)	> 4.0 kg (*n*=45)	Yes (*n*=211)	No (*n*=829)
Asthma or Wheezing	14.8	18.7	17.4	12.8	17.6	17.8	17.7	16.6
Frequent nighttime or early morning cough	5.9	6.0	4.6	8.1	5.1	4.4	5.8	5.2
Eczema	6.8	6.1	6.5	3.2	6.7	8.9	10.5	5.3
Hay fever sinus problem or some other allergy	16.5	20.6	24.4	15.5	21.1	15.6	27.4	18.3

Table 5. Odds ratios† and 95% confidence intervals (CI) of antenatal and perinatal risk factors for asthma or wheezing, and frequent nighttime or early morning cough

	Asthma or Wheezing (*n*=976)				Frequent nighttime or early morning cough (*n*=945)			
Factors	cOR	95% CI	aOR	95% CI	cOR	95% CI	aOR	95% CI
Antenatal outcomes								
Maternal oral contraceptive use	1.62*	1.15-2.26	1.81*	1.25-2.61	2.17*	1.21-3.89	2.72*	1.41-5.24
Maternal complications during pregnancy	1.43*	1.03-1.99	1.27	0.86-1.81	1.30	0.75-2.28	0.90	0.47-1.71
Maternal infections during pregnancy	1.21	0.84-1.74	1.05	0.71-1.56	1.15	0.62-2.14	1.28	0.66-2.47
Maternal hospital admission during pregnancy.	1.10	0.68-1.79	1.11	0.65-1.89	1.43	0.68-3.01	1.46	0.62-3.41
Smoking during pregnancy	0.63	0.31-1.28	0.70	0.33-1.46	0.99	0.35-2.82	1.07	0.35-3.20
Perinatal outcomes								
Induced labor	1.14	0.61-2.13	0.97	0.50-1.86	0.81	0.25-2.67	0.39	0.09-1.70
Analgesic or anesthetic given during labor	1.11	0.76-1.64	0.82	0.52-1.30	1.34	0.72-2.48	0.99	0.47-2.10
Maternal complications during delivery	1.10	0.70-1.74	1.10	0.67-1.82	1.74	0.89-3.39	1.70	0.80-3.64
Child's illness or health problems in the first week of life	1.08	0.73-1.62	0.97	0.61-1.55	1.13	0.58-2.19	0.83	0.38-1.80
Number of older siblings	0.93	0.80-1.08	0.87	0.71-1.07	0.99	0.77-1.26	0.86	0.60-1.23
Birth weight <2.5kg	0.69	0.39-1.20	0.64	0.36-1.17	1.66	0.81-3.41	1.58	0.72-3.47
Reference 2.5 kg – 4.0 kg.	1	1	1	1	1	1	1	1
>4.0 kg	1.01	0.46-2.22	1.07	0.48-2.41	0.87	0.20-3.73	0.50	0.07-3.85

† Adjusted for variables in the table and additionally for gender and maternal age, education and access to health care.
* p-value ≤ 0.05

Table 6. Odds ratios† and 95% confidence intervals (CI) of antenatal and perinatal risk factors for eczema, and hay fever or sinus problem or some other allergy

	Eczema (*n*=973)				Hay fever or sinus problem or some other allergy (*n*=968)			
Factors	cOR	95% CI	aOR	95% CI	cOR	95% CI	aOR	95% CI
Antenatal outcomes								
Maternal oral contraceptive use	1.35	0.81-2.24	1.21	0.69-2.12	0.98	0.72-1.33	1.01	0.71-1.43
Maternal complications during pregnancy	1.43	0.86-2.37	1.22	0.67-2.21	1.29	0.95-1.76	1.50*	1.05-2.16
Maternal infections during pregnancy	0.87	0.48-1.57	0.70	0.36-1.35	1.07	0.76-1.52	1.00	0.68-1.47
Maternal hospital admission during Pregnancy.	1.85	0.98-3.50	1.76	0.84-3.69	0.72	0.43-1.19	0.62	0.36-1.10
Smoking during pregnancy	1.01	0.39-2.60	1.25	0.42-3.74	0.50	0.25-1.03	0.70	0.33-1.49
Perinatal outcomes								
Induced labor	2.04	0.93-4.46	1.19	0.51-2.80	1.01	0.55-1.86	0.69	0.35-1.35
Analgesic or anesthetic given during labor	2.81*	1.68-4.71	1.54	0.83-2.87	1.96*	1.39-2.75	1.26	0.83-1.90
Maternal complications during delivery	1.19	0.61-2.34	0.78	0.36-1.68	1.10	0.72-1.68	0.82	0.50-1.34
Child's illness or health problems in first week of life.	2.12*	1.24-3.63	1.62	0.87-3.02	1.69*	1.19-2.40	1.41	0.92-2.15
Number of older siblings	0.74*	0.58-0.95	0.76	0.55-1.06	0.73*	0.63-0.85	0.70*	0.57-0.86
Birth weight <2.5kg	0.47	0.17-1.31	0.39	0.13-1.14	0.68	0.41-1.14	0.61	0.34-1.09
Reference 2.5 kg – 4.0 kg.	1	1	1	1	1	1	1	1
>4.0 kg	1.37	0.47-3.96	1.51	0.49-4.68	0.69	0.30-1.57	0.62	0.25-1.54

† Adjusted for variables in the table and additionally for gender and maternal age, education and access to health care.
* p-value ≤ 0.05

COMPONENT FOUR

***Postnatal exposure to SHD affects biomarkers of allergy* in infancy**

Immune function biomarkers in children exposed to lead and organochlorine compounds: a cross-sectional study

Wilfried Karmaus[1§], Kevin R. Brooks[1*], Thomas Nebe[2*], Jutta Witten[3*], Nadia Obi-Osius[4*], Hermann Kruse[5*]

[1] Department of Epidemiology, Michigan State University, B601 West Fee Hall, East Lansing, MI 48824, USA

[2] Central Laboratory, University Hospital Mannheim, Germany

[3] Ministry of Social Welfare Hesse, Department of Health, Wiesbaden, Germany

[4] Epidemiological Working Group of the Ministry of Environment and Health and the Institute for Mathematics and data management in Medicine, University Hospital Hamburg-Eppendorf and

[5] Institute of Toxicology, Christian-Albrecht University, Kiel, Germany

*These authors contributed equally to this work

[§]Corresponding author

Abstract

Background: Different organochlorines and lead (Pb) have been shown to have immunomodulating properties. Children are at greater risk for exposure to these environmental toxicants, but very little data exist on simultaneous exposures to these substances.

Methods: We investigated whether the organochlorine compounds (OC) dichlorodiphenylethylene (DDE), hexachlorobenzene (HCB), hexachlorocyclohexane (γ-HCH), the sum of polychlorinated biphenyls (∑PCBs)

and Pb were associated with immune markers such as immunoglobulin (Ig) levels, white blood cell (WBC), counts of lymphocytes; eosinophils and their eosinophilic granula as well as IgE count on basophils. The investigation was part of a cross-sectional environmental study in Hesse, Germany. In 1995, exposure to OC and Pb were determined, questionnaire data collected and immune markers quantified in 331 children. For the analyses, exposure (OC and Pb) concentrations were grouped in quartiles (γ-HCH into tertiles). Using linear regression, controlling for age, gender, passive smoking, serum lipids, and infections in the previous 12 months, we assessed the association between exposures and immune markers. Adjusted geometric means are provided for the different exposure levels.

Results: Geometric means were: DDE 0.32μg/L, ΣPCBs 0.50 μg/L, HCB 0.22 μg/L, γ-HCH 0.02 μg/L and Pb 26.8μg/L. The ΣPCBs was significantly associated with increased IgM levels, whereas HCB was inversely related to IgM. There was a higher number of NK cells (CD56+) with increased γ-HCH concentrations. At higher lead concentrations we saw increased IgE levels. DDE showed the most associations with significant increases in WBC count, in IgE count on basophils, IgE, IgG, and IgA levels. DDE was also found to significantly decrease eosinophilic granula content.

Conclusion: Low-level exposures to OC and lead (Pb) in children may have immunomodulating effects. The increased IgE levels, IgE count on basophils, and the reduction of eosinophilic granula at higher DDE concentrations showed a

most consistent pattern, which could be of clinical importance in the etiology of allergic diseases.

Background

Environmental toxicants such as organochlorine compounds (OC) and lead (Pb) may alter immune responses. There is a paucity of studies reporting associations between organochlorine [1-4] and lead [5-8] exposures and immune function biomarkers in children.

We conducted a large-scale environmental study of second-grade school children in three regions south of the Federal State of Hesse, Germany in 1995. Two of the regions are situated in the Rhine Valley with low mountains on both sides. One of these areas with several municipalities is located within a 10 km radius around an industrial waste incinerator and other industries, such as chemical plants. One plant was associated with dichlorodiphenylethylene (DDE), hexachlorobenzene (HCB), and hexachlorocyclohexane (γ-HCH) pollution [9]. The other region, also industrial, is 15 km north (downwind) of the incinerator. Both Rhine valley regions are also intensively used for the production of vegetables. The third study region is located in low mountains (about 0.4 km above sea level) that separate it from the industrial area. Blood concentrations of PCBs were shown to be higher in children living close to the toxic waste incinerator [10]. Results on PCBs and thyroid hormones, chromium and lymphocytes, DDE and breastfeeding and asthma have been published elsewhere [4, 11-15].

Considering infection and atopic disorder in children, we have previously shown an association between DDE blood levels; asthma and one immunoglobulin (Ig), namely IgE [4]. However, the potential effects of organochlorines on other Igs

and cellular defense were not reported. Hence, the focus of this paper is to investigate the impact of organochlorine compounds and Pb on humoral immune markers and cell-mediated immune responses. Specifically, for immune responses we focus on leukocytes, lymphocytes, B-cell, T-cells and their subsets. Assuming a concurrent effect of OC on immune markers, we conducted cross-sectional analyses of the data from the first of three surveys conducted in 1994/1995, 1996, and 1997. Only the first investigation included an extensive clinical assessment of immune markers.

Methods

Study population

After obtaining approval from the Data Protection Agency of Hamburg, Germany, the Ministry of Cultural Affairs of Hesse, Germany, and the local school committees, we invited the parents of 1,091 second-grade school children in 18 townships to participate in our study. We obtained informed consent from all participating parents, according to the requirements of the Ethical Committee of the Board of Physicians, the Helsinki Declaration, and the Data Protection Agency of the State of Hamburg. We asked each parent to allow their child to participate in phlebotomy only when passive smoking in the private household did not exceeded 10 cigarettes per day during the previous 12 months.

Questionnaires

We used four self-administered parental questionnaires in the survey: one regarding the living condition and nutrition of the family, one for each parent, and

one regarding information on the child. Duration of breastfeeding was recorded in weeks of total and in weeks of exclusive nursing. Environmental tobacco smoke (ETS) was graded as smoking in the child's home in the previous 12 months (no cigarettes, 1-10 cigarettes, 11-20 cigarettes, 21-30 cigarettes, more than 30 cigarettes per day). We recorded age, gender, and the number of infections, defined as cold, coughing, and sore throat, with or without fever, in the last 12 months (none, less than 5 infections, 5-10 infections, more than 10 infections).

Laboratory analyses of blood samples

One parent accompanied each child in the medical examination. For blood sampling, we used the 'Vacutainer System' (Becton, Dickinson & Company, San José, California,). Approximately 25 mL were drawn and separated into different aliquots. Immunoglobulin (Ig) E in serum was quantified at the Medical, Alimentary and Veterinary Institute for Research Middle Hesse, Division of Human Medicine, Dillenburg, Germany, using a florescence-immunoassay (CAP, Pharmacia, Uppsala, Sweden). To determine levels of specific IgE against inhalant allergens (aeroallergens), we incubated serum with immunocaps containing a mixture of aeroallergens and determined the reactivity using a fluorescence measurement (UNICAP Pharmacia, Uppsala, Sweden). Results from this method were provided in semi-quantitative format. We also measured IgA, G, and M by laser immunonephelometry (Dade Behring, Liederbach, Germany). The results for IgA, G and M were provided in mg/dL and for IgE in kU/L serum. Triglycerides and cholesterol were measured on a clinical chemistry analyzer according to IFCC methods (Hitachi 717, Boehringer Mannheim).

Leukocyte subsets

We collected 8 mL of blood in tubes containing EDTA and mixed them to prevent clotting. This aliquot was transported to the Central Laboratory of the University Clinic of Mannheim and analyzed on the same day. We used 200 μL of blood for the automated differential (laser-based hematology analyzer CD3500, Abbott Diagnostics, Santa Clara, California), and 100 μL for each of the nine three-color test tubes analyzed by flow cytometry (FACScan, Becton, Dickinson, & Company, San José, California, equipped with a 488 nm air-cooled argon ion laser). Eosinophils were determined according to their specific depolarisation characteristics and their eosinophilic granula content by the intensity of light scatter by flow cytometry. Basophils were identified by their high IgE density on the cell surface using immunofluorescence with a Phycoerythrin (PE) labeled anti-IgE antibody.

We used monoclonal antibodies directed against specific cell surface antigens to differentiate cell populations by multicolour immunofluorescence. Three antibodies were simultaneously applied with the fluorochrome combination FITC/PE/PE-Cy5. CD4/CD8/CD3 was used to detect absolute number of lymphocytes, T-helper cells and cytotoxic T-cells; CD19/CD5/IGE was used to differentiate B-cell subsets and basophils; CD3/CD16 and CD56/CD57 were used for natural killer cells. CD45RO defines memory T-helper cells. The CD nomenclature assigns the antibodies to clusters of differentiation, according to the International Workshop on Human Leukocyte Differentiation Antigens [16].

Organochlorine compounds (OC) in blood

OC including eight PCB congeners (101, 118, 138, 153, 170, 180, 183, 187), DDE, HCB, and three HCH congeners (α-, β- and γ) were determined (in μg/L) at the Institute of Toxicology, University of Kiel, Germany. OC were analyzed in 5 mL samples of whole blood by high resolution gas chromatography (HRGC, Model 3400 by Varian Inc., Palo Alto, California) with a 63Ni-electron-capture-detector. The detection limit (DL) (two times the signal/low-noise ratio) was 0.02 μg/L for β- and γ-HCH, DDE and each PCB congener, and 0.01 μg/L for HCB and α-HCH. For extraction and clean-up procedures, we used florisil and n-hexane for elution (9 g florisil were deactivated with 3% H_2O and placed in a chromatography column 22 mm in diameter and 48 mm in length). The capillary column amounted to 30 mm in length and 0.25 mm in diameter; nitrogen was used as a carrier gas. We determined the PCB congeners by retention times on the chromatograms and identified them by comparison with known standards. Additionally, we tested reliability with gas chromatography-mass spectroscopy (GC/MS). The laboratory successfully participated in nationwide quality assessments for the determination of these OC.

Lead in blood

Lead (Pb) analysis was done at the Institute of Toxicology, University of Kiel, Germany. The determination in whole blood samples was by flow injection atomic absorption spectroscopy (Perkin Elmer) after adding 0.1% Triton –X-1-solution and 15 mol nitric acid to from a solution. This solution was then centrifuged at 3000 rpm. The DL for Pb was 9 μg/L (48 nmol/l; atomic weight:

207.19).

Data analyses

Since the data for leukocytes (WBC) and their subsets (lymphocytes and eosinophils), immunoglobulins, DDE, PCB congeners, HCB, γ-HCH and Pb were not normally distributed, the geometric mean, 5-, 95-percentiles are provided. In order to obtain a multivariate normal distribution, we log-transformed the number of cells and immunoglobulins before testing associations with possible predictors by multiple linear regression models.

All statistical analyses were performed using SAS software [17]. We calculated the sum of the PCB congeners (ΣPCBs = sum of seven congeners, the congener PCB101 was not detected). For descriptive purposes, we substituted values of OC below detection limit with one half of the detection limit. The statistical procedure (PROC RANK) was used to group exposure variables into quartiles (DDE, PCBs, HCB and Pb) or tertiles (γ-HCH). All observations below the detection limit were part of the lowest level group (reference). To account for the influence of lipids on the concentration of OC, we controlled for the sum of triglycerides and cholesterol in the regression analyses. Further steps were taken to determine whether our results were different when lipids were represented as sum of triglycerides and cholesterol as opposed to triglycerides and cholesterol as individual variables.

We used linear regression models (PROC GLM) with immune markers as dependent variables and all organochlorine compounds and lead as independent

variables in each model. We also controlled for potential confounders (age, gender, environmental tobacco smoke (ETS), number of infections during the last 12 months, and lipid concentration). Information on passive smoking (ETS) in the child's home in the previous 12 months was divided into four categories (no cigarettes, 1-10 cigarettes, 11-20 cigarettes, 21 cigarettes per day and more). For the number of infections we considered four categories (none, less than 5, 5-10, more than 10). Age of the child was divided into three groups; 7, 8 and 9-10 years.

From the results of the regression analyses, we calculated adjusted geometric means for leukocyte subsets and immunoglobulins for increasing categories of exposure. T-tests were used to compare the statistical effect of higher exposure group to the lowest (reference).

Since one major route of exposure to the pollutants analyzed is breast feeding [18-21] and breastfeeding provides passive immunity [22-24], immune markers and pollutants could be spuriously correlated if breast feeding is not controlled for.

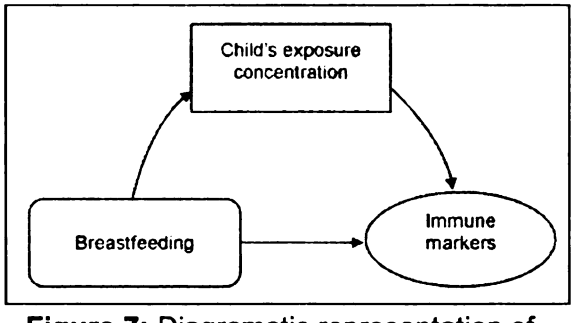

Figure 7: Diagramatic representation of the breastfeeding, childhood exposures and immune markers associations

However, this triangle (Figure 7) cannot be tested with linear regression models, as intervening variables do not qualify as confounders [25]. Controlling will

reduce the initial association between the risk factor and the marker, as one causal chain is split into two associations. Thus, we explored the relationship between childhood breastfeeding (total duration of breastfeeding in weeks), the concentration of OC, and immune response by path analysis [26], using the CALIS procedure SAS Institute [17].

Results

The proportion of participation was 61.5 % (671 of 1091). We obtained blood samples from 350 children, conducted OC and Pb analyses on 343 samples, and quantified immunoglobulins in 340. Overall, information (i.e., questionnaires, exposure biomarkers, and immune markers) was available for 331 children. Fewer girls than boys participated in phlebotomy; and 96 % of the children were 7 to 8 years of age (Table 1). Due to the inclusion criterion for blood sampling (passive smoking of less than 10 cigarettes in the child's home), the prevalence of passive smoking was also lower in the group with phlebotomy than in the total group (Table 1). Nevertheless, the fact that parents were separated or divorced and shared cohabitation for their child, resulted in a re-assessment of the passive smoking status after phlebotomy. Eligibility was determined on the information provided by one parent (mother or father) for their household. In the case of separate dwellings, we re-assessed the exposure by taking the average number of cigarettes smoked in both homes. As a consequence, 26 (7.9%) children who were exposed to more than 10 cigarettes per day at home had a phlebotomy and were included in the analyses.

For γ-HCH, 27.7 % of the observations were below the detection limit, 2.9% for Pb, whereas none for DDE and HCB. At least one of seven PCB congeners was detected in each sample. Whole blood concentration for the sum of PCB congeners (118, 138, 153, 170, 180, 183, 187), HCB and of Pb showed a decline with increasing age (Table 2). DDE, PCB, and HCB concentrations were lower in children with higher passive smoking exposure. Regarding infections, lead concentration was higher in children with more than 10 infections during the last 12 months, whereas DDE concentration was lower in this group (Table 2).

The concentrations of DDE, ΣPCBs (sum of PCBs), and HCB were all correlated (Table 3). However, we used categorized levels of OC, which were then only marginally correlated; the highest rank correlation was for the PCB and HCB groups ($r_{Spearman}$=0.46). These correlations did not result in multicollinearity since the tolerance (variance of OC not explained by other predictors) was at least 53%. The volume-based organochlorine concentrations were only marginally correlated with the lipid serum levels. To adjust for lipid concentrations, we included lipid concentrations as a confounder in the explanatory models for leukocyte subsets and immunoglobulins. Results derived from models using the sum of triglycerides and cholesterol compared to triglycerides and cholesterol as individual variables did not reveal any substantial difference (data not shown). We therefore reported results from models using the sum of triglycerides and cholesterol.

Regarding lead in whole blood, we found weak correlations with whole blood levels of OC (DDE: $r = 0.15$, $n = 331$, $p < 0.01$; HCB: $r = 0.14$, $n = 331$, $p < 0.01$; γ-HCH: $r = -0.02$, $n = 331$, $p < 0.70$; ΣPCBs: $r = 0.14$, $n = 331$, $p < 0.01$)

Increased white blood cell count (WBC; total leukocytes) was evident in the group with highest DDE level, whereas Pb, at the second, along with PCB at the highest level was associated with a reduction in WBC count. An increase in the number of eosinophils – a leukocyte subset – was identified in the highest DDE category, but not statistically significant, Table 4. However, eosinophilic granula content was significantly reduced at the upper DDE levels. In addition, IgE count on basophils was increased at higher DDE exposure, being statistically significant for the 0.3-0.43μg/L category.

Regarding lymphocytes and specific lymphocyte subsets (B-cells, T-cells), the number of T-cells (CD3+), cytotoxic T-cells (CD8+) and B-cells (CD19+) were all significantly reduced in the median Pb category (Table 5). Both natural killer (NK) cells (CD56+) and a NK cells subset (CD57+) were significantly associated with γ-HCH. However, these associations did not reveal dose-dependency.

All four immunoglobulins were associated in a virtually dose dependent fashion to either DDE, HCB or PCBs (Table 6). IgM serum levels increased with the concentration of PCBs (F-test, $p<0.01$) but decreased with increasing concentration of HCB (F-test, $p<0.01$). In the two upper quartiles of DDE exposures, IgA levels were significantly higher, but lower in the upper quartile of HCB. DDE was not associated in a dose-response mode with IgG (F-test,

p=0.14), however, compared to the reference, the highest DDE exposure group showed a significantly elevated IgG level (t-test, p=0.04). IgE levels more than doubled as DDE concentration increased (F-test, p=0.02). The Pb serum levels were related to a significant differences in IgE (F-test: p=0.028), but not in a dose dependent fashion (Table 6).

Figure 8 shows that both DDE and lead were associated with higher serum IgE levels in children. In groups with lower DDE blood concentrations, Pb concentrations above the median (28µg/L) were related to increase IgE levels. In groups with higher DDE, there was no additional effect of Pb. Statistically, the combined effect of DDE and Pb on IgE was not significant.

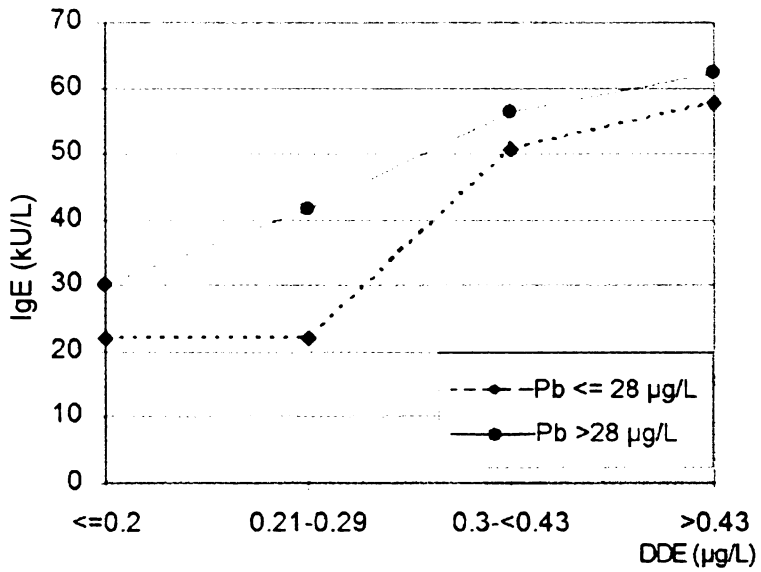

Figure 8: The combined effect of increasing DDE and lead (Pb) on IgE serum levels

In order to determine whether breastfeeding confounded the associations identified in linear regression models (Figure 7), we repeated our analyses using structural model (path analysis) for exposures determined as significant in linear regressions. Inclusion of breast feeding did not substantially change our findings.

Discussion

In 331 school children, age 7-10 years, we demonstrated significant relationships between OC and Pb whole blood concentration and cellular and humoral immune markers. First, modest associations were found between NK cells (CD3-CD16+CD56+) and a subset of natural killer cells (CD3-CD16+CD56+CD57+) and γ-HCH (Table 5). Second, HCB was inversely related to IgM. Third, ΣPCBs were directly related to IgM. Fourth, our data showed that Pb decreased the count of T-cells (CD3+), cytotoxic T-cells (CD3+CD8+), and B-cells (CD3+CD5+ CD19+). This reduction was most evident at the 22.1 – 28.3 µg/L Pb concentration, though not in a dose response fashion. Lastly, DDE was inversely related to all immunoglobulins, except IgM (Table 6). However, DDE was not associated with total serum protein (data not shown). The DDE effect was strongest for IgE – more than twofold increase - which also corresponded to an increased count of IgE on basophils. We did not detect a significant relationship between DDE and eosinophils, nevertheless, the number of eosinophils was positively correlated with IgE ($r_{Spearman} = 0.4$, $p < 0.01$). However, high DDE levels were found to be significantly associated with lower eosinophilic granula content. The granula contains basic proteins which are cytotoxic and part of the inflammatory response [27].

The cross-sectional nature of the study limits conclusions on whether exposure occurred before immune responses. We can assume that organochlorine concentrations do not vary substantially in childhood, post breastfeeding. There is a decline of PCBs and HCB with age (Table 2), however the assumption of the

stability is supported by a follow-up of the same children and OC determined in 1997. The Spearman rank correlation between the two successive measurements were high, with the exception of γ-HCH: DDE: $r = 0.86$, $n = 274$, $p < 0.01$; HCB: $r = 0.74$, $n = 274$, $p < 0.01$; γ-HCH: $r = 0.1$, $n = 270$, $p = 0.11$; ΣPCBs: $r = 0.82$, $n = 274$, $p < 0.01$.

The reported concentrations for OC were not lipid-based. In this cohort, there is a high correlation between lipid- and non lipid-based concentrations for OC (Table 3). Thus, our findings are independent of lipid- or wet weight-based determinations. In our models we controlled for lipids instead of dividing the concentration of OC by the lipid concentration for three reasons. First, a simple division assumes a monotonous linear relation between lipids and organochlorines. Although Phillips and co-workers reported for 20 adults that division by lipids reduces the difference between fasting and non-fasting concentration of OC [28], there is no data to justify a linear relation. Our data in children showed only weak correlations between OC and the sum of cholesterol and triglycerides (Table 3). This correlation did not increase when the sum of lipids were derived by using the 2nd formula proposed by Phillips et al. [28]. Second, there is no standard approach to adjust concentrations below the limit of detection for lipids. In particular, the probability of detection may be influenced by the individual lipid concentration of a child. Third, division by lipids does not take into account that they may confound the organochlorine – immune response relationships. Confounding is likely since lipids and OC are correlated, plus lipids

are, for example, associated with the count of lymphocytes [29, 30].

There is evidence that breast milk is a significant source of OC, Pb [18-21], and passive immunity [22-24]. Path analytical techniques (Figure 7) were used to verify whether breastfeeding as an intervening variable confounded our associations. The inclusion of breastfeeding in the path analysis did not reveal results different from the linear regression models. Hence, the associations between pollutants and immune markers were independent of breastfeeding.

We found whole blood concentrations of OC in our cohort comparable to similar children in Germany [31]. Compared to children in the United States, age 12-19 years (NHANES - 1999-2000) [32], our DDE values were lower though still within the 95% confidence interval. However, when comparing our results (in whole blood) with those of NHANES (in serum), we have to consider differences between serum and whole blood concentrations. Mes et al. reported that DDE was higher in sera and plasma than in whole blood samples [33]. Conversely, PCBs were higher in whole blood samples. No other comparison with NHANES data was possible as the values for PCB congeners and other OC were below the limit of detection.

Regarding lead (Pb), the geometric mean of 27µg/L in our investigation was similar to the 33µg/L found in a study of 797 East-German children 5-14 years of age [34]. Against that, the 1999-2000 NHANES study showed a lower geometric mean (15.1 µg/L) in 905 children 6-11 years of age [32]. However other studies in areas of higher exposure, reported average concentrations above the NHANES

value: 40 µg/L for children, 6 to 15 years of age in four communities with mining and smelting operations and two control groups in the United States [6], and 95µg/L in Chinese children 3-6 years old [8].

We selected a subgroup for blood analyses due to budget constraints. The group having a lower ETS exposure in their homes was selected to reduce the potentially confounding effect of ETS. This group did not significantly differ from other participating children (Table 1). Parents did not know the individual results of the blood analyses, when they provided information on their children, thus reducing recall bias.

The inverse association between DDE and the number of infections 12 months prior to the interview is surprising (Table 2). However, in a logistic regression model the number of infections reported did not show a significant protective effect of DDE. Additionally, when infection was eliminated from the models, there were no major changes in the OC - immune markers association.

The few existing studies estimating the immunotoxicity of lead (Pb) in children, measured by immune markers, are inconsistent in their findings. Regarding immunoglobulins, our positive relation between Pb and IgE was consistent with that of Lutz et al. [7]. However, Sun and co-workers had different results [8]. Concerning lymphocytes, we found that the number of B-cells was significantly reduced with increased Pb concentration. Conversely, Sarasua et al. reported an increase in the number of B-cells for children less than 3 years old [6].

Studies assessing the relation between organochlorine and immune markers, determined in our study, also showed conflicting results and focused mostly on adults [35-37]. In comparison with these adult studies, Vine et al. reported similar modest findings for immunoglobulins and DDE. However, only results for IgA showed statistical significance [35]. Our findings regarding IgE and eosinophilic granula suggest that DDE shifts the immune response into a T helper (Th) 2 direction [38]. Mechanistically, immune responses have been polarized into Th1 and Th2 reactions. Th1 responses lead to the secretion of immunoglobulin G (IgG) and removal of the allergen. The allergic Th2 phenotype is characterized by secretion of cytokines that promote immunoglobulin E (IgE) production resulting in allergies. This suggestion is in agreement with findings of Daniel and co-workers, who reported an association between DDE and interleukin-4, a Th2 cytokine [39]. In addition, our interpretation that DDE may be associated with an allergy-like response is supported by the distribution of aeroallergen-specific IgE results over the four DDE exposure levels. In the lowest DDE exposure group 11.3% of the children showed a positive specific IgE, 10.9% and 12.2% in the two intermediate groups, but 23.0% in the highest DDE exposure group ($p=0.03$).

Interestingly, the effects of lead (Pb) and DDE on IgE seems to be competitive. At lower DDE exposure, Pb seems to increase IgE concentrations (Figure 8). There was no additional effect of the other pollutant if one is high; therefore it is possible that both pollutants are involved in the same mechanism. Indeed, studies have surmised that Pb may also shift the immune responses in a Th2

direction [40-42].

There are only few studies on OC blood/serum concentration and immune responses in children. Weisglas-Kuperus et al. reported that prenatal PCB exposure was associated with an increase in the T-cell markers CD3CD8+ and CD4+CD45RO+ [2]. Our data did not support these findings. In another study with prenatal exposures to PCBs, HCB, and DDE, Dewailly et al. did not identify significant associations with immune markers including CD3+, CD4+, CD8+ lymphocytes nor with IgA, IgG, and IgM [3]. However, we found significant relationships between PCBs and HCB with IgM (Table 6). Reichrtova et al. have shown that in utero exposure to DDE is positively correlated with cord serum IgE [43]. No other study of children has investigated the relationship between DDE determined postnatally and Th2 markers such as IgE and eosinophilic granula. This is the second publication showing an association between DDE and serum IgE [4] and the first to report associations between Pb, and DDE and IgE count on basophils and eosinophilic granula.

Conclusion

In conclusion, our study suggests a non-linear association between IgE and Pb concentration. Regarding OC, our data indicated an increase of IgE related to DDE serum concentrations. A parallel association between DDE, IgE count on basophils, and reduction of eosinophilic granula contents further supports a potential stimulation of a Th_2 response related to DDE exposure.

Prospective studies should determine more than one OC in a scenario with multiple exposures in order to prevent spurious correlations and include repeated determinations of immune responses to determine changes in immune development during childhood. Furthermore, studies are warranted that determine allergic susceptibilities following DDE and Pb exposure in children.

Acknowledgements

This study was supported by the Ministry of Environment, Energy, Youth, Family and Health Hesse, Germany. We acknowledge Dr. Rauterberg for the analyses of immunoglobulins. The analyses were conducted in preparation of an EPA STAR grant (R830825). KRB was a graduate assistant in the EPA grant while preparing the final manuscript.

References

1. Marth E, Sixl W, Bencko V, Medwed M, Lapajne S, Voncina E, Brumen S: People on the garbage dumps of Cairo: a toxicological in vivo model? *Cent Eur J Public Health* 1995, 3:154-157.

2. Weisglas-Kuperus N, Patandin S, Berbers GA, Sas TC, Mulder PG, Sauer PJ, Hooijkaas H: Immunologic effects of background exposure to polychlorinated biphenyls and dioxins in Dutch preschool children. *Environ Health Perspect* 2000, 108:1203-1207.

3. Dewailly E, Ayotte P, Bruneau S, Gingras S, Belles-Isles M, Roy R: Susceptibility to infections and immune status in Inuit infants exposed to organochlorines. *Environ Health Perspect* 2000, 108:205-211.

4. Karmaus W, Kuehr J, Kruse H: Infections and atopic disorders in childhood and organochlorine exposure. *Arch Environ Health* 2001, 56:485-492.

5. Rabinowitz MB, Allred EN, Bellinger DC, Leviton A, Needleman HL: Lead and childhood propensity to infectious and allergic disorders: is there an association? *Bull Environ Contam Toxicol* 1990, 44:657-660.

6. Sarasua SM, Vogt RF, Henderson LO, Jones PA, Lybarger JA: Serum immunoglobulins and lymphocyte subset distributions in children and adults living in communities assessed for lead and cadmium exposure. *J Toxicol Environ Health A* 2000, 60:1-15.

7. Lutz PM, Wilson TJ, Ireland J, Jones AL, Gorman JS, Gale NL, Johnson JC, Hewett JE: Elevated immunoglobulin E (IgE) levels in children with exposure to environmental lead. *Toxicology* 1999, 134:63-78.

8. Sun L, Hu J, Zhao Z, Li L, Cheng H: Influence of exposure to environmental lead on serum immunoglobulin in preschool children. *Environ Res* 2003, 92:124-128.

9. Forth W: Hexachlorcyclohexan - Gift in den Lebensmitteln? *Deutsches Aerzteblatt* 1980, 83:2169-2176.

10. Holdke B, Karmaus W, Kruse H: [Body burden of PCB in whole human blood of 7-10-year-old children in the vicinity of a hazardous waste incinerator]. *Gesundheitwesen* 1998, 60:505-512.

11. Osius N, Karmaus W, Kruse H, Witten J: Exposure to polychlorinated biphenyls and levels of thyroid hormones in children. *Environ Health Perspect* 1999, 107:843-849.

12. Karmaus W, Huang S, Osius N, Nebe T: Chromium urine concentration and effects on lymphocyte subpopulations in children. *J Environ Med* 1999, 1:153-161.

13. Karmaus W, DeKoning EP, Kruse H, Witten J, Osius N: Early childhood determinants of organochlorine concentrations in school-aged children. *Pediatr Res* 2001, 50:331-336.

14. Karmaus W, Davis S, Chen Q, Kuehr J, Kruse H: Atopic manifestations, breast-feeding protection and the adverse effect of DDE. *Paediatr Perinat Epidemiol* 2003, 17:212-220.

15. Obi-Osius N, Misselwitz B, Karmaus W, Witten J: Twin frequency and industrial pollution in different regions of Hesse, Germany. *Occup Environ Med* 2004, 61:482-487.

16. International workshop on human leukocyte differentiation antigens [http://www.ncbi.nlm.nih.gov/prow/guide/45277084.htm]

17. SAS Institute. Statistical Analysis System, Version 9.1. Gary, NC, 2002-2003.

18. Abraham K, Papke O, Gross A, Kordonouri O, Wiegand S, Wahn U, Helge H: Time course of PCDD/PCDF/PCB concentrations in breast-feeding mothers and their infants. *Chemosphere* 1998, 37:1731-1741.

19. Lackmann GM, Schaller KH, Angerer J: Organochlorine compounds in breast-fed vs. bottle-fed infants: preliminary results at six weeks of age. *Sci Total Environ* 2004, 329:289-293.

20. Rabinowitz M, Leviton A, Needleman H: Lead in milk and infant blood: a dose-response model. *Arch Environ Health* 1985, 40:283-286.

21. Ettinger AS, Tellez-Rojo MM, Amarasiriwardena C, Bellinger D, Peterson K, Schwartz J, Hu H, Hernandez-Avila M: Effect of breast milk lead on infant blood lead levels at 1 month of age. *Environ Health Perspect* 2004, 112:1381-1385.

22. Hanson LA: Breastfeeding provides passive and likely long-lasting active immunity. *Ann Allergy Asthma Immunol* 1998, 81:523-533; quiz 533-524, 537.

23. Hanson LA, Korotkova M, Lundin S, Haversen L, Silfverdal SA, Mattsby-Baltzer I, Strandvik B, Telemo E: The transfer of immunity from mother to child. *Ann N Y Acad Sci* 2003, 987:199-206.

24. Van de Perre P: Transfer of antibody via mother's milk. *Vaccine* 2003, 21:3374-3376.

25. Rothman KJ: *Modern Epidemiology.* Boston/Toronto: Little Brown and company; 1986.

26. Hatcher L: *A step-by-step Approach to Using SAS for Factor Analysis and Structural Equation Modeling.* 6 edn. Cary, NC: SAS Institute Inc.; 1994.

27. Leung DY: Molecular basis of allergic diseases. *Mol Genet Metab* 1998, 63:157-167.

28. Phillips DL, Pirkle JL, Burse VW, Bernert JT, Jr., Henderson LO, Needham LL: Chlorinated hydrocarbon levels in human serum: effects of fasting and feeding. *Arch Environ Contam Toxicol* 1989, 18:495-500.

29. Moreno LA, Sarria A, Lazaro A, Lasierra MP, Larrad L, Bueno M: Lymphocyte T subset counts in children with hypercholesterolemia receiving dietary therapy. *Ann Nutr Metab* 1998, 42:261-265.

30. Ogawa Y, Imaki M, Yoshida Y, Shibakawa M, Tanada S: An epidemiological study on the association between the total leukocyte and neutrophil counts, and risk factors of ischemic heart disease by smoking status in Japanese factory workers. *Appl Human Sci* 1998, 17:239-247.

31. Landesgesundheitsamt Baden-Wuerttemberg: Projekt Beobachtungsgesundheitsaemter. Belastungs- und Wirkungsmonitoring. Stuttgart; 2000.

32. Second national report on human exposure to environmental chemicals [http://www.cdc.gov/exposurereport/2nd/pdf/secondner.pdf]

33. Mes J, Marchand L, Turton D, Lau P, Ganz P: The determination of polychlorinated biphenyls congeners and other chlorinated hydrocarbon residues in human blood, serum and plasma. A comparative study. *Intern J Environ Anal Chem* 1992, 48:175-186.

34. Jacob B, Ritz B, Heinrich J, Hoelscher B, Wichmann HE: The effect of low-level blood lead on hematologic parameters in children. *Environ Res* 2000, 82:150-159.

35. Vine MF, Stein L, Weigle K, Schroeder J, Degnan D, Tse CK, Backer L: Plasma 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE) levels and immune response. *Am J Epidemiol* 2001, 153:53-63.

36. Daniel V, Huber W, Bauer K, Suesal C, Conradt C, Opelz G: Associations of dichlorodiphenyltrichloroethane (DDT) 4.4 and dichlorodiphenyldichloroethylene (DDE) 4.4 blood levels with plasma IL-4. *Arch Environ Health* 2002, 57:541-547.

37. Cooper GS, Martin SA, Longnecker MP, Sandler DP, Germolec DR: Associations between plasma DDE levels and immunologic measures in African-American farmers in North Carolina. *Environ Health Perspect* 2004, 112:1080-1084.

38. Mosmann TR, Sad S: The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol Today* 1996, 17:138-146.

39. Daniel V, Huber W, Bauer K, Suesal C, Conradt C, Opelz G: Associations of blood levels of PCB, HCHs, and HCB with numbers of lymphocyte subpopulations, in vitro lymphocyte response, plasma cytokine levels, and immunoglobulin autoantibodies. *Environ Health Perspect* 2001, 109:173-178.

40. McCabe MJ, Jr., Lawrence DA: Lead, a major environmental pollutant, is immunomodulatory by its differential effects on CD4+ T cells subsets. *Toxicol Appl Pharmacol* 1991, 111:13-23.

41. Heo Y, Lee BK, Ahn KD, Lawrence DA: Serum IgE elevation correlates with blood lead levels in battery manufacturing workers. *Hum Exp Toxicol* 2004, 23:209-213.

42. Heo Y, Lee WT, Lawrence DA: In vivo the environmental pollutants lead and mercury induce oligoclonal T cell responses skewed toward type-2 reactivities. *Cell Immunol* 1997, 179:185-195.

43. Reichrtova E, Ciznar P, Prachar V, Palkovicova L, Veningerova M: Cord serum immunoglobulin E related to the environmental contamination of human placentas with organochlorine compounds. *Environ Health Perspect* 1999, 107:895-899.

Table 1: Descriptive characteristics of the study cohort.

		Total group	Subgroup with OC and immune markers
		(N=671)	(n=331)
		%	%
Boys		53.1	56.8
Age	7 years	45.8	46.2
	8 years	50.2	50.2
	9-10 years	4.1	3.6
Passive smoking in the child's home during the last 12 months(cigarettes per day)			
	None	52.2	66.5
	1-10	23.4	24.8
	11-20	14.3	5.3
	more than 30	10.1	2.4
Number of infections during the last 12 months			
	None	6.0	5.7
	Less than 5	74.7	74.8
	5 to 10	17.2	17.4
	more than 10	2.1	2.1
Duration of total breastfeeding (weeks)			
	0	19.1	15.1
	less than 5	17.9	15.4
	5 – 8	12.5	12.1
	9- 12	10.6	11.8
	more than 12	34.7	41.1
	Missing	5.2	4.5
Serum cholesterol concentration (mean, 5-95%-value, mg/dL)		186 (143-235)	
Triglyceride concentration (mean, 5-95%-value, mg/dL)		130 (53-262)	

Table 2: Geometric mean and 5-, 95% values for whole blood OC and Pb by covariates.

Covariate	Category (n)	DDE (µg/L)	∑ of PCBs (µg/L)	HCB (µg/L)	γ-HCH (µg/L)	Pb (µg/L)
Gender	Girls (143)	0.32 (0.13 – 1.07)	0.43 (0.16 – 1.39)	0.21 (0.11–0.48)	0.02 (0.01–0.06)	25.4 (11.0 – 4 3.8)
	Boy (188)	0.31 (0.13 – 0.96)	0.54 (0.19 – 1.66)	0.23 (0.11–0.54)	0.02 (0.01–0.04)	27.8 (14.8 – 48.2)
Age-groups	7 years (153)	0.32 (0.13 – 0.97)	0.54 (0.18 – 1.90)	0.23 (0.11–0.56)	0.02 (0.01–0.06)	27.3 (13.9 – 48.2)
	8 years (166)	0.31 (0.13 – 0.98)	0.47 (0.18 – 1.29)	0.21 (0.11–0.48)	0.02 (0.01–0.05)	26.4 (10.7 – 47.8)
	9-10 years (12)	0.31 (0.20 – 0.84)	0.33 (0.10 – 0.99)	0.17 (0.10–0.46)	0.02 (0.01–0.06)	25.4 (16.6 – 39.4)
Passive smoking in the child's home during the last 12 months (cigarettes per day)	None (220)	0.35 (0.14 – 1.08)	0.57 (0.21 – 1.70)	0.24 (0.11–0.55)	0.02 (0.01–0.06)	26.5 (10.1 – 47.4)
	1 – 10 (84)	0.26 (0.12 – 0.88)	0.39 (0.17 – 1.02)	0.19 (0.11–0.45)	0.02 (0.01–0.05)	26.0 (16.0 – 43.0)
	11 – 20 (18)	0.27 (0.09 – 0.69)	0.40 (0.13 – 1.29)	0.18 (0.10–0.49)	0.02 (0.01–0.04)	33.5 (18.9 – 113.7)
	21 – 30 (8)	0.23 (0.13 – 1.11)	0.27 (0.18 – 0.34)	0.15 (0.11–0.21)	0.02 (0.01–0.04)	30.1 (19.4 – 47.3)
Number of infections during the last 12 months	None (19)	0.60 (0.16 – 4.02)	0.49 (0.10 – 2.24)	0.21 (0.10–0.58)	0.02 (0.01–0.08)	28.8 (15.9 – 58.7)
	< 5 (247)	0.31 (0.13 – 0.94)	0.49 (0.18 – 1.39)	0.22 (0.11–0.48)	0.02 (0.01–0.06)	26.2 (10.7 – 46.7)
	5-10 (57)	0.29 (0.13 – 0.79)	0.53 (0.19 – 2.21)	0.23 (0.11–0.70)	0.02 (0.01–0.04)	27.9 (16.0 – 47.8)
	>10 (7)	0.25 (0.16 – 0.43)	0.56 (0.34 – 0.87)	0.21 (0.15–0.27)	0.02 (0.01–0.05)	33.4 (26.2 – 48.5)

Table 3: Spearman correlation coefficients between organochlorine compounds (wet-based and lipid-based, n=331) and their geometric means.

	∑PCBs	HCB	γ-HCH	Lipids ψ	Lipids §	DDE/lipid	∑PCBs/lipid	HCB/lipid	γ-HCH/lipid	Geometric mean
DDE (μg/L)	0.61 p<0.01	0.55 p<0.01	0.16 p<0.01	0.08 p=0.09	0.06 p=0.25	0.86 p<0.01	0.51 p<0.01	0.46 p<0.01	0.14 p<0.01	0.32
∑PCBs (μg/L)		0.76 p<0.01	0.04 p=0.4	0.04 p=0.65	0.05 p=0.34	0.59 p<0.01	0.90 p<0.01	0.70 p<0.01	0.09 p=0.11	0.50
HCB (μg/L)			0.07 p=0.19	0.03 p=0.63	0.04 p=0.47	0.54 p<0.01	0.74 p<0.01	0.83 p<0.01	0.09 p=0.11	0.22
γ-HCH (μg/L)				0.15 p=0.01	0.11 p<0.05	0.13 p=0.02	0.05 p=0.34	0.03 p=0.64	0.88 p<0.01	0.02
DDE, lipid-based Ψ (ng/g)							0.63 p<0.01	0.63 p<0.01	0.23 P<0.01	103.03
∑PCBs, lipid-based Ψ (ng/g)								0.81 p<0.01	0.14 p=0.01	164.99
HCB, lipid-based Ψ (ng/g)									0.16 p<0.01	70.72
HCH, Lipid-based Ψ (ng/g)										6.65

Ψ total lipids calculated as sum of cholesterol and triglycerides

§ total lipids calculated using formula 2 of Phillips et al.[28].
rank correlation between total lipids from both formulae was ($r_{Spearman}$=0.95).

∑PCBs sum of PCB congeners 101, 118, 138, 153, 170, 180, 183, 187

Table 4: White blood cell, eosinophilic characteristics, and basophilic surface IgE by OC and Pb (geometric mean)

	DDE (μg/L)				Sum of PCBs (μg/L)				HCB (μg/L)				γ-HCH (μg/L)				Pb (μg/L)			
Outcome	≤ 0.2	0.21–0.29	0.30– 0.43	>0.43	≤0.30	0.31–0.48	0.49– 0.75	>0.75	≤ 0.15	0.16 –0.2	0.21 – 0.27	>0.27	0.01	0.02	>0.02	<22.0	22.1– 28.3	28.4– 34.1	>34.1
N	78	89	79	85	80	86	82	83	84	77	86	84	91	130	110	82	81	86	82
White blood cells, x10^3/μL																			
Total‡ crude	8136	8579	8131	8555	8373	8456	8528	8074	8456	8354	8383	8233	8062	8512	8421	8612	7907	8376	8552
Adjusted §	7782	8275	7970	8564*	8354	8318	8447	7488*	8260	8037	8141	8131	7920	8264	8247	8397	7730*	8167	8290
		F-test: p=0.09				F-test: p=0.02				F-test: p=0.92			F-test: p=0.09				F-test: p=0.33		
Eosinophil cell count, x10^3/μL																			
Total‡ crude	162	206	188	207	176	182	197	210	192	187	193	190	186	206	177	176	166	210	214
Adjusted §	176	220	208	223	181	194	217	235	245	217	196	172	199	225	194	190	185	233	218
		F-test: p=0.37				F-test: p=0.61				F-test: p=0.31			F-test: p=0.32				F-test: p=0.22		
Eosinophilic granula																			
Total‡ crude	902	903	901	893	897	901	896	904	899	897	905	897	906	896	898	890	904	900	904
Adjusted §	919	913	910	895*	902	907	905	923	908	907	916	906	915	906	906	898	913	911	914
		F-test: p=0.22				F-test: p=0.37				F-test: p=0.65			F-test: p=0.47				F-test: p=0.27		
IgE count on basophils																			
Total‡ crude	810	822	863	859	807	842	838	866	808	841	839	867	845	838	833	836	811	845	860
Adjusted §	851	851	896*	884	860	884	861	877	852	879	866	886	880	868	863	871	845	878	888
	F-test: p=0.67				F-test: p=0.50					F-test: p=0.41			F-test: p=0.60				F-test: p=0.10		

‡ absolute number of cells/μL.
* $p \leq 0.05$ based on a t-test compared with the lowest exposure category as the reference.
§ Adjusted for all exposures in the table (OC & Pb) in addition to gender, age, number of infections in the last 12 months, passive smoke exposure in the child's home in the last 12 months, and lipids (sum of cholesterol and triglycerides)

Table 5: Lymphocyte phenotypes by whole blood DDE, PCBs, HCB, γ-HCH and Pb concentration (geometric mean)

	DDE (μg/L)				Sum of PCBs (μg/L)				HCB (μg/L)				γ-HCH (μg/L)			Pb (μg/L)			
Cells	≤ 0.2	0.21- 0.29	0.30- 0.43	>0.43	≤ 0.30	0.31 -0.48	0.49- 0.75	>0.75	≤ 0.15	0.16 - 0.2	0.21- 0.27	>0.27	0.01	0.02	>0.02	<22.0	22.1-28.3	28.4-34.1	>34.1
N	78	89	79	85	80	86	82	83	84	77	86	84	91	130	110	82	81	86	82
T-cells (CD3+)																			
Total‡ crude	2193	2183	2139	2242	2286	2179	2144	2156	2289	2170	2155	2146	2131	2212	2213	2318	2101	2160	2184
Adjusted §	1950	2005	1980	2076	2092	1998	1990	1932	2082	1975	1986	1968	1967	2020	2021	2118	1919*	1979	1999
		F-test: p=0.71				F-test: p=0.74				F-test: p=0.76				F-test: p=0.77			F-test: p=0.17		
T-helper cells CD3+CD4+)																			
Total ‡ crude	1204	1209	1205	1251	1276	1212	1198	1189	1293	1190	1209	1180	1155	1232	1255	1297	1182	1203	1192
Adjusted §	1087	1138	1147	1200	1183	1145	1141	1100	1220	1113	1139	1100	1101	1150	1176	1214	1106	1128	1123
		F-test: p=0.51				F-test: p=0.83				F-test: p=0.37				F-test: p=0.37			F-test: p=0.24		
Cytotoxic T-cells (CD3+CD8+)																			
Total‡ crude	753	743	727	778	773	740	739	752	769	744	740	751	750	753	748	799	711	746	750
Adjusted §	660	665	649	692	697	660	668	642	678	658	662	669	671	669	659	712	634*	661	662
		F-test: p=0.78				F-test: p=0.80				F-test: p=0.97				F-test: p=0.93			F-test: p=0.23		
Memory T-helper cells (CD4+CD45RO+)																			
Total‡ crude	315	249	352	325	316	277	308	325	265	349	311	307	331	274	325	317	304	327	341
Adjusted §	332	323	363	362	343	342	347	347	354	363	345	317	348	341	345	358	321	348	351
		F-test: p=0.18				F-test: p=1.00				F-test: p=0.34				F-test: p=0.91			F-test: p=0.22		
Natural killer cells (CD16+CD56+)																			
Total‡ crude	384	367	350	372	382	378	350	364	369	362	394	348	328	395	373	371	348	365	389
Adjusted §	371	338	334	362	378	366	317	345	326	348	390	342	322	368*	305*	350	322	354	378
		F-test: p=0.48				F-test: p=0.24				F-test: p=0.16				F-test: p<0.01			F-test: p=0.20		
Natural killer cells subset (CD16+CD56+CD57+)																			
Total‡ crude	156	153	148	148	159	156	142	147	155	144	164	142	137	162	151	154	148	148	156
Adjusted §	168	157	158	162	177	171	142	156	148	153	183	162	143	175*	167	162	152	161	169
		F-test: p=0.89				F-test: p=0.21				F-test: p=0.18				F-test: p=0.03			F-test: p=0.69		
B-cells (CD3+CD5+CD19+)																			
Total‡ crude	457	474	456	468	463	461	464	469	473	482	458	446	445	474	469	505	423	462	469
Adjusted §	378	398	382	393	369	372	395	416	420	411	375	349	371	395	397	418	353*	389	393
		F-test: p=1.00				F-test: p=0.67				F-test: p=0.26				F-test: p=0.53			F-test: p=0.10		

‡ absolute number of subtype cells/μL based on percent of lymphocytes x total lymphocyte count.

* $p \leq 0.05$ based on a t-test compared with the lowest exposure category as the reference.

§ Adjusted for all exposures in the table (OC & Pb) in addition to gender, age, number of infections in the last 12 months, passive smoke exposure in the child's home in the last 12 months and lipids (sum of cholesterol and triglycerides)

Table 6: Immunoglobulins by whole blood DDE, PCBs, HCB, γ-HCH and Pb concentration in children (geometric mean)

	DDE (μg/L)				Sum of PCBs (μg/L)				HCB (μg/L)				γ-HCH (μg/L)			Pb (μg/L)			
Outcome	≤0.2	0.21-0.29	0.30-0.43	>0.43	≤ 0.30	0.31-0.48	0.49-0.75	>0.75	≤ 0.15	0.16-0.2	0.21-0.27	>0.27	0.01	0.02	>0.02	<22.0	22.1-28.3	28.4-34.1	>34.1
N	78	89	79	85	80	86	82	83	84	77	86	84	91	130	110	82	81	86	82
IgG (mg/dL)																			
	1121	1116	1102	1159	1168	1105	1103	1125	1159	1147	1090	1106	1121	1111	1144	1122	1112	1151	1112
Crude																			
Adjusted §	1177	1197	1199	1295*	1229	1187	1217	1232	1246	1266	1196	1160	1221	1204	1224	1210	1214	1241	1201
		F-test: p=0.14				F-test: p=0.78				F-test: p=0.29			F-test: p=0.84				F-test: p=0.83		
IgA (mg/dL)																			
	128	136	140	134	148	135	124	133*	149	138	128	124	139	135	131	129	126	139	146
Crude																			
Adjusted §	112	125	138*	141*	141	127	119	127	145	134	126	111*	130	131	125	123	121	133	136
		F-test: p=0.05				F-test: p=0.34				F-test: p=0.08			F-test: p=0.71				F-test: p=0.27		
IgM (mg/dL)																			
Crude	122	121	125	123	125	122	115	131	126	129	124	114	123	118	129	125	116	129	120
Adjusted §	148	146	153	147	134	138	144	184*	160	164	151	123*	153	142	150	150	143	153	148
		F-test: p=0.92				F-test: p<0.01				F-test: p<0.01			F-test: p=0.42				F-test: p=0.74		
IgE (kU/l)																			
Crude	23	27	33	51	28	34	28	39	26	32	31	43	30	32	35	31	20	46	45
Adjusted §	30	37	53	80*	48	53	41	45	49	51	43	45	41	49	51	46	30	59	59
		F-test: p=0.02				F-test: p=0.84				F-test: p=0.95			F-test: p=0.59				F-test: p=0.03		

* $p \leq 0.05$ based on a t-test compared with the lowest exposure category as the reference.

§ Adjusted for all exposures in the table (OC & Pb) in addition to gender, age, number of infections in the last 12 months, passive smoke exposure in the child's home in the last 12 months and lipids (sum of cholesterol and triglycerides)

CHAPTER 4

DISCUSSION

The pieces of the sex hormone disrupters – childhood allergy puzzle presented in this monograph suggest an immunomodulating role of sex hormone disrupters in the etiology of childhood allergic disorders. First, evidence was presented in support of DDE playing a role in the prenatal priming of allergic diseases through the alteration of cord plasma cytokines. Second, an association between prenatal exposure to exogenous sex steroid hormones and biomarkers of allergic susceptibility was demonstrated. Third, prenatal exposure to exogenous sex steroid hormones appears to be associated with allergic phenotypes such as asthma; and fourth, postnatal organochlorine exposure seems to alter serum biomarkers of allergy.

The motivation for this work stems from my curiosity to investigate the speculation that sex steroid hormones disrupters may be important in the etiology of childhood allergy. This effort was represented by the use of both questionnaire and lab data for various exposures and outcomes. In addition, the data used were from cohorts of varying backgrounds (Caucasian and African-Caribbean) and study designs (cross-sectional and follow-up).

The pathways explored in the first three manuscripts highlights the importance of *in utero* exposure by 'connecting the dots' showing a link between: 1) placental *p,p'* DDE and cord plasma IL-4, IL-13, and INF-γ (cytokines suggesting allergy susceptibility); 2) Maternal oral contraceptive use before pregnancy (a proxy for *in utero* progesterone/estrogen exposure) and serum IgE, IgA, and basophilic surface IgE counts; 3) Maternal contraceptive use and asthma and hay fever in

offspring. These three pieces of work suggest that *in utero* exposure to sex steroid hormones may alter the immune status at birth, which may result in childhood allergic disorders. This suggests that the *in utero* environment may play a major role in the pathogenesis of childhood allergy.

To the best of our knowledge, there is no report of an association between placental p,p' DDE and cord plasma cytokines. Noakes and co-workers [1] were unable to detect placental p,p'-DDE and used maternal levels instead. Though their results for cord IL-13 and INF-γ were not significant, they tended in the same direction (increased IL-13, decreased INF-γ) as our findings for these cytokines.

This overall sex hormone – childhood allergy puzzle was made clearer with findings from the fourth manuscript. In children ages 7 to 10 years, serum DDE was shown to significantly increase humoral WBC count, IgE levels, IgE count on basophils, IgG, and IgA: all considered important immune markers of allergy.

This work would have benefited from the use of actual blood concentrations of estrogen and progesterone since previous findings on OC use and hormone levels are not consistent [2,3]. However, since DDE is known to have weak estrogenic properties, the association between placental DDE and cord cytokines provides useful clues of possible results. Another limitation is the fact that, the duration between OC use and pregnancy was not taken into consideration. The

effect of OC use on childhood allergy may be minuscule if the period between OC use and pregnancy is long.

The fact that data from studies of different ethnic groups and study designs was used is noteworthy. Data from an African-Caribbean cohort (component 3) provided consistent findings to those from Caucassian cohorts. Another strength of this work is the different time-windows in which exposures and outcomes were assessed.

On a whole, the effects of sex hormone disrupters on childhood allergic disorders are pervasive but unexplained. This compilation of four studies from three diverse cohorts suggests possible explanations. The first component (*In utero* exposure to SHD affects **perinatal** biomarkers of allergy) implies that placental DDE (a SHD) concentration may influence the priming of cord blood immune marker towards an allergic state. Whilst important, on its own this component may not be sufficient to make the leap from placental DDE to childhood allergy. This gap was narrowed by findings from component two (*In utero* SHD affects **postnatal** biomarkers of allergy), which indicate a possible relation between maternal oral contraceptive use before pregnancy and serum immune markers of allery in offspring. Furthermore, the data suggest that maternal OC use may have sex-related differences of the immunomodulating effects in offspring. The first two components breached the gap between the in utero and postnatal environment. However, though immune markers (especially IgE levels) are good predictors of disease outcome they do not always provide consistent results [4-8].

The investigation was therefore taken to another level with component three (In utero exposure to SHD affects allergic outcomes in infancy) providing evidence of a possible involvement of maternal oral comtraceptive use in the etiology of childhood allergic diseases including asthma. By connecting the clues embedded in these three studies, a picture is painted that implies that *in utero* exposure to SHD may be a risk factor for childhood asthma (for example) by first primining the fetal immune response. This priming may be sustained into childhood, evident by altered serum allergic markers, and ultimately clinical allergic manesfestations. The findings of component four (Postnatal exposure to SHD affects biomarkers of allergy in infancy) was consistent with previous studies that inferred a link between postnatal HOC exposure and serum immune markers. These results should be interpreted with caution since HOC concentrations were measured once at 7-10 years of age. Hence we do not know whether the concentrations were higher before this age and are therefore responsible for the observed immune response. For example, it is possible that the increased serum immune levels may be the result of *in utero* and not postnatal exposures (see component 2).

It would be advantageous to test all four components in one study; collecting all relevant biologic samples and questionnaire data at the appropriate time. However, since no such study exist, and may not be realistic, the present work provides important stepping stones along the path of better understanding the role of SHD (sex hormone disrupters) in the etiology of childhood allergy.

Future work

The mechanism through which these compounds (organochlorines, exogenous estrogen and progesterone) disrupt normal sex hormone function is unclear. The epigenetic concept has been proposed as a credible explanation. This concept posits that adverse effects can result from enduring changes in gene expression [9]. This means that the active portion of the genetic information is altered without a change in the genetic blueprint (epigenesis). The expression or silencing of genes then results in cells/tissue with permanent (irreversible) committed activities.

Epigenetic mechanisms have been suggested as the underlying reason for the observed increased risk of several chronic diseases in adulthood associated with xenobiotic exposures early in life [10-12]. For example, Fukuzawa et al. reported that (coplanar) PCB affects the expression of steroidogenic enzyme genes [13]. In addition, Goldman et al. found that PCBs (Aroclor 1254) inhibit the cytochrome P-450 21-hydroxylase activity, thus blocking the branch from progesterone to glucocorticoid production [14]. Furthermore, Chen et al. reported that DDT and its metabolites (including DDE) down-regulate the gene expression of the estrogen receptor [15].

Future studies aimed at explicating the mechanism through which sex hormones are disrupted should embrace the epigenetic concept. Since gene expression can be detected as early as in fetal tissues of the placenta, it may be prudent to

assess the variations in gene expression in neonates with varying concentrations of exposure to DDE, PCB, exogenous estrogen, and progesterone. The effect of sex hormone disrupters on allergy may be from their direct impact on the gene expression of allergy related cytokines such as IL-4, IL-13, and INF-γ.

In summary, it is of personal and public health importance to consider the potential impact of sex hormone disrupters on the pathogenesis of allergy. The prenatal period is of particular importance since the fragile fetal immune system is more vulnerable to exogenous exposures. Therefore, intervention through education regarding exposure prevention should be implemented before pregnancy. Further exploration of the epigenetic concept is a fertile area for future research.

References

1. Noakes PS, Taylor P, Wilkinson S, Prescott SL. The relationship between persistent organic pollutants in maternal and neonatal tissues and immune responses to allergens: A novel exploratory study. Chemosphere 2006;63(8):1304-1311.

2. Barbieri RL, Gao X, Xu H, Cramer DW. Effects of previous use of oral contraceptives on early follicular phase follicle-stimulating hormone. Fertility and Sterility 1995;64(4):689-92.

3. Moore JW, Key TJ, Wang DY, Bulbrook RD, Hayward JL, Takatani O. Blood concentrations of estradiol and sex hormone -binding globulin in relation to age at menarche in premenopausal British and Japanese women. Breast Cancer Research and Treatment 1991;18 Suppl 1:S47-50.

4. Eiriksson TH, Sigurgeirsson B, Ardal B, Sigfusson A, Valdimarsson H. Cord blood IgE levels are influenced by gestational age but do not predict allergic manifestations in infants. pediatric allergy and immunology 1994;5(1):5-10.

5. Klink M, Cline MG, Halonen M, Burrows B. Problems in defining normal limits for serum IgE. J Allergy Clin Immunol 1990;85(2):440-4.

6. Bergmann RL, Edenharter G, Bergmann KE, Guggenmoos-Holzmann I, Forster J, Bauer CP, Wahn V, Zepp F, Wahn U. Predictability of early atopy by cord blood-IgE and parental history. Clin Exp Allergy 1997;27(7):752-60.

7. Perkin MR, Strachan DP, Hc W, Lack G, Golding J. The predictive value of early life total immunoglobulin E measurement in identifying atopic children in a population-based birth cohort study. Pediatr Allergy Immunol 2006;17(2):118-24.

8. Croner S, Kjellman NI, Eriksson B, Roth A. IgE screening in 1701 newborn infants and the development of atopic disease during infancy. Arch Dis Child 1982;57(5):364-8.

9. Tchernitchin AN, Tchernitchin NN, Mena MA, Unda C, Soto J. Imprinting: perinatal exposures cause the development of diseases during the adult age. Acta Biol Hung 1999;50(4):425-40.

10. Jirtle RL, Sander M, Barrett JC. Genomic imprinting and environmental disease susceptibility. Environ Health Perspect 2000;108(3):271-8.

11. Li S, Hursting SD, Davis BJ, McLachlan JA, Barrett JC. Environmental exposure, DNA methylation, and gene regulation: lessons from diethylstilbesterol-induced cancers. Ann N Y Acad Sci 2003;983:161-9.

12. Li S, Washburn KA, Moore R, Uno T, Teng C, Newbold RR, McLachlan JA, Negishi M. Developmental exposure to diethylstilbestrol elicits demethylation of estrogen-responsive lactoferrin gene in mouse uterus. cancer research 1997;57(19):4356-9.

13. Fukuzawa NH, Ohsako S, Nagano R, Sakaue M, Baba T, Aoki Y, Tohyama C. Effects of 3,3',4,4',5-pentachlorobiphenyl, a coplanar polychlorinated biphenyl congener, on cultured neonatal mouse testis. Toxicol In Vitro 2003;17(3):259-69.

14. Goldman D, Yawetz A. The interference of aroclor 1254 with progesterone metabolism in guinea pig adrenal and testes microsomes. Journal of Biochemical Toxicology 1990;5(2):99-107.

15. Chen CW, Hurd C, Vorojeikina DP, Arnold SF, Notides AC. Transcriptional activation of the human estrogen receptor by DDT isomers and metabolites in yeast and MCF-7 cells. Biochem Pharmacol 1997;53(8):1161-72.