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Risks of allergic sensitization and diseases associated with tap and recreational water: Findings in epidemiological studies among young schoolchildren

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SUMMARY

Over the past three decades, most developed countries have witnessed a dramatic increase in the prevalence of allergic diseases, including eczema, asthma, allergic rhinitis or food allergies. More than a simple progression, the temporal pattern and the development of the "atopic march" are strongly influenced by genetic, environmental and lifestyle factors. These disorders may develop sequentially along an atopic pathway or there may be a causal link between eczema and these later-onset atopic respiratory disorders.

This study aimed at evaluating the impact of environmental factors and more particularly of exposure to chlorination products in swimming pools on the development of the atopic disease. A child cohort was recruited in order to conduct a prospective study on young children, which will allow the identification of indoor pollutants contributing to the development of the atopic diseases during childhood.

Our study was able to trace risk factors for respiratory health and allergy in young children. Some of them are personal characteristics and unavoidable such as the atopic status of the parents, other determinants were related to lifestyle and environment. Our results confirm that the influence of chlorinated pool attendance on the development of allergic diseases is not limited to interactions promoting the clinical manifestation of atopy. Results of our prospective study clearly show that chlorinated pool attendance may also increase the risk of eczema, bronchiolitis and allergic sensitization, which are strong predictors of asthma and allergic rhinitis later during childhood. Two different modes of action of chlorine-based irritants in swimming pools could promote the development of allergic diseases: an interaction with atopic status promoting the clinical expression of atopy or an interaction between chlorinated pool attendance and allergen exposure promoting allergic sensitization. These two modes of action probably share the same basic mechanism i.e. a disruption by chlorine-based oxidants of airways epithelial barriers, with a possible consequences a facilitated transepithelial passage of allergens and viruses and a decreased of down regulation of the Th_2 response by the airways epithelial cells.

The identification of early life disease in the atopic march as predictors for the development of lifelong chronic diseases offers entry points for primary and secondary disease prevention. They are modifiable and interventions that could be developed to halt the progression along the atopic march in a young children with atopy, eczema or early wheeze. In this context, the chlorine levels and temperature in swimming pools should be carefully regulated.

RESUME

Au cours des trente dernières années, et ce, plus particulièrement dans les pays industrialisés, nous observons une augmentation dramatique de la prévalence des maladies allergiques, tant au niveau de l'asthme, de la rhinite allergique, de la dermatite atopique qu'au niveau des allergies alimentaires. Plus qu'une simple progression, le modèle temporal et le développement de la marche atopique sont fortement influencés à la fois par la génétique et mais aussi par les facteurs liés à l'environnement et au mode vie. Ces maladies peuvent se développer séquentiellement le long d'une voie atopique mais on pourrait également incriminer un lien causal entre la dermatite atopique et les affections respiratoires atopiques ultérieures.

L'objectif de cette étude fut d'évaluer l'impact des facteurs environnementaux et plus particulièrement l'exposition aux produits chlorés dans le développement des maladies atopiques. Une cohorte d'enfants fut initiée afin de conduire une étude prospective qui permettrait d'identifier les polluants de l'air intérieur qui contribuent au développement des maladies atopiques au cours de l'enfance.

Notre étude mit en évidence des facteurs de risque pour la santé respiratoire et allergique des jeunes enfants. Certains d'entre eux sont des caractéristiques personnelles et inévitables comme le statut atopique des parents ; d'autres déterminants sont liés au style de vie et à l'environnement. Nos résultats confirment que l'influence de la fréquentation des bassins chlorés sur le développement de maladies allergiques n'est pas limitée aux interactions favorisant la manifestation clinique de l'atopie. Les résultats de notre étude prospective montrent clairement que la fréquentation des piscines chlorées peut aussi augmenter le risque de dermatite atopique, la bronchiolite et la sensibilisation allergique, présages d'asthme et de rhinite allergique ultérieurement au cours de l'enfance.

L'exposition aux irritants à base de chlore présents dans des piscines pourrait promouvoir le développement de maladies allergiques selon deux pistes distinctes : d'une part, en interaction avec le statut atopique afin de promouvoir l'expression clinique de rhinite allergique et l'asthme ou d'autre part, en une interaction avec l'exposition aux allergènes, pour promouvoir la sensibilisation allergique; les deux modes d'action partageant probablement le même mécanisme de base c'est à dire une rupture de la barrière épithéliale cutanée et des voies respiratoires sous l'effet oxydant des produits chlorés facilitant par conséquence le passage transepithelial des allergènes et des virus ainsi qu'un affaiblissement de la régulation de la réponse Th_2 au niveau des cellules épithéliales.

Pouvoir identifier les premières étapes de la maladie atopique et leurs manifestations cliniques en tant que signe annonciateur de maladies chroniques ultérieures permet de définir des moyens de préventions primaires et secondaires de la maladie. Ces interventions permettraient d'interrompre la progression de la marche atopique chez les très jeunes enfants présentant de la dermatite atopique ou encore du sifflement. Dans ce contexte, les bassins de natation doivent être scrupuleusement entretenus et les taux de chlore adaptés.

ABBREVIATIONS

AD	Atopic dermatitis
AR	Allergic rhinitis
ATS	American Thoracic Society
BALF	Bronchio-alveolar lavage fluid
Ca	Calcium
$CaCO_3$	Calcium carbonate
CC16	Clara cell protein
COPD	Chronic obstructive pulmonary disease
$CP_{(s)}$	Chlorinated compound(s)
CPA	Cumulated pool attendance
CTL	Cytoxic T lymphocyte
DC	Dendritic cell
DEHP	Di (2-ethylhexyl) phtalate
ECRHS	European Community Respiratory Health Survey
EIB	Exercise-induced bronchoconstriction
ELF	Epithelial lining fluid
ELISA	Enzyme-linked immune-absorbent assay
ERS	European Respiratory Society
ETS	Environmental tobacco smoke
FeNO	Fractional exhaled nitric oxide
FEV1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
HDM	House dust mite
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	Interleukin
INF-γ	Interferon gamma
ISAAC	International study on asthma and allergy in children
kDA	Kilo-Dalton
LRTI	Lower respiratory tract infection
Mg	Magnesium
NAL	Nasal lavage
NALF	Nasal lavage fluid
NK	Natural killer
NO_2	Nitric oxide

O_3	Ozone
PEF	Peak expiratory flow
PM	Particulate matter
RSV	Respiratory syncytial virus
RV	Rhinovirus
SES	Socio-economic status
SHC	Superior Health Council
SO2	Sulphur dioxide
SP-D	Surfactant pulmonary-associated protein D
Th	T Helper cell
TNF	Tumour necrosis factor
$VOC_{(s)}$	Volatile organic compound(s)
WHO	World Health Organization

1. INTRODUCTION

Ranked sixth in the world of the big pathologies, as classified by the World Health Organization (WHO), allergies are becoming a real phenomenon of society, affecting an ever-increasing population in all industrialized countries of the Northern hemisphere. The increase in the incidence of allergic manifestations is particularly noticeable amongst children. There is clear evidence that children are more susceptible to some stressors in the environment.

Personal and/or familial tendency to produce Immunoglobulin E (IgE) antibodies and sensitization in response to ordinary exposure [1], the atopy may evolve from an atopic dermatitis, to the rhino-conjunctivitis and to asthma characterized as the "atopic march". The exact relationship to each other is debated and is certainly not as simple as one disease progressing to another.

Abnormal and excessive reaction of the immune system is generated by contact with a substance generally foreign to the body. The allergy shows itself under various forms according to the allergen and to the particular sensibility of the individual: respiratory, cutaneous, eye or even gastronomic-intestinal.

Although the allergy can arise at any age, it mostly appears from childhood; first years of life being a critical period for the development of postnatal atopy in relation to exposure to environmental agents [2]. The origins and symptoms of allergy will vary with age. Up to the age of 3, allergies are mostly of food origin with intestinal disorders and atopic dermatitis. Up to the age of 4, food is responsible for food allergies. Beyond the age of 5, allergies of respiratory origin are more frequent, with bronchial asthma, allergic rhinitis, or rhino-conjunctivitis.

Most researchers agree that this epidemic of allergic diseases is driven by factors specifically linked to the Western lifestyle. The phenomenon is indeed much too rapid to result from changes in genetic predisposition. Nor can it be ascribed solely to changes in allergen exposure since most common allergens (pollen, dust mite, furry animals, etc.) have always existed in the human surroundings. The hypothesis currently raising most interest relates the rise of allergies to the hygiene practices of Westernized societies. According to the hygiene hypothesis formulated in 1989, the increase of allergic diseases in developed countries would be the consequence of the reduced exposure to infective agents, especially during early infancy and childhood.

In 2003, however, a variant of the hygiene hypothesis was proposed that links the rise of allergies not to the declining exposure to microbial agents during early life but to the increasing exposure to products and by-products of chlorination, the most widely used method to disinfect tap and recreational water. Basically, the chlorine hypothesis postulates that chlorine-based oxidants used or formed during water chlorination cause a disruption of epithelial airways barriers that promotes allergic sensitization and diseases in genetically predisposed individuals. Airway barriers, of course, can be damaged by a variety of other environment or lifestyle stressors, including infectious agents, tobacco or wood smoke, endotoxin, ambient ozone, strenuous exercise or cold air. Exposure of children to most of these stressors has, however, not really increased in

affluent countries affected by the allergies epidemic, or if it has increased, it is certainly not in the same proportion as for chlorination products.

This study aimed at evaluating the impact of environmental factors and more particularly of exposure to chlorination products on the development of the atopic disease. A child cohort was initiated in order to conduct a prospective study on young children, which will allow the identification of indoor pollutants contributing to the development of the atopic diseases during childhood and to evaluate the predictive value of new non-invasive indicators of airway inflammation and damage with respect to the development of asthma and respiratory allergies.

2. LITTERATURE REVIEW

1. The atopic/allergic disease

Developed nations show an important increase in the prevalence of allergic diseases, which are often called atopic diseases. They include atopic dermatitis, asthma and allergic rhinitis.

1.1. Definitions

1.1.1. Allergy

An allergy is defined by a reaction of hypersensitivity caused by immunological mechanisms [3]. Allergy can be anti-body mediated or cell-mediated [1]. In most patients, the antibody typically responsible for an allergic reaction belongs to the IgE isotope [3], this type of allergy being related to both the presence of IgE and a significant exposure to allergens [4]. An allergic person will react to the common allergens of the environment only under the influence of a break in the natural defences: infectious, toxic or by abundance of allergens.

In a non-IgE-mediated allergy, the antibody may belong to the IgG isotope as an anaphylaxis due to immune complexes containing dextran and the classical serum sickness [3].

Allergies are classified in 4 types [5] according to the mode of answer of the body:

- The hypersensitivity of type I: includes atopic diseases caused by immunoglobulin E antibody leading to the release of chemical mediators [6]
- The hypersensitivity of type 2 or allo-immunization is the consequence of the introduction in the body of one of the allo-antigens erythrocytic, leukocytic or serum during pregnancy, during blood transfusions or during transplantations
- The hypersensitivity of type 3 or immune complex triggers the creation of precipitant antibodies, which settle within the target tissue (the disease of the farmer lung and of breeder of birds)
- The hypersensitivity of type IV or delayed allergy, reaction of cellular infiltration. The most common type is contact dermatitis, cell-mediated allergy, in which immunologically sensitized lymphocytes play a major role.

1.1.2. Atopy

The term "atopy" appears at the beginning of the twentieth century to group under the same term all the clinical manifestations of exaggerated IgE-mediated immune response. The atopy or the atopic status refers to the predisposition to develop allergic reactions, a partly inherited predisposition to have elevated total serum IgE and to mount a Th_2 response with allergen-specific IgE [7].

The terms atopy and allergy are often used interchangeably but are different. All atopic disorders are considered allergic, but many allergic disorders (eg, hypersensitivity pneumonitis) are not atopic.

1.2. Physiopathology

1.2.1. Allergy

Within the allergic population, the physiopathology is based on passive immunity. Our body is protected from the environment by a cutaneous interface and a mucous interface. Skin and mucous membrane play the role of a physiological barrier, which is the basis of the immunity because this barrier is fundamental to bind the one to the non-one. It is the role of the mucous and sebaceous secretions, the tears, the ciliary mobility, the cellular renewal, the desquamation, the keratinization, the commensal flora, the transit and the cough to protect the body during a prolonged contact with the outside environment and to thwart the implementation of an active immunizing reaction.

These interfaces can be broken by a congenital, toxic, infectious deficit or by an abundance of antigens allowing the implementation of a specific immune answer [8]. This specific immune answer is based on a production of lymphocyte T helper 1 (Th_1) or T helper 2 (Th_2). In the case of a Th_1 answer, these lymphocytes secrete IL2, 3, interferon gamma (INF-γ) and the tumour necrosis factor (TNF) beta. These cytokines are responsible for an activation of cells B, T, cytoxic T lymphocytes (CTL) and NK, of a proliferation and a differentiation of cells B and of stimulation at the presentation of antigens. The INF gamma favours an immunizing cellular answer to IgG, IgM, IgA and inhibits the proliferation of Th_2.In the case of a Th_2 answer, these lymphocytes produce IL3, 4, 5, 6 and 10. These cytokines in turn stimulate the synthesis of the IgE (IL4 and 10), the development and the attraction of eosinophils (IL5 and 6), and an eosinophilia inflammation. Furthermore, IL10 inhibits the proliferation of Th_1. An imbalance Th_1 /Th_2 response favours the appearance of an answer Th_2 and the production of specific IgE antibodies [9].

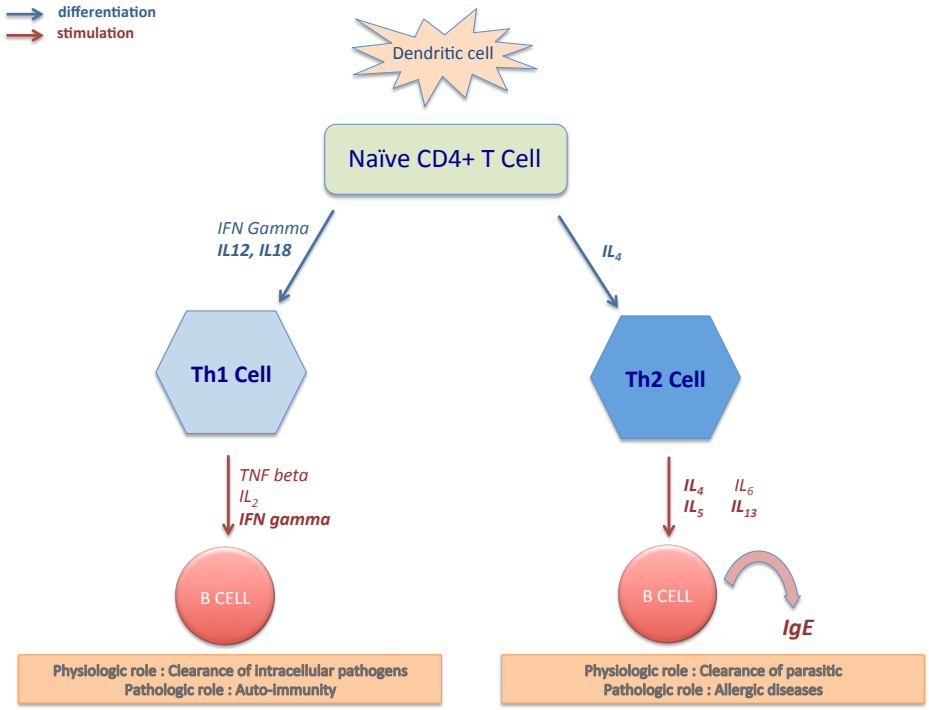
Once the antibody E specific develops, it will join the general blood circulation to go and settle on the membranes of mastocytes (tissular cells) and basophiles (circulating cells). The ensuing contact of the allergen, presented by dendritic cells (Langerhans cells in epithelium of skin and mucosal membranes), with the body triggers a reaction of activation of these cells with a liberation of histamine and other numerous mediators, thus realizing the initial phase characterized by the acute phase of allergy (secretion, vasodilatation, pruritus) and followed by a particular inflammatory phase with polynuclear eosinophils.

1.2.2. Atopy

Atopy begins during early life and develops sequential along the "atopic march" [10-12]. Although the links between these different types of allergic disease are not fully understood, the prevailing paradigm assumes that the atopic march starts with atopic dermatitis, and then proceeds to asthma to end with allergic rhinitis [13].

The imbalance from a Th_1 to a Th_2-mediated immune response profile would be the result of a reduced production of interleukin-12 and IFN-γ by a natural immunity cells, which are stimulated by bacterial products via their toll-like receptor [14]

Figure 1: T helper cell differentiation [6] and role of Th2-like cytokines, B cells and IgE in the development of allergy [15]



1.3. Expression places of allergy

The specific IgE, synthesized by lymphocytes clones B, are distributed via the general circulation. The binding of IgE to receptors on the basophiles and mastocytes induces the release potent inflammatory mediators, such as histamine, proteases, chemotactic factors, cytokines and metabolites of arachidonic acid that act on the vasculature, smooth muscle, connective tissue, mucous glands and inflammatory cells. These mediators are responsible for the inflammatory response that characterises allergic diseases.

1.4. The atopic march

The concept of atopic march describes the progression of atopic disorders from atopic dermatitis (AD) in infants to allergic rhinitis and asthma in children [16]. Although there are important interpersonal variations, the demonstrations of the atopy are especially present during the first 20 years of life. The production of IgE begins during the 11th week of gestation. The rates of total IgE are very low but increase strongly during childhood.

Atopic dermatitis pathogenesis includes disrupted epidermal barrier function, immune dysregulation, and IgE-mediated sensitization to food and environmental allergens. AD takes part in the atopic march, a progression from AD to asthma and allergic rhinitis. It is interesting to observe that the conditions may not occur simultaneously but occur and disappear over time and a 'reverse' allergic march may occur, e.g. asthma appears first and atopic dermatitis later on [17].

-SYMPTOMS-

The symptoms of the atopy are similar to the symptoms of the allergy, and some of them are often premature, beginning with atopic eczema (or atopic dermatitis), sometimes evolving over time to asthma and then rhino-conjunctivitis, occasionally punctuates by acute episodes: escalation of the asthma, nettle rash, oedema, anaphylactic shock (allergic reaction engraves). The evolution and the preservation of the symptoms in adulthood however seem to concern only a minority of the sick children, between 10 and 15 %.

1.4.1. *Atopic dermatitis*

The common term "dermatitis" or "eczema" indicates a local inflammation of the skin [3]. Atopic dermatitis generally represents the first atopic manifestation and has the highest incidence during the first 6 months of life and the highest period of prevalence during the first 3 years of life. Atopic dermatitis pathogenesis includes disrupted epidermal barrier function. It is an inflammatory skin disorder characterized by severe pruritus, a chronically relapsing course, a distinctive distribution of atopic dermatitis and skin lesions, and a personal or family history of atopic diseases [18]. The skin disease is most often associated with the existence of environmental or food allergen-specific IgE. This extrinsic form of atopic dermatitis is found in 80% of diseases. The intrinsic variant is found in 20% with the typical clinical appearance of atopic dermatitis but without specific IgE (Holgate).

The common term indicating a local inflammation of the skin should be the one of dermatitis. The most appropriate term is the one of atopic dermatitis. The subgroup of dermatitis in connection with the presence of allergic asthma and rhino-conjunctivitis is known as atopic dermatitis. Close contact with chemical substances of low molecular weight can activate a dermatitis of contact, mediated by lymphocytes T essentially Th_1. The non-allergic dermatitis can be described by the term of dermatitis of contact of toxic type or irritating [3;19].

1.4.2. *Food allergy*

At birth, the IgE answer to food allergens is rare and would increase when baby food is diversified [18]. A food allergy is a reaction of immediate hypersensitivity of type I. It is caused by the ingestion of food, which also includes food additives. Over the past years, the prevalence of food-related anaphylaxis allergy has significantly increased. AD and food allergy frequently co-exist and that, especially amongst people having an early onset, a severe or persistent atopic dermatitis.
Food allergy is a well-known provoking cause of AD. "The prevalence of IgE-mediated food allergy amongst children with AD appears in about 35% of the affected children" [20].

Sensitization to food allergens comes first. It is then followed by the development of sensitivity towards aeroallergens. In several studies, sensitization to common food allergens and to inhalant allergens in the first year of life was found to be a strong predictor for the development of atopic disease by the age of 6 in children with a positive family history of atopy [21]. According to recent studies children with high levels of blood IgE during early childhood, undergoing a change for IgE directly targeted against the indoor and outdoor allergens are at higher risk of bronchial hyper reactivity and persistent allergic asthma [18]. In the Maas study, twice as many children who, at the age of seven, were diagnosed as having asthma or who presented an airway hyper-responsiveness had measurable IgE antibodies towards food allergens at the age of one, compared with non-asthmatic children [21;22].

1.4.3. *Allergic asthma*

Asthma is an airway chronic disease related to bronchial hyperresponsiveness. It leads to recurrent wheezing, dyspnoea, chest tightness and cough, especially at night and in the early morning [4].

Allergic asthma refers to asthma mediated by immunologic mechanisms [3]. Asthma, one of the most serious allergic diseases in developed nations, has been characterized by an increased responsiveness of the tracheobronchial tree to a multiplicity of stimuli, an increased infiltration of various inflammatory cells especially eosinophils into the airway, by epithelial damage and airway smooth-muscle hypertrophy [23], constriction, by variable airway obstruction usually associated with inflammation in the conducting airways of the lungs [24]and by mucous hyper-secretion in the bronchiolar walls of the lung [25]. Asthma is critically dependent on a series of cell adhesion molecule-mediated interactions between vascular endothelium and leukocytes, leading to symptoms and to an increase in total serum IgE [26].

Asthma is clinically classified according to the frequency of its symptoms, forced expiratory volume in 1 second (FEV1), and peak expiratory flow rate. Asthma may also be classified as atopic or non-atopic. Respiratory allergy is a special sensitiveness to substances that are generally tolerated by the main population. The organism reacts in an exaggerated way producing specific IgE against the

allergen. A while after the exposure, chemical mediators are liberated and produce bronchial hyper-responsiveness, oedema, mucus secretion…

Asthma most often begins as a wheezing combined with respiratory infections. Some risk factors such maternal smoking during pregnancy, birth before term can affect the development of the airways and favour obstructive respiratory diseases among infants [27].

The incidence increases up to the age of 8 and is stable beyond the age of 15 [28]. After infancy, young patients with allergic asthma often enjoy a transient or even a permanent remission; incidences fall and asthma will persist for life in some % of cases. Allergic asthma most often develops in the second decade of life and frequently persists into adult years.

Although asthma and Chronic Obstructive Pulmonary Disease (COPD) share similar characteristics, they are different in terms of disease onset, frequency of symptoms, and reversibility of airway obstruction. In the case of COPD, in the most cases a sequel to many years of active smoking, it is usually not related to allergy [18].

1.4.4. *Rhino-conjunctivitis*

A rhinitis is defined as a nasal inflammatory disease, which causes symptoms like nasal obstruction, nasal pruritus with sternutatory crises, sneezing, anterior and/or posterior rhinorrhea, disorder or disturbances of the sense of smell, with, sometimes, eye, otologic and pharyngeal symptoms [29]. The seasonal or perennial allergic rhino-conjunctivitis rarely manifests itself amongst children younger than 2. Generally, at least 2 seasons of exposure to pollens are necessary before the appearance of the symptoms. The prevalence of allergic rhino-conjunctivitis affects more than 20% in young European teenagers [18].

The causes of the rhinitis are variable and it is considered that between 30 and 50 % of cases find an allergic aetiology. In sensitized individuals, this allergic response can result from the exposure to allergens such as pollens, moulds and animal dander [12]. Almost all allergic rhinitis are IgE-mediated. As for asthma, either occasional or persistent, it can be differentiated according to the duration of the symptoms [19]. In many cases, an allergic rhinitis is associated with lower airway hyperreactivity or bronchial hyperresponsiveness [30;31]. Several studies point out that allergic rhinitis is a risk factor for developing asthma and can precede asthma in the atopic march [12].

2. Epidemiological data

All the epidemiological studies mainly led in Europe indicate a fast increase of the prevalence of the diseases connected with the atopy. The atopic dermatitis affects 10 to 25 % of children according to the recent Northern European studies, while the inquiries of the sixties indicated a prevalence in the neighborhood of 5 %. The interpretation of these data reflects the influence of the environment on the genotype. In countries with high prevalence, it is stabilizing, which may correspond to a saturation of the achievement of the population genetically at risk [32].

An important increase of allergy has been reported in children and young adults whereas no major increase in the prevalence of allergy has been found in adults [33]. The prevalence of childhood asthma has increased tremendously from the 1960s to the 1990s and nowadays it is the most common chronic disease affecting children in Western Europe, Australia and Zealand. In addition to this variation over time, recent studies have shown a geographical variation with higher prevalence rates in industrialized countries and an east-west gradient within Europe [34]. In the International *Study* of Asthma and Allergies in Childhood (ISAAC), the proportion of children with symptoms of multiple disorders (AD, allergic rhino-conjunctivis or asthma) rose slightly from Phase 1 (1999) to Phase 3 (2006). In Belgium, in the 6 to 7 year-old group, the proportion rose from 7.7 to 11.6 for atopic dermatitis, from 4.9 to 5.8 for allergic rhino-conjunctivitis and from 7.3 to 7.5 for asthma symptoms. In the 13 to 14 year-old group, the proportion rose from 6.7 to 7.2 for atopic dermatitis, from 14.5 to 16.9 for allergic rhino-conjunctivitis and decreased from 12.0 to 8.3 for asthma symptoms [35]. This progress has been noticed in several European countries where studies carried out according to the same methodology were repeated overtime [36].

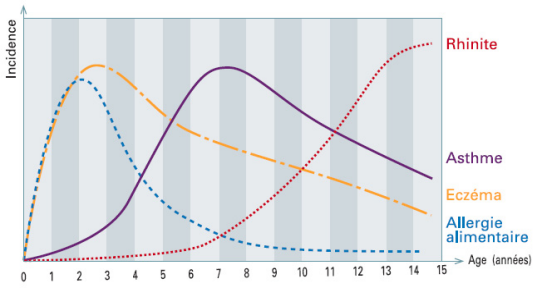
According to the estimations of the WHO, there are now 235 million asthmatics in the world and this is the most common chronic disease amongst children. Asthma is not a problem of public health limited to countries with high income; it rages in all countries, whatever their level of development. Most of the deaths attributable to asthma happen in low-and middle-income countries or intermediary [37]. Asthma is also one of the most common diseases of childhood causing substantial morbidity (hospital admissions for asthma in childhood [38]).

3. Potential mechanisms underlying the allergic march

Two international epidemiological studies have identified a link between the various types of allergies: the study ECRHS (European Community Respiratory Health Survey)[39], which concerns the prevalence of asthma, was carried out on adults aged 20-45, and the study ISAAC which was led in 50 countries on 700 000 children (aged 6-7) and on teenagers (aged 13-14)[35;40].

Describing the natural history of the allergic manifestations, the allergic march shows that atopic demonstrations follow one another over time and can possibly evolve towards graver forms, appearing during early childhood and persisting for several years [17]. Generally, the atopic dermatitis will be the first demonstration and will precede the development of asthma or the allergic rhinitis, as illustrated in figure 2. Atopic dermatitis would be "the entry point" for possible allergies happening in late childhood. Positive tests for atopy in a wheezing child also increase the likelihood of asthma. By example, the risk of hay fever is increased by up to 5 times in case of a history of atopic disease; and the risk of asthma is increased by 3 to 4 times. In children up to the age of 14, a positive skin test for allergies and an increase in IgE enhances the risk of having asthma [41]. In the adult population, the more one reacts positively to in a skin test, the higher the odds of having asthma [42]. If sensitization to one or several allergens is a risk factor for asthma, this sensitization and the allergen exposure levels are less clearly correlated to asthma severity [4].

Figure 2: Evolution of allergic infants diseases related with age [40;43]



Can environmental changes and of life style be responsible of this allergic march?

3.1. Risk factors

3.1.1. Genetic factor

Allergic diseases are certainly better known today than they were 50 years ago; the diagnosis is more reliable and done earlier. Besides, since the first description of the atopy, a genetic determinism has been established. The heritability of allergic diseases has been recognised since the early 20th century. A parental history of allergy is one of the major risk factors for childhood asthma [44] and it is also the case for atopic dermatitis and allergic rhinitis.

Numerous studies have shown that when both parents are allergic, there is a 50% probability that children develop allergies, a probability even higher if parents present the same type of allergy. When none of the parents is affected, the proportion falls to 10 %. At least one aero-allergy in one parent confers additional risk in the presence of asthma in the other parent [45]. In most studies, maternal asthma confers a greater risk than paternal asthma [46] although some studies indicate either a stronger paternal effect or no differences [44]. A stronger maternal effect might be explained by a stronger maternal parent-of-origin effect, the effects of maternal environmental exposure during pregnancy, or immune interactions between mothers and their offspring *in utero*. For childhood atopic dermatitis, several studies have reported a greater maternal effect than paternal effect. For allergic rhinitis, only a few studies have investigated the parent-of-origin effect and they have found no significant difference between maternal or paternal rhinitis.

Several studies on gender differences in asthma have shown that during childhood, prevalence and incidence rates of asthma are higher among boys but then shift in the opposite direction from puberty. Male preponderance is also observed with most allergies [47]. Adult women tend to have a more severe disease, often recalcitrant to treatment. In different studies, atopic dermatitis and rhinitis in preschool children show no significant gender difference or male preponderance. More adult females suffer from atopic dermatitis. Sex-specific differences have also been reported for bronchial hyper-responsiveness, allergic sensitization, serum IgE levels and developmental cytokine response profiles [48]. In the PIAMA study, the prevalence of wheezing was significantly higher in boys than in girls at the age of 1 and this higher prevalence persisted up to the age of 7. The prevalence of asthma is higher amongst boys in the first 3 years of life but not thereafter [49]. But these factors only cannot explain by themselves the increase of the frequency of these diseases.

3.1.2. Epigenetic factors

DNA modifications, chromatin modifications, regulatory RNA molecules, and other processes affecting gene expression, epigenetic changes, including DNA methylation and histone modifications, play an essential role in foetal development, gene-environment interactions, and the maintenance of cell lineage. The prenatal environment can have lasting effects on the phenotype of the offspring, mediated at least in part through epigenetic modifications. A link between epigenetics and the developmental origins of increased allergic risk has been demonstrated in murine studies but evidence in humans is still limited. The histone acetylation/deacetylation may be dysregulated in asthma and that DNA methylation could play an important role in T-cell differentiation. Currently available literature supports the importance of epigenetic alterations in the development and manifestations of atopic diseases, such as asthma and allergic rhinitis [50].

3.1.3. Individual factors

a) Perinatal factors

Prematurity and caesarean section have been associated with higher risk of asthma and allergy among children before the age of three [51]. Nevertheless, among children diagnosed at 3 years old or later, relationships between these risk factors and the development of asthma tend to disappear. Perinatal factors play a role in the development of asthma in childhood but their implication appear to differ between early and late onset of asthma [51].

b) Socio demographic and individual factors

Several social, economic, demographic and individual factors are significantly associated with the development of atopic symptoms and diseases [52;53]. Studies have demonstrated the racial disparities in asthma morbidity among those in extreme poverty and suggested a great role for differential patterns of social and environmental exposure rather than of genetic risks[54]. The studies exploring the influence of socio-economic status (SES) have provided conflicting results regarding asthma but they have shown that atopy is usually more prevalent among high SES groups[55].

Obesity and the development of asthma appear to show a strong correlation, which is not recent [56;57]. Current asthma severity and wheezing are associated with high Body mass index (BMI) and the relation is particularly obvious among overweight children [58]. An increase in body weight, however, does not appear to affect all asthmatic subjects alike. Recent studies have shown that having a larger BMI is part of the asthma phenotype characterized by adult-onset asthma, female preponderance and less atopy [59;60]. Several factors associated with obesity might thus play a role in the pathogenesis of asthma, including low physical activity, diet, hormonal influence, immune modification and mechanical factors [60].

3.1.4. Exposure to allergens

There is no allergy without exposure to allergens. Allergens that are the most frequent causes of allergic diseases are found in food and in the indoor or outdoor air.

a) Food allergens

For a long time, human milk has been considered as being the least allergic food. In reality, in spite of the numerous advantages of breastfeeding, its protective potential towards the risks of allergic sensitization and diseases is more and more debated [6]. The numerous studies that have addressed this issue have provided conflicting results. At infancy, when at risk and carrier of atopic dermatitis, food allergies appear from the moment when food diversification is too premature. The increase of the incidence of the atopic dermatitis at world level is considerable: some evoke a 250 % factor during these last 3 decades. In 80 % of cases, it reveals itself before the first birthday and it is

related to food origin. Allergies to proteins of cow's milk do not however seem to account for all food allergies, which are steadily growing. Practically all foods can be concerned: egg, groundnut and nuts, wheat, soya, fish and shellfish, kiwi, etc. Within minutes and up to 2 hours of food ingestion, IgE-mediated food allergy starts. The major food allergens are glycoproteins. They are generally water soluble and resistant to heat, proteases, and acids. Food allergens that escape to proteolysis are taken up by intestinal epithelial cells and presented to primed T cells. This leads to the generation of T-helper type 2 (Th_2) cells. Tolerance can be acquired with >70% of children becoming tolerant to cow's milk and eggs by the age of 16 while allergies to peanuts, tree nuts, and seafood are frequently lifelong [61]. However, if cow's milk allergy disappears, other atopic conditions tend to develop [62]. A higher prevalence of specific IgE to wheat or egg white predicts later childhood asthma [63]. Further, the American Academy of Pediatrics found no convincing protective effect of breastfeeding on the development of atopic disease with delaying the introduction of solid food, including common allergens, beyond 4-6 months [61].

b) Indoor air allergens

The air we inhale can contain allergens of animal or vegetable origins, which, for sensitive persons, may cause reactions affecting the respiratory system, the skin and the eyes. The increasing need to save energy from the 1970s led to more airtight houses, which was translated by an increase of the allergenic load of air: house dust mite, allergens of animal origin, moulds and cockroaches. Draught proofing leads to rising internal temperatures and humidity favours the production of internal allergens. In infancy and early childhood, these allergens are a primary cause of the rise in asthma and allergy [64;65]. Reducing the exposure of children to airborne allergens at home may however not necessarily reduce the risks of allergic diseases. For example, if a reduction of house dust mite (HDM) exposure indoors can decrease the risk of HDM allergic sensitisation, the risk of developing childhood asthma is only modestly decreased [66]. Reducing the exposure to cat and dog allergens even exerts opposite effects [67]. Indeed, several recent studies have reported that early childhood exposure to pets or farm animals, are associated with a lower prevalence of asthma, hay fever, and inhalant allergen sensitization [25].

Exposure to fungi produces respiratory disease in humans through both allergic and non-allergic mechanisms too. Occupants of homes with excess dampness and moulds growth often complain of aero-irritant symptoms. The preponderance of epidemiological data supports/ points to a link between exposure to dampness or excess mould growth and the development of aero-irritant symptoms.

c) Outdoor air allergens

For the subjects at risk, the main outdoor organic pollution is pollen. The "pollinose" is defined as the set of visible allergic manifestations caused by the contact of pollens with the skin or mucous membranes, mainly those of the eyes, the nose and bronchi. The correlation between the intensity of the symptoms and the quantity of present grains of pollen in the atmosphere has been known for

a long time. Previously, the majority of the symptoms caused by the contact with pollens were due to grasses. At present, pollen from many other plants can cause respiratory allergies.

3.1.5. Microbial exposure

Studies on endotoxin in asthma provide a paradox. Endotoxin is a potent immune-stimulatory component of the bacterial cell wall of all gram-negative bacteria. Endotoxin exposure is well present in our environment and has emerged as a frequent cause of asthma-like symptoms in a wide range of occupational settings. Asthmatics are particularly sensitive to inhaled endotoxin, and inhalation induces both immediate and sustained airflow obstruction. The paradox of endotoxin exposure is that higher levels of exposure in early life might mitigate the development of allergy and persistent asthma [25]. A meta-analysis integrating the results of studies examining the association of endotoxin exposure to wheezing and asthma in children showed that endotoxin is a risk factor for wheezing in younger children, but a protective factor for asthma in older children [68].

Viral respiratory infections may increase one's risk of developing asthma especially in young children. Respiratory infections such as rhinovirus, Chlamydia pneumonia and Bordetella pertussis are also correlated with asthma exacerbations. Lower respiratory tract infections (LRTIs), caused by viruses such as respiratory syncytial virus (RSV) and rhinovirus (RV), are a leading cause of bronchiolitis in infants. Viral respiratory infections are the most common cause of acute illness and wheezing during infancy and infections with RSV has been associated with a subsequent increased risk of recurrent wheezing and asthma [69]. Among these infections, acute bronchiolitis is a common cause of lower respiratory disease in young children. In infants, the occurrence, of acute bronchiolitis is strongly correlated with the later development of asthma. Infants hospitalized with bronchiolitis are at significantly increased risk for both recurrent wheezing and childhood asthma [70].

At the opposite, exposure to parasitosis could be protective factor against allergy. Epidemiological and interventional human studies, as well as experiments in animal models, strongly indicate that helminth parasitic infections can confer protection from immune dysregulatory diseases such as allergy, autoimmunity and colitis [71].

3.1.6. Physical and chemical pollution

a) Environmental Tobacco Smoke (ETS)

Environmental tobacco smoke (ETS) is a major cause of respiratory problems in children. The foetus can be exposed either by mother's active smoking or by her exposure to ETS during pregnancy. Chemicals substances of tobacco smoke can be transferred across the placenta to the foetus [72]. A smoking mother can increase the risk of low respiratory infections of her child, during the first 3 years of his life by more than 50 % [51]. So, at least 15 to 26 % of the episodes of low respiratory infections affecting European young children would be attributable to an exposure to tobacco smoke at home [73]. In epidemiological studies, several perinatal factors have been suggested as being associated with the risk of asthma. One of the most common is the exposure to

tobacco smoke. Studies have shown that adverse effects of tobacco smoke on asthma and chronic respiratory symptoms are the strongest when smoking takes place during pregnancy. The relation is weaker for exposure during both early-life and beyond 2 years old and is even weaker or non-existent for later exposure [72].

b) Ambient air pollution

A potential driver of the rise in allergic respiratory diseases and bronchial asthma is the increased exposure to ambient air pollutants in particular those emitted by vehicles. The most abundant air pollutants in urban areas are particulate matter (PM), nitrogen dioxide, ozone (O_3) and sulphur dioxide (SO_2) [74]. The increasing temperature and changing climate alter the concentrations and the distribution of these air pollutants and interfere with the seasonal presence of allergenic pollens in the atmosphere [75]. Ambient air pollutants may have some impact, at various levels of depth on the respiratory system, depending on their physico-chemical characteristics, their concentrations, the duration of exposure and the susceptibility of exposed individuals. Coarse particles affect the upper airways and alveoli and water-soluble gases react with the mucus layer of the upper airways while less soluble gases are more likely to reach the alveoli [76].

Pollutants can compromise the respiratory system defences. Pollutants may affect the composition or the production of the mucus (first line of defences) and/or degrade the function of the airways epithelial cells. They may also affect sensory cells endings leading to hyper-reactive airways or increased mucus secretion [76]. Damage caused by air pollution may facilitate access of inhaled allergens to the cells of the immune system and promote the sensitization [75]. Several studies have shown that both the development and the exacerbation of respiratory allergies including childhood asthma are affected by ambient air pollutants. In Germany, the prevalences of allergies, on the eve of the reunification, was twice as high in West of Germany than in the East Germany, the relationship that reversed for the irritative bronchial affections. An association has also been reported between traffic density close to the place of residence of children and the prevalence of respiratory symptoms, and more specifically the asthma and allergic symptoms [77].

The prevalence of the symptoms of bronchitis and the alteration of the lung function of children are associated with an exposure to particles in suspension at annual average levels superior to 20 $\mu g/m^3$under the shape of $PM_{2.5}$ (particle matter having a diameter lower than 2,5 µm) or in 30 $\mu g/m^3$ under the shape of $PM_{0.1}$ (less than 0,1 µm). Exposure to traffic exhausts during infancy is associated with a higher risk of developing asthma during childhood [77]. Moreover, the first years of life are a critical period for the development of atopy related to exposure to environmental agents [77]. In particular, children's hospitalization from 4 months to 4 years old affected by obstructive bronchitis is related to an increase in the density of the road traffic [73]. If diesel particles might consolidate the allergen specific Th_2 immunity leading to imbalance between Th_1 and Th_2 cells, among children with familial history of atopy [77], it has been shown recently that diesel emissions may be responsible for allergic reactions among non atopic people without any genetic predisposition [77].

c) Volatile Organic Compound (VOCs)

Consumer products, including synthetic building materials, carpets, wallpaper, cleaning chemicals and cosmetics, emit an array of volatile organic compounds (VOCs) that predominantly exist in the vapour phase in the atmosphere and may persist from several months to years. Some, as formaldehyde, are useful for their bactericidal action but is also known as a potential upper respiratory irritant [78]. Frequent use of chemical based products in the prenatal period has been associated with persistent wheezing in young children [79] and with lung abnormalities in non-atopic children [80]. Observational studies have found that indoor exposure to VOCs may be one of the triggers of asthma, however experimental studies have not confirmed these observations [81]. Even VOC exposure at low levels has been associated with an increase in the risk of paediatric asthma. Because there are so many VOCs in the air, measuring total VOC concentrations in the indoor environment may not represent the exposure of individual compounds. There is a significant association between asthma-like symptoms (wheezing) among preschool children and the concentration of DEHP (di-ethylhexyl-phthalates) in indoor environment. Phthalates may have an adjuvant effect on basic mechanism in allergic sensitization [82]. In infants and children, the role of indoor VOCs as allergens, adjuvants … are associated with the development of allergic asthma, and rhinitis is still controversial. Recent reviews have identified that indoor residential chemicals, emitted from particle board, plastic materials, recent painting, home cleaning agents, air freshener, pesticide, and insecticide, are consistently associated with increased risks of allergic and asthma-like symptoms [30].

d) Hardness of water

Water hardness, mainly determined by calcium and magnesium compounds in varying proportions is expressed as the equivalent amount of calcium carbonate that could be formed from the calcium and magnesium in solution. Hard water is highly charged with calcium (Ca^{++}) and magnesium (Mg^{++}) ions and is expressed in French degrees (°F). One French degree corresponds to a level of calcium and magnesium equivalent to 10 mg of calcium carbonate per litre (CaCO3). Water hardness may be associated with the risk of atopic dermatitis among children [83;84].

e) Chlorination products

It is well known that the water chlorination process is intimately linked to hygiene and is considered as one of the greatest advances of the 20th century in public health. In the developed world, the routine chlorination of water supplies has indeed led to the virtual eradication of waterborne diseases such as thyphoid fever, cholera or dysentry, which has undoubtdly contributed to increase human longevity. Nowadays, chlorine-based disinfectants are used in a wide variety of applications such as sanitation of drinking and recreational water, disinfection in the food industry or cleaning of surfaces in public and private buildings. Advantages of chlorine include low cost, easy use, residual protection, deodorising and a strong germicide activity against a wide spectrum of microorganisms[85].

* *Sodium hypochlorite (chlorine bleach)*

Chlorine bleach is the most commonly used disinfecting and cleaning agent. Professional use of hypochlorite solution (bleach) has been associated with respiratory symptoms. High-level exposure to irritants may induce a reactive airway dysfunction syndrome. Cleaning workers may also have a greater relative risk of developing asthma due to a prolonged low-to-moderate exposure to respiratory irritants. In addition, asthmatic symptoms without confirmed asthma are also common after exposure to cleaning agents. People who clean their home with it are less likely to be atopic but more likely to have respiratory symptoms [86]. On the other hand, bleach is capable of inactivating allergens and some studies have showed the protective effect of cleaning chlorinated products [87]. The observations of these studies showed that the prevalence of asthma and allergies were less high in children leaving in a house which was cleaned with chlorinated products. Treatment with common household bleach containing hypochlorite destroys dust mites and denatures protein allergens. Emphasis on cleaning and cleaning education combined with hypochlorite-based cleaning supplies resulted in a significantly improved quality of life for families with asthmatic children [88].

But chlorine bleach is unstable and a highly reactive chemical. When reacting with organic matter or other agents, it can release trichloramine, which is a strong irritant to the respiratory tract and eyes [89]. When mixed with acid, bleach also releases chlorine gas, another strong irritant frequently responsible for acute poisoning at home or in indoor swimming pools.

* *Chlorinated pool attendance*

Several case reports have shown that the accidental release of chlorine gas in indoor swimming pools can cause lung injuries in recreational swimmers and in other pool attendees [90]. Lung damage observed in these circumstances is fortunately transient in most cases with a recovery of the lung function within a period of a few weeks.

The possibility, however, that the chlorine-laden atmosphere of indoor swimming pools can cause chronic effects on the lungs of recreational swimmers appeared in the early 2000s when new non-invasive tests of lung damage were applied for the first time to children. In a cross-sectional study primarily designed to assess the chronic effects of air pollution on the respiratory tract of schoolchildren [91;92]it was unexpectedly found that the serum concentrations of the surfactant-associated proteins A and B increased in a dose-dependent manner with the cumulated time the children had spent with school in an indoor chlorinated pool. Repeated exposure to chlorination products used in swimming pools has adverse effects on the Clara cells function in children [93]. Children who regularly attended chlorinated swimming pools had a significant lower CC16 level in serum compared to non-swimming children. The CC16 concentration in serum reflects both the epithelial permeability and the integrity of the Clara cells.

Especially when pool attendance is cumulated from birth up until the first grade of primary school, cumulated pool attendance (CPA) by children emerged among the most consistent predictors of asthma (doctor-diagnosed or screened with the EIB test) and of elevated fraction of exhaled NO (FeNO) immediately after atopy and family history of asthma or hay fever [91].

Baby swimming

As suggested by some of these studies, one of the most critical factors in determining the risks of CPs for children appears to be the timing of exposure. This unavoidably raises the question of the safety of baby swimming practice, especially as the higher water temperature and the greater organic pollution in swimming pools attended by young children are conditions favoring the formation of chlorination by-products. Baby swimming practice is associated with an increased risk of recurrent respiratory tract infections and otitis media in the first year of life concerning mainly children from atopic parents [94]. Children who had been swimming as babies showed a significant decrease of the serum Clara cell protein (CC16), a marker of the integrity of Clara cells lining terminal airways [95;96]. Baby swimming emerged as a significant predictor of serum CC16 and the strongest determinant of the CC16/Surfactant protein-D (SP-D) ratio. These effects were associated with higher risks of asthma and of recurrent bronchitis. Interestingly, passive exposure to tobacco alone had no effect on these outcomes but appeared to interact with baby swimming practice to increase the risks of asthma (parental smoking at home, or of recurrent bronchitis (maternal smoking during pregnancy)[92;97]. Baby swimming practice in chlorinated pools may be associated with distal airways alterations predisposing infants to the development of respiratory diseases [95].

3.2. Hypothesis

The atopic disease rises rapidly without specific explanation and stimulates thus investigation to identify the cause, so the preventive measures can be devised. Although genetic predisposition is an essential factor leading susceptibility to atopic disease, the period of time in which these changes have occurred has been too short for genetic shifts to be playing a major role. Research suggests that some genetic variants may only cause asthma when they are combined with specific environmental exposures, and otherwise may not be risk factors for asthma.

Although genetic predisposition is an essential factor leading susceptibility to atopic disease, they cannot explain the rate at which prevalences of allergic diseases have increase over the last decades. Such a rapid rise must necessarily be driven by changes in our environment or lifestyle.

3.2.1. Hygiene hypothesis or the "microbial exposure" hypothesis

Currently, the hypothesis generating the most interest is the "hygiene hypothesis" postulating that the rise in asthma and allergy is due to the decreased exposure to microbiological products and/or infectious agents, especially in early life [98]. During the 1990s, there was a higher prevalence of hay fever, atopic dermatitis, skin prick positivity, and allergen specific IgE in individuals brought up in smaller and more affluent families [99]. Asthma shows a different epidemiological pattern from these indicators of atopy. The hygiene hypothesis suggests that the rise in the prevalence of allergies and asthma comes as a direct and unintended result of reduced exposure to a wide variety of different bacteria and virus types in modern societies, or modern hygienic practices preventing childhood infections.

Several studies showed lower incidences of asthma and allergic diseases among children living in less hygienic environments (East Germany vs. West Germany, families with many children, day care environments) [99]. Endotoxin exposure being significantly higher in homes with animals and in farming households, where allergy and asthma are less likely to develop, endotoxin and other microbial exposures in early life may keep allergen sensitization and asthma from developing by promoting Th1-type immune development. This protective effect of infection, however, has not been confirmed by other studies [100;101], and some features of the childhood asthma epidemic remain unexplained by the hygiene hypothesis. For instance, asthma has also increased among inner-city children who live in very poor housing conditions presumably somewhat dirty [102].

To account for these conflicting results, researchers in the fields of epidemiology and immunology regularly reformulate the hygiene hypothesis. After having initially attributed the protective effect to overt viral and bacterial infections, the protagonists of the hygiene hypothesis have linked the protective effect with an exposure to some microbial compounds or have postulated the existence of narrow windows of opportunity during early life. Other supporters of this hypothesis invoke the existence of a complex interplay between the immune responses of the host, the characteristics of the invading microorganism, the level and variety of the environmental exposure, and the interactions of all these factors with the genetic background [103;104]

This hypothesis is in contradiction with the logic that viruses are often causative agents in exacerbation of asthma. And in other studies, viral infections of the lower airway may in some cases *induce* asthma, as a history of bronchiolitis with respiratory syncytial virus in early childhood is a predictor of asthma risk in later life. And this hypothesis is subject to numerous controversies. Other studies have shown by example that the presence of endotoxin is positively associated with an asthmatic child living at home [105]. In this study, the asthmatic children were found to be exposed to higher levels of endotoxin than the non-asthmatic matched control subjects.

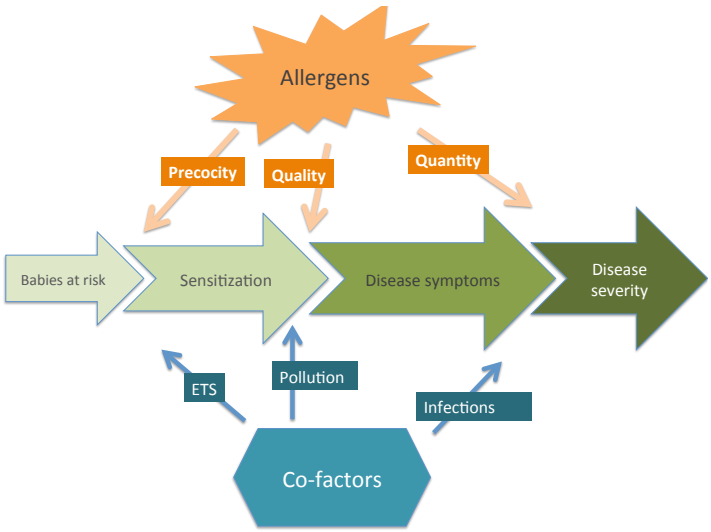
Antibiotic use in early life has been linked to the development of asthma and in several instances; it is thought that antibiotics make children who are predisposed to atopic immune responses susceptible to develop asthma because they modify gut flora, and thus the immune system (as described by the hygiene hypothesis). Caesarean sections have been also associated with asthma, possibly because of the modifications of the immune system. This association is supported by epidemiologic data for asthma and negatively affects exposure to beneficial bacteria that are important during development, and thus may cause an increased risk for asthma and allergy.

Nevertheless, recent studies seem to demonstrate that a decrease in intensity of helminth infections may have contributed to the reduced capacity of immune-modulation by helminths in this paediatric population.

3.2.2. Nutritional and environmental hypothesis

The rising incidence and prevalence of allergic diseases and asthma during the past few decades has drawn attention to the environmental factors that may be influencing these outcomes.

Figure 3:Risk factors of allergic sensitization and respiratory diseases [40;43]



In allergic sensitization, allergens must cross epithelial barriers to interact with antigen-presenting cells (dendritic cells). Tight junctions occluding the paracellular routes are the main barriers

preventing this penetration of allergens. The way these efficient barriers are disrupted in order to allow the delivery of allergens is still poorly understood.

a) Allergens skills

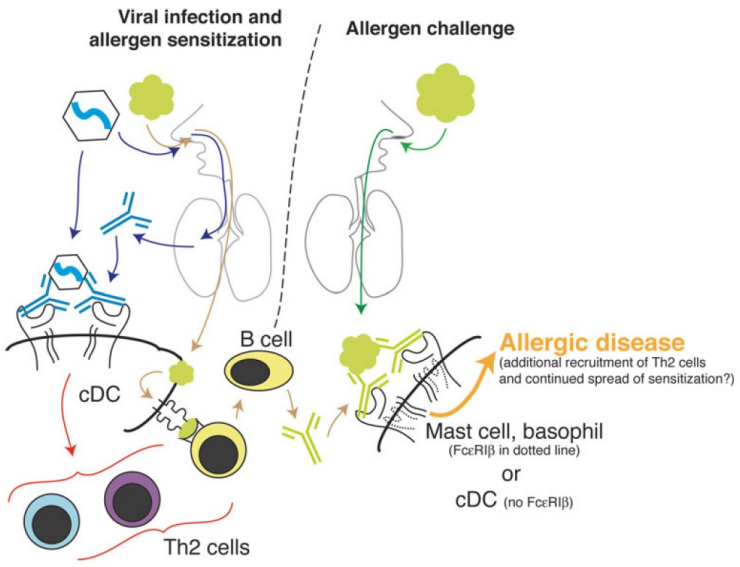
Because of modern and low energy consuming houses and of climate changes, an increase in exposure to allergens such as house dust mites, molds and pollen, and then trigger attacks, has been observed. Evidence from recent *in vitro* studies suggests that because of their proteolytic activity some allergens (the house dust mite Derp 1 and Derp 2 allergens and the Penicillium, Aspergillum Pen ch 13 allergens and pollen peptidase can facilitate their passage by opening the tight junctions and inhibiting the proteolytic activity of the allergen enzymes blocks that delivery [106].

It is unknown to what extent this phenomenon effectively occurs *in vivo*[107;108] and, these changes do not seem adequately to explain this increase [106]. Exposure to outdoor allergens depends on the number of airborne particles, the time spent outdoor and the efficiency with which the indoor environment is isolated from the outdoors. For hay fevers sufferers, and children, it is possible to effectively protect from exposure [54].

b) The "Viral Hypothesis"

It has also been speculated that viral infections could play a role by damaging the airways epithelium. RNA viral infections have been identified from epidemiological studies among children as a cause of development of recurrent wheezing, asthma and allergic sensitization [109]. Respiratory syncytial virus (RSV) is a major cause of lower respiratory viral infections and patients with RSV-induced low respiratory infection (LRI) have an increased risk for asthma and allergic sensitization through 18 years of age [110]. Moreover, infants with RSV bronchiolitis are more susceptible to recurrent wheezing at 3 years of age [111]. The following figure suggests mechanism that might drive the antiviral immune response to atopic disease.

Figure 4 : Potential mechanism for viral induced atopy[109]



The figure 4 *illustrates lessons from mouse studies. A respiratory RNA viral infection (blue) leads to production of IgE against the virus. Crosslinking FceRI on dendritic cells (cDC) by antiviral IgE and virus leads to recruitment of Th2 cells in an antigen nonspecific fashion (red arrow). If a non-viral antigen (brown arrow) is inhaled during the antiviral immune response, it can be processed by a cDC and presented to the appropriate Th2 cell, which instructs antigen-specific B cells to produce IgE against the antigen. This IgE can load onto mast cells, basophils and cDC in the respiratory mucosa. A subsequent exposure to the non-viral antigen (green arrow) can crosslink the antigen-specific IgE on mast cells and basophils, leading to allergic disease. If the IgE is cross-linked on a cDC there could be recruitment of additional Th2 cells, and potentially spreading of allergic sensitivity as outlined above* [109].

Respiratory viral infections in early childhood have a direct etiologic role in the development of asthma and atopy but cannot explain the increasing prevalence observed since more than 30 years.

c) Pollutants exposure

During the last decades, more than 100 000 new chemicals have been introduced to the environment. Outdoor, the main sources of pollutants are fuel combustion from vehicles, construction and ozone (O_3). Many of these chemicals and many consumer products are impacting human health. During the same period of time that the prevalence of these modern chemicals has increased, there has been a remarkable increase in several chronic illnesses, including asthma and allergy in children. The integrity of the mucociliary epithelium can be severely disrupted by many agents such as the inhalation of irritating gases or pollutant gaseous, as the smoke of cigarette, the sulphur dioxide (SO_2), the nitrogen dioxide (NO2), the ozone (O_3), and the acid or alkaline sprays. Major pollutants as phthalates have been identified adjuvant effects on Th_2 differentiation, production of Th_2 cytokines and enhanced levels of Th_2 promoted immunoglobulins (mainly IgG but also Ig E) in mice [82].

3.2.3. The Chlorine hypothesis

By contrast with this hygiene hypothesis, and according with relations observed between atopy and chemical pollutants exposure, the recently proposed chlorine hypothesis suggests that the increasing attendance of swimming pools could be implicated in the increasing prevalence of respiratory diseases. This variant of the hygiene hypothesis links the rise of allergies not to the declining exposure to microbial agents during early life but to the increasing exposure to products and by-products of chlorination, the most widely used method to disinfect tap and recreational water.

Human exposure to chlorination products has considerably increased during the 20th century especially after the 1950s with the development of public and leisure pools and other water recreational areas. When attending these aquatic environments, the population of the

industrialized countries have been increasingly exposed to powerful chlorine-based oxidants, either through direct contact with chlorinated water or by inhaling them in the form of gases (trichloramine and chlorine gas) or of aerosols (hypochlorite/hypochlorous acid and chloramines). The existence of respiratory and allergic problems in competitive swimmers training in the chlorine-laden atmosphere of swimming pools has also been known for more than two decades. Serious concern about these chemicals arose, however, only recently when it was found that they could affect the lung epithelial barrier of recreational swimmers and increase the risks of atopic diseases such as asthma or hay fever. Furthermore, studies focusing on occupational exposures have demonstrated an increased risk of asthma and respiratory problems among swimming pool workers while ecological studies have brought to light associations between the prevalence of childhood asthma or atopic dermatitis and swimming pool accessibility [112].

A variety of experimental studies have shown that hypochlorous acid and chloramines are membrane permeable oxidants capable of rapidly opening the tight junctions of epithelial layers. This led to the suggestion, supported by some epidemiological and experimental observations, that these oxidants closely linked to our Western lifestyle might act as adjuvant in the development of atopic diseases by facilitating the transepithelial penetration of allergens [113].
Whereas the risk of elevated FeNO increased with CPA independently of total or specific serum IgE, the probability of developing asthma increased with CPA only in children with serum IgE > 100 k/[92]. These findings suggest that CPs contaminating the air and water of indoor pools can act as an adjuvant promoting the development of asthma in atopic children, especially in young children attending small heavily polluted pools. A study in Germany [97]provides further evidence that risks of hay fever increased in adults who were exposed to swimming pools at school age, or during the past 12 months or ever exposed.

Evidence from *in vitro* and *in vivo* studies reviewed here indicates that the acute or chronic epithelial hyperpermeability induced by CPs might constitute a mechanism by which allergens could have access to the dendritic cells. These chlorine-based oxidants are indeed produced by inflammatory cells possessing a myeloperoxidase activity that releases them to fight infections or to facilitate cell recruitment. These chemicals are powerful membrane-penetrating oxidants that react rapidly with the sulfhydryl groups of proteins of the cytoskeleton and extracellular matrix, thereby causing cell retraction, disruption of cellular junctions and an almost immediate increase of endothelial or epithelial permeability [114;115]. A repeated exposure to disinfection by products formed by hypochlorite and organic matter in pools may decrease the CC16 secretion because of Clara cells dysfunction or damage [93]. Their properties would be also valuable for the skin barrier. Recreational swimming could lead to significant changes in skin properties [116]. Even though CPs probably represent the strongest triggers of the above mechanisms because of their strong oxidizing potential and their high concentrations in recreational environments, other stressors have the potential to cause similar epithelial effects and thus to act additively or synergistically with CPs. Ambient air pollutants (such as ozone or diesel exhaust particles), tobacco smoke, endotoxins, dry or cold air, or strenuous exercise are all factors that could compromise the

epithelial barriers of the respiratory tract and thereby facilitate the penetration of allergens and thus the allergic sensitization [93;117]

4. Investigations

Although evidence suggests that the onset of an allergic disease occurs in early childhood, many standard outcome measures are either impractical or unreliable in preschool-aged children, especially for asthma. Several clinical studies have used various techniques to measure symptoms, pulmonary function, and cellular mediators of inflammation. Some measures, such as inflammatory marker analysis, may be suitable options for assessing pulmonary function and predicting susceptibility to asthma in preschool-aged children. Indeed, altered levels of inflammatory markers, including immunoglobulin-E (IgE), CC16, and exhaled nitric oxide (FeNO), may be useful tools in diagnosing asthma, and assessing future risks for asthma symptomatology in very young children [118].

Sensitivity and specificity

In statistics and in epidemiology, the ***sensitivity*** of a test or a diagnostic examination is its capacity to give a positive result when the disease (or the condition) is present. It is opposed to the ***specificity***, which is the capacity of a test or an examination to give a negative result when the disease is not present. Together, the sensitivity and the specificity of a test give an appreciation of its intrinsic validity. The positive predictive value is the probability that the condition is present when the test is positive. The negative predictive value is the probability that the condition is not present when the test is negative. The predictive value is a function of the sensibility and the specificity of the test, as well as the prevalence of the condition in the study.

4.1. Exhaled No

The nitric oxide (NO) is an endothelium-derived relaxing factor acting like a free radical. It has a very short half-life and functions as a messenger in processes including the regulation of peripheral circulation, immune responses, platelet function and neurotransmission [119]. NO is a gaseous signalling molecule, generated by three iso-enzymes of NO synthase (NOS) that are differentially regulated and expressed in the peripheral airways and alveoli and appear to play different pathophysiologic roles [120].

Nasopharynx, nose and mouth are the anatomical site of nitric oxide formation. Endogenous NO is derived from L-arginine by the enzyme NO synthase (NOS), of which at least three distinct isoforms exist. Two are constitutively and activated by small rises in intracellular calcium concentrations, the third is inductibe (iNOS), NOS2) and is independent of calcium concentration and may be induced by inflammatory cytokines [121]. Increased NOS2 expression is found in the airways epithelial cells of asthmatic patients and is reduced by inhaled corticosteroids (ICS) and also in the peripheral lung and small airways in patients with COPD. Oxidative stress generates superoxide anions, and in combination with NO, may result in the formation of the highly reactive species peroxynitrite, which is increased in the exhaled breath condensate of COPD patients, removing NO from de gaseous phase so that its concentration in the airways is reduced in the case of high level of

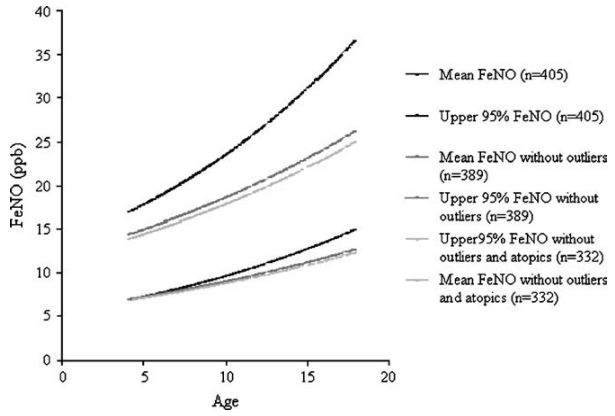
oxidative stress [120].

Endogenous NO has the following properties and functions that affect the respiratory system [122]:

- a weak bronchodilator effect,
- a strong vasodilator effect,
- a non-cholinergic and non-adrenergic neurotransmitter activity,
- an antibiotic activity,
- a role as a modulator of cell differentiation and
- a role as an amplifier of airway inflammation. Fractional exhaled NO (FeNO) is universally considered as an indirect marker of eosinophilia airway inflammation, playing an important role in the physiopathology of childhood asthma. When pathological oxidative stress occurs, NO inhibits Th1 cells and IFN-gamma production, acting as a modulator in diseases such as asthma, amplifying the inflammatory response.

Possible confounders of the exhaled NO test include ambient NO, passive or active smoking, age, sex, ethnicity, height, weight.

Figure 5: Individual values of FeNO in healthy children [123]



FeNO measurements add a new dimension to the traditional clinical tools (symptoms scores, lung function tests) in the assessment of asthma [124].The exhaled NO is an early marker of bronchial inflammation. It is thus useful to detect asthma at an infra-clinical stage but this is not specific to asthma. Interestingly, FeNO can be used as a sensitive marker of inflammation in young children[118]. Indeed, advances in technology and standardization have allowed a wider use of FeNO in clinical practice on children from the age of four years. A systematic review summarizing the influence of allergic rhinitis and atopy on FeNO values, these appear higher in children with atopy or in children with allergic rhinitis, when compared with children without rhinitis, atopic or with allergic rhinitis[125]. FeNO appears also to be higher among children with atopic dermatitis [126].

The sensitivity and the specificity of the method is dependent on the selection of an appropriate cut-off point. A concentration of exhaled NO superior to 15ppb from the expiratory flow of 200ml would have a 90 % specificity and a positive predictive value of 93,3 % to differentiate the

asthmatic children from the healthy subjects [127]. Exhaled No (solid line in the figure 6) was more accurate than lung function tests for the diagnosis of asthma [128].

Figure 6 : Comparison of specificity and sensitivity between eNO and lung function tests in the diagnosis of asthma [161]

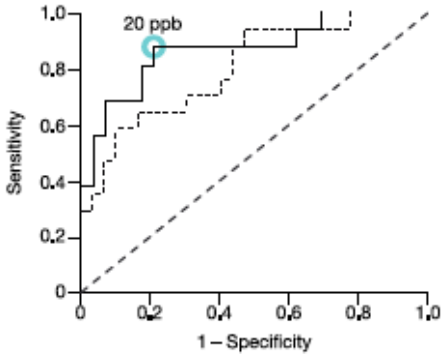


Table 1: Reference values of FeNO[123]

	N	Geometric mean, ppb	Individual 95% upper limit	N	Geometric mean, ppb	Individual 95% upper limit	N	Geometric mean, ppb	Individual 95% upper limit
Age, y	With outliers			Without outliers			Without outliers and atopics*		
4	29	7.1	15.7	29	7.1	15.7	27	7.0	15.0
5	35	7.9	16.6	35	7.9	16.6	33	8.0	17.1
6	49	8.2	19.3	48	8.0	17.1	42	7.5	15.5
7-9	107	8.1	18.4	106	8.0	17.2	89	7.8	17.1
10-13	105	11.2	28.2	98	10.1	19.2	85	9.8	18.0
14-17	80	13.7	39.2	73	11.9	24.2	56	11.6	22.4
Total	405	9.7	25.2	389	9.0	19.4	332	8.8	18.5

*Positive answer to rhinitis/conjunctivitis or hay fever symptoms.
(The outliers were characterized by having more reports of rhinitis symptoms ever or within the last 12 months, or itchy-watery eyes)

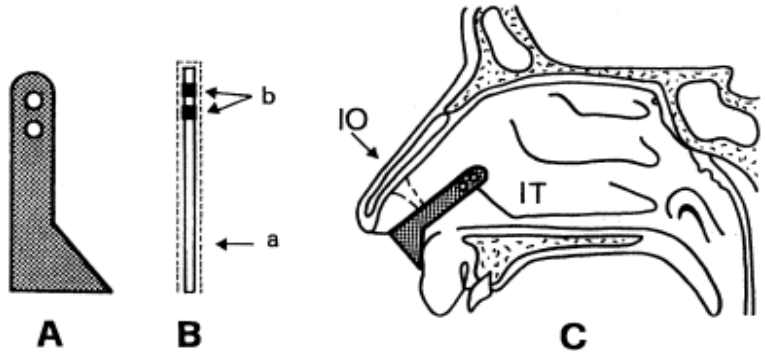
Using NIOX to measure FENO is safe, has a good short-term repeatability and is feasible among school-age children [123].

Picture 1: The Aerocrine NIOX monitoring system

4.2. Rhinosticks: Nasal specific IgE measurements

The Rhinostick test developed Professor Marcucci from Perugia is non-invasive method to screen atopy based on the measurement in specific IgE in nasal mucosa [129]. The incubation of the solid-phase coupled allergen with IgE antibody is performed, *in situ*, directly on the mucosal surface by means of a proper applicator instead of *in vitro* in/on collected secretion. A paper disc coupled to allergen is put in contact with septum nasal mucosa behind the internal ostium.

Figure 7: Details of rhinostick and its application on the nasal mucosa [129]



(A: Applicator, B: Rhinostick (a) with sponges (a), C: Position of applicator on nasal mucosa. IT: inferior turbinate)

After *in situ* incubation, the solid-phase is removed from the container and put into a test tube with 50µl of saline solution (NaCl 0.9%) and stored at -20°C until use. After an exhaustive washing procedure, specific IgE determination is performed according to the Phadezym RAST method. We have obtained values of specific IgE with 5 min incubation on septal mucosa about two times higher than with 3 hours in-vitro incubation with native secretion [129]. Nasal IgE have shown sensibility significantly higher than SPT (98% vs. 83%) and a excellent specificity, negative results being obtain in all non-atopic controls for the all tested allergens [129]. The reproducibility tests showed a between-subject variability significantly higher than that within-subject variability ($p<0.0001$).

4.3. Clara Cell 16kDa protein (CC16)

Biomarkers associated with allergic diseases have been more and more studied over the last decades. Among those, Clara cell 16kDa protein (CC16) appears like an interesting one related with pulmonary inflammation.

CC16 is produced by the epithelial Clara cells (non-ciliated cells), which are mainly present in the human respiratory bronchioles. The Clara cells have been shown to repair damaged epithelium, detoxify xenobiotics and secrete proteins with important biological activities like CC16. The production of CC16 is mostly abundant in the respiratory epithelium, but some also takes place in nasal mucosal epithelial cells, the male urogenital tract, the endometrium, the foetal lung, the foetal kidney, the amniotic fluid and the female urogenital tract.

Increased permeability of a damaged respiratory epithelium is thought to increase the amounts of CC16 in serum. CC16 has been thought to be able to diffuse through the air-blood barrier of the lung epithelium because of its small size. Both anti-inflammatory and immuno-modulatory qualities have been attributed to CC16. Furthermore, CC16 may be able to handle oxidative stress in the respiratory tract and has been reported to have anti-tumoral qualities.

The highest concentrations of CC16 are found in pulmonary fluids (ELF, Broncho-lveolar lavage fluid (BALF), sputum...). Although of great potential for evaluating the extent of inflammation or tissue damage, markers in BAL are not applicable for monitoring the child/ juvenile population exposed to air pollutants in the environment. But CC16 is also present in human amniotic fluid, serum and urine. CC16 is at the origin of another approach for assessing the integrity of the respiratory epithelium, based on the assay in serum of lung-specific proteins. Serum CC16 can increase for several reasons including increased leakage across the pulmonary epithelium due to increased epithelial permeability, up-regulated CC16 due to anti-inflammatory qualifications, and decreased renal clearance. Among them, transient exposure to tobacco smoke, ozone, trichloramine or to swimming pool disinfection by products can provoke a transient increase of serum CC16 levels [130]. But serum CC16 could be a biomarker of permanent pulmonary effects. Chronic changes in serum CC16 concentrations have been explored in relation to specific disease states and chronic exposure to pulmonary irritants and toxicants (tobacco smoke, bioaerosols, particulate matter, nitrogen oxides, swimming pool disinfection by products) [130]. There have been several investigations into the relationship between serum or BALF levels of CC16 and various respiratory diseases. Intermittent allergic rhinitis is associated with decreased levels of CC16 in nasal lavage fluids compared with healthy controls [131]. Asthma, bronchitis obliterans or chronic obstructive pulmonary disease (COPD) are associated with decreased levels of CC16 in serum among children with one of these pathologies compared with healthy children [132;133].

-NON-INVASIVE TESTS IN CHILDREN-

4.3.1. In the urine

Both serum and urinary CC16 can be measured using enzyme-linked immune-absorbent assay (ELISA) techniques that are now commercially available. So far urinary CC16 has been used mainly as a marker of proximal tubular dysfunction especially in pre-puberty children who have no post-renal secretion of the protein. Because of their non-invasiveness tests on urine samples are readily accepted and can be applied for very young children. Urinary CC16 might be used as a surrogate indicator of airways integrity after adjustment for the fractional uptake of the protein on the basis of urinary RBP, a reliable marker of the capacity of reabsorption of the proximal tubule [134].

4.3.2. In the nasal lavage

Although CC16 has also been seen in nasal lavage fluid (NALF), only a few studies on nasal levels have actually been reported. The technique of nasal lavage (NAL) enables to collect in a completely

painless way the proteins and other molecules that leak or are secreted at the surface of the nasal epithelium. The concentration of albumin or other plasma-derived proteins can be used to detect an acute or chronic disruption of the nasal epithelium associated with inflammation (rhinitis) or exposure to some irritants (e.g. ozone).The technique consists in an instillation at a constant flow using a peristaltic pump, 2,5 ml of saline (distilled water + NaCl 0.9%) at 37°C in each nostril while holding the head in a downward position. During 20 seconds, the fluid is recovered by returning the head in the upward position. The technique is easily applicable on children. The recovery of proteins such as CC16 and albumin in the nasal lavage sample is checked. The 16 kDA Clara cell protein, which is secreted throughout the airways and predominantly by the bronchiolar Clara cell, could be identified in NAL. CC16 is a very sensitive marker of increased airway permeability.

When comparing the amounts of albumin and CC16 measured in the nasal lavage fluid collected in children from the left and the right nostrils these appear to be correlated. Some studies have analysed the CC16 levels in NALF related to the exposure to air pollution. Decreased levels have been found in a group of epoxy workers with chronic exposure to an irritating chemical, contrary to increased levels after acute exposure to a hot and humid ozone-polluted environment in combination with physical exercise. In intermittent allergic rhinitis due to pollen allergy the levels observed on patients outside the pollen season were lower than those observed during the pollen season. An inverse relation between nasal CC16 levels, symptoms and signs of rhinitis were observed after an allergen-challenge. Another recent study measured albumin and CC16 in the NALF from 474 adolescents. The NALF CC16/albumin ratio, integrating the permeability and cellular integrity of the nasal epithelium, decreased mostly with/ according to the amount of time spent in chlorinated pools. In boys, a lower CC16/albumin ratio in NALF was associated with an increased risk of house dust mite sensitization. The results suggest that the CC16/albumin ratio in NALF can be used to detect nasal epithelium alterations linked to allergic sensitization [135].

3. OBJECTIVES

The aim of this work was to study the impact of environmental factors and lifestyle on the allergic march among children. It aims at identifying the avoidable risks involved in the increase of allergies in the « allergic march » and thereby provides a basis for implementing preventive actions.
The main objectives of our studies were:

- To further test the chlorine hypothesis postulating that early exposure to chlorinated pools may promote allergic sensitization and later the development, alone or in interactions with other risk factors
- To examine whether the early exposure to chlorinated pools might increase the risks of respiratory infections and in particular of bronchiolitis, which is a risk factor for the development of allergic diseases.
- To examine whether skin irritation cause by chlorine and its by-products may increase the risk of eczema while adjusting for the influence of other risk factors and in particular water hardness.

The protocol consisted to recruit a cohort of 5-years-old children, and to examine twice these children, at baseline when they were in third kindergarten and two-years later when they were in second primary schools. Information about the child health and the risk factors for studied outcomes was obtained via a parent self-administered questionnaire. We measured exhaled NO and we screened atopy by the Rhinostick test.

4. STUDY PROTOCOL

1. Ethical aspects

The ethics committee of the Faculty of Medicine of the Catholic University of Louvain approved the study protocol that complied with all applicable requirements of international regulations.

2. Target population

The respiratory health of children is a priority of the environmental health programs for different raisons. First, the respiratory affections are a major cause of disease for children (30 % in certain groups) and represent a real problem for Public health. Moreover, we can observe an increase of the respiratory disease seriousness.

Secondly, the premature exposure to perinatal risk factors and internal environment play a prevailing role in the development of respiratory disease. Organs and tissues, rapidly expanding, are much more sensitive to certain factors of the environment. Furthermore, because of their specific behaviours, their small weight and their wide pattern of activities, children are more exposed to certain environmental pollutants.

An early screening would allow a more adequate, more effective prevention of respiratory and allergic diseases.

3. Studied population

3.1. Recruitment

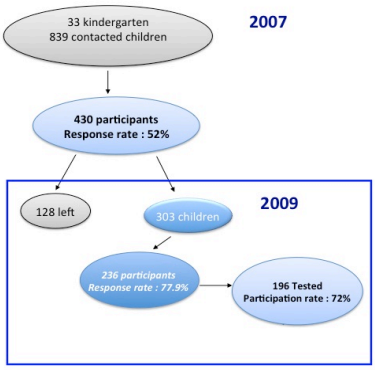
The population of the study was recruited on a volunteer basis. The children were informed through a meeting with a member of the study team in each class of the schools where the director had agreed to take part to the study. These children were recruited in the framework of a prospective study on the respiratory impact of indoor air pollution.

Figure 8: Map of Belgium with the main cities of participant schools

3.2. Group description

The first phase of the study was conducted in 2008 in 30 kindergarten schools located in the areas of Brussels, Louvain-La-Neuve, Saint Vith and Liège. A questionnaire and an informed consent document were distributed to all children in third kindergarten in these schools. Of the 839 children who received these documents, 430 returned the questionnaire with the informed consent document filled by the parents (appendix 2). The overall participation rate was 52% but showed a large variation between schools (between 20 and 90%). Since participation rate did not correlate with the prevalence of the main outcomes and risk factors, we decided to retain all schools for the statistical analysis and to add the participation rate to the list of potential confounders.

Figure 9: Population of the follow-up study



The children were then re-examined two years later by visiting schools in the same order. The baseline total population included 431 children but two years later, 128 of them had left the schools. Among remaining children, 236 (77.9%) participated to the study with the agreement of their parents and of them 196 performed successfully all the tests. we invited again the children to take part study (figure 9).

4. Protocol

4.1. Phases of the study

The study was conducted among 196 schoolchildren in 30 schools located in the areas of Brussels and Liège in Belgium. The first examination took place between December 2007 and March 2008 when children were all in the third kindergarten (mean age, 5.7 years; SD, 0.37). The children were then re-examined two years later by visiting schools in the same order between December 2009 and March 2010. The baseline total population included 431 children but two years later, 128 of them had left the schools. Among remaining children, 236 (77.9%) participated to the study with the agreement of their parents and of them 196 performed successfully all the tests.

4.2. Informed Consent

Informed consent documents (appendix n°1 and 2) with the questionnaire (appendix n°3) were distributed to children.

4.3. Examination

Children were examined in schools from 9h to 13h. Examination included the following tests and measurements:

a) Measurement of height and body weight

b) Measurement of nitric oxide (NO) in exhaled air with the NIOX analyzer (Aerocrine AB, Sweden). FeNO measurements were performed before exercise testing. Single-breath, on-line measurement of FeNO was performed in accordance with ATS10 and European Respiratory Society11 recommendations at an exhalation flow of 50 ml/s (Aerocrine NO system; Aerocrine AB; Stockholm, Sweden; and CLD 77 am chemiluminescence analyzer; Eco Physics AG; Duernten, Switzerland). The use of this equipment is well documented.

c) Spirometric tests with a Spirostar 2000 (Medriko OY, Finland). FEV1, FVC and PEF measurements were performed according to the standards of the American Thoracic Society.

d) Collection of an untimed urine sample of measurement Clara cell protein (CC16), creatinine and retinol-binding protein (to adjust for variations in the tubular reabsorption of CC16.

e) Collection of a nasal lavage (NAL) sample from the two nostrils separately. The test consists in instillating with a peristaltic pump 1 ml of physiologic water per nostril during 15 second and then to collect the saline with the dissolved protein in it after another 10 seconds. These samples will be used for the assays of albumin, CC16 and urea.

f) Screening of sensitization to the eight most prevalent aeroallergens using the Rhinostick test. As the examination of children were done in schools, screening for respiratory allergies was performed noninvasively by measuring specific immunoglobulin E in nasal mucosa using the Rhinostick test [129]. This test was successfully performed with 5 and 7 years old children, with whom the following allergens were screened for cat epithelium, *Dermatophagoides Pteronyssinus,*

Anthoxanthu modoratum, Parietaria officinalis and a mix of threes (allergens containing *Betulaodorata, Corylusavellana* and *Alnusincana)*.

Children were considered as atopic when they were sensitized to at least one of the above allergens (specific IgE>0.35 kIU/l). Children with a negative Rhinostick test but under medication for allergy, as specified in the questionnaire, were also classified as atopics.

4.4. Questionnaire

* Parents completed a detailed questionnaire inquiring about the health of their child, respiratory symptoms, family antecedents, care during early life and all lifestyle or environmental factors known or suspected to influence the risk of respiratory diseases. Questions about respiratory symptoms and allergic diseases were those of the International Study of Asthma and Allergy in Children [38;136]. Wheezing was identified as a positive answer to the question "Has your child had episodes of wheezing during the 12 last months?". The questionnaire asked parents if their child had ever been diagnosed by a physician for bronchiolitis, asthma, hay fever or allergic rhinitis and bronchiolitis. In case of a positive answer, the parents were asked to indicate the exact age when the disease was diagnosed. The questionnaire also comprised questions about sport and recreational activities. For swimming practice, parents were asked to specify the type of pool attended by their child, the type of disinfection method (even though almost all of them use chlorine), the frequency of attendance and the age when their child started to regularly attend the pool. Thanks to this information, we could calculate the cumulative pool attendance at indoor or outdoor chlorinated pools, separately or combined, before the age of two years (during infancy) or later over lifetime (during childhood).

For the second phase of the study, an additional questionnaire (appendix n°5) completed the information and inquired about both past years. Regarding sport and recreational activities organized by the schools, we asked the school directors to complete a questionnaire that allowed us to crosscheck and complete the information given by the parents.

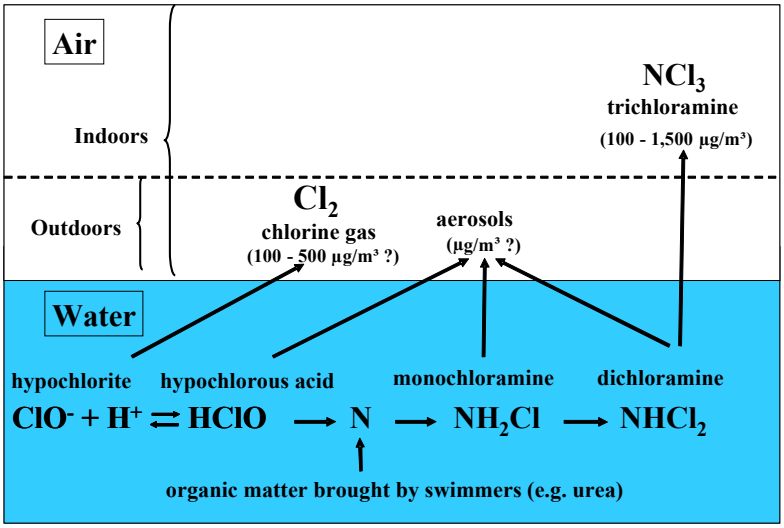
We also asked to the school directors to complete a questionnaire (Appendix n°4) inquiring about school environment and characteristics and sport activities organized in the schools.

5. Chlorinated and tap water

5.1. Swimming pools

In Belgium, if some pools use the copper/silver ionization method for sanitizing water (in Louvain-La-Neuve and in Saint Vith), most swimming pools are disinfected by using chlorine, the cheapest way. Chlorine is added in the water in the form of chlorine gas or of hypochlorite, which constitutes the free chlorine. The active chlorine is hypochlorous acid, a strong oxidant that kills bacteria and other pathogens. Hypochlorous also reacts with the organic matter brought by swimmers to release among other by-products, the chloramines. The water-soluble mono- and dichloramine form the combined chlorine. The trichloramine is by contrast a water insoluble gas that is released in pool air and gives indoor pools their typical smell. The combined chlorine and trichloramine are well-known irritants of the skin, eyes and the airways.

Figure 10: Major chlorine-based oxidants in the water or air of chlorinated recreational environments



The figure 10 shows the major chlorine-based oxidants in the water or air of chlorinated recreational environments such as swimming pools, whirlpools or hot tubes. The concentrations of these oxidants above water surface greatly fluctuate depending on a number of physicochemical variables related to the chlorine dosing, bathing load, mode of swimming, air temperature and ventilation of the hall or aerosols spraying by recreational equipments (cannons, jets and waterfalls). The most powerful and concentrated oxidants in these environments are trichloramine in the gaseous phase and the hypochlorous acid and mono-and dichloramine in the aerosols. . It was impossible of course to obtain the concentrations of chlorine and its derivatives in all swimming pools attended by the study participants. Nevertheless, the survey of air quality of indoor swimming pools has started only recently and in a few European countries only (e.g. France, Belgium, Germany and Netherlands) and mean levels of trichloramine in the air of public indoor

pools were found to vary between 300 and 500 µg/m^3 with extreme values attaining 2,000 µg/m^3 (concentrations averaged over a period of two hours and measured at a height of 1.5 m) [91;137-139].

Trichloramine gas appears thus as one of the most concentrated air pollutants to which children of industrialized countries are regularly exposed (mean concentrations of other indoor or outdoor air pollutants in Europe seldom exceed 300 µg/m^3). However, just above the water surface, pool air contains a number of other CPs such as chlorine gas and chlorinated microaerosols that also contribute to the amount of oxidants inhaled by swimmers. The only study that attempted to assess the levels of these other oxidants at the surface of swimming pools is the one from Drobnick *et al.*[140]. Studying the air at the surface of different pools, these authors obtained a mean concentration of 420 µg/m^3 for chlorine gas. This study, however, provided no evidence indicating that their measurements were specific of chlorine gas and free of interference by trichloramine or chlorinated aerosols.

Swimmers and especially competitive swimmers training intensively in indoor chlorinated pools represent one of the populations the most exposed to CPs. Swimmers are mainly exposed to CPs when actively inhaling air and aerosols floating just at the surface of the water. When the pulmonary ventilation level exceeds about 30 l/min, there is a shift from nose breathing to combined mouth and nasal breathing, allowing water-soluble gases and aerosols to by-pass the nasopharynx filter and to penetrate more deeply in the lung. An elite swimmer training intensively for 30 hours per week is exposed to these CPs 20 times more than a lifeguard working in the same pool and over a 100 times more than a recreational swimmer [141].

Among recreational swimmers, exposure to CPs probably culminates in young children who are probably also the most vulnerable to these chemicals. Because most children cannot really swim before the age of 6 or 7 years, they have indeed to attend the small pool, which is shallow, hot and more polluted than the large pool. For instance, concentrations of trichloramine in the air around the small pool are on average 50% higher than on the side of the large pool. In addition, when children play or learn to swim, they inhale and swallow more aerosols and water droplets containing hypochlorous acid and soluble chloramines. These can be carried more or less deeply into the respiratory tract depending on the size of aerosols and the respiration pattern (oral vs. nasal breathing)[141]. Because of their greater surface area-to-body mass ratio, children also absorb more water-soluble CPs through the skin in proportion to their body weight.

In Belgium, each public swimming pool is legally required to regularly check the microbial and chemical quality of water by measuring several parameters including active (0.5-1.5 ppm) and combined chlorine (<0.8 ppm) and the setting of a standard for trichloramine in pool air (<500 µg/m^3 in air sampled at 1.5 m above pool surface). In Belgium like in most countries, there are no specific regulations for privately owned swimming pools, which are disinfected according to the instructions of the chlorine supplier (active chlorine between 1 and 2 ppm).

5.2. Tap water

An additional potential source of exposure to chlorination products for children is the use of tap water, in particular for showering or bathing.

The Public Department of Wallonia, the Vivaqua Company and the Brussels Institute for Management of the Environment provided Data about water quality for years 2003 and 2007. According to the postcode of residence of the 63 municipalities studied, a value of water hardness was allocated to each child. Water hardness was expressed as mg of $CaCO_3$ L^{-1}. The free chlorine concentration in tap water from all the municipalities was below the standard for tap water in Belgium (0.2 mg/L). The concentration of THM, the major chlorination by-product in tap water did not exceed 10 µg/l.

5.3. Sample collection and statistical analysis

All the information collected by the questionnaire and all the measures made on the ground or in laboratory are encoded in a single database. The subjects are only identified by a code to guarantee the confidentiality of the results. All the statistical analyses are done by means of the statistical software Statview 5 release 5.0.1, business unites of SAS (AIRLOCK,SIEVE,AIRLOCKS), third Edition(Publishing), Cary, NC, SAS(AIRLOCK,SIEVE) Institute Inc ., on 2001.

All the data were obtained from the written or oral anthropometric, respiratory and biological questionnaires, the measures are encoded in a database from which several statistical analyses can be realized: 1) a descriptive analysis of all the data, 2) the examination of the correlations between the anthropometric, nasal lavage, urinary and respiratory parameters, 3) a comparison of the various parameters studied by the children distributed according to criterion such as the attendance of the chlorinated or non-chlorinated swimming pools, 4) a search by logistic regression of the predictors of the respiratory symptoms or the diseases including allergies, asthma, and other respiratory diseases, 5) an analysis of the dose-response relationships .All the statistical analyses were made by checking of the normality of variables and applying a logarithmic transformation to variables whose distribution deviated from the normal law. Furthermore, as certain analysed parameters show a nycthemeral variation, the hours in which medical examinations and sampling of blood and urine were made must be introduced.

5. RESULTS

The results are presented in 4 papers. Each one considers a different step of the atopic march. This study contains two phases.

1. Phase 1: Transversal study of a 5 years old children cohort

The first phase was realized when children were 5 years old. We took into account early exposure to life style and environmental factors and family history to evaluate the impact of some of those on the development of respiratory diseases.

1.1. Interactions between domestic water hardness, infant swimming and atopy in the development of childhood eczema

(Chaumont A., Voisin C., Sardella A. and Bernard A.)
published in : Environ Res. 2012 Jul;116:52-7.

1.2. Infant swimming in chlorinated pools and the risks of bronchiolitis, asthma and allergy

(Voisin C., Sardella A., Marcucci F. and Bernard A.)
published in : EurRespir J. 2010 Jul;36(1):41-7.

2. Phase 2: Prospective study of the cohort when children were 7 years old

The second phase took place when children were 7 years old. We observe evolution of the atopic status during these 2 years according to the exposure to several environmental factors. Introduction and conclusion of this part of the project are shared.

2.1. Risks of allergic sensitization and airway inflammation after chlorinated pool attendance in early life: a prospective study on young children

(Voisin C., Sardella A., Dumont X. and Bernard A.)
submitted in Int J Hygiene environ Health

2.2. Allergic sensitization and airway inflammation after early swimming

(Voisin, C., Sardella A., Bernard, A.)
Accepted in Am J Respir Crit Care Med

Phase 1: Transversal study of a 5 years old children cohort

1. Interactions between domestic water hardness, infant swimming and atopy in the development of childhood eczema

(Chaumont A, Voisin C, Sardella A and Bernard A, 2012)

Very frequent disease among young children, the atopic dermatitis is generally considered as the first clinical demonstration of the atopy and the start of the atopic march. The skin would be the first site of raising sensitization because of the epithelial barrier changes or systemic immune disorder leading to excessive Th_2 response at epithelial surface exposed to allergens. Animal studies showed in many cases that the epithelial barrier dysfunctions can be caused by a raising sensitization repeated by the skin to aeroallergens which can lead to atopic dermatitis, to systematic raising sensitization and greater risk of developing afterward an allergic rhinitis, lung inflammation or another bronchial hyperreactivity [142;143]. The risk of developing all these atopic diseases is complex. The temporal pattern described in the atopic march cannot be a simple progress of an allergic disease to another one. The development of these diseases is not only strongly influenced by the genetics but also by the environmental factors. The prevalence of atopic dermatitis could be related to the skin barrier disruption caused by environmental stressors. Among these, quality of tap or recreational water might be especially relevant. Association between hard water and atopic dermatitis prevalence has been observed in different studies among children [142;144]. The exposure to chlorine based irritants when attending swimming pool could be also responsible of skin diseases. We conducted a cross-sectional study on 358 children aged 5-6 years (54% of boys) in 30 kindergarten schools. Parents completed a questionnaire about the child's health, chlorinated pool attendance and potential confounders. Water companies provided data about tap water quality. Atopy was defined as a sensitization to at least one aeroallergen or as a medication for allergy. Multivariate logistic models assessed the effects of water hardness and infant swimming practice. In addition, the effects of these risk factors combined with atopy were evaluated using two measures of biological interaction: the attributable proportion of interaction (AP) and the synergy index (S). AP>0 and S>1 indicate biological interaction between the two risk factors.

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Interactions between domestic water hardness, infant swimming and atopy in the development of childhood eczema

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ABSTRACT

Aim: Recent studies suggest that domestic water hardness and swimming in chlorinated pools may increase the prevalence of childhood eczema. The combined influence of these two factors as well as their interaction with atopic status has not been investigated.
Methods: We conducted a cross-sectional study on 358 children aged 5–6 years (54% of boys) in 30 kindergarten schools. Parents completed a questionnaire about the child's health, chlorinated pool attendance and potential confounders. Data about tap water quality were provided by water companies. Atopy was defined as a sensitization to at least one aeroallergen or as a medication for allergy. The effect of water hardness and infant swimming practice were assessed by multivariate logistic models. In addition, the effects of these risk factors combined with atopy were evaluated using two measures of biological interaction: the attributable proportion of interaction (AP) and the synergy index (S). AP > 0 and S > 1 indicate biological interaction between the two risk factors.
Results: Water hardness was linearly associated to the prevalence of eczema whereas the relationship of eczema with infant swimming was not linear. We observed a biological interaction between hard water (> 150 mg/L $CaCO_3$ L^{-1}) and atopic status that increases the prevalence of eczema with an odds ratio (OR) of 3.30 and a 95% confidence interval (CI) of 1.34–8.15 (AP, 0.41; 95% CI 0.15–0.66 and S, 2.4; 95% CI 0.96–6.01). Infant swimming practice combined with atopy also increased the prevalence of eczema (OR, 2.72; 95% CI 1.29–5.74) although none of the interaction measures was significant. However, when water hardness and infant swimming were combined, there was no further increase of the eczema prevalence due to some form of antagonistic interaction between these two factors (AP, −0.56; 95% CI −1.12 to −0.01 and S, 0.54; 95% CI 0.33–0.87).
Conclusions: Our study shows that exposure to hard water and infant swimming interact with atopic status to increase the prevalence of childhood eczema. A breaching of the epidermal barrier by detergents or salts in hard water and by chlorine-based oxidants in swimming pool water might explain these interactions.

1. Introduction

Eczema is a chronic inflammatory disease characterized by alterations of the epidermal barrier function and an immunoglobulin E-mediated (IgE-mediated) sensitization to food and environmental allergens (Bieber, 2008). The disease develops as a result of complex interactions between various host (gender, family predisposition) and environmental factors (allergens, irritants and infectious agents). In these processes, skin barrier dysfunction appears as one of the most important components of eczema not only as a consequence of the skin inflammatory response but probably also as a factor promoting allergic sensitization and the resulting inflammatory response. The IgE-mediated sensitization often occurs several weeks or months after the first skin lesions appear, suggesting that the skin is the primary site of sensitization (Spergel and Paller Amy, 2003). The disease usually begins during infancy and frequently represents the first step of the atopic march that leads to the development of allergic rhinitis and asthma (Leung et al., 2004). The prevalence of eczema, like that of other atopic disorders, has steadily increased in industrialized countries over the last decades. Current estimates indicate that 10% to 20% of children in developed countries suffer from this condition (Williams et al., 2008). Although important, genetic factors cannot explain such a rapid rise of eczema, which can arise only from changes in our environment or lifestyle.

The hygiene hypothesis postulates that the increase of allergic diseases in most developed countries is the consequence of the declining exposure to microbial agents, especially during early life (Sherriff et al., 2002). An alternate hypothesis, which has been suggested more recently, relates the prevalence of eczema to the

Abbreviations: OR, odds ratio; CI, confidence interval; IgE, Immunoglobulin E; AP, attributable proportion of interaction; S, synergy index

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skin barrier disruption caused by environmental stressors. Of these stressors, those linked to the quality of tap or recreational water might be particularly relevant because exposure concerns the population at large and culminates precisely during infancy or early childhood. An association between water hardness and eczema prevalence has been observed in different ecological studies among schoolchildren (Arnedo-Pena et al., 2007; McNally et al., 1998; Miyake et al., 2004). Another potential causative factor linked to water is the exposure to chlorine-based irritants when attending swimming pools. Skin diseases, including eczema, are indeed more frequent in swimmers and workers exposed to swimming water (Lazarov et al., 2005; Basler et al., 2000; Font-Ribera et al., 2009; Bernard, 2007). All these studies, however, were focused on the effects of water hardness or swimming pool water taken separately and have not considered the possible interactions between these two factors, nor between these factors and the atopic status, which is the major driver of eczema risk.

We report here the results of a cross-sectional study focused on school-children in which we investigated the separate and combined effects of exposure to hard water, chlorinated pool attendance and atopy on the prevalence of eczema during childhood.

2. Material and methods

2.1. Study population

The study was conducted in 2008 on children recruited in 30 kindergarten located mainly in the areas of Brussels and Liege in Belgium. These schools belonged to two school networks who agreed to participate in the project: the school network of the municipality of Auderghem and the education network of the Liège area. All parents having children in third kindergarten in the schools of these two networks were invited to participate. A questionnaire and an informed consent document were distributed to a total of 839 children. The overall median participation rate was 51.3% and varied largely between schools (range 20–90%). Since there was no correlation between the participation rate and the prevalence of eczema, we decided to retain all schools for the statistical analysis and to include the participation rate among the potential predictors. The study protocol described in details previously (Voisin et al., 2010) was approved by the Ethics Committee of the Faculty of Medicine of Catholic University of Louvain.

Parental questionnaires were used to gather information about care during early life and all lifestyle or environmental factors susceptible to influence atopic diseases. The questionnaire included specific questions asking whether the child had ever been diagnosed by a doctor for most common childhood diseases, including eczema. For each question, the parents were asked to reply "yes" or "no". In the case of a positive reply, parents were asked to indicate the exact age at which the disease (thus eczema) was diagnosed. There were also questions about allergy medication ("Is your child under allergy medication?", "What is the name of the medication?"). The questionnaire also comprised specific questions intended to estimate the total time spent in outdoor or indoor chlorinated pools before the age of two years.

Data about water quality for years 2003 and 2007 were provided by the Public Department of Wallonia, the Vivaqua Company and the Brussels Institute for Management of the Environment. A value of water hardness was allocated to each child according to the postcode of residence of the 63 municipalities studied. Since data on water quality provided for the year 2007 were more completed, we used these data after having checked the good correlation with data of 2003 (Spearman's correlation test, $r=0.91$). When one municipality was supplied with distinct sources of water, the value for water hardness was estimated as the mean of the yearly averages of these different sources. Water hardness was expressed as mg of $CaCO_3\ L^{-1}$. The free chlorine concentration in tap water from all the municipalities was below 0.2 mg/L, which is the standard for tap water in Belgium.

Because the examination of children in schools precluded any provocation or blood test, screening for allergies was performed non-invasively by measuring specific IgE in nasal mucosa with the Rhinostick test. In comparison with the skin prick test, this test offers a similar sensitivity and a greater specificity (Marcucci et al., 2004). Among children for who water hardness data were available, the test was successfully performed in 358 children. The following allergens were screened: cat epithelium, *Dermatophagoides pteronyssinus*, *Anthoxanthum odoratum*, *Parietaria officinalis* and a mix of four allergens containing *Betula odorosa*, *Corylus avellana*, *Carpinus betulus* and *Alnus incona*. Children were considered as atopic when they were sensitized to at least one of the above allergens (specific IgE >0.35 kIU/l). Children with a negative Rhinostick test but under medication for allergy, as specified in the questionnaire, were also classified as atopics.

2.2. Statistical analysis

Multiple logistic regression analysis was used to assess the adjusted odds ratio (OR) and their 95% confidence intervals (CI) of eczema associated with water hardness and chlorinated pool attendance during infancy. Age, gender, parental eczema and/or allergy, breastfeeding, parent's education level (high, minimum one parent in possession of a bachelor educational level or higher), presence of older siblings, passive smoking, maternal smoking during pregnancy, participation rate (above 50% or not) were selected as *a priori* potential confounding factors. To study these associations, we first assessed whether or not water hardness and infant swimming practice were linearly related to the log-odds of eczema using a restricted cubic spline model (Software R 2.12.1). The null hypothesis that the log-odds of eczema was a linear function was accepted only for the water hardness but rejected for infant swimming. Water hardness was thus tested as a continuous variable. However, to exploit the wide range of water hardness of our study with values up to 420 mg/L, which is quite uncommon (WHO, 1996), we also analyzed this variable stratified in three categories of increasing values (soft: <150 mg/L $CaCO_3\ L^{-1}$, moderately hard: 150–350 mg/L $CaCO_3\ L^{-1}$and very hard: >350 mg/L $CaCO_3\ L^{-1}$). These cut-off points were selected because they corresponded to the standard water hardness categories used in Belgium. Of interest, these limits also corresponded to the 25th and 50th percentiles of water hardness distribution of our database. To examine the nonlinear relationship between infant swimming and prevalence of eczema, we modeled infant swimming by restricted cubic spline with four knots at percentiles 5%, 35%, 65%, and 95% in a logistic regression. Unadjusted trends for increasing eczema prevalence with increasing water hardness were assessed with the Cochran–Armitage trend test.

We tested potential statistical and biological interactions between atopy, water hardness and infant swimming practice on the prevalence of eczema. Statistical interactions were examined by including the appropriate product interaction term in the multivariate logistic regression. Biological interactions in the regression models were tested as departure from additivity. We use the two indices proposed by Rothman and Greenland (1998) to measure these interactions from logarithmic models: the attributable proportion of interaction (AP) and the synergy index (S). The AP estimates the attributable proportion of eczema, which is due to the interaction in children with both exposures. The S examines whether the proportion of children with eczema is significantly different from the one obtained among children exposed to the additive effect of the two risk factors taken separately. First, atopy, infant swimming and water hardness were combined two by two in three new variables with four levels summarized as follows: a level of joint exposure to both risk factors (A^+B^+), a level of exposure to one of the risk factors only (A^+B^- or A^-B^+) and a level of reference of no exposure (A^-B^-). On the basis of adjusted odds ratios obtained in the logistic regression models, we calculated the two measures of interaction on an additive scale and their corresponding confidence intervals by using an Excel spreadsheet set up by Andersson et al. (2005). There is a biological interaction when AP (95% CI) does not include 0 and S (95% CI) does not cross 1.

3. Results

The study population included 358 schoolchildren (54% of boys) with a mean age of 5.7 years (standard deviation, 0.4). Main characteristics of these children are summarized in Table 1 for the whole population and separately for children who had a doctor-diagnosed eczema ($n=94$, 26%) and those who had not. Table 1 also shows the odds ratios and their 95% confidence interval for eczema associated with the described characteristics. A significant increase of eczema prevalence was associated to atopic status, parental allergies or eczema, maternal education, domestic water hardness and cumulative pool attendance before the age 2 years. There were no significant differences between the two groups in the proportions of children sensitized to allergens, children who had been breastfed or those who had been exposed to older siblings or tobacco smoke. Although the hardness of tap water was on average higher in children with reported eczema, the proportions of children having access to hard water (>150 mg/L $CaCO_3\ L^{-1}$) did not differ between the two groups. Proportion of infant swimmers was higher in the eczema group but the hours spent in chlorinated pool by these infant swimmers were not higher. There was no association between the prevalence of atopy and the exposure to hard water or infant swimming practice. Last, the proportion of infant swimmers was lower in the soft water group than in the hard water group (31% vs 51%,), meaning that the specific influence of hard water and infant

swimming can be accurately assessed only by the mutual exclusion of these factors.

Table 2 shows the crude and adjusted odds ratio of eczema associated with water hardness and infant swimming in the whole population and separately for non-atopic and atopic children. When tested as a continuous variable, water hardness was associated with an increased eczema prevalence independently of the atopic status. However, when testing water hardness as a categorical variable, the odds for eczema was significantly increased only among atopic children exposed to very hard water (>350 mg/L $CaCO_3$ L^{-1}, OR, 3.36; 95% CI 1.02–11.1). The adjusted odds ratio for eczema associated to the upper *quartile* of infant swimming compared with lower was 1.41 (95% CI 0.98–2.04) in all children and was statistically significant among non atopic children (OR, 1.78; 95% CI 1.09–2.89).

In order to assess the specific influence of water hardness and infant swimming, we repeated the logistic analyses by mutually excluding each of these factors. The results shown in Table 3 revealed that these two factors were mutually confounding their associations with prevalence of eczema. Non-infant swimmers showed indeed a strong association between water hardness and eczema prevalence (OR, 3.76; 95% CI 1.31–10.76), which completely disappeared in their peers who had swum during infancy (OR, 0.76; 95% CI, 0.27–2.14). Similarly, in children using soft water, infant swimming and prevalence of eczema showed a tendency to be associated (OR, 2.13; 95% CI 0.98–4.64), which was not seen in those having access to moderately or very hard water (OR, 1.26; 95% CI 0.79–2.02).

Given the above influence of water hardness, infant swimming and atopy on the prevalence of eczema, we further assess the possible interactions between these three factors. When introducing the interactive terms in the logistic regression models, there was no evidence of statistical interaction (OR (atopy *x* hard water), 1.38; 95% CI 0.43–4.36, OR (atopy *x* infant swimming), 0.86; 95% CI, 0.31–2.36, OR (infant swimming *x* hard water), 0.40; 95% CI 0.12–1.33). By contrast a series of biological interactions as a departure from additivity were found by calculating the AP and S interaction measures. Table 4 shows the odds ratios for each factor tested separately and in combination with each other. The interaction measures calculated on the basis of these odds showed a significant synergistic interaction between atopy and water hardness (AP, 0.41; 95% CI 0.15–0.66 and S, 2.40; 95% CI 0.96–6.01). Although the odds for eczema associated to infant swimming combined with atopy was higher than the odds for each factor alone, the additive interaction measures were not statistically significant (AP, 0.04; 95% CI −0.34 to 0.42 and S, 1.07; 95% CI 0.57 to 2.0). The same analysis revealed a significant antagonistic effect between hard water and infant swimming practice on eczema (AP, −0.56; 95% CI −1.12 to −0.01 and S, 0.54; 95% CI 0.33–0.87). Indeed, children with both exposures reported a prevalence of eczema that was lower than the additive effect of each risk factor.

4. Discussion

While confirming the increased prevalence of childhood eczema associated with exposure to hard water and chlorinated pools, our study shows that this increase in prevalence largely stems from their biological interactions with the atopic status. Both infant swimming and exposure to hard water were indeed significantly associated with prevalence of eczema only when combined with atopic status. Our study also shows that by interacting with each other, hard water exposure and infant swimming mutually weaken their association with eczema, resulting in a complex pattern of confounding effects. Clearly, the failure to consider all these interactive effects may lead to an underestimation of the prevalences of eczema associated with hard water or infant swimming.

Interactions reported in epidemiological studies should always be interpreted with caution. We are, however, rather confident that the interactions found in this study arise from the biological interactions between the exposure to skin irritating substances and the genetic

Table 1
Characteristics of study participants according to eczema and associations of covariates with the prevalence eczema.

	All ($n=358$)	Eczema		OR for eczema (95% CI) [b]
		No ($n=264$)	Yes ($n=94$)	
Age, mean (SD [a])	5.7 (0.4)	5.7 (0.4)	5.7 (0.4)	0.86 (0.47–1.59)
Boys, *n* (%)	294 (54.2)	139 (52.7)	55 (58.5)	1.27 (0.79–2.04)
Body mass index, mean (SD [a])	20.7 (3.2)	20.7 (3.2)	20.8 (3.2)	1.01 (0.94–1.09)
Mother and/or father with eczema, *n* (%)	63 (17.6)	38 (14.4)	25 (26.6)	2.16 (1.22–3.82)
Parents with allergies or eczema, *n* (%)	164 (45.8)	109 (41.3)	55 (58.5)	2.01 (1.24–3.23)
Older sibling ≥ 1, *n* (%)	203 (56.7)	154 (58.3)	49 (52.1)	0.78 (0.49–1.25)
Parental education level (high, bachelor level)				
Maternal education, *n* (%)	240 (67.0)	168 (63.6)	72 (76.6)	1.87 (1.09–3.21)
Paternal education, *n* (%)	218 (60.9)	160 (60.6)	58 (61.7)	1.05 (0.65–1.70)
Passive smoker at home, *n* (%)	114 (31.8)	90 (34.1)	24 (25.5)	0.66 (0.39–1.13)
Maternal smoking during pregnancy, *n* (%)	46 (12.8)	32 (12.1)	14 (14.9)	1.27 (0.64–2.50)
Breastfeeding, *n* (%)	279 (77.9)	206 (78.0)	73 (77.7)	0.98 (0.56–1.72)
Domestic water hardness,				
Median (IQR [a]), mg/L $CaCO_3$ L^{-1}	34.5 (9.1–36.2)	34.5 (9.1–36.2)	36.2 (19.3–36.5)	1.02 (1.00–1.04)
Water hardness >150 mg/L $CaCO_3$ L^{-1}, *n* (%)	265 (74.0)	190 (72.0)	75 (79.8)	1.54 (0.87–2.72)
Cumulative pool attendance before 2 years,				
Median (IQR [a]), hours	24.0 (10.8–59.0)	23.9 (8.8–48.4)	27.2 (13.0–69.0)	1.00 (1.00–1.01)
Infant swimmers, *n* (%)	164 (45.8)	116 (42.4)	52 (55.3)	1.68 (1.05–2.70)
Atopic status,				
At least 1 aeroallergen >0.35 kIU/L, *n* (%)	86 (24.0)	60 (22.7)	26 (27.7)	1.30 (0.76–2.22)
Current Medication for allergy, *n* (%)	59 (16.5)	34 (12.9)	25 (26.6)	2.45 (1.37–4.39)
Sensitization to at least 1 aeroallergen or medication for allergy, *n* (%)	121 (33.8)	80 (30.3)	41 (43.6)	1.78 (1.10–2.89)
Doctor-diagnosed eczema, *n* (%)	94 (26.3)	/	/	/

[a] IQR: interquartile range; SD: standard deviation.
[b] OR, odds ratio; 95% CI, 95% confidence interval.

Table 2
Prevalences and odds ratio for eczema associated with water hardness and infant swimming practice according to atopic status.

	All children				Non atopic				Atopic			
	N	(%)	Crude OR (95% CI) [a]	Adjusted OR (95% IC) [b]	*N*	(%)	Crude OR (95% CI) [a]	Adjusted OR (95% IC)[b]	*N*	(%)	Crude OR (95% CI) [a]	Adjusted OR (95% IC) [b]
Water hardness (mg/L $CaCO_3$ L^{-1})												
continuous	358	26.3	1.02 (1.00 –1.04)	1.02 (1.00–1.05)	237	22.4	1.02 (0.99–1.05)	1.03 (1.00–1.06)	121	33.9	1.03 (1.00–1.06)	1.04 (1.00–1.07)
< 150	93	20.4	1.00	1.00	55	18.2	1.00	1.00	38	23.7	1.00	1.00
150–350	96	25.0	1.30 (0.66 –2.57)	1.39 (0.67–2.88)	65	21.5	1.24 (0.5–3.05)	1.15 (0.44–3.02)	31	32.3	1.53 (0.53–4.44)	2.05 (0.63–6.65)
> 350	169	30.2	1.68 (0.92 –3.07)	1.97 (0.97–3.96)	117	24.8	1.48 (0.66–3.31)	1.77 (0.70–4.43)	52	42.3	2.36 (0.93–5.98)	3.36 (1.02–11.1)
P for trend	–	0.08	–	–	0.32	–	–	0.06	–	–	–	–
Infant swimming												
RCS [c]	358	26.3	1.51 (1.06–2.16)	1.41 (0.98–2.04)	237	22.4	1.96 (1.24–3.10)	1.78 (1.09–2.89)	121	33.9	1.05 (0.50–2.18)	0.94 (0.42–2.10)

[a] OR, Odds ratio; 95% CI, 95% confidence interval.
[b] Adjustment for age, gender, parental eczema and/or allergy, breastfeeding, parent's education level, presence of older siblings, passive smoking, maternal smoking during pregnancy and participation rate.
[c] The OR were estimated for the upper quartile by using restricted cubic spline (RCS) with the lowest quartile as the reference group.

Table 3
Prevalences and odds ratios for eczema associated with water hardness and infant swimming practice after stratification for these two risk factors.

Water hardness (mg/L $CaCO_3$ L^{-1})	Non infant swimmers				Infant swimmers			
	N	(%)	Crude OR (95% IC)[a]	Adjusted OR (95% IC)[b]	*N*	(%)	Crude OR (95% IC) [a]	Adjusted OR (95% IC)[b]
continuous	194	21.6	1.02 (1.00–1.05)	1.04 (1.01–1.08)	164	31.7	1.02 (0.99–1.05)	1.03 (1.00–1.06)
< 150	64	14.1	1.00	1.00	29	34.5	1.00	1.00
150–350	51	27.5	2.31 (0.91–5.89)	3.62 (1.26–10.4)	45	22.2	0.53 (0.19–1.54)	0.45 (0.15–1.38)
> 350	79	24.1	1.94 (0.81–4.64)	3.76 (1.31–10.76)	90	35.6	1.05 (0.44–2.53)	0.76 (0.27–2.14)
P for trend			0.17				0.56	
Infant swimming	Soft water (< 150 mg/L $CaCO_3$ L^{-1})				Hard water (> 150 mg/L $CaCO_3$ L^{-1})			
	N	%	Crude OR (95% IC)[a]	Adjusted OR (95% IC)[b]	*N*	Prevalence	Crude OR (95% IC)[a]	Adjusted OR (95% IC)[b]
RCS [c]	93	20.4	2.01 (0.98–4.09)	2.13 (0.98–4.64)	265	28.3%	1.40 (0.90–2.18)	1.26 (0.79–2.02)

[a] OR, Odds ratio; 95% CI, 95% confidence interval.
[b] Adjustment for age, gender, parental eczema and/or allergy, breastfeeding, parent's education level, presence of older siblings, passive smoking, maternal smoking during pregnancy and participation rate.
[c] The OR were estimated for the upper quartile by using restricted cubic spline (RCS) with the lowest quartile as the reference group.

Table 4
Biological interactions between atopy, water hardness and infant swimming.

Interaction categories	*N*	Prevalence (%)	Crude OR (95% CI)[a]	Adjusted OR (95% IC)[b]	AP (95% IC)[a]	S (95% IC)[a]
Interaction atopy + hard water [c]						
No atopy + soft water	55	18.2	1.00	1.00	0.41	2.40
Atopy	38	23.7	1.40 (0.51–3.85)	1.37 (0.48–3.92)	(0.15 to 0.66)	(0.96 to 6.01)
Hard water	182	23.6	1.39 (0.65–2.99)	1.59 (0.69–3.64)	–	–
Atopy + hard water	83	38.6	2.82 (1.25–6.38)	3.30 (1.34–8.15)	–	–
Interaction atopy + infant swimming						
No atopy + no infant swimming	126	17.5	1.00	1.00	0.04	1.07
Atopy	68	29.4	1.97 (0.98–3.95)	1.89 (0.93–3.86)	(−0.34 to 0.42)	(0.57 to 2.00)
Infant swimming	111	27.9	1.83 (0.99–3.40)	1.67 (0.88–3.19)	–	–
Atopy + infant swimming	53	39.6	3.10 (1.51–6.36)	2.72 (1.29–5.74)	–	–
Interaction hard water + infant swimming [c]						
Soft water + no infant swimming	64	14.1	1.00	1.00	–0.56	0.54
Hard water	130	25.4	2.08 (0.93–4.66)	2.36 (0.98–5.68)	(–1.12 to –0.01)	(0.33 to 0.87)
Infant swimming	29	34.5	3.22(1.14–9.12)	2.95 (1.01–8.58)	–	–
Hard water + infant swimming	135	31.1	2.76 (1.25–6.10)	2.82 (1.19–6.70)	–	

[a] OR, Odds ratio; 95% CI, 95% confidence interval; AP, attributable proportion of interaction; S, synergy index.
[b] Adjustment for age, parental education level, parental allergy and/or eczema, maternal smoking during pregnancy, presence of older siblings, gender, breastfeeding, participation rate and passive smoker.
[c] Soft water < 150 mg/L $CaCO_3$ L^{-1}; hard water > 150 mg/L $CaCO_3$ L^{-1}.

predisposition to atopic diseases. Although it is known since long that hard water can irritate the skin, the involved mechanisms are still unclear. The explanations commonly forwarded are the deposition of calcium salts on the skin and the use of larger amounts soap and shampooing to lather the water (Warren et al., 1996; White et al., 1987; Ananthapadmanabhan et al., 2004). Chlorine used to disinfect

swimming pool water and its by-products such as chloramines are potent oxidants that can also cause skin irritation (Lazarov et al., 2005; Basler et al., 2000; Font-Ribera et al., 2009; Bernard, 2007). For instance, Seki et al. (2003) reported that the water holding capacity of the *stratum corneum* decreased after immersion in chlorinated water. Gardinier et al. (2009) have shown that a single swimming session leads to a substantial removal of the sebum from the skin surface. This degreasing action of pool water increases skin pH from acidic to neutral pH, which in turn can alter the skin homeostasis and permeability (Mauro et al., 1998). Furthermore, water *per se* is a well-known skin irritant that can disrupt skin barriers as revealed by an increased transepidermal water loss (Ramsing and Agner, 1997; Tsai and Maibach, 1999). All these chemical stressors might quite conceivably increase the permeability of skin epithelial barriers and thereby facilitate the penetration of allergens and the development of eczema (Löffler et al., 2003; Boralevi et al., 2008; Mattila et al., 2011). This hyperpermeability mechanism triggered by skin irritation would be basically the same as that proposed for the increased risks of asthma and respiratory allergies observed among atopic swimmers regularly attending chlorinated swimming pools (Bernard, 2007).

Our study suggests that atopic subjects might benefit from washing or bathing with soft water or from swimming in non-chlorinated pools. At this stage, however, it is unknown whether avoidance of irritants in tap or swimming water could be beneficial for preventing childhood eczema or for alleviating eczema skin symptoms. With regard to hard water, a recent trial testing the effect of water softeners on eczema severity in children, showed no additional benefit after the installation of an ion-exchange water softener (Thomas et al., 2011). If these findings are confirmed, this would suggest that the association between eczema and water hardness reported by us and other studies (Arnedo-Pena et al., 2007; McNally et al., 1998; Miyake et al., 2004) will be related more to the initiation of eczema and in particular to the early steps of the skin sensitization. The same reasoning could be held for pool chlorine, which might also act more as an adjuvant of skin sensitization than as an irritant exacerbating the symptoms of eczema.

Our study presents several limitations. The lack of a detailed characterization of the cumulative exposure to hard water and swimming pool water is the main limitation of this study. For the exposure to hard water, we had a good measure of the hardness of tap water supplied to each participant but we had no estimate of the contact time while for infant swimming we had just the opposite information. It looks to us unlikely that this lack of information could have distorted our analyses. Skin contact with tap water during infancy is indeed mainly related to washing or bathing, i.e., activities that should not greatly vary between infants. As to the irritating potential of chlorinated pools, it is probably mainly determined by the concentrations of active and combined chlorine, which in public pools at least, are maintained within a narrow range of concentrations to comply with the legislation. Moreover, our data on time spent in chlorinated pool might be subjected to recall bias and could lead to a potential exposure misclassification bias. The association, however, also emerges when considering ever swimming, for which memory bias is less likely. Another limitation is the relatively low participation rate, which was almost unavoidable given the very young age of participants and the fact that our study required the application of medical tests in schools. The participation, however, was unrelated to the outcome and the main risk factors, making participation biases rather improbable.

In conclusion, our study shows that exposure to hard water and, to a lesser extent, infant swimming practice, interact with atopy to increase the prevalence of childhood eczema. A breaching of the epidermal barrier by detergents or salts in hard water and by chlorine-based oxidants in swimming pool might explain these interactions.

Conflict of interest

None of the authors has a conflict of interest to disclose.

Acknowledgments

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Interactions between domestic water hardness, infant swimming and atopy in the development of childhood eczema-What to remember?

1. Eczema is a chronic inflammatory disease characterized by alterations of the epidermal barrier function and an immunoglobulin E-mediated (IgE-mediated) sensitization to food and environmental allergens
2. Skin barrier dysfunction appears as one of the most important components of eczema not only as a consequence of the skin inflammatory response but probably also as a factor promoting allergic sensitization and the resulting inflammatory response.
3. Prevalence of eczema could be related to skin barrier disruption caused by environmental stressors like tap (hardness) or recreational (chlorinated) water
4. Our results confirm the increased prevalence of childhood eczema associated with exposure to hard water and chlorinated pools.
5. This prevalence increase mainly comes from their biological interactions of the substances with the atopic status.
6. By interacting with each other, hard water exposure and infant swimming mutually weaken their association with eczema.
7. The involved mechanisms are unclear. The most common explanation is the deposition of calcium salts on the skin and the use of larger amounts of soap and shampoo to lather the water. Chlorine used to disinfect swimming pool water and its by-products are potent oxidants that can also cause skin irritation.

2. Infant swimming in chlorinated pools and the risks of bronchiolitis, asthma and allergy

(Voisin C., Sardella A., Marcucci F. and Bernard A.)

The bronchiolitis is a viral infection of the low respiratory tracts characterized by a major obstruction of lower airways accompanied with wheezing. The RSV infects almost 70 % of children during their first year. Responsible of most of bronchiolitis cases, the cytopathogenic effect of the RSV leads to an epithelial necrosis with desquamations of the epithelium, at the origin of the mucociliary device destruction, reducing ciliary clearance. RSV bronchiolitis and asthma have many similarities. Disease affecting 1 on 4 children, the bronchiolitis is the most frequent low respiratory tracts infection amongst children. Evidence has shown that wheezing episodes early in life with the common cold, RSV is a major risk for later diagnosis of asthma at 6 years old. Several factors may increase the frequency or severity of bronchiolitis. Among them: being male, low gestational age, young mother, infant condition at birth (Apgare score), lack of breastfeeding, congenital heart or lung diseases and a family history of atopic diseases. Bronchiolitis is also more frequent among infants living with older siblings, attending a day-care centre or exposed to environmental tobacco smoke. Chlorine compounds used to disinfect swimming pools may also cause airway changes making the lungs more sensitive to infection and asthma inducing agents.

We hypothesised that the infant swimming practice could be associated with a higher risk of bronchiolitis. We assessed the influence of chlorinated pool attendance on bronchiolitis risk and its further consequences. A total of 430 children around 5 years old in 30 kindergartens were examined while parents completed a questionnaire on the child's health history, swimming practice and potential confounders.

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Infant swimming in chlorinated pools and the risks of bronchiolitis, asthma and allergy

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ABSTRACT: Recent studies suggest that swimming in chlorinated pools during infancy may increase the risks of lower respiratory tract infection. The aim of the present study was to assess the influence of swimming in chlorinated pools on the risks of bronchiolitis and its late consequences.

A total of 430 children (47% female; mean age 5.7 yrs) in 30 kindergartens were examined. Parents completed a questionnaire regarding the child's health history, swimming practice and potential confounders.

Attendance at indoor or outdoor chlorinated pools ever before the age of 2 yrs was associated with an increased risk of bronchiolitis (odds ratio 1.68; 95% confidence interval 1.08–2.68; p=0.03), which was exposure-dependent for both types of pool (p-value for trend <0.01). Associations persisted, and were even strengthened, by the exclusion of other risk factors. Among children with no parental antecedents of atopic disease or no day-care attendance, odds ratios for bronchiolitis amounted to 4.45 (1.82–10.9; p=0.001) and 4.44 (1.88–10.5; p=0.007) after >20 h spent in chlorinated pools during infancy. Infant swimmers who developed bronchiolitis also showed higher risks of asthma and respiratory allergies later in childhood.

Swimming pool attendance during infancy is associated with a higher risk of bronchiolitis, with ensuing increased risks of asthma and allergic sensitisation.

KEYWORDS: Bronchiolitis, infant swimming, respiratory infection, swimming pool

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Bronchiolitis is an acute infection of the small airways that primarily affects young infants, most often those aged 2–24 months. The main causative agent is respiratory syncytial virus (RSV), although other viruses may sometimes be involved. The disease occurs with a seasonal pattern, peaking during winter in temperate climates and the rainy season in tropical climates [1, 2]. The disease burden of bronchiolitis is substantial and seems to have increased in most developed countries since the 1960s–1970s [3]. In the USA and Europe, the annual incidence of bronchiolitis during the first year of life is estimated to be 10–20%, but incidences as high as 30% have been reported in some urban areas [3, 4]. Since bronchiolitis increases the risk of childhood asthma [5, 6], it also contributes to the rising incidence of chronic respiratory diseases in children.

Factors that may increase the frequency or severity of bronchiolitis are male sex, low gestational age, young or unmarried mothers, infant condition at birth (Apgar score), lack of breastfeeding, early weaning, congenital heart or lung diseases, and a family history of atopic diseases. The risk of bronchiolitis is also higher for infants living with older siblings, attending a day-care centre or exposed to environmental tobacco smoke [2, 7]. Since ambient air pollution appears to play, if any, a marginal role [8], factors driving the rise of the disease in industrialised countries remain largely unknown.

Infant swimming is a practice that has been greatly popularised in most industrialised countries. NYSTAD and co-workers [9, 10] were among the first to draw attention to the risks that this practice may pose to the airways of infants. These authors noted that infants of atopic parents were more likely to develop wheezing or lower respiratory tract infections when they were attending swimming pools. In a cross-sectional study of schoolchildren aged 10–13 yrs, it was also found that infant swimmers were more likely to develop asthma and recurrent bronchitis than their peers who had never attended a chlorinated pool before the age of 2 yrs [11, 12]. Interestingly, this poorer respiratory health of infant swimmers was associated with lower serum levels of Clara cell protein (CC16), a protein protecting from inflammation in acute RSV infection [13]. These findings led to the postulate that the chlorine used to disinfect

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swimming pools can cause airway changes making the lungs more sensitive to infection- and asthma-inducing agents [12]. A Swedish study also described decreased levels of serum CC16 in children who swam during infancy compared to those who did not [14], providing further evidence that terminal airways and, in particular, Clara cells may be damaged during infant swimming.

Given these associations between infant swimming, serum CC16 and RSV infection, it was hypothesised that the infant swimming practice should be associated with a higher risk of bronchiolitis. In order to test this hypothesis, the associations between infant swimming, bronchiolitis and its sequelae were explored in a cross-sectional study focused on young school-children. Since both outdoor and indoor pools may affect the airways of swimmers [15], the present study was not limited to baby swimming lessons in indoor pools, but considered all kinds of swimming activity during infancy, regardless of the type of chlorinated pool and the conditions of attendance.

METHODS

Study population

The study participants were 5–6-yr-old children from 30 schools located mainly in the areas of Brussels and Liège (Belgium). These children were recruited in the framework of a prospective study on the respiratory impact of air pollution. A questionnaire and an informed consent document were distributed to children in the third year of kindergarten. Of the 839 children who received these documents, 430 returned the questionnaire with the informed consent document filled in by the parents. The overall participation rate was 51.3%, but showed great variation between schools (20–90%). Since the participation rate did not correlate with the prevalence of the main outcomes and risk factors, it was decided to retain all schools for the statistical analysis and to add the participation rate to the list of potential confounders. The ethics committee of the Faculty of Medicine of the Catholic University of Louvain (Louvain, Belgium) approved the study protocol, which complied with all applicable requirements of the international regulations.

Protocol

Parents completed a detailed questionnaire inquiring about the health of their child, respiratory symptoms, family antecedents, care during early life and all lifestyle or environmental factors known or suspected to influence the risk of bronchiolitis. Questions regarding respiratory symptoms were those of the International Study of Asthma and Allergies in Childhood [16]. Wheezing was identified by a positive answer to the question ''Has your child had episodes of wheezing during the last 12 months?''. The questionnaire included specific questions asking whether the child had ever been diagnosed for most common childhood diseases. Among these, there were seven distinct questions about the following respiratory diseases: asthma, bronchitis, bronchiolitis, pneumonia (or bronchopneumonia), hay fever, allergic rhinitis, and sinusitis. For each question, the parents were asked to reply ''yes'' or ''no''. In the case of a positive reply, parents were then asked to indicate the exact age at which the disease was diagnosed. The questionnaire also comprised questions about sport and recreational activities. For swimming practice, parents were asked to specify the type of pool attended by their child, the type of disinfection method used (even though almost all of them use chlorine), the frequency of attendance and the age at which their child started to attend the pool regularly. This information served to calculate the cumulative pool attendance (CPA) at indoor or outdoor chlorinated pools, separately or combined, before the age of 2 yrs (during infancy) or later in life (during childhood). Children were examined in schools during 09:00–16:00 h. Height and body weight were measured. Since the examination of children in schools precluded any provocation test, screening for respiratory allergies in school was performed noninvasively by measuring specific immunoglobulin E in nasal mucosa using the Rhinostick test (made by F. Marcucci) [17]. This test was successfully performed in 372 children, in whom the following allergens were screened for: cat epithelium, *Dermatophagoides pteronyssinus*, *Anthoxanthum odoratum*, *Parietaria officinalis* and a mix of tree allergens containing *Betula odorata*, *Corylus avellana*, *Carpinus betulus* and *Alnus incana*. Regarding sport and recreational activities organised by the schools, the school directors were asked to complete a questionnaire that permitted the information given by the parents to be cross-checked and completed.

Swimming pools

In Belgium, every public swimming pool is legally required to regularly check the microbial and chemical quality of the water by measuring several parameters, including active (0.5–1.5 ppm) and combined chlorine (<2 ppm). In 2003, the legislation was strengthened by a lowering of the standard for combined chlorine (<0.8 ppm) and the setting of a standard for trichloramine in pool air (<500 $\mu g \cdot m^{-3}$ in air sampled 1.5 m above the pool surface). Since there were no public outdoor pools in the areas studied, the open-air swimming pools attended by infant swimmers were mostly residential. In Belgium, as in most countries, there are no specific regulations for these privately owned swimming pools, which are disinfected according to the instructions of the chlorine supplier (active chlorine 1–2 ppm).

Statistical analysis

Continuous variables were described using the median with interquartile range. Differences between infant swimmers and their controls were assessed using the two-sided Mann–Whitney U-test for the CPA indices and an unpaired t-test for the other variables. Binary variables were compared using the Chi-squared test, or with a Chi-squared test for trend for the analysis of exposure–response relationships. Logistic regression models were used to assess the associations between swimming and bronchiolitis while adjusting for potential confounders. CPA at outdoor or indoor pools before the age of 2 yrs was categorised as never, >0–20 h and >20 h. Crude and adjusted odds ratios (ORs) for the outcomes were calculated using as reference level the occurrence of the outcome among children who never attended an indoor or outdoor chlorinated pool before the age of 2 yrs. A backward approach was used, including all potential control variables and removing the least significant predictor until the model only contained variables with a p-value of <0.20. A total of 24 potential predictors were tested, including, among others, age, sex, parental asthma and/or respiratory allergies (hay fever or allergic rhinitis), high parental educational level (father and/or

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TABLE 1 Characteristics of children who swam during infancy and their controls

	Swimming infants	Controls	p-value
Subjects n	195	235	
Males	105 (53.9)	124 (52.8)	0.82
School participation rate %	54.9±19.4	58. 3±23.0	0.19
Age yrs	5.60±0.38	5.68±0.38	0.79
BMI kg·m^{-2}	20.9±3.8	20.6±3.5	0.39
Parents			
Higher educational level	154 (79.0)	156 (66.4)	0.004
Asthma	38 (19.5)	25 (10.6)	0.01
Asthma and/or respiratory allergies	86 (44.1)	88 (37.4)	0.16
Early life			
Birthweight g	3206±639	3436±2396	0.30
Breastfeeding	161 (82.6)	169 (71.9)	0.01
Day-care centre attendance	127 (65.1)	102 (43.4)	<0.001
Exposure to tobacco smoke			
During pregnancy	31 (16.0)	32 (13.6)	0.51
Parental smoking at home	55 (28.2)	74 (31.5)	0.46
CPA before age of 2 yrs h			
Indoor	6 (0–21)		
Outdoor	8 (0–31)		
Total	22 (8–50)		
CPA over lifetime h			
Indoor	53 (17–129)	18 (0–48)	<0.001
Outdoor	50 (4–146)	0 (0–28)	<0.001
Total	132 (52–202)	35 (5–91)	<0.001
Environment and lifestyle			
Number of older siblings	0.87±0.79	0.92±1.12	0.66
House cleaning with bleach	43 (22.1)	66 (28.1)	0.15
Living <100 m from a busy road	63 (32.3)	79 (33.6)	0.78
Exposure to pets since birth	85 (43.6)	99 (42.1)	0.76
Aeroallergen-specific nasal IgE[#]			
House dust mite	21 (12.2)	22 (11.0)	0.71
Cat	15 (8.7)	14 (7.0)	0.54
Pollen	27 (15.7)	27 (13.5)	0.55
At least one aeroallergen	42 (24.4)	48 (24.0)	0.92
Respiratory symptoms and diseases[¶]			
Wheezing[+]	36 (18.5)	40 (17.0)	0.70
Asthma	16 (8.2)	15 (6.4)	0.47
Hay fever	17 (8.7)	27 (11.5)	0.35
Allergic rhinitis	27 (13.9)	25 (10.6)	0.31
Bronchiolitis	71 (36.4)	56 (23.8)	0.004
Bronchitis	93 (47.7)	111 (47.2)	0.92
Sinusitis	28 (14.4)	30 (12.8)	0.63
Pneumonia	20 (10.3)	28 (11.9)	0.59

Data are presented as mean±SD, n (%) or median (interquartile range) unless otherwise indicated. BMI: body mass index; CPA: cumulative pool attendance; Ig: immunoglobulin. [#]: 172 children were tested in the swimming infant group and 200 in the other group; [¶]: defined as doctor-diagnosed diseases at any time; [+]: episodes of wheezing during the last 12 months.

mother graduate of university or high school), maternal smoking during pregnancy, parental smoking at home, season of birth, birthweight, number of older siblings, house cleaning with bleach, breastfeeding, day-care centre attendance, area of current residence (Liège *versus* Brussels area), having spent infancy in a urban or rural area, living in the vicinity of a polluting industry or within a distance of 100 m of a busy road, and cumulative time spent in indoor or outdoor chlorinated swimming pools before the age of 2 yrs, separately or in combination (CPA). A classification of CPA in tertiles was also used. For outcomes other than bronchiolitis, CPA at outdoor or indoor swimming pools after the age of 2 yrs was added to the potential predictors. Statistical analyses were performed using SAS version 9.1.3 (SAS, Cary, NC, USA).

RESULTS

Table 1 compares the characteristics of infant swimmers in indoor or outdoor chlorinated pools (n=195) and children who never swam before the age of 2 yrs (n=235). On average, infant swimmers had spent 6 h in indoor and 8 h in outdoor chlorinated pools before the age of 2 yrs. These infant swimmers also showed a greater lifetime CPA at indoor or outdoor chlorinated swimming pools (total indoor and outdoor CPA 132 *versus* 35 h in other children) (table 1). There were no significant differences between the two groups regarding most risk factors of respiratory disease, including sex, age, body mass index, birthweight, exposure to tobacco smoke and parental allergies. The social class or socioeconomic status, as evaluated on the basis of parental education, and the proportions of children with parental asthma, who had been breastfed or attended a day-care centre were higher in the group of swimmers than in non-swimmers. The two groups did not differ with respect to the prevalences of wheezing and ever-diagnosed asthma, respiratory allergies, bronchitis, sinusitis and pneumonia. The prevalence of bronchiolitis was, however, significantly greater in infant swimmers than in their controls (36.4 *versus* 23.8%; p=0.004).

The logistic regression analysis confirmed the association between infant swimming and the risk of bronchiolitis (OR 1.68; 95% confidence interval (CI) 1.08–2.68; p=0.03). Other risk factors of bronchiolitis identified included male sex (OR 1.65; 95% CI 1.05–2.59; p=0.03), parental asthma and/or respiratory allergies (OR 1.73; 95% CI 1.20–2.74; p=0.02), number of older siblings (OR 1.69; 95% CI 1.07–2.67; p=0.02), maternal smoking during pregnancy (OR 1.80; 95% CI 1.01–3.37; p=0.05) and day-care centre attendance (OR 1.87; 95% CI 1.17–3.00; p=0.009). Breastfeeding, by contrast, was associated with a protective effect towards bronchiolitis (OR 0.58; 95% CI 0.34–0.97; p=0.04). The parental educational level, a surrogate of socioeconomic status, the use of bleach for house cleaning or parental smoking at home did not emerge as significant predictors of bronchiolitis (p>0.20 for all).

The analysis was pursued by examining whether or not the risk of bronchiolitis was different when attending indoor or outdoor chlorinated pools. As shown in table 2, the OR for bronchiolitis increased dose-dependently with CPA at both types of swimming pool considered separately (p-value for trend <0.05 for both). Stratification of infant swimmers into tertiles of CPA at indoor or outdoor chlorinated pools led to similar patterns of significant increases (p-value for trend 0.009 for indoor and 0.03 for outdoor pools; data not shown). The same pattern of exposure-related increases was found when

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TABLE 2 Risk of bronchiolitis according to cumulative pool attendance (CPA) at indoor or outdoor chlorinated swimming pools during infancy[#]

CPA category	CPA h	Subjects n/N (%)	OR (95% CI)		p-value	p_{trend}
			Unadjusted	Adjusted		
Indoor pool total[¶]						
0 h	0	76/287 (26.5)	1.0 (1.0–1.0)	1.0 (1.0–1.0)		
>0-20 h	6 (2–12)	27/92 (29.4)	1.15 (0.69–1.94)	0.79 (0.44–1.42)	0.44	
>20 h	32 (25–45)	24/51 (47.1)	2.47 (1.34–4.54)	2.02 (1.01–4.02)	0.05	0.006
Outdoor pool total[¶]						
0 h	0	79/308 (25.7)	1.0 (1.0–1.0)	1.0 (1.0–1.0)		
>0-20 h	9 (5–14)	21/54 (38.9)	1.85 (1.01–3.38)	1.91 (0.99–3.68)	0.05	
>20 h	47 (30–84)	27/68 (39.7)	1.91 (1.1–3.3)	1.59 (0.85–2.98)	0.14	0.006
Indoor pool alone						
0 h	0	53/235 (22.6)	1.0 (1.0–1.0)	1.0 (1.0–1.0)		
>0-20 h	6 (2–11)	11/50 (22.0)	0.9 (0.43–1.88)	0.58 (0.25–1.32)	0.19	
>20 h	36 (24–66)	12/23 (52.2)	3.49 (1.46–8.34)	3.49 (1.30–9.34)	0.01	0.01
Outdoor pool alone						
0 h	0	59/235 (25.1)	1.0 (1.0–1.0)	1.0 (1.0–1.0)		
>0-20 h	9 (4–12)	9/27 (33.3)	1.6 (0.68–3.76)	1.69 (0.66–4.3)	0.27	
>20 h	50 (39–76)	11/25 (44.0)	2.51 (1.08–5.85	2.08 (0.81–5.34)	0.13	0.03

CPA is presented as median (interquartile range). Odds ratios (ORs) were adjusted for sex, breastfeeding, day-care centre attendance, number of older siblings, parental asthma and/or respiratory allergies, maternal smoking during pregnancy, and, when the analysis was performed on all children, attendance of the other type of swimming. CI: confidence interval; N: number of subjects in group; p_{trend}: p-value for trend. [#]: before the age of 2 yrs; [¶]: all children.

the analysis was based on children attending only indoor or only outdoor pools to the exclusion of the other type of pool.

It was also ascertained that these associations were not confounded by differences in participation rate between schools or by other risk factors for bronchiolitis, especially those linked to the socioeconomic status of the children, which, indeed, was higher in infant swimmers than in controls. This analysis was conducted by combining the CPA at indoor and outdoor pools in order to obtain sufficient subjects in the different pool attendance categories. As shown in table 3, associations between bronchiolitis and infant swimming persisted, and were very consistent (OR for CPA of >20 h was 2.2–2.7, $p \leqslant 0.03$) across categories created by excluding children from schools with a low participation rate (<50%), children from parents with a lower educational level, children who had not been breastfed, children who had been exposed to tobacco smoke or else who had lived with older siblings or in a house cleaned with bleach. Quite interestingly, exclusion of children of parents with asthma or respiratory allergies, or of children having attended a day-care centre, two well-known risk factors for bronchiolitis, noticeably strengthened the associations between bronchiolitis and infant swimming (OR for CPA of >20 h 4.45 and 4.44; $p=0.001$ and 0.007, respectively), which emerged through a particularly remarkable dose–response relationship among children who had never attended a day-care centre ($p<0.001$).

Lastly, the temporal coherence of these associations was checked by comparing the age of bronchiolitis occurrence with the age at which the child started to attend swimming pools. Of the 71 infant swimmers who had bronchiolitis, there were 54 cases for which the parents provided the exact dates when their child started swimming and developed bronchiolitis. On average, these infant swimmers developed bronchiolitis at the age of 9.6 ± 5.9 months and were diagnosed with asthma at the age of 21.8 ± 18.6 months. Among these, bronchiolitis occurred after the start of infant swimming in 35 (65%) cases, a number consistent with the excess of bronchiolitis cases observed in the infant swimming group. Children who never swam during infancy had bronchiolitis at about the same age as infant swimmers (9.3 ± 5.5 months), but were diagnosed with asthma later, at the age 30.6 ± 10.0 months.

Since bronchiolitis is known to increase the risk of wheezing, asthma or allergic sensitisation in subsequent years, the late consequences of bronchiolitis were also compared between infant swimmers and nonswimmers. This comparison was made by adjusting the ORs for CPA at indoor or outdoor pools after the age of 2 yrs, which, indeed, was very different between the two groups. Table 4 clearly shows that bronchiolitis was associated with an increased risk of wheezing, doctor-diagnosed asthma and hay fever only among children who had attended chlorinated pools during their infancy. Interestingly, bronchiolitis and infant swimming also interacted to increase the risk of sensitisation to house dust mite ($p=0.04$) and pollen ($p=0.05$). There were no differences in the ORs for cat allergy and allergic rhinitis between the two groups. When considering children who never developed bronchiolitis, there were no significant differences in the prevalences of respiratory diseases and sensitisation to aeroallergens between children who swam during infancy and those who did not ($p>0.07$ for all; Chi-squared test) (table 4).

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TABLE 3 Risk of bronchiolitis with increasing cumulative pool attendance (CPA) at indoor and/or outdoor chlorinated pools during infancy# by risk-factor category

	0-h CPA		>0–20-h CPA			>20-h CPA			p_{trend}
	Subjects n/N (%)	OR (95% CI)	Subjects n/N (%)	OR (95% CI)	p-value	Subjects n/N (%)	OR (95% CI)	p-value	
Total	56/235 (23.8)	1.0 (1.0–1.0)	29/95(30.5)	1.24 (0.69–2.23)	0.46	42/100 (42.0)	2.25 (1.32–3.85)	0.003	<0.001
Schools with >50% response rate	35/146 (24.0)	1.0 (1.0–1.0)	17/59(28.8)	1.24 (0.57–2.7)	0.59	23/58 (39.7)	2.25 (1.10–4.59)	0.03	0.03
High parental educational level	36/156 (23.1)	1.0 (1.0–1.0)	22/72(30.6)	1.31 (0.67–2.57)	0.43	34/82 (41.5)	2.26 (1.22–4.18)	0.009	0.003
Parents with no asthma/respiratory allergies	17/115 (14.8)	1.0 (1.0–1.0)	13/38 (34.2)	4.31 (1.55–12.0)	0.005	18/45 (40.0)	4.45 (1.82–10.9)	0.001	0.001
No breastfeeding	19/66 (28.8)	1.0 (1.0–1.0)	7/12 (58.3)	1.06 (0.55–2.06)	0.86	12/22 (54.6)	2.10 (1.11–3.98)	0.02	0.008
No day-care centre attendance	21/133 (15.8)	1.0 (1.0–1.0)	9/32 (28.1)	2.35 (0.87–6.38)	0.09	15/36 (41.7)	4.44 (1.88–10.5)	0.007	<0.001
No maternal smoking during pregnancy	46/203 (22.7)	1.0 (1.0–1.0)	21/77 (27.3)	1.27 (0.65–2.49)	0.48	34/87 (39.1)	2.16 (1.20–3.90)	0.01	0.005
No exposure to parental smoking	38/161 (23.6)	1.0 (1.0–1.0)	19/65 (29.2)	1.16 (0.57–2.35)	0.61	32/75 (42.7)	2.21 (1.18–4.17)	0.01	0.004
No older siblings	16/99 (16.2)	1.0 (1.0–1.0)	13/45 (28.9)	2.26 (0.87–5.88)	0.09	16/47 (34.0)	2.72 (1.13–6.56)	0.03	0.01
No use of bleach for house cleaning	48/201 (23.9)	1.0 (1.0–1.0)	28/87 (32.2)	1.37 (0.75–2.51)	0.30	36/87 (41.4)	2.22 (1.25–3.95)	0.007	0.003

Odds ratios (ORs) were adjusted for breastfeeding, sex, number of older siblings, area of residence, day-care centre attendance, maternal smoking during pregnancy and parental antecedents of atopic disease (except when the factor was excluded). A high level of education means that the father and/or mother graduated from a university or high school. N: number of subjects in group; CI: confidence interval; p_{trend}: p-value for trend. #: before the age of 2 yrs;

DISCUSSION

The present study shows that swimming in indoor and outdoor pools during infancy is associated with an exposure-related increase in the risk of bronchiolitis. This effect is independent of other known risk factors for bronchiolitis, such as day-care attendance, exposure to tobacco smoke or parental antecedents of atopic diseases. The present study also shows that, among children who had bronchiolitis, only those who were infant swimmers were at greater risk of asthma and respiratory allergies in subsequent years.

These results are in concordance with the observation of NYSTAD and co-workers [9, 10] that swimming before the age of 2 yrs increases the prevalence of lower respiratory tract infection. They are also consistent with a prior study on schoolchildren [11, 12], suggesting that infant swimming may cause airway changes predisposing to asthma and recurrent bronchitis. In the present study focusing on younger children, no significant differences were found between infant swimmers and other children regarding the risk of asthma and allergies. This lack of association between infant swimming and asthma, reported by other authors for children of the same age [18–20], does not necessarily argue against the hypothesis of a causal link between infant swimming and poorer respiratory health later during childhood. Children aged 5–6 yrs are probably too young to detect associations with chronic respiratory diseases that develop and are correctly diagnosed later during childhood. This might explain why studies linking swimming pool attendance to childhood asthma were all based on children with a mean age of >9 yrs [21–23]. The follow-up of the present cohort of infant swimmers should provide more conclusive data regarding the long-term consequences of infant swimming.

The swimming pool factor responsible for the risk of bronchiolitis is hard to identify with certainty given the multiplicity of potentially harmful agents in the swimming pool environment [20, 24]. Currently, one of the most plausible explanations is that the airways of infant swimmers are made more sensitive to infections because of the irritating effects of the chlorine used to disinfect swimming pools. The fact that bronchiolitis is increased by outdoor, as well as by indoor, pools means that chloramines are not the sole and probably not the main irritants that might be implicated in the risk of bronchiolitis. The first reason for this is that the outdoor pools attended by infant swimmers in the present study were mainly residential pools. Compared to public pools, residential pools are, indeed, much less polluted by organic matter from bathers, in particular by urine, which is the main source of nitrogen leading to the formation of chloramines. The second reason is that trichloramine, the ultimate chlorination by-product, is a highly volatile gas that is very quickly dispersed into the atmosphere once released at the surface of open-air pools, which, therefore, do not have the characteristic chlorine smell of indoor pools. As for the risks of asthma and respiratory allergies associated with outdoor pools [15], we suspect that the major burden of oxidants irritating the airways of infant swimmers comes from the microaerosols or small volumes of water that they inhale while actively playing and having their head under water [12]. The risk of inhaling small volumes during submersion exercise is especially important since infants cannot control their breathing. If the infant

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TABLE 4 Risk of respiratory diseases and sensitisation to aeroallergens associated with bronchiolitis in swimming infants and their controls

	Controls					Swimming infants				
	Bronchiolitis n (%)		OR (95% CI)		p-value	Bronchiolitis n (%)		OR (95% CI)		p-value
	No	Yes	Unadjusted	Adjusted		No	Yes	Unadjusted	Adjusted	
Subjects n	179	56				124	71			
Wheezing	28 (15.6)	12 (21.4)	1.47 (0.69–3.13)	1.29 (0.47–3.54)	0.62	18 (14.5)	18 (25.4)	2.00 (0.96–4.16)	2.49 (1.13–5.49)	0.03
Asthma	11 (6.2)	4 (7.1)	1.18 (0.36–3.85)	0.91 (0.23–3.70)	0.90	5 (4.0)	11 (15.5)	4.36 (1.45–13.1)	8.27 (2.30–29.6)	0.001
Allergic rhinitis	16 (8.9)	9 (16.1)	1.95 (0.81–4.70)	2.08 (0.79–5.40)	0.14	13 (10.5)	14 (19.7.)	2.10 (0.92–4.76)	4.65 (1.42–15.2)	0.01
Hay fever	19 (10.6)	12 (21.4)	1.40 (0.58–3.41)	0.96 (0.34–2.70)	0.94	6 (4.8)	11 (15.5)	3.61 (1.27–10.2)	4.70 (1.52–14.5)	0.007
Nasal IgE										
Pollen[#]	24 (15.7)	3 (6.4)	0.39 (0.11–1.38)	0.57 (0.15–2.10)	0.40	14 (12.8)	13 (20.6)	1.86 (0.80–4.30)	2.63 (1.01–6.80)	0.05
HDM[#]	15 (9.8)	7 (14.9)	1.62 (0.62–4.25)	2.05 (0.62–6.75)	0.24	9 (8.3)	21 (33.3)	2.59 (1.02–6.55)	2.86 (1.07–7.80)	0.04
Cat[#]	11 (7.2)	3 (6.4)	0.89 (0.24–3.32)	0.90 (0.20–4.18)	0.90	8 (7.3)	7 (11.1)	1.56 (0.54–5.54)	1.26 (0.40–3.96)	0.69

Outcomes are defined in table 1. Bronchiolitis was diagnosed at a mean ± SD age of 9.6 ± 5.9 months in infant swimmers and 9.3 ± 5.5 months in controls. The prevalences of respiratory diseases and sensitisation to aeroallergens among children who never developed bronchiolitis did not differ significantly between swimming infants and their controls ($p>0.07$ for all; Chi-squared test). OR: odds ratio; CI: confidence interval; Ig: immunoglobulin; HDM: house dust mite. [#]: a total of 153 and 47 controls and 109 and 63 swimming infants, respectively, who did not or did develop bronchiolitis, were tested.

swimming practice is considered to be safe, it is because of the laryngeal or gag reflex (closure of the larynx with the epiglottis) that is triggered when water gets into the infant's mouth. This gag reflex disappears, however, when the infant gets older (>6 months), and, anyway, this reflex cannot prevent small amounts of chlorinated water deposited or trapped in the upper respiratory tract being conducted more deeply into the lungs when the infant surfaces to breathe [12].

The most reactive and concentrated chlorine compound present in the water and microaerosols of both outdoor and indoor pools is hypochlorite/hypochlorous acid, *i.e.* the active chlorine itself (concentrations ranging 1–2 ppm). Depending on the level of organic pollution of pool water, infant swimmers have also been exposed to chlorination by-products, among which the most irritating and concentrated are mono-, dichlor- and trichloramine, as well as dichloromethylamine [19, 20, 24, 25]. Since public pools are usually more polluted by nitrogenous substances from bathers (urine, sweat and saliva) than residential pools, irritating effects caused by the chloramines might explain why the ORs for bronchiolitis are higher with indoor than with outdoor pools.

Chlorine-based oxidants, such as hypochlorous acid or chloramines, are known to be potent oxidants capable of damaging the endothelial and epithelial barriers [26, 27]. Studies based on lung injury markers have shown that, at concentrations commonly found in indoor pools, these chemicals can affect the permeability or the cellular integrity of the deep lung epithelium. Regular attendance at chlorinated pools by schoolchildren has been associated with an exposure-dependent increase in lung epithelium permeability (lung hyperpermeability), resulting in intravascular leakage of surfactant-associated proteins A and B [28]. A decrease in serum CC16 has also been described in children who use indoor pools during their infancy or later in childhood, which is a reflection of a decrease in Clara cell numbers and an ensuing decrease in CC16 production [11, 12, 14]. The latter observation is particularly relevant to the present study given the experimental evidence that CC16 downregulates inflammation during acute RSV infection [13].

Even though airways irritation by chlorine-based oxidants appears to be a very likely explanation for the increased risk of bronchiolitis associated with infant swimming, other causative factors might also play a role. As a result of inadequate disinfection with chlorine, the swimming pool environment can be contaminated by viruses (adeno-, noro- and echovirus), which may cause outbreaks of water-borne illnesses, such as gastroenteritis, dermatitis and respiratory infections [29]. Respiratory illnesses in these incidents are rather uncommon, and, in most cases, limited to the upper airways. Nevertheless, we have no evidence permitting us to exclude the possibility that the swimming pool environment may increase the risks of cross-infection with RSV [30, 31]. Another possibility that should be considered is the inhalation of swimming pool water when infants perform submersion activities. Owing to its hypotonicity, swimming pool water deposited in airways might, perhaps, cause some airway alterations, aggravating the toxic effects of chlorination products [32].

The present study has certain limitations. The most important one concerns exposure assessment, since it was obviously not possible to retrieve exposure data regarding the levels of chlorine in the indoor or outdoor swimming pools that the children attended during infancy. There was no choice but to use the information provided by the parents. The existence of exposure–response relationships for both outdoor and indoor

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pools suggests that the lack of data regarding the precise levels of chlorine in swimming pools has not been critical to the point of distorting the present analysis. Another limitation concerns the participation rate, which only reached 51% overall and varied greatly between schools. The young age of the children combined with the fact that the medical examination, though based on noninvasive tests, was performed in schools probably deterred a significant proportion of parents from participating in the study. This low participation rate does not seem to have biased the study since there were no significant correlations between participation rate and the prevalences of the main outcomes and risk factors. The response rate, tested along with other potential confounders, did not emerge as a significant predictor of bronchiolitis either. This lack of confounding by the participation rate probably stems from the fact that parents were blinded to the tested hypothesis. Since the study was conducted in the framework of a prospective study on environmental factors affecting the respiratory health of children, infant swimming was only one of the many items addressed by the questionnaire.

In summary, the present study shows that infant swimming is associated with a dose-dependent increase in the risk of bronchiolitis. Exposure to chlorinated pools during infancy also interacts with bronchiolitis to increase the risks of asthma and respiratory allergies later during childhood, which suggests that the infant swimming practice may have a more long-standing impact on the respiratory health of children.

SUPPORT STATEMENT

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STATEMENT OF INTEREST

None declared.

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Infant swimming in chlorinated pools and the risks of bronchiolitis, asthma and allergy-What to remember?

1. Bronchiolitis is an acute infection of the small airways affecting young children before the age of 2 years old. Prevalence of bronchiolitis seems to have increased in most developed countries since 1960-1970. Since bronchiolitis increases the risk of childhood asthma, it also contributes to the rising incidence of chronic respiratory diseases in children.
2. Among several factors increasing the frequency or the severity of bronchiolitis, lack of breastfeeding, parental history of asthma and day care attendance appear to have an important impact.
3. Our study shows association between swimming pool and bronchiolitis risk. The effect is independent.
4. Among children who had bronchiolitis, only those who were infant swimmers were at greater risk of asthma and respiratory allergies later in childhood.
5. One of the most plausible explanations of the impact of swimming pool attendance on bronchiolitis risk could be the higher sensitivity of airways of infant swimmers to infections because of irritating effects of chlorine used to disinfect swimming pools.

Phase 2: Prospective study of the cohort when children were 7 years old

3. Risks of allergic sensitization and airway inflammation after chlorinated pool attendance in early life: a prospective study on young children.

(Voisin C, Sardella A, Dumont X, Bernard A)

Atopic eczema, allergic rhinitis and asthma are the most prevalent chronic diseases in children of the developed world. Having usually their onset during early life, they develop sequentially along an "atopic or allergic march" pathway. Factors driving the atopic march and the allergies epidemic are largely unknown but new hypotheses postulate that allergic diseases are primarily epithelial disorders driven by epithelial barrier dysfunction caused by environmental insults. Among alternative hypotheses for hygiene hypothesis, several studies have indeed shown that exposure to chlorination products in swimming pools is associated with increased risks of allergic diseases including allergic rhinitis and asthma. These risks develop dose-dependently with the cumulative pool attendance and appear to come mainly from interactions with the atopic status. To be triggered these interactive effects require indeed a sensitization to aeroallergens but usually also cumulative pool attendance over some years. However, studies focused on infant swimmers have shown that this practice is associated with an increased risk of eczema. But of more concern, the chlorinated pools attendance during infancy or early childhood has been associated with an increased risk of sensitization to house-dust mite, which is one of strongest predictor of childhood asthma. In this prospective study, we assessed whether chlorinated pools attendance early in life was associated with increased risks of IgE sensitization and of increased exhaled NO, a subclinical marker of airways inflammation, a strong predictor of allergic rhinitis and wheeze later in childhood.

Risks of new-onset allergic sensitization and airway inflammation after early swimming in chlorinated pools

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Conflict of interest: none of the authors has a conflict of interest to disclose

Running title: Allergies, airways inflammation and early swimming

Key words: chlorine, swimming pool, IgE, allergy, house dust mite, exhaled nitric oxide

Abbreviations: CPA, cumulative pool attendance; HDM, house dust mite; IgE, immunoglobulin E; IQR, interquartile range; MOA, mode of action; SD, standard deviation; OR, odds ratio; aOR, adjusted OR

Author contributions: CV recruited the children, organized their examination in schools, performed the collection and analysis of data and contributed to the writing of the manuscript. AS was responsible for the Rhinostick tests both in the field and in the laboratory. AB provided the scientific guidance of the project, participating to the study design, the data analysis and interpretation and the writing of the manuscript.

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Abstract

Rationale: Irritant chlorination products in swimming pools can cause respiratory problems in swimmers but their possible implication in allergies development is still unclear.

Objectives: To assess prospectively whether early-life attendance at chlorinated pools increases the risks of IgE sensitization and of airways inflammation later during childhood.

Methods: We conducted a two-year prospective study among 196 kindergarten children (mean age of 5.7 years, 54% of boys). We measured exhaled nitric oxide (eNO) and aeroallergen-specific IgE in nasal mucosa. Parents completed a questionnaire about the child's health, chlorinated pool attendance and potential confounders.

Main Results: Ever swimming at indoor or outdoor chlorinated pools before the age of three years was associated with higher odds for new-onset IgE sensitization to house dust mite (adjusted odds ratio [aOR] 2.93, 95% confidence interval [CI] 1.14-7.55) and for new-onset increased eNO (>15 ppb; aOR, 4.54, 95% CI 1.48-13.9). For both outcomes, aORs increased dose-dependently with time spent in chlorinated pools with values reaching, respectively, 3.60 (95% CI 1.21-10.7) and 5.92 (95% CI 1.72-20.5) when the cumulative pool attendance exceeded 60 hours. These risks appeared independently of each other, of parental history of allergies and of pre-existing diseases, including eczema, which at baseline was more prevalent in early swimmers (aOR, 3.17, 95% CI 1.29-7.81). Such associations were not seen with IgE sensitization to pollen or cat allergens.

Conclusion: Attendance at chlorinated swimming pools in early life is associated with higher risks of new-onset airways inflammation and IgE sensitization to house dust mite, independently of other risk factors.

Introduction

Atopic eczema, rhinitis and asthma are the most prevalent chronic diseases in children of the developed world (Mallol et al., 2012). These diseases have usually their onset during early life and then they develop sequentially along a pathway called the allergic or atopic march (Ker and Hartert, 2009; Spergel, 2010; Zheng et al., 2011). Although the links between these different types of allergic disease are not fully understood, the prevailing paradigm assumes that the atopic march starts with atopic dermatitis, then proceeds to allergic rhinitis to end with asthma (Martin et al., 2011).

Factors driving the atopic march and the epidemic of allergies are largely unknown. Current hypotheses postulate that these factors are related to the hygiene practices of the Western world. According to the classical hygiene hypothesis (Strachan, 1989), the increase of allergic diseases in developed countries would be the consequence of the reduced exposure to certain microbial agents during early life when the immune system is maturating (Strachan, 1989, 2000). The hygiene hypothesis postulates that an insufficient stimulation of the immune system early in life shifts the immune system from a Th1- to a Th2-cell response, which leads to IgE-mediated diseases such as eczema, asthma and hay fever (McGeady, 2004). Some recent studies, however, give reasons for questioning the validity of the hygiene hypothesis as a unifying explanation for the worldwide rise of allergies. Among these, there is the emerging concept that allergic diseases are primarily epithelial disorders driven by airway barrier dysfunction caused by environmental insults (Holgate et al., 1999; Cookson, 2004). Also challenging the hygiene hypothesis, recent studies have failed to confirm the protective role of infectious exposures, older siblings or Bacillus Calmette-Guèrin (BCG) vaccination towards the risk of allergic outcomes (Cramer et al., 2012; Flohr et al., 2012; Brunekreef et al., 2012).

Another hypothesis closely linked to hygiene is the "chlorine or chlorination hypothesis". This hypothesis proposes that the rise of allergic diseases in Westernized countries is, at least partly, driven by the increasing and largely uncontrolled exposure of children to chlorination products, especially in swimming pools (Bernard et al., 2003; Bernard, 2007). Because of their strong oxidizing potential, chlorine and its derivatives such as chloramines can open tight junctions of epithelial barriers and thereby promote the allergen delivery to dendritic cells and the mounting of the Th2 immune response. Several studies have indeed shown that chlorinated pool attendance is associated with increased risks of allergic diseases including hay fever, allergic rhinitis and asthma (Bernard, 2007; Bernard et al., 2006; Kohlhammer et al., 2006; Bernard et al., 2009; Cotter and Ryan, 2009; Ferrari et al., 2011). These risks, which increase dose-dependently with the cumulative pool attendance (CPA), appear to largely stem from interactions with the atopic status (Bernard et al., 2006, 2009). To be triggered, these pool chlorine/atopy interactions require thus a pre-sensitization to aeroallergens as well as a cumulative exposure to chlorinated pools lasting at least a few years. These two conditions, with perhaps some misclassification biases (Bernard et al., 2011; Klootwijk and Krul, 2011), might explain the apparent inconsistencies between studies in young

children (Schoeffer et al., 2008; Font-Ribera et al., 2009, 2011) and those conducted in adolescents or adults (Bernard et al., 2009; Cotter and Ryan, 2009; Ferrari et al., 2011).

The influence of chlorinated pools on the development of allergic diseases might, however, not be limited to interactions promoting the clinical manifestations of atopy (Bernard et al., 2011). Some observations suggest that chlorinated pool attendance might also increase the risks of allergen sensitization and therefore contribute to the development of the atopic status. The attendance at chlorinated pools during infancy or early childhood has been associated with an increased risk of house dust mite (HDM) sensitization (Bernard et al., 2007, 2008; Jacobs et al., 2012), which is one of strongest predictors of childhood rhinitis and asthma (Lodge et al., 2011). Studies among infant swimmers have also shown that this practice is associated with an increased risk of eczema, one of the earliest signs of the atopic march (Font-Ribera et al., 2009; Chaumont et al., 2012).

As all these findings were based on cross-sectional studies with a retrospective assessment of swimming pool attendance, the possibility of recall bias, exposure misclassification or even of reverse causation cannot be formally excluded. The aim of this prospective study was thus to further assess whether attendance at chlorinated pools early in life may increase the risks of aeroallergen sensitization as well as of increased exhaled nitric oxide (eNO), a strong predictor also of rhinitis and asthma in children (Olin et al., 2010; Malinovschi et al., 2012).

Materials and methods

Study population

The study was conducted among 196 schoolchildren in 30 schools located in the areas of Brussels and Liège in Belgium. The first examination took place between December 2007 and March 2008 when children were all in the third kindergarten (mean age, 5.7 years; SD, 0.37). The children were then re-examined two years later by visiting schools in the same order between December 2009 and March 2010. The baseline total population included 431 children but two years later, 128 of them had left the schools. Among remaining children, 236 (77.9%) participated to the study and of them 196 performed successfully all the tests. The children were examined only with the written informed consent of their parents. In addition, just before performing the tests in schools, we verbally assured to have the assent of each child. The ethics committee of the Faculty of Medicine of the Catholic University of Louvain approved the study protocol, which complied with applicable requirements of the international regulations.

Questionnaire

Parents completed a self-administered questionnaire addressing aspects related to social and medical characteristics of the child and its family, the in- and out-house environment and recreational activities including swimming. For swimming practice, parents were asked to specify

the type of swimming pool attended by their child, the type of disinfection method used (some children had access to copper-silver sanitized pools), the frequency of attendance and the age at which their child started to attend the pool on a regular basis. This information served to calculate the cumulative attendance of indoor and/or outdoor chlorinated pools, before the age of 3 years (what was referred to as early swimming), during the two-years follow-up and over lifetime, at the mean age of 5.7 or 7.5 years. The questionnaire also included questions asking whether the child had ever been diagnosed for most common respiratory or allergic diseases, including bronchiolitis, eczema, asthma, hay fever and allergic rhinitis.

Exposure to chlorination and its by-products

In Belgium, public swimming pools (all indoor in the studied areas) are legally required to monitor the microbial and chemical quality of the water by maintaining several parameters within regulatory limits, including free chlorine (0.5-1.5 ppm) and combined chlorine (<0.8 ppm) in water and trichloramine in pool air (<500 µg/m^3 in air sampled 1.5 m above the pool surface). There are no specific regulations for privately owned swimming pools, which are disinfected according to the instructions of the chlorine supplier (recommended free chlorine, 1-2 ppm). An additional potential source of exposure to chlorination products for our children was via the use of tap water, in particularly for showering or bathing. Data chlorine levels in tap water for the years 2003 were provided by the Public Department of Wallonia, the Vivaqua Company and the Brussels Institute for Management of the Environment. Data were allocated to each child according to the postcode of residence of the 63 municipalities studied. The mean concentration of free chlorine in tap water (regulatory limit, 200 µg/l) of our study participants was 65 µg/l, a value more than 10 times lower than levels in swimming pools. The concentration of THM, the major chlorination by-product in tap water, did not exceed 10 µg/l.

Samples collection and analyses

Children were examined in their schools between 9:00 and 13:00. The protocol comprised the measurement of height and body weight, a screening of aeroallergen sensitization using the Rhinostick test, a non-invasive test having a similar sensitivity but a greater specificity than skin-prick tests (Marcucci et al., 2004). The concentration of nitric oxide (NO) was measured in exhaled air with the NIOX™ analyzer (Aerocrine AB, Solna, Sweden) by following the guidelines of the American Thoracic Society (1999).

Statistical analyses

Results were reported as mean ± SD or as median with interquartile range for non-normally distributed variables. Differences between early swimmers and controls were assessed using the Chi-squared test for binary variables and the Student's t-test or the Mann–Whitney U-test for continuous variables. Associations between early swimming and outcomes were assessed by

logistic regression analyses. We adopted a backward approach that consisted to include all potential control variables and to remove the least significant predictor until the model only contained variables with a p-value of <0.20. We tested 17 potential predictors including age, gender, maternal smoking during pregnancy, preterm birth, delivery by cesarean, breastfeeding, day care attendance, number of older siblings, probiotics, raw milk, parental asthma, parental allergies, high parental educational level (father and/or mother graduate of university or high school), parental smoking, house cleaning with bleach, double-glazed windows, mold on bedroom walls, air fresheners and chlorinated pool attendance (all types of pools combined). We analyzed exposure-response relationships by stratifying children in categories of increasing cumulative pool attendance (CPA). For the attendance at chlorinated pools before the age of three (referred to early swimming), we used as controls children having never attended any kind of chlorinated pool and divided early swimmers in a low versus high or low versus very high CPA using as cutoff values a CPA of 30 hours or 60 hours, respectively. These cut offs were adopted because they approximated respectively the median (32 hours) or twice the median of the CPA values of early swimmers before the age of three. We also selected these two cutoff values because they were respectively equivalent to a 30 minutes swimming session fortnightly or weekly during three first years of life. For the attendance later or during the follow-up, we stratified children in tertiles of increasing CPA as there were only 10 children who had never attended a chlorinated pool after the age of three. Results were considered as statistically significant at p-values below 0.05. All statistical analyses were performed using StatView (v5.0.1, 3rd ed., a business unit of SAS, 2001).

Results

Table 1 compares the characteristics of children who had attended chlorinated pools before the age of three with their peers who did not, both at baseline and at the end of the two-year follow up period. Early swimmers had on average spent a total of 33 hours in indoor or outdoor chlorinated pools, which over a period of three years corresponds approximately to a 30 minutes swimming session fortnightly. These early swimmers continue to swim more frequently than their peers later so that their CPA during the follow-up and over lifetime was more than twice that of controls. Except for chlorinated pool exposure, early swimmers and their controls were well matched for gender, parental history of atopic diseases and the exposure to indoor or outdoor pollutants. Proportions of children who had been breastfed or had attended a day care center were however higher among early swimmers than in their peers. Another difference concerned the consumption of raw milk, which was less frequent in early swimmers than in controls (8.9% vs 25%, respectively).

At baseline, early swimmers and controls were well matched for sensitization to aeroallergens, the two groups having almost the same prevalences of sensitization to HDM (13.6 vs. 14.3 %) and pollen (17.9 vs. 16.1 %). There prevalence of elevated eNO in early swimmers (12.1 %) was almost twice lower than that of controls (23.2 %) but after adjustment for other covariates this difference was not statistically significant. The two groups also did not show any meaningful difference in the baseline prevalences of doctor-diagnosed respiratory or allergic diseases except for eczema which much more frequent in early swimmers (aOR 3.17, 1.29-7.81, p=0.01).

Table 3 shows the prevalences of studied outcomes at the end of the two-year follow-up period. Compared to controls, early swimmers were significantly more likely to develop new-onset HDM sensitization (aOR, 2.93, 95% CI 1.14-7.55, p=0.03) and increased eNO (aOR, 4.54, 95% CI 1.48-13.9, p=0.008). These associations were largely independent of each other, being moderately affected by the mutual exclusion of the two outcomes (aOR for new-onset HDM without increased eNO; 2.95, 0.96-9.02, p=0.06; aOR for new-onset increased eNO with no HDM sensitization, 2.96, 0.93-9.44, p=0.07). Excluding children with a history of eczema at baseline who were overrepresented among early swimmers did not weaken either these associations between early swimming and new-onset HDM sensitization (aOR, 3.27, 95% CI 1.04-10.3, p=0.02) or increased eNO (aOR, 3.52, 95% CI 1.07-11.5, p=0.04). Nor were these associations weakened by the exclusion of bronchiolitis, asthma and other pre-existing diseases. There was a trend towards a lower prevalence of new-onset sensitization to pollen among early swimmers but this trend was largely abolished by the exclusion of eczema cases (aOR, 0.47, 95 % CI 0.12-1.77, p=0.26). Because of the pre-existing differences at baseline, this pattern of changes did really emerge when considering all cases at the end of follow-up. The increase in the prevalence of HDM sensitization in early swimmers was of borderline significance (p=0.056) and the increase in eNO completely lost its statistical significance. The only significant difference found with all cases was again a lower prevalence of pollen sensitization among early swimmers but as for the new-onset cases this

difference disappeared with the exclusion of children with eczema at baseline (aOR, 0.66, 95% CI, 0.21-2.06, p=0.47). Whether considering all or new-onset cases, there were no significant differences between early swimmers and controls in the prevalences of doctor-diagnosed allergic rhinitis, hay fever and asthma at the end of the follow-up.

We pursued our analyses by examining the exposure-response relationships between new-onset cases of allergic sensitization or increased eNO and the early swimming practice using as exposure metric the number of hours children had spent in chlorinated pools before the age of three years. We categorized early swimmers in low versus high or low versus very high CPA using as cutoff points the values of 30 h or 60 h, which approximated respectively the median (32 hours) or twice the median of the CPA values of early swimmers before the age of three years. Table 4 shows that for both types of stratification, the adjusted prevalences of new-onset HDM sensitization and elevated eNO increased dose-dependently with the cumulative exposure to chlorinated pools during early life. There were yet some differences between the two outcomes in that the exposure-response relationship emerged at higher CPA for elevated NO than for HDM sensitization. Quite remarkably, all these associations were strengthened by the exclusion of children with reported eczema at baseline or with a parental history of allergic diseases. The same analyses done with pollen sensitization did not reveal any significant exposure-related relationship for this outcome (all p for trend >0.20, results not shown). Because early swimmers continued to attend more frequently chlorinated pools than their peers later during their childhood, we repeated the above analyses by testing as independent variable the time spent in chlorinated pool during the follow-up period or after the age of three years. Whether tested alone or together with the CPA before the age of three years, the attendance at chlorinated pools during the follow-up or after the age of three years was never retained as a significant predictor for any studied outcome at a p-value threshold of 0.20.

Discussion

The results of this prospective study show that early-life attendance at chlorinated pools increases dose-dependently the risks of HDM sensitization and of airways inflammation during childhood. Children having spent more than 30 hours in chlorinated pools before the age of three, i.e. the equivalent of a swimming session fortnightly, were respectively 3.3 and 4.7 times more likely to be sensitized to HDM and to have elevated eNO than their peers who had never attended chlorinated pools at the same age. Odds of developing these two outcomes increased up to 3.6 and 5.9, respectively, when the CPA before the age of three exceeded 60 hours, which roughly corresponds to a weekly swimming session. These respiratory effects were largely independent of each other and also of traditional risks factors of allergic diseases, including parental allergies and eczema.

This prospective study confirms the observations of cross-sectional studies suggesting that the early attendance at chlorinated pools increases the risk of HDM sensitization (Bernard et al., 2007, 2008; Jacobs et al., 2012). Selection bias is unlikely to explain our results as at baseline early

swimmers and their controls were well matched for HDM sensitization as well as for the parental history of allergic diseases. Regarding the risk of elevated eNO, the situation is less clear-cut in that early swimmers at baseline showed a non-significant trend towards a lower prevalence for this outcome. Explanation for this difference, which persisted after adjustment for all possible confounders, is unclear. This difference might arise by chance but it might also be linked to early swimming since a decrease of exhaled NO has been observed after a swimming session in a chlorinated pool (Carbonnelle et al., 2002). This would mean that exhaled NO could present a biphasic response to chlorinated products, an early decrease due to the inhibition of NO production followed after prolonged exposure by an increase as a consequence of the airways inflammation. The case for implicating chlorinated pools in the higher prevalence of eczema in early swimmers at baseline is by contrast much stronger as this has already been reported in two cross-sectional studies (Font-Ribera et al., 2009; Chaumont et al., 2012). Furthermore, it is well known that chlorine and its by-products can irritate the skin and cause epithelial barrier defects that might facilitate skin sensitization or exacerbate eczema symptoms (Chaumont et al., 2012).

Our findings highlight the importance of conducting prospective studies and also of using appropriate exposure and outcome indicators to investigate the respiratory effects of chlorinated pools. The comparison between early swimmers and non-swimmers at the mean age of 5.7 or 7.5 years did not reveal any significant increase in the prevalences of allergic sensitization or elevated eNO in early swimmers, despite the fact that the risks for these two outcomes were multiplied by 3 to 4 in the course of the follow-up. But even in the prospective analysis of our data, the effects of chlorinated pools would have passed undetected with endpoints based on diagnosed allergic diseases or on the overall prevalence of allergic sensitization. The explanation for these findings probably lies in the fact that allergic diseases develop in several steps, each step requiring a certain time to be realized, depending on the exposure to the triggers and the natural course of the disease. Current evidence suggests that chlorine-based irritants in swimming pools promote the development of allergic diseases by two different modes of actions (MOA). The first MOA is an interaction with atopic status promoting the clinical expression of allergic rhinitis and asthma. This interaction, which by definition can be triggered only in sensitized swimmers, requires a CPA of at least several hundreds of hours. Such a CPA is unlikely to be met before early adolescence in most recreational swimmers, explaining why early swimming is not associated with clinically diagnosed allergic diseases in young children, in our study as well as in studies conducted by others (Schoeffer et al., 2008 Font-Ribera et al., 2009, 2011). The second mode of action is an interaction between chlorinated pool attendance and allergen exposure, which promotes allergic sensitization. This second MOA can be triggered in early life after a CPA of a few tens of hours. However, to be effective, this MOA requires a concomitant and sufficient exposure to allergens, two conditions that are more easily met with perennial allergens such as HDM than with seasonal allergens linked to pollination. This probably explains why the increased HDM sensitization associated with early swimming is observed after a time lag time of a few years and also why sensitization to pollen is not increased in early swimmers. Even though they operate at different stages of the allergic march,

these two MOA probably share the same basic mechanism i.e. which is a disruption of the airways epithelial barriers by chlorine-based oxidants (Bernard et al., 2003; Bernard 2007; Carbonnelle et al., 2002; Lagerkvist et al., 2004; Bougault et al., 2011). The types of epithelial defects in epithelial barrier enhancing allergic sensitization and its clinical manifestations are largely unknown. There is, however, some evidence from biomarker studies that epithelial defects promoting allergic sensitization might consist in an increased epithelial hyperpermeability facilitating the allergen delivery to dendritic cells associated with a dysfunction or dedifferentiation of epithelial cells secreting immune-modulatory proteins, in particular the Clara cells (Sardella et al., 2012, 2013).

The main limitation of our study is the relatively small size of the cohort and in particular of the group used as reference. Because of the increasing popularity of swimming in affluent countries and the widespread use of chlorine to sanitize swimming pools, it has become increasingly difficult to recruit children having never attended chlorinated pools during early life. This limitation, however, has been compensated by the prospective design of our study and the use of accurate and sensitive effect and exposure indicators. The fact that tap water in Belgium is disinfected with very low level of chlorine has probably also eliminated a source of confounding. These methodological strengths along with the relatively high prevalences of studied outcomes explain why our study could evidence highly significant exposure-response relationships despite a relatively limited sample size.

In conclusion, attendance at chlorinated swimming pools in early life is associated independently with higher risks of developing IgE sensitization to HDM and an increased exhaled NO, which are among the strongest predictors of rhinitis and asthma (Olin et al., 2010; Malinovschi et al., 2012). If one adds to these findings the evidence from previous studies suggesting that pool chlorine interacts with atopic status to increase the risks of eczema, allergic rhinitis and asthma, this suggests that exposure to chlorine-based oxidants in swimming pools is a potent driver of the allergic march, promoting IgE sensitization at an early stage and later the clinical manifestations of atopy.

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Table 1 Characteristics of children at baseline and follow-up

	Baseline			Follow-up		
	Controls	Early swimmers	P	Controls	Early swimmers	
N	56	140		56	140	
Boys, N (%)	30 (53.6)	77 (55.0)	0.86			
Age, mean ± SD, years	5.78 ± 0.43	5.64 ± 0.34	0.02	7.54 ± 0.42	7.45 ±0.35	0.08
BMI, mean ± SD, kg/m^2	21.2 ± 3.80	20.8 ± 3.45	0.45	16.8 ± 2.24	16.7 ± 2.45	0.64
Parents						
Higher education, N (%)	39 (69.6)	112 (80.0)	0.12			
Parental allergies, N (%)	23 (41.1)	63 (45.0)	0.62			
Parental asthma, N (%)	7 (12.5)	18 (12.9)	0.95			
Early life care						
Smoking during pregnancy, N (%)	4 (7.1)	20 (14.3)	0.17			
Preterm birth, N (%)	12 (21.4)	35 (25.0)	0.60			
Cesarean delivery, N (%)	9 (16.0)	35 (25.0)	0.18			
Breastfeeding, N (%)	40 (71.4)	119 (85.0)	0.03			
Number of older siblings, mean ± SD	1.12 ± 1.11	0.87 ± 0.94	0.11			
Day-care attendance, N (%)	21 (37.5)	96 (68.6)	<0.001			
Environment and lifestyle						
Living close a busy road, N (%)	28 (50)	89 (63.6)	0.08	28 (50)	81 (74.3)	0.32
Exposure to furry pets, N (%)	29 (51.8)	71 (50.7)	0.89	27 (48.2)	76 (54.3)	0.44
Probiotics consumption, N (%)	27 (48.2)	54 (38.6)	0.22	35 (66.0)	81 (60.9)	0.51
Raw milk, N (%)	14 (25)	12 (8.6)	<0.001	18 (32)	13 (9.3)	<0.001
Parental smoking at home, N (%)	16 (28.6)	38 (27.1)	0.84	14 (25.0)	38 (27.1)	0.76
House cleaning with bleach, N (%)	12 (21.4)	24 (16.1)	0.48	18 (32.1)	34 (24.3)	0.26
Chlorinated pool attendance						
Before 3 years, median (IQR), hours		33 (14.0-81.2)			33 (14.0-81.2)	
Lifetime, median (IQR), hours	26.5 (7.8-70.5)	139 (61.0-240)	<0.001	98.5 (31.5-286)	305 (166-533)	<0.001

Table 2 Prevalences and adjusted odds ratios for aeroallergen sensitization, airways inflammation and allergy or respiratory diseases in controls and early swimmers at the mean age of 5.7 years

	Controls (N=56)	Early swimmers (N=140)	Adjusted ORs (95 % CI)	P
Sensitization to aeroallergens				
At least one aeroallergen [a]	17 (30.4)	37 (26.4)	0.90 (0.43-1.86)	0.77
House dust mite [b]	8 (14.3)	19 (13.6)	1.07 (0.42-2.75)	0.89
Pollen [c]	9 (16.1)	25 (17.9)	1.18 (0.48-2.93)	0.72
Cat [d]	7 (12.5)	12 (8.6)	0.78 (0.28-2.21)	0.64
Airway inflammation				
Exhaled NO>15 ppb [e]	13 (23.2)	17 (12.1)	0.45 (0.19-1.09)	0.08
Allergic and respiratory diseases				
Eczema [f]	9 (16.1)	45 (32.1)	3.17 (1.29-7.81)	0.01
Allergic rhinitis [g]	8 (14.3)	16 (11.5)	0.79 (0.29-2.09)	0.63
Hay fever [h]	7 (12.5)	13 (9.3)	0.64 (0.23-1.79)	0.39
Asthma [i]	6 (10.7)	10 (7.1)	0.55 (0.17-1.77)	0.32
Bronchiolitis [j]	12 (21.4)	47 (33.6)	1.68 (0.73-3.86)	0.22

Letters indicate covariates for which ORs were adjusted
[a] preterm birth
[b] preterm birth, older siblings, raw milk, probiotics, furry pets
[c] preterm birth, living close a busy road, day care attendance, gender, parental smoking
[d] breastfeeding, parental smoking, furry pets
[e] preterm birth, furry pets, older siblings, day care attendance, raw milk
[f] older siblings, parental smoking, furry animals, mold in bedroom, raw milk, probiotics
[g] parental asthma, parental allergies, furry pets, day care attendance, bleach, doubled-glazed windows
[h] preterm birth, BMI, bleach, parental asthma
[I] preterm birth, parental smoking, parental asthma
[j] BMI, cesarean delivery, probiotics, living close a busy road, breastfeeding, day care attendance, parental asthma

Table 3 Prevalences and adjusted odds ratios for aeroallergen sensitization, airways inflammation and allergic diseases in controls and early swimmers at the mean age of 7.7 years

	New-onset cases				All cases			
	Controls	Early swimmers	Adjusted OR (95% CI)	P	Controls	Early swimmers	Adjusted OR (95% CI)	P
Allergic sensitization								
At least one aeroallergen [a]	9/39 (23.0)	39/103 (37.9)	1.58 (0.63-3.93)	0.33	17/56 (30.4)	51/140 (36.4)	1.45 (0.73-2.89)	0.29
House dust mite [b]	7/48 (14.6)	35/121 (28.9)	2.93 (1.14-7.55)	0.03	11/56 (19.6)	43 /140 (30.7)	2.15 (0.98-4.71)	0.056
Pollen [c]	9//47 (19.1)	10/115 (8.7)	0.40 (0.15-1.09)	0.07	11/56 (19.6)	16/140 (11.4)	0.36 (0.14-0.92)	0.03
Cat [d]	5/49 (10.2)	10/128 (7.8)	0.69 (0.17-2.81)	0.61	6/56 (10.7)	10/140 (7.1)	0.74 (0.23-2.33)	0.60
Airway inflammation								
Exhaled NO>15 ppb [e]	5/43 (11.6)	37/123 (30.1)	4.54 (1.48-13.9)	0.008	13/56 (23.2)	47/140 (33.6)	1.74 (0.81-3.72)	0.15
Allergic diseases								
Eczema [f]	3/47 (6.4)	4/95 (4.2)	0.61 (0.08-4.63)	0.63	6/56 (10.7)	24/140 (17.1)	1.60 (0.57-4.50)	0.38
Allergic rhinitis [g]	2/48 (4.2)	3/123 (2.5)	0.49 (0.07-3.21)	0.45	3/56 (5.4)	9/139 (6.5)	1.72 (0.37-7.92)	0.49
Hay fever [h]	5/49 (10.2)	7/127 (5.5)	0.75 (0.20-2.83)	0.67	10/56 (18.2)	14/140 (10.0)	0.55 (0.22-1.37)	0.20
Asthma [i]	2/50 (4.0)	2/130 (1.5)	0.35 (0.05-2.67)	0.50	5/56 (8.9)	9/140 (6.4)	1.6 (0.37-6.94)	0.53

Letters indicate the covariates for which ORs were adjusted:

[a] New-onset cases: older siblings, BMI, raw milk, double-glazed windows, gender, probiotics; all cases: probiotics

[b] New-onset cases: preterm birth, mold in bedroom; all cases: probiotics, mold in bedroom, living close a busy road

[c] New-onset cases: probiotics; all cases: mold in bedroom, double-glazed windows, house cleaning with bleach, raw milk, probiotics

[d] New cases: probiotics, mold in bedroom, urban area, double-glazed windows, bleach, breastfeeding, raw milk, day care attendance, smoking during pregnancy, parental asthma; all cases: bleach, probiotics; day care attendance

[e] New cases: preterm birth, older siblings, mold in bedroom, house cleaning with bleach, smoking during pregnancy, parental allergies, parental smoking; all cases, urban area, house cleaning with bleach, cesarean delivery, preterm birth, parental allergies, parental smoking

[f] New cases: BMI, raw milk, probiotics, bleach, day care, gender, parental allergies; all cases, bleach, smoking during pregnancy, parental allergies, raw milk, breastfeeding, rural vs urban, probiotics

[g] New cases, birth at term, furry pets; all cases, mold, traffic pollution, gender, parental asthma

[h] New cases, BMI, rural vs urban, day care; all cases: parental asthma, furry pets, parental smoking, traffic pollution, rural vs urban, probiotics

[i] New cases, parental asthma; all cases, parental asthma, BMI, raw milk, day care, birth at term, probiotics

Table 4 New-onset cases of sensitization to HDM and of increased exhaled NO according to the total time spent in chlorinated pools before the age of three years in all children and in children with no eczema at baseline and no parental history of allergic diseases

	All		No eczema at baseline		No parental history of allergies	
	n/N (%)	Adjusted OR (95% CI)	n/N (%)	Adjusted OR (95% CI)	n/N (%)	Adjusted OR (95% CI)
New-onset HDM sensitization[a]						
Never	7/48 (14.6)	1.00	4/41 (9.8)	1.00	4/28(14.3)	1.00
1-30 h	15/57 (26.3)	2.60 (0.91-7.38)	8/39 (20.5)	2.38 (0.65-8.78)	7/33 (21.2)	2.35 (0.53-10.4)
>30 h	20/64 (31.3)	3.27 (1.18-9.07)	15/47 (31.9)	4.08 (1.21-13.7)	13/37 (35.1)	4.12 (1.00-17.0)
P for trend		*0.047*		*0.01*		*0.049*
1-60 h	21/76 (27.6)	2.63 (0.98-7.12)	12/52 (23.1)	2.63 (0.77-8.98)	11/46 (23.9)	2.55 (0.63-10.3)
>60 h	14/45 (31.1)	3.60 (1.21-10.7)	11/34 (32.4)	4.46 (1.24-16.0)	9/24 (37.5)	5.02 (1.11-22.7)
P for trend		*0.06*		*0.02*		*0.053*
New-onset exhaled NO>15 ppb[b]						
Never	5/43 (11.6)	1.00	4/37 (10.8)	1.00	2/25 (8.0)	1.00
1-30h	17/59 (28.8)	4.41 (1.33-14.7)	11/40 (27.5)	3.34 (0.92-12.1)	11/33 (33.3)	11.8 (1.62-85.7)
>30 h	20/64 (31.3)	4.66 (1.42-15.2)	14/45 (31.1)	3.68 (1.02-13.2)	14/37 (37.8)	15.1 (2.04-111)
P for trend		*0.03*		*0.04*		*0.01*
1-60 h	21/78 (26.9)	3.88 (1.21-12.4)	13/51 (24.5)	2.92 (0.83-10.3)	13/45 (28.9)	9.54 (1.32-68.8)
>60 h	16/45 (35.2)	5.92 (1.72-20.5)	12/34 (35.3)	4.64 (1.23-17.5)	12/25 (48.0)	27.9 (3.36-332)
P for trend		*0.01*		*0.02*		*0.002*

[a] adjusted for preterm birth, mold in bedroom

[b] adjusted for preterm birth, older siblings, mold in bedroom, bleach, smoking during pregnancy, parental allergies, parental smoking

4. Allergic sensitization and airway inflammation after early swimming
(Voisin C, Sardella A, Dumont X, Bernard A)

Allergic sensitization and airway inflammation after early swimming

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Author contributions: CV recruited the children, organized their examination in schools, performed the collection and analysis of data and contributed to the writing of the manuscript. AS was responsible for the screening of aeroallergen-specific IgE in nasal mucosa. AB provided the scientific guidance of the project, participating to the study design, the data analysis and interpretation, and the writing of the manuscript.

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To the Editor

There is increasing evidence that irritant chlorination products in swimming pools can cause respiratory problems in pool workers and competitive swimmers (1,2). Whether these risks may extend to recreational swimmers is an important question that, to date, remains unresolved. Several studies among adults or school adolescents (5-8) have shown that recreational swimming in outdoor or indoor chlorinated pools is associated with increased risks of allergic diseases, in particular asthma. In adolescents, the asthma risk associated with chlorinated pool attendance appears to be largely the consequence of an interaction with atopy, increasing only among subjects sensitized to aeroallergens (3-7). Other studies found no increase of asthma risk with swimming pool attendance (8-10). These studies, however, were more focused on children who perhaps were too young to detect associations with atopic asthma, a chronic disease developing mostly during late childhood (11). Further complicating the picture, some observations suggest that early exposure to chlorinated pools might promote allergic sensitization and thus contribute to the development of atopy itself. Attendance at chlorinated pools during infancy or early childhood has indeed been associated with an increased risk of eczema (9) and house dust mite (HDM) sensitization (12, 13), two outcomes that are predictive of rhinitis and asthma later in life. We here report the results of a two-year prospective study comparing the development of new-onset allergic sensitization and diseases and increased exhaled nitric oxide (eNO) between children who started to swim in early life and those who did not.

The study was conducted among 196 schoolchildren in 30 schools located in the areas of Brussels and Liège in Belgium. The first examination took place between December 2007 and March 2008 when children were all in the third kindergarten (mean age, 5.7 years; SD, 0.37). The children were then re-examined two years later by visiting schools in the same order between December 2009 and March 2010. The baseline total population included 431 children but two years later, 128 of them had left the schools. Among remaining children, 236 (77.9%) participated to the study with the agreement of their parents and of them 196 performed successfully all the tests. Participants and non-participants did not differ significantly regarding studied outcomes, chlorinated pool attendance and the main risk factors of allergies. Parents filled out a self-administered questionnaire inquiring about the health of the child and factors likely to influence the development of respiratory and allergic diseases. The questionnaire included questions intended to calculate the total time (cumulative pool attendance, CPA) the child had spent in indoor or outdoor chlorinated pools, before the age of three (referred to hereinafter as “early swimmers”), during the two-year follow-up and over lifetime. The examination of children, made in schools between 9:00 and 13:00, included a screening of aeroallergen-specific IgE in nasal mucosa by the Rhinostick test (14) and the measurement of eNO (NIOX™ analyzer Aerocrine AB, Solna, Sweden). The Rhinostick test was calibrated with serum standards and considered positive at specific IgE ≥0.35 kIU/l (14). For eNO, we used a cut-off of 15 ppb (15), which in our study corresponded to the 95th percentile of baseline values in non-atopic children. The study protocol was approved by the Institutional Review Board of the Catholic University of Louvain. We used backward logistic regression analyses

to calculate the crude and adjusted odds ratios (aORs) of outcomes according to increasing CPA while adjusting for confounders that remained in the model at a *P* value <0.20. For these analyses, we used as referents children who never attended chlorinated pools before the age of 3 and we dichotomized early swimmers at CPA cut-off values (30 or 60 hours) that corresponded approximately to the median (32 hours) or twice the median of their CPA. As there were only 10 children who never visited chlorinated pools, the CPA during the follow-up or over lifetime was stratified in tertiles. Results were considered as statistically significant at a *P* value <0.05.

Early swimmers and their controls were well matched with respect to gender, parental history of allergies and other traditional risk factors of atopic diseases. At baseline, the two groups did not show any significant difference in the prevalences of increased eNO and of allergic sensitization or diseases, except for eczema that much more frequent in early swimmers (aOR 3.17, 95% confidence interval [CI], 1.29-7.81, *P* = 0.01). When children were re-examined two years later, we found no significant differences in the prevalences of new-onset allergic diseases between the two groups. By contrast, there was a marked increase in the prevalence of new-onset sensitization to HDM (aOR 2.93, 95% CI, 1.14-7.55, *P* = 0.03) and new-onset increased eNO (aOR, 4.54, 95% CI 1.48-13.9, *P* = 0.008) among early swimmers as compared to controls. As shown in the Table, the adjusted ORs for these two outcomes increased dose-dependently with the time spent in indoor or outdoor chlorinated pools before the age of three. These associations were independent of each other, of parental allergies and of pre-existing diseases, including eczema. Such associations were not seen with IgE sensitization to pollen or cat allergens. Whether tested alone or with the CPA before the age of three, the CPA during the follow-up (median [hours], early swimmers, 161; controls, 77) or over lifetime (early swimmers, 309; controls, 99) was never retained as a significant predictor for any of the studied outcomes.

This prospective study shows that children having regularly attended indoor or outdoor chlorinated pools during their infancy are more likely to develop airways inflammation and a sensitization to HDM. Attendance at chlorinated pools later during childhood did not emerge as a significant predictor for these risks. These specific associations with early swimming, also observed in cross-sectional studies (12, 13), suggest that not surprisingly developing airways of infants are highly vulnerable to injury by chlorination products. Our study has some limitations in particular regarding the exposure assessment based on questionnaire and the lack of a control group attending non-chlorinated pools. However, as at the age of 3 and even later, children cannot really swim and do not actively practice a sport, physical exercise is unlikely to be the cause of our findings. The most plausible explanation is that the exposure to chlorine-based irritants in swimming pools causes epithelial barriers defects predisposing to allergic sensitization and airways inflammation (11,12). To enhance sensitization, these epithelial defects logically require a concomitant and sufficient exposure to allergens, a condition more easily met with perennial allergens like HDM than with seasonal allergens linked to pollination. This probably explains why in early swimmers sensitization to pollen was not increased and also why the risk of HDM

sensitization was increased only after a time lag of a few years. Combined with data from previous studies showing that pool chlorine can interact with atopic status to increase the risks of allergic rhinitis or asthma (3,4), our findings suggest that chlorine used to disinfect swimming pools is a significant driver of the atopic march, promoting allergic sensitization at an early stage and later the clinical manifestations of atopy.

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Table New-onset cases of sensitization to HDM and of increased exhaled NO according to the total time spent in indoor or outdoor chlorinated swimming pools before the age of three years

	Cumulative attendance at chlorinated pools before the age of three years (hours)				
	Never	1 - 30	> 30	1 - 60	> 60
New-onset HDM sensitization [a]					
n/N (%)	7/48 (14.6)	15/57 (26.3)	20/64 (31.3)	21/76 (27.6)	14/45 (31.1)
Crude OR (95% CI)	1.00	2.09 (0.77-5.66)	2.66 (1.02-6.96)	2.24 (0.87-5.76)	2.65 (0.95-5.76)
Adjusted OR (95% CI)	1.00	2.60 (0.91-7.38)	3.27 (1.18-9.07)	2.63 (0.98-7.12)	3.60 (1.21-10.7)
P for trend		0.047		0.06	
New-onset exhaled NO>15 ppb [b]					
n/N (%)	5/43 (11.6)	17/59 (28.8)	20/64 (31.3)	21/78 (26.9)	16/45 (35.6)
Crude OR (95% CI)	1.00	3.08 (1.04-9.15)	3.46 (1.18-10.1)	2.80 (0.97-8.07)	4.19 (1.38-12.8)
Adjusted OR (95% CI)	1.00	4.41 (1.33-14.7)	4.66 (1.42-15.2)	3.88 (1.21-12.4)	5.92 (1.72-20.5)
P for trend		0.03		0.01	

[a] adjusted for preterm birth, mold in bedroom

[b] adjusted for preterm birth, number of older siblings, mold in bedroom, use of bleach for house cleaning, smoking during pregnancy, parental allergies and parental smoking.

N represents the number of subjects in the different groups that at baseline were not sensitized to HDM or were negative in the eNO test.

Risks of allergic sensitization and airway inflammation after chlorinated pool attendance in early life: a prospective study on young children-What to remember?

1. Early-life chlorinated pools attendance increases dose-dependently the risks of HDM sensitization and airways inflammation during the subsequent years of childhood.
2. Children having spent more than 30 hours in chlorinated pools before the age of three, (the equivalent of a swimming session fortnightly), were respectively 3.3 and 4.7 times more likely to be sensitized to HDM and to have elevated FeNO than their peers who were never exposed to chlorinated pools.
3. Risks of these two outcomes were even increased up to 3.6 and 5.9 respectively among with a CPA> 60 hours, the equivalent of a weekly swimming session.
4. These respiratory effects were largely independent of each other and also of traditional risks factors of allergic diseases, including parental allergies and eczema.
5. An association between infant swimming and eczema prevalence has been observed. It is also known for long that chlorine and its by-products can irritate the skin and cause epithelial barrier defects that might facilitate skin sensitization or exacerbate eczema symptoms.

6. GENERAL DISCUSSION

For the last 30 years, we have observed an important increase in the prevalences of allergic diseases including asthma, allergic rhinitis, atopic dermatitis or food allergies. There is ample evidence indicating that heredity alone cannot explain this fast increase, especially when more and more children become allergic in the absence of any parental antecedents of allergies [10]. More than a simple progression, the temporal pattern and the development of the "atopic march" are strongly influenced by both genetic and environmental and lifestyle factors. These disorders may develop sequentially along an atopic pathway or there may be a causal link between eczema and these later-onset atopic respiratory disorders [12].

The main objective work was to further assess to what extent the development of atopic diseases is influenced by stressors linked to hygiene and in particular the chlorinated pool attendance and the use of hard tap water.

Our results confirm that the influence of chlorinated pool attendance on the development of allergic diseases is not limited to interactions promoting the clinical manifestation of atopy. Results of our prospective study clearly show that chlorinated pool attendance may also increase the risk of eczema, bronchiolitis and allergic sensitization, which are strong predictors of asthma and allergic rhinitis later during childhood. Other protective or risk factors identified in our studies. Some of them are personal characteristics and unavoidable such as the atopy status of the parents. Other determinants were related to lifestyle and environment. We confirmed protective determinants such as birth at term and day-care attendance. This has been observed also in other interesting international studies and gives support for the robustness of our study results. In addition we identified environmental risk factors such as exposure to environmental tobacco smoke, living near an industry, exposure to air fresheners, humidity at home and swimming pool attendance.

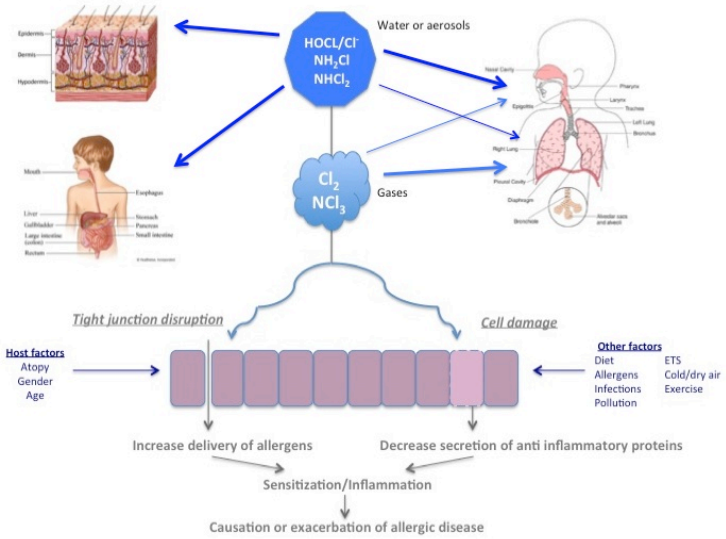
Baby swimming is very popular and in Belgium doesn't differ from another countries. Even we observed a very high frequency, around 50% of baby swimmers in our study population; this prevalence is confirmed by other studies conducted in Germany [145] or in the Netherlands [146].

As to the possible mechanisms underlying the adjuvant effects of chlorine-based irritants in swimming pools on the development of allergies, two different modes of action appear to be operate: an interaction with atopic status promoting clinical expression of allergic rhinitis and asthma or an interaction between chlorinated pool attendance and allergen exposure, which promotes allergic sensitization.

The two modes of action probably share the same basic mechanism (figure 11). Chlorine and its by-products can irritate the skin and cause epithelial barrier defects. Chlorine-based oxidants in water and air could thus play the role of chemical adjuvant facilitating the transepithelial passage of allergens and thereby amplifying the sensitization in atopic subjects and the increased risk of allergic diseases observed in recreational or elite swimmers [91;97;137;147-153]. Following excessive or repeated exposures, CPs have also been found to damage the epithelial cells. Consequences of this

damage can be manifold since epithelial cells are known to synthesize and secrete a number of molecules regulating allergic or inflammatory responses such as cytokines/chemokines, growth factors, lipid mediators or anti-oxidant/inflammatory proteins [154]. This decreased secretion by adding to the intravascular leakage of proteins across the disrupted epithelial barrier, contributes to further reduce the intrapulmonary pools of these secretory products. An example is the reduced secretion of the anti-inflammatory CC16 that has been found in children having regularly attended indoor [13;149].

Figure 11: Scheme illustrating how chlorine-based oxidants in water or air can disrupt the epithelial barriers of exposed organs and thereby facilitate the penetration of allergens and the allergic sensitization [155] for those swimming during their early childhood [93;95;96].



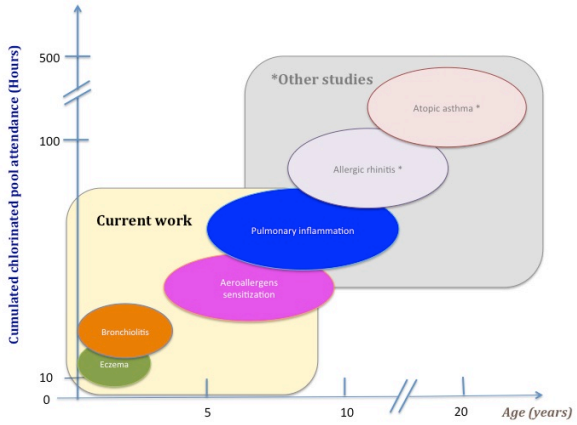
While children are exposed to chlorinated pools during infancy, higher allergic sensitization prevalences appear not so early in life. Indeed, although the prevalences of allergic sensitization are very similar at the age of 5 years, they increase significantly among infant swimmers when they are 7 years old. The explanation for these findings could be found in the fact that allergic diseases develop in several steps, each step requiring a certain time to be realized depending on the exposure to the triggers and the natural causes of the disease. Numerous epidemiological studies underlined the necessity of a preliminary raising sensitization associated with an exposure for the development of the symptoms.

The age at the time of the exposure also has an influence and an exposure in perannuels allergens in the early childhood, defined as the first three years of life, would seem to contribute to the chronicity of the asthma. It could be one explanation to the absence of observed relations with hay fever or asthma in our study. Hay fever is a seasonal disease, depending on an intermittent exposure; children should need more time to be sufficiently exposed to the allergens and young babies and infants spend less time outside than those older. In the case of asthma, most of them need a previous allergic sensitization. Even if pets and especially cats are known to be asthma

triggers among sensitized individuals, the role early exposure to pets in the development of sensitization and allergic disease is less clear and remains a subject of debate. If some studies suggest that early childhood exposure might prevent the development of atopic disorders [156], observations in longitudinal studies are inconsistent

Previous works showed relation between chlorinated pool attendance and the development of asthma and allergies among teenagers and adults [96;97;147;157]. These relations requiring important lifestyle cumulated pool attendance while we observe that in early life low cumulated time of exposure are sufficient to promote the disruption of the epithelial barrier and favour the development of allergic sensitization. Babies and toddlers represent a population at high risk because of the vulnerability of their developing respiratory tract, immature lungs and immunological system. Observations of the current work and of the previous studies are synthesized in the figure 12.

Figure 12: Temporal pattern of development of respiratory diseases related with cumulated pool attendance.



Even if we did have any access to the individual physic-chemical characteristics of each pool attended by the children such as pH, levels of chlorine compound in the water and the air, we calculated as precisely as we could the time spend in water at each age separately for each type of attendance. In contrast, if we had very sharped data about the quality of tap water, the use was less precisely defined and after the first phase of the project, we had information about water softener use.

Our study has some limitations in particular regarding the exposure assessment based on questionnaire and the lack of a control group attending non-chlorinated pools. However, as at the age of 5 and even later, children cannot really swim and do not actively practice a sport, physical exercise is unlikely to be the cause of our findings. Even we tried to use objective information from non-invasive test applied in participants, eczema and bronchiolitis were outcomes obtained from questionnaires filled by the parents with the inherent risks of recall biais. In the case of bronchiolitis, we checked by telephone that exact nature of the diagnosis (bronchiolitis and not

bronchitis) as well as the time when the diagnosis . There might be also some biases such as a grape-effect due to the fact that children from the same school could share same characteristics. The prevalence of risk factors and of studied outcomes did not vary with the participation rate in the individual schools. In the follow-up study, there were also no significant between participants and non-participants.

If the Superior Health Council (SHC) does not recommend the baby swimming practice before the age of 1, our work would encourage avoiding chlorinated pool water before the age of 3. According to the Superior Health Council (SHC), there are no real benefits in attendance of swimming pools for infants. First, babies are a particularly vulnerable population (more disposed to infections, hyperreactive mucous membranes, immature lungs, etc.) and secondly, interlimbs coordination is not acquired before the age of 3-4 [158].

Baby swimming practice takes place in pools with relatively high water and air temperatures, which in turn results in significant humidity and increased micro-organism growth. Furthermore, baby swimming practice is often organised in privately owned pools for public use that are not systematically monitored or in which there are no systematic inspections [158].

Regarding recommendations for older children and related with health considerations, it is necessary for swimming pools to be properly disinfected. Chlorine doesn't make water sterile and some organism can resist disinfection. Both air and water qualities should be monitored and inspections should be carried out [158] while minimizing the exposure to chlorination products. For this purpose, there are two possible approaches. The first is to use alternative methods of disinfection such as the copper-silver method, ozone, UV,… ,… The copper-silver method is an electro-physical method that allows to achieve a sanitary quality of the water comparable to that of chlorine[159]. The second is to strengthen the regulations regarding the concentrations of chlorination products in water and air as this has been already done in some countries (Germany, Switzerland).

The chlorine levels and temperature in swimming pools should be carefully regulated. Indoor pools should be properly ventilated and swimmers should be informed about the good personal practice reducing the irritant nature of swimming pool environments.

Both the quality of the air as well as that of the water should be monitored and inspections should be carried out. As regards the water quality, this should concern the microbiological and physicochemical aspects of the water, as well as the presence of chlorinated organic by-products. As is the case of water, the air needs to be renewed and/or filtered. The swimming pool hall should be properly ventilated and the water filtered to remove chlorinated-organic compounds.

Unfortunately, there are no regulations that govern ventilation requirements in Belgian swimming pools. Particular attention should be paid to privately owned pools for public use that are visited by

small children, who inevitably produce more organic material.

At present, there are no standard regulations that apply to the whole of Belgium. Rather, they differ from one region to another (table 2). Given its popularity, the problem of the health hazards related to swimming pool attendance is currently a major issue at international level. It therefore seems essential that these regulations should be harmonised and/or revised on the basis of norms or regulations that currently apply in other European countries, e.g. Germany and the United Kingdom (UK) (table 3).

Table 2: Standards for various physicochemical parameters in Belgium [158]

Parameters measured	**Brussels**		**Wallonia**		**Flanders**
threshold values	Large pool	Small pool	Type 1 pools (indoor)	Type 2 pools (outdoor)	
pH	7,0 - 7,6		7,0 -7,6		7,0-7,6
Free chlorine	0,5-1,5 mg/l		0,5-1,5 mg/l	0,8-3,0 mg/l	0,5-1,5 mg/l*
Combined chlorine	0,8 mg/l		0,8 mg /l		≤ 1,0 mg/l
Water temperature	28°C	30°C			≤ 32°C
Urea	2,0 mg /l		2,0 mg/l		2,0 mg/l
Chloroform	0,1 mg/l				
TCA in the air	0,5 mg/m³ (sampling over 2 hours)		0,5 mg/m³ (at 1,5m above the floor/once a year)	-	-

* As regards outdoor pools and hot whirlpools, the threshold value is 3,0 mg/ml.

Table 3: Standards for various physicochemical parameters in neighbouring countries [158]

Parameters measured (threshold values)	**Germany** (norms)	**France** (norms)	**UK** (regulation)	**Italy** (norms)	**Netherlands** (norms)
pH	6,5-7,6	6,9-8,2	7,2-7,4	6,5-7,5	6,8-7,8
Free chlorine	0,30-0,60 mg/l	0,4-1,4 mg/l	< 2 mg/l	0,7-1,5 mg/l	0,5-1,5 mg/ml
Combined chlorine	0,20 mg/l	0,6 mg/l	< 1 mg/l	0,4 mg/l	< 1,0 mg/
Water temperature			27-30°C	24-30°C	
TCA in the air		0,3 mg/m³			

If Belgium applied the standards of chlorine and current chloramines established in Germany and in Switzerland, practically all the public swimming pools should be closed. Germany and Switzerland lowered the standard of trichloramine in the air in 0,2 mg / m^3, while the standard in the Walloon Region and in Brussels is still 0,5 mg / m^3.

At least, it is interesting to see that in swimmers who stopped high-level training, BHR and asthma attenuated or even disappeared while airways inflammation (eosinophils in sputum) did not change significantly while in highly trained swimmers who remained active during the follow up, the three indicators persisted and even tended to be aggravated [150].

7. CONCLUSIONS

The atopic march is a useful paradigm to describe the clinically observed progression of atopic diseases in certain children and refers to the progression from atopic eczema to allergic asthma in children.

The first years of life are a critical period for the development of atopy in relation to exposure to environmental agents. If the skin sensitization occurring in eczema appears to be the trigger for the subsequent development of other allergic diseases [17], the airways of infants are still in development and more sensitive to the exposure to many environmental factors, contributing to the atopic march in young children.

Environmental factors can modify the immune-inflammatory processes that occur early in life [77]. Among these, the chlorine and its by-products contaminating the water and the air of swimming pools are powerful oxidizers, which can damage the epithelial barriers protecting the respiratory tracts of the swimmers. Early exposure to chlorine by products used for the disinfection of pools appears to alter the epithelial barrier of the skin and of the airways, favouring the entry of allergens and the development of atopic diseases. Among infants or young children, the epithelial changes led by the products of chlorination in swimming pool can exercise an adjuvant-role in the raising allergic sensitization and thus in the development of the atopic ground.

The identification of early life diseases in the atopic march as predictors for the development of lifelong chronic diseases offers entry points for primary or secondary disease prevention [10]. They are modifiable and interventions that could be developed to halt the progression along the atopic march in a young child with atopy, eczema or early wheeze. Primary prevention measures for atopic diseases involve the avoidance of early allergen exposure to certain foods and inhalants [160]. The prevention crosses also by standards and stricter controls of the quality of the water and the air of swimming pools and if possible by the use of alternative disinfection methods. While waiting for these measures, the implementation of which for the public swimming pools will doubtless take time, we can only recommend to be cautioned with the attendance of establishments with a very strong chlorine smell. The study of the toxic effects of chlorination products on children is only a beginning and given the toxic properties of these substances, we cannot exclude other noxious effects.

8. PERSPECTIVES

Our study illustrates the great potential of non-invasive biomarkers of airways inflammation or allergic sensitization to explore lifestyle or environmental factors that might be implicated in the rise of allergic diseases. This project represents a first step in this approach. The study of children at young ages by means of non-invasive biomonitoring is feasible and provides useful information. We have various biological samples (serum, nasal lavage fluid, urine) from different cohorts including competitive swimmers and adolescents. The analysis of biomarkers of airways inflammation or damage in these cohorts is currently under progress. Promising results have been obtained but confirmation is needed in independent study populations. Expanded studies are needed with larger patient groups that are clinically well characterized.

Next to diagnostic markers, there is also a strong need for early warning biomarkers. Early warning biomarkers are sensitive signals, which allow taking measures before diseases become manifest. Some exposure ways are considered in this study but we need more information to know exactly how the sensitization occurs. To develop these markers, the study has started with the prospective approach but the study needs to be expanded and should include a larger study population that is followed up over a longer time period of time.

Results available to date provide additional evidence that the attendance of chlorinated swimming pools can cause chronic epithelium defects increasing the risks of asthma and allergic rhinitis in subjects sensitized to allergens. More interestingly, these results suggest that these airways defects may promote the process of allergic sensitization i.e. the development of atopy itself (Bernard et al, manuscript under preparation). Of course, all these observations are based on cross-sectional studies so that the direction of causality cannot be determined with certainty. In the future, efforts should be made to apply such tests in prospective studies comparing for instance swimmers in chlorinated –pools with those attending non-chlorinated pools. Further research is also needed to identify the chemicals or chemical mixtures in the air or water of chlorinated pools that are responsible for the airways defects. In that respect, it would be particularly useful to determine under which form chlorination products are the most detrimental to airways, whether it is in the form of gases (e.g. trichloramine), aerosols (active chlorine, monochloramine…;) or even of water that these chemicals are the most toxic to airways epithelium. This information is of course needed to implement preventive strategies based on ventilation, water renewal or treatment or the use of alternative disinfection methods (copper-silver, ozone,..).

The specification of the critical time window would be also interesting in order to identify the period and the mechanism in the course of which children are made most vulnerable to the exposure to these risk factors. The project illustrates again that early life exposures may be among the most vulnerable stages of life for the determination of health at an older age. Protecting the child and its environment may have a significant effect on the disease burden of the population.

9. SCIENTIFIC VALORISATION

1. Publications

Published articles

1. Voisin C, Sardella A, Marcucci F, Bernard A. Infant swimming in chlorinated pools and the risks of bronchiolitis, asthma and allergy. European Respiratory Journal. January, 2010 (Article 2)
2. Voisin C, Bernard A. Risques d'affections allergiques associés aux produits de chloration en piscine. Environnement, Risques et Santé, 2008
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In press

Voisin C, Bernard A. Allergic sensitization and airway inflammation after early swimming. Research letter. Am J Respir Crit Care Med (Article 4)

Submitted articles

1. Voisin C, Sardella A, Bernard A. Risks of new-onset allergic sensitization and airway inflammation after early swimming in chlorinated pools. Int J Hygiene environ Health (Article 3)
2. Bloemen K, Van Den Heuvel R, Koppen G, Peirsman E, Nelen V, Voisin C, Witters E, Bernard A, Desager K, Schoeters G. Non-invasive biomarker measurements in children with asthma and allergies. European Respiratory Journal

2. Oral Communications

Abstracts

1. "*Indoor risk factors for childhood respiratory diseases: application of non-invasive biomarkers in a prospective study with schoolchildren*". ANIMO-COPHES Workshop Non-invasive sampling techniques for human biomonitoring, Brussels, December 2010.
2. "*Risks of allergic sensitization associated with infant swimming*". American Thoracic Society-New Orleans-USA, May, 2010. Am J RespirCrit Care Med 181;2010:A6238.
3. "*Swimming during infancy increases the risk of bronchiolitis*". European Respiratory Symposium-Vienna-Austria, September 2009.
4. "*Horse riding, pool attendance and the risks of asthma and respiratory allergies*". European Respiratory Symposium-Stockholm-Sweden, September 2008.
5. "*Risks of allergic and respiratory diseases associated with early swimming in indoor chlorinated pools*". Pool and Spa Conference-Munich-Germany, March 2007.

2.1. Indoor risk factors for childhood respiratory diseases: application of non-invasive biomarkers in a prospective study with schoolchildren

Catherine Voisin.

Abstract

Children's respiratory health is among the priorities of environmental health programs. There is clear evidence that children are more susceptible to some stressors in the environment. Respiratory diseases are a major cause of illness in children in developed countries and asthma and allergies are increasing even up to 30% in certain age groups. Environmental factors are thought to affect a child's likelihood to develop these diseases but are still largely unknown.

The ANIMO project addresses children's respiratory health by developing non-invasive Biomarkers easily applicable in children that enable us to detect adverse effects in an early stage allowing preventive measures to be taken before disease outbreak, and which can be used in environmental research. We conducted an epidemiological study in 5 years-old children since September 2007 to May 2010. At 5 and 7 years old, a agreement form and a questionnaire - inquiring about health, family history, environment and life style- were filled by the parents. Children were examined and tested twice with the following measurements: collection of exhaled breath condensate, spirometric tests, measurement of weight and length and of exhaled NO, collection of urine sample and of a nasal lavage, screening of sensitization to the most prevalent aeroallergens using the Rhinosticks. We have recruited and examined a cohort of 394 young children originated from schools located in urban and rural areas. The protocol of the study could be applied successfully to almost all children at the exception of the rhinostick test that a few children (less than 10%) refused to perform. The exhaled NO, nasal lavage and EBC test did not pose any problem. Almost all children could also provide a sample or urine.

If the results obtained with EBC were not conclusive, exhaled NO values, rhinosticks and measurement of CC16 in urine and in the nasal lavage provide interesting information about the environmental exposure and the screening of some respiratory diseases or symptoms.

Acknowledges

This study was supported by the Belgian Science Policy (Contract number SD/HE/05A: ANIMO project). We thank the patients and volunteers for sample donation.

2.2. Risks of allergic sensitization associated with infant swimming

C.F. Voisin, A. Sardella, F. Marcucci, A. Bernard.

Introduction

Recent studies suggest that attendance at indoor or outdoor chlorinated swimming pools during childhood may increase the risk of developing allergic diseases such as asthma and hay fever. This has led to the chlorine hypothesis postulating that by disrupting airways epithelial barriers, chlorination products might exert an adjuvant effect in the clinical manifestation of atopy or in the allergic sensitization itself. In the present study, we have further explored this hypothesis by examining whether infant swimming in chlorinated pools is associated with an increased risk of sensitization to aeroallergens.

Methods

We examined 430 children (47% of girls, mean age, 5 years) in 30 Belgian kindergarten schools. Allergic sensitization against pollen, cat and house dust mite was screened by using the rhino-sticks. Information about attendance at indoor or outdoor chlorinated pools before two years and traditional risk factors of allergies was obtained by parental self-administered questionnaire. Logistic regression analyses were used to evaluate associations between pool attendance and allergic sensitization while adjusting for other risk factors (parental asthma, smoking, day care...).

Results

There was no difference in the rates of aeroallergen sensitization between boys and girls (P values > 0.10). Risk factors for allergic sensitization varied with the type of aeroallergen and were noticeably different between girls and boys. Among boys, ever attendance at outdoor chlorinated during infancy was associated with higher risks of sensitization to house dust mite (OR, 3.83, 95 % CI 1.54-9.52), cat (OR, 4.21, 95 % CI 1.22-14.5) and pollen (OR, 2.46, 95 %CI 1.02-6.26). Boys in the highest tertile of cumulative outdoor pool attendance were approximately 7 (OR, 7.02, 95 % CI 1.81-27.2), 3 (OR, 2.61, 95% CI 1.04-6.59) and 4 (OR, 3.79, 95% CI 1.02-14.1) times more likely to be sensitized against house dust mite, pollen and cat allergens, respectively, as compared with their peers in the lowest tertile. Such associations were not found among girls nor with the attendance at indoor chlorinated pools.

Conclusions

Boys regularly attending outdoor chlorinated pools during infancy show higher risks of being sensitized to common aeroallergens. The fact these risks emerge with outdoor but not with indoor pools might be explained by differences in time spent in the pool and/or in exposure levels to chlorine or aeroallergens.

Supported by the Belgian Federal Government (ANIMO project).

2.3. Swimming during infancy increases the risk of bronchiolitis

C. Voisin, A. Bernard.

Introduction

Recent studies have suggested that swimming during infancy may increase the risk of wheezing and lower respiratory tract infections. The possible association of infant swimming with bronchiolitis specifically has not been explored yet.

Objective

We assessed whether chlorinated pool attendance before the age of two is related to higher risks of bronchiolitis.

Methods

We examined 425 children (47% of girls, mean age, 5 yrs) in 30 kindergarten schools. Information about medical history, chlorinated pool attendance and other risk factors was obtained by questionnaire. Logistic regression analyses were used to evaluate associations between pool attendance and bronchiolitis after adjustment for other risk factors (parental asthma, smoking, day care...). Children who had never attended a swimming pool during infancy served as referents.

Results

The risk of bronchiolitis was increased among children who had ever attended an indoor pool (OR 1.71, 95% CI 1.15-2.64), an outdoor pool (OR 1.37, 95% CI 0.84-2.25) or either type of pools (OR 1.87, 95% CI 1.18-2.97). For both types of pools, analyzed separately or in combination, the risk of bronchiolitis increased with the number of hours spent in the pool. The ORs associated with the attendance at either type of pool increased to 1.54 (95% CI 0.85-2.80) after 1-19 h and to 2.07 (95% CI 1.24-3.44) after more than 20 h spent in pools (p for trend, p=0.02). Excluding children with parental asthma or maternal smoking during pregnancy strengthened these associations (p for trend < 0.01).

Conclusion

Attendance at chlorinated pools during infancy is associated with an increased risk of bronchiolitis.

This study is supported by the Belgian Science Policy (ANIMO project).

2.4. Horse riding, pool attendance and the risks of asthma and respiratory allergies

Catherine Voisin, Marc Nickmilder and Alfred Bernard.

Background

Living in contact with farm animals appears to protect children against the development of asthma and allergic diseases.

Objective

To investigate whether the protective effect of farming environment extends to horse riding while taking into account the influence of other sport activities.

Methods

Cross-sectional study of 857 adolescents aged 13-19 years from three secondary schools, including 77 horse-riding adolescents. Examination of adolescents included a questionnaire, an exercise-induced bronchoconstriction test (FEV15) and the measurement of total and specific serum IgE.

Results

The prevalences of asthma and respiratory allergies were not significantly different between adolescents participating to horse riding and the others. While no influence of other sports was found, a significant interaction emerged between horse riding and the attendance of an open air chlorinated swimming pool. The risk of doctor-diagnosed asthma or of total asthma (diagnosed and/or FVE15) was significantly increased in horse riding adolescents who had regularly attended an outdoor pool (n = 42, cumulated pool attendance > 50 hr) (OR, 2.53, 95th CI 1.02-6.3 and OR, 2.12, 95th CI 1.00-4.65, respectively). These adolescents had also a higher risk of being sensitized against house-dust mite (OR, 2.26, 95th CI 1.04-4.91) but not against pollen and pets.

Conclusion

Horse riding and swimming attendance appear to interact to increase the risks of asthma and HDM allergy.

Supported by the Afsset, France and the FNRS, CFWB-MRW, Belgium

2.5. Risks of allergic and respiratory diseases associated with early swimming in indoor chlorinated pools

Catherine Voisin, Marc Nickmilder, Alfred Bernard

Background

Irritant gases and aerosols contaminating the air of indoor swimming pools can cause detrimental effects on the airways and increase the risks of asthma. We have conducted a questionnaire-based survey in order to further assess these risks in a population of schoolchildren.

Methods

A total of 372 schoolchildren, aged 10 to 12 years, participated to the study. They were recruited from 10 primary school in the city of Liège in Belgium (mean response rate, 80%). Information about the respiratory health of children, their familial antecedents and their exposure to various environmental or lifestyle risk factors including swimming pool attendance was obtained by a questionnaire filled by the parents. Logistic regression analyses were used to study the effects of swimming practice by adjusting for possible confounders.

Results

The baby swimming practice was associated with an increased risk of wheezing (OR 6.6, $p<0.001$), hay fever (OR 4.6, $p=0.01$) and allergy to pets (OR 3.23, $p=0.02$). Stratification of children according to their attendance of swimming pools from different age showed that children having swum as baby had the highest prevalence of wheezing (20%), followed by those having started swimming during kindergarten (6.3%) or primary school (6.0%) and those having never attended a swimming pool (3.3%) (Chi^2 for trend, $p=0.04$). There was also a significant association between the risk of doctor-diagnosed asthma and the lifetime cumulated pool attendance (OR, 1.1, $p=0.04$ for each 100 hours cumulated pool attendance). These effects persisted when considering only children without a family history for the studied endpoint.

Conclusions

Children regularly attending indoor chlorinated swimming pools, particularly during early childhood, appear to be at higher risk of developing some respiratory and allergic diseases.

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11. APPENDICES

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Woluwe, 18 janvier 2008

A l'attention de chaque parent,

Madame, monsieur,

Nous nous permettons de solliciter votre collaboration à notre projet de recherche. Notre équipe de recherche participe à un projet fédéral en collaboration avec le VITO et l'université d'Anvers qui va permettre d'étudier les risques d'affections respiratoires et d'allergies chez l'enfant liés à la qualité de l'air intérieur. Vous trouverez ci-dessous les détails et informations relatives à ce projet mais nous restons bien entendu disponibles pour tout renseignement complémentaire.

ANIMO

Risques d'affections respiratoires chez l'enfant liés à la qualité de l'air intérieur : développement et application de biomarqueurs non-invasifs

1. QUEL EST LE BUT DE CETTE ETUDE ?

Notre environnement est par définition notre lieu de vie, qu'il s'agisse de l'atmosphère qui nous entoure, de l'air de nos espaces intérieurs, l'eau que nous buvons ou encore notre alimentation. Il a un impact incontestable sur nous et sa détérioration peut potentiellement altérer notre santé. L'étude de ses impacts sur notre santé est ainsi devenue une des préoccupations primordiales de notre société. Actuellement, des outils de plus en plus performants sont développés pour mieux comprendre ces relations entre santé et environnement.

L'objectif de l'étude que nous menons actuellement s'inscrit dans ce cadre pour une meilleure compréhension de ces relations chez les enfants et elle a pour but d'identifier les sources de pollution (polluants de l'air, contaminants alimentaires, …) pouvant être impliquées dans l'augmentation moderne d'affections allergiques telles que l'asthme, l'eczéma, le rhume des foins,…. Pour la mener à bien, nous sollicitons votre participation ainsi que celle de votre enfant et nous vous en remercions d'avance.

Cette étude a reçu un avis favorable de la commission d'éthique biomédicale de l'Université catholique de Louvain et est soutenue par le service public fédéral de programmation politique scientifique de Belgique.

2. EN QUOI CONSISTE LA PARTICIPATION DE VOTRE ENFANT?

Si vous êtes d'accord que votre enfant à cette étude, on vous demandera de compléter un questionnaire sur son état de santé, ses antécédents familiaux, votre habitation, votre environnement et ses activités sportives.

Votre enfant sera également invité à effectuer quelques tests pendant une partie de matinée scolaire au cours de laquelle :

- Il sera pesé(e) et mesuré(e)
- On lui demandera d'effectuer des tests respiratoires ; c'est-à-dire de souffler dans des appareils. (Spirométrie de base, Niox, EBC)
- Un tampon sera aussi appliqué pour collecter du liquide nasal afin de dépister les allergies
- On recueillera également un échantillon d'urine

Ces tests seront réalisés au sein de l'école de votre enfant au cours d'une matinée scolaire et en présence d'un médecin.
Nous insistons vraiment sur le fait que tous ces tests sont absolument non invasifs et tout à fait indolores.

Même si vous ne désirez pas que votre enfant participe, pourriez-vous nous rendre le questionnaire complété afin que nous puissions nous assurer qu'il n'y aura aucun biais de sélection entre les participants et les non participants (même sans nom ni prénom si vous le préférez)?

3. QUELS SONT LES RISQUES ET INCONVENIENTS EVENTUELS ASSOCIES A L'ETUDE ?

Votre enfant ne prend aucun risque à participer à cette étude. Les tests respiratoires sont des examens indolores qui n'entraînent aucun inconvénient particulier.

4. QUELS SONT LES BENEFICES ASSOCIES A L'ETUDE ?

Grâce à cette étude scientifique, vous bénéficierez gratuitement d'une évaluation de la fonction respiratoire et d'un dépistage des allergies les plus communes (acariens, chat et pollens) à partir du liquide récolté au niveau nasal. Les résultats des mesures seront envoyés à votre domicile.

Comme nous l'avons déjà signalé plus haut, le but de l'étude est d'identifier des facteurs de risque de maladies respiratoires liés aux polluants présents dans l'environnement intérieur et extérieur. Ces observations contribueront à établir un ensemble de recommandations (normes et dispositifs pour des groupes à risques, …) et à accroître l'efficacité d'une politique de prévention contre ces maladies.

5. QU'EN EST-IL DU RESPECT DE LA CONFIDENTIALITE ?

Le secret médical et les exigences légales en matière de vie privée seront respectés (en conformité avec la loi belge du 8 décembre 1992).

Votre identité et les données vous concernant seront traitées de façon confidentielle. L'ensemble des informations figurant dans le questionnaire, le prélèvement d'urine et les mesures respiratoires seront identifiés par un numéro anonyme. Les informations ainsi codifiées seront traitées par les chercheurs qui analyseront l'ensemble des réponses au questionnaire et les résultats des mesures effectuées. Si vous le désirez, vous pourrez, à tout moment de l'étude, avoir accès ou modifier les données concernant votre enfant.

6. COUTS ?

La participation à cette étude ne vous coûtera rien financièrement. Votre enfant sera remercié(e) de sa participation par un petit cadeau.

7. LA PARTICIPATION EST VOLONTAIRE

Votre enfant et vous avez le droit de refuser de participer à cette étude et il pourra la quitter à tout moment.

8. QUELLE UTILISATION SERA FAITE DES RESULTATS DE CETTE ETUDE ?

Dans un but d'information, le rapport final de l'étude sera publié dans une revue scientifique internationale. L'article ne comportera aucune donnée personnelle vous concernant.

9. CONTACTS

N'hésitez pas à nous contacter si vous aviez la moindre question ou inquiétude, nous nous ferons un plaisir d'en discuter avec vous.

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ANIMO

Risques d'affections respiratoires chez l'enfant liés à la qualité de l'air intérieur : développement et application de biomarqueurs non-invasifs

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FORMULAIRE DE CONSENTEMENT (A RENDRE COMPLETE ET SIGNE)

Nous, soussignés, ... (nom et prénoms du père et de la mère en majuscules ou des personnes responsables de l'enfant), parents ou responsable(s) de …….. (nom et prénom de l'enfant en majuscules), confirmons que l'équipe de recherche de toxicologie de l'Université Catholique de Louvain a demandé que notre enfant participe à l'étude intitulée «Risques d'affections respiratoires chez l'enfant liés à la qualité de l'air intérieur : développement et application de biomarqueurs non-invasifs ».

Cette étude est soutenue par le service public fédéral de programmation politique scientifique de Belgique. La Commission d'Ethique de l'Université catholique de Louvain a donné un avis favorable à la réalisation de cette étude.

Nous comprenons le but de l'étude à laquelle il est demandé que notre enfant participe. Nous comprenons que si notre enfant participe à cette étude, nous acceptons de remplir le questionnaire. Notre enfant accepte d'avoir des tests respiratoires et de donner un peu d'urine et nous n'y sommes pas opposés. Nous avons été informés que la participation à cette étude ne nous occasionnera aucun frais mais ne nous donne droit à aucune indemnité.

Notre consentement ne dégage pas les chercheurs de leurs responsabilités. Notre enfant garde tous les droits qui lui sont garantis par la loi.

A tout moment, la participation de notre enfant peut être arrêtée, selon son désir ou le nôtre. Nous en informerons le Professeur Bernard. Les données qui concernent notre enfant resteront confidentielles.

Nous pourrons à tout moment demander toute information complémentaire au Professeur Bernard.

◊ **Nous sommes d'accord que notre enfant participe à cette étude dans les conditions précisées ci-dessus : OUI / NON (entourer la réponse qui convient).**

◊ Signature des parents ou responsable(s) de l'enfant :

Fait à ……, le ……

ANIMO : Environnement et santé des enfants : étude épidémiologique des facteurs de risque

Questionnaire sur la santé de votre enfant et son environnement

Pour chaque question, entourez la mention adéquate ou noircissez le carré.

Son identité

Nom : …… Prénom : ……

Taille : ……cm Poids : ……kg

1. Date de naissance : ……
2. Adresse : rue : …… N° : ……

Commune : …… Code Postal : ……

Téléphone : …… @-mail des parents : ……

3. Sexe : ☐ Masculin ☐ Féminin
4. Nationalité : ☐ belge
   ☐ non belge

Si non belge, précisez : 4.1 Pays d'origine : ……

4.2 Date d'arrivée en Belgique : ……

Sa Santé

5. Au cours des 12 derniers mois,
   - 5.1 A-t-il eu une respiration sifflante ? Oui ☐ Non ☐
   - 5.2 A-t-il été réveillé(e) par une sensation de poids sur la poitrine ? Oui ☐ Non ☐
   - 5.3 A-t-il été réveillé(e) par une sensation de manque d'air ? Oui ☐ Non ☐
   - 5.4 A-t-il été réveillé(e) par une crise de toux ? Oui ☐ Non ☐
   - 5.5 A-t-il été réveillé(e) par une crise d'asthme ? Oui ☐ Non ☐
   - 5.6 A-t-il eu des éternuements, le nez qui coule ou le nez bouché (sans avoir de rhume, de rhino-pharyngite ou grippe) Oui ☐ Non ☐
   - 5.7 Quelle(s) maladie(s) a-t-il eue(s) :……
   - 5.8 A-t-il eu des diarrhées ? : Oui ☐ Non ☐
   - 5.9 A-t-il eu de la température ? (supérieure à 38.5°) : Oui ☐ Non ☐

6. Depuis sa naissance, a-t-il eu les maladies suivantes (diagnostic fait ou confirmé par un médecin) ?
   - 6.1 Asthme : Oui ☐ Non ☐
   - 6.2 Bronchite : Oui ☐ Non ☐
   - 6.3 Bronchiolite : Oui ☐ Non ☐
   - 6.4 Pneumonie ou broncho-pneumonie : Oui ☐ Non ☐
   - 6.5 Eczéma : Oui ☐ Non ☐
   - 6.6 Verrue : Oui ☐ Non ☐
   - 6.7 Mycose (ex : champignon aux pieds) : Oui ☐ Non ☐
   - 6.8 Conjonctivite : Oui ☐ Non ☐
   - 6.9 Rhume des foins : Oui ☐ Non ☐
   - 6.10 Rhinite allergique : Oui ☐ Non ☐
   - 6.11 Rhume (plus de 4 fois/an) : Oui ☐ Non ☐
   - 6.12 Sinusite (au moins 1fois/an) : Oui ☐ Non ☐
   - 6.13 Otite (au moins 1fois/an) : Oui ☐ Non ☐
   - 6.14 Infection urinaire : Oui ☐ Non ☐

Appendix 3- Questionnaire phase 1

6.15 Diabète : Oui □ Non □
6.17 Hépatite : Oui □ Non □
6.18 Méningite : Oui □ Non □
6.19 Démangeaisons : Oui □ Non □
6.19 Autres affections : ……

7. Si une des maladies suivantes a été diagnostiquée, pouvez-vous préciser à quel âge ?
   7.1 Asthme : ……
   7.2 Eczéma : ……
   7.3 Rhinite allergique : ……
   7.4 Diabète : ……
   7.5 Méningite : ……
   7.6 Hépatite : ……

8. Quels sont les différents vaccins que votre enfant a eus et quand ?
   8.1 ……en ……
   8.2 …… en ……
   8.3 …… en ……
   8.4 …… en ……
   8.5 …… en ……
   8.6 …… en ……

9. A-t-il ou a-t-il eu des caries ? Oui □ Non □
   Si oui, combien d'entre elles ont été soignées (plombages) ? ……

10. Depuis sa naissance, a-t-il eu des allergies ?
    10.1 Alimentaires ? Oui □ Non □
    10.2 Aux œufs ? Oui □ Non □
    10.3 Aux acariens ? Oui □ Non □
    10.4 Aux poils d'animaux ? Oui □ Non □
    10.5 Aux pollens ? Oui □ Non □

11. A-t-il pris ou prend-il des médicaments ?
    11.1 Pour les allergies ? Oui □ Non □
    11.2 Pour l'asthme ? Oui □ Non □
    11.3 Si oui, lesquels ? ……
    11.4 Des antibiotiques ? : Oui □ Non □
    11.5 si oui, quand et lesquels ?: ……

12. Depuis sa naissance, a-t-il été hospitalisé ?
    12.1 Pour infection respiratoire ? Oui □ Non □
    12.2 Pour asthme ? Oui □ Non □

13. Pendant la nuit, combien de fois votre enfant se réveille-t-il ? : ……

14. Sa maman a-t-elle fumé pendant sa grossesse? Oui □ Non □

15. Quel était son poids à la naissance ? ……grammes

16. Est-il né(e) à terme (entre 39 et 41 semaines de grossesse) ? Oui □ Non □
    Si non, à combien de semaines gestationnelles est-il né(e) ? ……

17. A-t-il été allaité(e) ? Oui □ Non □
    Si oui, pendant combien de mois ? ……

18. Ses biberons étaient-ils préparés avec :

□ De l'eau du robinet ? □ De l'eau du robinet filtrée (Brita) ? □ De l'eau en bouteille ?

19. Pendant sa petite enfance :

19.1 A-t-il mangé des petits pots ? Jamais □ 1 fois/sem □ 2 fois et plus/sem □

19.2 A-t-il eu une tétine ? Oui □ Non □

20. Entre 0 et 2 ans, allait-il à la crèche ? Oui □ Non □

20.1 Si oui, de quel âge à quel âge ? ……

20.2 A quelle fréquence ?

□ 1 jour/sem □ 2 jours/sem □ 3 jours/sem □ 4 jours/sem □ 5 jours/sem

21. Entre 0 et 2 ans, vivait-il ? : □ En ville □ A la campagne

22. Depuis son enfance, boit-il l'eau du robinet ?

□ Oui □ Rarement □ Jamais

23. Votre enfant consomme-t-il les aliments suivants ?

23.1 Pain : Oui □ Non □
Si oui, à quelle fréquence ? :……
23.2 Probiotiques (Actimel, Yakult, …) : Oui □ Non □
Si oui, à quelle fréquence ? :……
23.3 Produits laitiers enrichis au bifidus : Oui □ Non □
Si oui, à quelle fréquence ? :……
23.4 Yaourt, fromage blanc : Oui □ Non □
Si oui, à quelle fréquence ? :……
23.5 Lait frais non pasteurisé : Oui □ Non □
Si oui, à quelle fréquence ? :……
23.6 Produits à base de soja : Oui □ Non □
Si oui, à quelle fréquence ? :……
23.7 Légumes : Oui □ Non □
Si oui, à quelle fréquence ? :……
23.8 Pâté : Oui □ Non □
Si oui, à quelle fréquence ? :……
23.9 Viande : Oui □ Non □
Si oui, à quelle fréquence ? :……
23.10 Fruits frais : Oui □ Non □
Si oui, à quelle fréquence ? :……
23.11 Poisson : Oui □ Non □
Si oui, à quelle fréquence ? :……
23.12 Œufs : Oui □ Non □
Si oui, à quelle fréquence ? :……
23.13 Lait : Oui □ Non □
Si oui, à quelle fréquence ? :……
23.14 Chips : Oui □ Non □
Si oui, à quelle fréquence ? :……
23.15 Gâteaux, biscuits ? : Oui □ Non □
Si oui, à quelle fréquence ? :……
23.16 Produits Beneo ? : Oui □ Non □
Si oui, à quelle fréquence ? :……
23.17 Noix ? : Oui □ Non □

Appendix 3- Questionnaire phase 1

Si oui, à quelle fréquence ? :……

23.23 Cacahuètes ? : Oui □ Non □

Si oui, à quelle fréquence ? :……

Environnement et habitation

24. A proximité de chez vous, y a-t-il ?

24.1 Une installation industrielle (usine, ….) : Oui □ Non □

Si oui, laquelle ? ……, à quelle distance ? …… km

24.2 Un incinérateur, décharge, déchetterie, traitement des déchets : Oui □ Non □

Si oui, quel est son nom ? ……, à quelle distance ? …… km

24.3 Un aéroport à moins de 5 km : Oui □ Non □ Si oui, lequel ? ……

25. Votre habitation se trouve-t-elle près d'une route avec un trafic important (ex : chaussée, boulevard, nationale, autoroute) ? Oui □ Non □

Si oui, à quelle distance ? : □ moins de 10 mètres
□ moins de 100 mètres
□ plus de 100 mètres

26. Y a-t-il ou y avait-il un ou plusieurs animaux à la maison ?

□ Aucun animal
□ Un ou des animal(aux) à poils : □ chat □ chien
□ Un ou des animal(aux) à plumes
□ Autre à préciser : ……

26.1 Depuis quel âge, vit-il avec lui (eux) ? ……

26.2 Laissez-vous entrer l'(es) animal(aux) dans sa chambre ? Oui □ Non □

27. De combien de chambres votre habitation est-elle constituée ?

□ 1 □ 2 □ 3 □ 4 □ 5 □ 6 □ 7 □ 8 ou plus

28. Les chambres sont-elles ventilées ou aérées ?

1 fois/jours □ 1 fois/2 jours □ 1fois/sem □ 1 fois/mois □ jamais □

29. Utilisez-vous des aérosols ou des parfums d'ambiance ?

29.1 Si oui, à quelle fréquence ? :

□ 4fois/mois □ 3 fois /mois □ 2 fois /mois □ 1fois/mois □ jamais

30. Pour le nettoyage du sol sans tapis, utilise-t-on des produits à base d'eau de javel ?

Oui □ Non □

30.1 Si oui, à quelle fréquence ? :

□ 4fois/mois □ 3 fois /mois □ 2 fois /mois □ 1fois/mois □ jamais

30.2 Si non, quel produit utilise-t-on ? ……

31. Y a-t-il une piscine chez vous ? Oui □ Non □

31.1 Si oui, est-elle ? □ A l'intérieur □ A l'extérieur

31.2 Est-elle ? □ gonflable □ construite dans le sol

31.3 Est-elle désinfectée au chlore ? Oui □ Non □

31.4 Quand y va-t-il ? □ en été □ toute l'année

31.5 Depuis quel âge va-t-il dans cette piscine ? ……

31.6 Combien de mois par an ? ……

31.7 Combien d'heures par semaine pendant la période où il y va ? ……

Ses activités sportives

32. Quel(s) type(s) d'activité extrascolaire fait-il régulièrement (au moins une heure par semaine) ?
a) …… b) …… c) ……

33. Fait-il de l'équitation ? Oui □ Non □
Si oui, depuis quel âge ? : ……

34. Allait-il à la piscine avant l'âge de 2 ans ? : Oui □ Non □
Si oui, 34.1 Etait-ce des cours de « bébé nageur » ? Oui □ Non □
De quel âge (mois) à quel âge ? ……
A quelle fréquence ? □ 1 fois/sem □ 1 fois /15 jours □ 1 fois/mois
Combien de temps par séance ? □ 10 min □ 20 min □ 30 min □ 1h
Quel était le nom de la piscine ? ……
34.2 Etait-ce comme loisir avec ses parents ? Oui □ Non □
De quel âge à quel âge ? ……
A quelle fréquence ? □ 1 fois/sem □ 1 fois /15 jours
□ 1 fois/mois □ 1 fois/3 mois
Combien de temps par séance ? □ 10 min □ 20 min □ 30 min □ 1h
Quel était le nom de la piscine ? ……

35. Depuis l'âge de deux ans, va-t-il ou est-il allé à la piscine dans un club, avec ses parents ou avec des amis ? Oui □ Non □
Si oui, 35.1 De quel âge à quel âge ? ……
35.2 A quelle fréquence ? □ 1 fois/sem □ 1 fois/15 jours □ 1 fois/mois
□ 1 fois/3mois □ 1 fois/ans □ jamais
35.3 Combien de temps par séance ? □ 30 min □ 1 h □ 1 h 30 □ 2 h
35.4 Quel est le nom de la piscine ? ……

36. Est-il déjà allé dans des lieux de villégiature avec piscine ?
36.1 Si oui, la piscine était-elle ? □ A l'intérieur □ A l'extérieur
36.2 Est-elle désinfectée au chlore ? Oui □ Non □
36.3 Combien de fois a-t-il bénéficié de ce type de vacances ? ……
36.4 Depuis quel âge ? ……
36.5 Combien de semaines par an ? ……
36.6 Combien d'heures par semaine pendant la période où il y va ? ……

Sa famille

37. De combien de personnes son foyer est-il constitué (en le comptant) ? :
□ 2 □ 3 □ 4 □ 5 □ 6 □ 7 □ 8 ou plus

38. A-t-il des frères et/ou des sœurs ? □ Oui □ Non
Si oui, 38.1 Combien ?……
38.2 Combien de sœurs et de frères sont-ils plus âgés que lui ?
□ Aucun □ 1 □ 2 □ 3 □ 4 ou plus

39. Parmi ses frères et/ou sœurs, certains ont-ils ou ont-ils eu ? :
    39.1 De l'asthme : Oui □ Non □
    39.2 Des allergies : Oui □ Non □

40. A la maison,
    40.1 Sa maman fume-t-elle ? Oui □ Non □
    40.2 Son papa fume-t-il ? Oui □ Non □

41. D'autres personnes à la maison fument-elles ? : Oui □ Non □
    Si oui, combien ? □ 1 □ 2 □ 3

42. Sa maman souffre-t-elle de ?
    42.1 Eczéma : Oui □ Non □
    42.2 Asthme : Oui □ Non □
    42.3 Rhume des foins : Oui □ Non □
    42.4 Allergies : □ Acariens □ Poils d'animaux □ Pollen □ Alimentaires

43. Son papa souffre-t-il de ?
    43.1 Eczéma : Oui □ Non □
    43.2 Asthme : Oui □ Non □
    43.3 Rhume des foins : Oui □ Non □
    43.4 Allergies : □ Acariens □ Poils d'animaux □ Pollen □ Alimentaires

44. Concernant les études et les activités de ses parents ?
    44.1 Maman : quelle est son activité professionnelle ? ……
    quelles études a-t-elle terminées ?
    □ L'école primaire □ L'enseignement secondaire
    □ Etudes supérieures non universitaires □ Etudes universitaires
    44.2 Papa : quelle est son activité professionnelle ? ……
    quelles études a-t-il terminées ?
    □ L'école primaire □ L'enseignement secondaire
    □ Etudes supérieures non universitaires □ Etudes universitaires

Date et Signature ……

Glissez le questionnaire dans l'enveloppe que vous fermerez et sur laquelle vous écrirez son nom et son prénom ainsi que son école. Un grand merci pour votre collaboration !!!!

Environnement et santé des enfants : étude épidémiologique des facteurs de risque - PHASE 2

Pour chaque question, entourez la mention adéquate ou noircissez le carré.

Son identité

Nom : …… Prénom : ……

1. Date de naissance : ……
2. Adresse : rue : …… N° : ……
   Commune : …… Code Postal : ……
   Téléphone : …… @-mail des parents : ……
3. Sexe : □ Masculin □ Féminin
4. Nationalité : □ belge
   □ non belge
   Si non belge, précisez : 4.1 pays d'origine : ……
   4.2 date d'arrivée en Belgique : ……
5. Poids : ……kg
   Taille : ……cm
6. Date de naissance de la maman : ……

Sa santé

7. Au cours des 12 derniers mois,
   - 7.1 A-t-il eu une respiration sifflante ? Oui □ Non □
   - 7.2 A-t-il été réveillé(e) par une sensation de poids sur la poitrine ? Oui □ Non □
   - 7.3 A-t-il été réveillé(e) par une sensation de manque d'air ? Oui □ Non □
   - 7.4 A-t-il été réveillé(e) par une crise de toux ? Oui □ Non □
   - 7.5 A-t-il été réveillé(e) par une crise d'asthme ? Oui □ Non □
   - 7.6 A-t-il eu des éternuements, le nez qui coule ou le nez bouché (sans avoir de rhume, de rhino-pharyngite ou de grippe) Oui □ Non □
   - 7.7 A-t-il pris des médicaments pour l'asthme Oui □ Non □
8. Au cours de 2 dernières années, quelle(s) maladie(s) a-t-il eue(s) :
   ……
9. Au cours des 2 dernières années, votre enfant a-t-il été vacciné ? Oui □ Non □
   Si oui, quels vaccins et quand? ……
10. Si une des maladies suivantes a été diagnostiquée, pouvez-vous préciser à quel âge ?
    - 10.1 Asthme : ……
    - 10.2 Eczéma : ……
    - 10.3 Rhinite allergique : ……
    - 10.4 Diabète : ……
    - 10.5 Méningite : ……
    - 10.6 Hépatite : ……
11. Combien de plombages votre enfant a-t-il en bouche ? ……
12. A-t-il des allergies ?
    - 12.1 Alimentaires ? Oui □ Non □
      Si oui, pour quel aliment ……

Appendix 4- Follow-up questionnaire

12.2 Aux acariens ? Oui □ Non □

12.3 Aux poils d'animaux ? Oui □ Non □

Si oui, pour quel animal ? ……

12.4 Aux pollens ? Oui □ Non □

12.5 Autres allergies ? Oui □ Non □

Si oui, lesquelles ? ……

13. Prend-il des médicaments ?

13.1 Pour les allergies ? Oui □ Non □

13.2 Pour l'asthme ? Oui □ Non □

13.3 Pour une autre maladie ? Oui □ Non □

Si oui, pour quelle maladie ? ……

13.4 Nom(s) des médicaments : ……

14. Depuis votre naissance, votre enfant a-t-il été hospitalisé(e) ?

14.1 Pour infection respiratoire ? Oui □ Non □

14.2 Pour asthme ? Oui □ Non □

14.3 Autre ? ……

15. L'accouchement a-t-il eu lieu par césarienne ? Oui □ Non □

Mode de vie et habitudes alimentaires

16. Durant les 12 derniers mois, à quelle fréquence votre enfant a-t-il consommé les aliments suivants :

	Jamais	1-3 x/mois	1x/sem	2-4 x/sem	Plus de 4x/sem	1x/jour	Plus d'1x/jour
Pain							
Probiotiques (Actimel, Yacult,…)							
Produit laitier enrichi au bifidus							
Yogourt							
Fromage en tranche							
Lait frais non pasteurisé							
Babeurre							
Produits au soja							
Pâté							
Viande							
Abats (rognons, foie,..)							
Fruit frais							
Poisson							
Crustacés							
Œufs							
Lait							
Chips							
Gâteaux							
Produits Beneo							
Noix							
Cacahuètes							
Fastfood							

(Quick, AC Do, ..)							
Aliment « bio »							

17. Utilisez-vous un adoucisseur d'eau ? Oui □ Non □
    15.1 Depuis quand ? ……..
    15.2 Lequel ? ……..

18. Votre enfant, boit-il l'eau du robinet ?
    □ Oui □ Rarement □ Jamais

19. Au déjeuner, que mange-t-il généralement ?
    □ Des céréales (ex : kellog's) □ Tartine(s) + beurre □ Tartine(s) + margarine
    □ Tartine(s) + choco □ Tartine + fromage □ Tartine(s) + autre chose

20. En moyenne, combien de tartines mange-t-il par jour ? ……

21. Avec quel type de matière grasse cuisine-t-on chez vous ?
    margarine □ beurre □ huile d'olive □ huile de tournesol □ autre □

22. Quel type de viande consomme-t-il le plus ?
    porc □ boeuf □ volaille □

Environnement et habitation

23. Entre 0 et 2 ans, vivait-il ? : □ En ville □ A la campagne
    S'agit-il de votre résidence actuelle ? Oui □ Non □
    Si non, précisez votre ancienne adresse : …….. Votre habitation se trouvait-elle près d'une route avec un trafic important (ex : chaussée, boulevard, nationale, autoroute) ? Oui □ Non □
    Si oui, à quelle distance ? : □ moins de 10 mètres
    □ moins de 100 mètres
    □ plus de 100 mètres

24. Votre habitation actuelle :
    24.1 est-elle située ? □ En ville □ A la campagne
    24.2 a-t-elle été construite ? □ Avant 1950 □ Après 1950
    24.3 a-t-elle des canalisations en plomb ? □ Oui □ Non
    24.4 comporte-t-elle du double-vitrage ? □ Oui □ Non
    24.5 est-elle la même qu'il y a 2 ans ? □ Oui □ Non
    24.6 comporte-t-elle un conditionnement d'air ? □ Oui □ Non
    24.7 A-t-elle été rénovée (ex peinture) depuis la naissance de votre enfant ? Oui □ Non□

25. Y a-t-il un potager chez vous ? Oui □ Non □
    Si oui, mangez-vous les légumes du potager ? Oui □ Non □

26. Y a-t-il un ou plusieurs animaux à la maison ?
    □ Aucun animal
    □ Un ou des animal(aux) à poils : □ chat □ chien
    □ Un ou des animal(aux) à plumes

□ Autre à préciser : ……

26.1 Depuis quel âge, votre enfant vit-il avec lui (eux) ? ……

26.2 Les animaux vont-ils dans la chambre de votre enfant ? ……

26.3 Vous êtes-vous séparés d'1 animal en raison d'une réaction allergique de votre enfant? Oui □ Non □

26.4 Si oui, de quel animal s'agissait-il et à quelle époque ?……

26.5 Votre enfant a-t-il ou avait-il des contacts réguliers avec des animaux de la ferme ?

Oui □ Non □

26.6 Si oui, de quel animal s'agissait-il et à quelle époque ?……

27. De combien de chambres votre habitation était-elle constituée ?

□ 1 □ 2 □ 3 □ 4 □ 5 □ 6 □ 7 □ 8 ou plus

28. Les chambres étaient-elles ventilées ou aérées ?

1 fois/jour □ 1 fois/2 jours □ 1fois/sem □ rarement □

29. Dans les chambres ou le living, utilisait-on régulièrement :

29.1 Des insecticides ? Oui □ Non □
Si oui, à quelle fréquence ? ……

29.2 Des antifongiques ? Oui □ Non □
Si oui, à quelle fréquence ? ……

29.3 De la naphtaline ? Oui □ Non □
Si oui, à quelle fréquence ? ……

29.4 Des assouplissants ? Oui □ Non □
Si oui, à quelle fréquence ? ……

30. Sur les murs ou le plafond, dans les pièces de vie (chambre, living, salle de jeu), y-a-il ?

30.1 Des moisissures ? Oui □ Non □
30.2 Des cafards ? Oui □ Non □
30.3 Des souris ? Oui □ Non □
30.4 De l'humidité ? Oui □ Non □

31. Votre maison comporte-t-elle un feu ouvert ? Oui □ Non □
31.1 Si oui de quel type ? ……
31.2 A quelle fréquence l'utilisez-vous ?……

32. Dans votre maison, utilise-t-on *et à quelle fréquence:

	Spray:oui/ non	**Jamais**	**Moins d'1x/sem**	**1x/sem**	**Plus d'1x/sem**	**Chaque jour**
Désodorisants						
Parfums d'ambiance						
Bâtons d'encens						
De la cire						
Solvants, détachants						

Appendix 4- Follow-up questionnaire

Nettoyant pour meubles						
Nettoyant fenêtres						
Nettoyant tapis, rideaux						
Nettoyants pour le four						
Détachants à sec						

(*Mettre une croix dans la case de la réponse appropriée)

33. Y avait-il du tapis plein sur le sol de :
    33.1 votre chambre Oui □ Non □
    33.2 votre living Oui □ Non □

34. Pour le nettoyage du sol (ailleurs que dans les toilettes), utilisait-on des produits à base :
    34.1 De l'eau de Javel ? Oui □ Non □
    Si oui, à quelle fréquence ?
    □ au moins 1 fois /semaine □ 1 fois /15 jours □ 1 fois /mois
    34.2 D'ammoniaque ? Oui □ Non □
    Si oui, à quelle fréquence ?
    □ au moins 1 fois /semaine □ 1 fois /15 jours □ 1 fois /mois
    34.3 De décalcifiants ? Oui □ Non □
    Si oui, à quelle fréquence ?
    □ au moins 1 fois /semaine □ 1 fois /15 jours □ 1 fois /mois

35. Y avait-il une piscine chez vous ? Oui □ Non □
    35.1 Si oui, était-elle ? □ A l'intérieur □ A l'extérieur
    35.2 Etait-elle désinfectée au chlore ? Oui □ Non □
    35.3 Quand y allez-vous ? □ en été □ toute l'année
    35.4 Depuis quel âge allez-vous dans cette piscine ? ……
    35.5 Combien de mois par an ? ……
    35.6 Combien d'heures par semaine pendant la période où vous y allez ? …….

Les activités sportives de votre enfant

36. Votre enfant pratique-t-il régulièrement une ou plusieurs activités sportives (au moins une heure par semaine) ?: Oui □ Non □
36.1 Si oui, lesquels et à quelle fréquence ? a) ……
b) ……

37. Votre enfant va-t-il aux sports d'hiver ? Oui □ Non □
    Si oui, depuis quel âge ? ……

38. Fait-il partie d'un/de club(s) sportif(s) ? Oui □ Non □
Si oui, le(s)quel(s) ? …… ……

39. Fait-il partie d'un club de natation ? Oui □ Non □
Si oui, 38.1 De quel âge à quel âge ?……
38.2 Combien de séances par semaine ? ……
38.3 Combien de temps dans l'eau ? □ 1 h □ 1 h 30 □ 2h
38.4 Quel est le nom de la piscine ? ……

39. Va-t-il à la piscine avec l'école :
39.1 en maternelle ? Oui □ Non □
Si oui, A partir de quelle année ? ……
A quelle fréquence ? □ 1 fois/sem □ 1 fois/15 jours
□ 1 fois/mois □ 1 fois/2 mois
Combien de temps dans l'eau ? □ 20 min □ 30 min □ 1 h
Quel était le nom de la piscine ? ……
Quel était le nom de votre école maternelle ……

39.2 en primaire ? Oui □ Non □
Si oui, A partir de quelle année ? ……
A quelle fréquence ? □ 1 fois/sem □ 1 fois/15 jours
□ 1 fois/mois □ 1 fois/2 mois
Combien de temps dans l'eau ? □ 20 min □ 30 min □ 1 h
Quel était le nom de la piscine ? ……
Quel était le nom de votre école primaire ……

40. A-t-il fait des stages de natation ? Oui □ Non □
Si oui, 40.1 De quel âge à quel âge ? ……
40.2 Combien de périodes d'une semaine ? ……
40.3 Combien de temps dans l'eau par jour ? ……

41. Va-t-il régulièrement à la piscine pour une raison médicale ? :
Oui □ Non □
Si oui, précisez la raison : ……

Votre famille

42. De combien de personnes votre foyer était-il constitué (en comptant votre enfant) ?
□ 2 □ 3 □ 4 □ 5 □ 6 □ 7 □ 8 ou plus

43. Votre enfant a-t-il des frères et/ou des sœurs ? □ Oui □ Non
Si oui, 43.1 Combien ?……
43.2 Combien de sœurs et de frères sont-ils plus âgé(e)s que lui ?
□ Aucun □ 1 □ 2 □ 3 □ 4 ou plus

44. Parmi ses frères et/ou sœurs, certains ont-ils ou ont-ils eu ? :
44.1 De l'asthme : Oui □ Non □
44.2 Des allergies : Oui □ Non □
44.3 De l'eczéma : Oui □ Non □

45. A la maison,
45.1 Madame, fumez-vous ? Oui □ Non □
45.2 Monsieur, fumez-vous ? Oui □ Non □

46. D'autres personnes à la maison fument-elles ? Oui □ Non □

46.1 Si oui, combien ? □ 1 □ 2 □ 3

47. Les grands parents paternels fument-ils ? Oui □ Non □

48. Les grands parents maternels fument-ils ? Oui □ Non □

Date et Signature ……..

Un grand merci pour votre collaboration !
Merci de remettre ce questionnaire complété à l'enseignant de votre enfant.

Appendix 4- Follow-up questionnaire

ANIMO

Risques d'affections respiratoires chez l'enfant liés à la qualité de l'air intérieur : développement et application de biomarqueurs non-invasifs

Madame, Monsieur,

Nous vous remercions de votre accueil et de votre collaboration à notre projet. Pourriez-vous compléter ce questionnaire dans son entièreté et nous le faire parvenir soit par fax, soit par mail ?

Nom de votre école :
Nom du directeur :
Adresse :
Tél/fax :
Mail :

Au sujet de l'établissement

1. Quelle est l'année de construction du bâtiment ?

2. Quel est le volume des classes de maternelles ?

3. Combien y a-t-il d'enfants en 3ème maternelle ?

4. Combien d'enfants y a-t-il par classe en maternelles ?

5. Les classes sont-elles ventilées et si oui, à quelle fréquence et de quelle façon?

6. Quels sont la fréquence et le mode de nettoyage des classes ?

7. Utilise-t-on des produits chlorés ? Pour quel usage ? Et à quelle fréquence ?

8. Quels autres produits d'entretien sont-ils utilisés ?

9. Quel est le type des conduites d'eau ?

10. Votre établissement bénéficie-t-il d'un jardin ou d'un parc ?

11. Si oui, quels types d'arbres et de végétation en général y a-t-il ?

12. Y a-t-il des animaux dans les classes ou dans les pièces communes ?

13. Si oui, de quel type d'animaux s'agit-il ?

Au sujet des activités sportives proposées

1. Quelles sont les activités sportives auxquelles les enfants participent ?

2. A partir de quelle année scolaire?

3. A quelle fréquence ?

4. les enfants de maternelle vont-ils à la piscine ?

5. Si oui, à partir de quand ?

6. Dans quelle piscine ?

7. Combien de temps restent-ils dans l'eau ?

Au niveau du mode de vie

1. Les enfants consomment-ils l'eau du robinet ?

2. Les repas sont-ils cuisinés au sein de l'école ou par une société extérieure ?

Contacts

N'hésitez pas à nous contacter si vous aviez la moindre question, nous nous ferons un plaisir d'en discuter avec vous.

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