

Outdoor Swimming Pools and the Risks of Asthma and Allergies during Adolescence

Alfred Bernard, PhD, Marc Nickmilder, PhD and Catherine Voisin, MSc

Department of Public Health, Catholic University of Louvain, Belgium

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

Address correspondence to A. Bernard, Unit of Toxicology, Faculty of Medicine, Catholic University of Louvain, Avenue E. Mounier 53.02, B-1200 Brussels Belgium Phone: 32-2-7645334 Fax: 00-32-2-7645328

E-mail: bernard@toxi.ucl.ac.be

Running Title: Swimming pool, asthma and allergy

Key Words: chlorine, trichloramine, nitrogen trichloride, swimming pool, childhood asthma, atopy, total IgE, aeroallergens, exhaled nitric oxide, exercise-induced asthma

Acknowledgements:

Alfred Bernard is Research Director of National Fund for Scientific Research. This work was supported by the National Fund for Scientific Research in Belgium, the Agency for Environmental and Occupational Health Safety in France and the Governments of the Walloon Region and of the French Community of Belgium. The authors declare that they have no financial competing interests.

ABSTRACT

Background

Exposure to indoor chlorinated swimming pools can be detrimental to the airways of swimmers and increase asthma risks but it is unknown whether these effects concern outdoor pools.

Methods

We studied 847 secondary school adolescents who had attended at a variable rate residential or non-residential outdoor chlorinated pools. Main outcomes were ever asthma (physician-diagnosed at any time), current asthma (ever asthma under medication and/or with exercise-induced bronchoconstriction), elevated exhaled nitric oxide and aeroallergens-specific IgE in serum.

Results

The prevalences of ever and current asthma increased with the lifetime number of hours spent in outdoor pools by up to four and eight times, respectively, among adolescents with the highest attendance (>500 hours) and a low exposure to indoor pools (<250 hours) (all P for trend <0.001). Odds for asthma were significantly increased among adolescents with total serum IgE above 25 kIU/l, on average by one to two units for each 100 hours-increase in pool attendance. Use of residential outdoor pools was also associated with higher risks of elevated exhaled nitric oxide and sensitization to cat or house-dust mite allergens.

Conclusions

Outdoor chlorinated pools attendance is associated with higher risks of asthma, airways inflammation and some respiratory allergies.

INTRODUCTION

Over the last decades, outdoor swimming pools have become increasingly popular, especially in countries with a warm climate¹. Global warming will probably see many more people installing private pools for exercising or refreshing on hot days. Most swimming pools worldwide are disinfected with chlorine-based disinfectants that in water release hypochlorous acid, a powerful oxidant destroying pathogenic microorganisms². The type and form of chlorine used in swimming pools vary with the size of the pool and its level of attendance. Residential outdoor pools are usually sanitized with chlorinated isocyanurates, which are stabilized forms of chlorine easy to handle and resistant to ultraviolet degradation. Public outdoor pools use cheaper forms of chlorine such as chlorine gas or sodium hypochlorite. Chlorine as a swimming pool disinfectant presents, however, two major drawbacks. The first is that when oxidizing organic substances brought by swimmers or from other sources, hypochlorous acid generates a mixture of harmful breakdown products including potent irritants such as chloramines, haloacetic acids or haloacetonitriles^{2,3}. The second drawback, often overlooked, is that hypochlorous acid is a non-selective biocide that inevitably also reacts with the organs of the bather in contact with pool water or aerosols, causing irritation of the skin, eyes and of the upper respiratory tract⁴⁻⁶.

Paradoxically, while the acute toxicity of chlorination products has been known for more than one century and populations of industrialized countries have been increasingly exposed to these chemicals, in particular with the development of swimming pools, studies evaluating their effects on swimmers have started only recently. Studies on elite swimmers were among the first to suggest that the chlorine-laden atmosphere of indoor pools could be detrimental to the lungs, increasing the risks asthma, bronchial hyperreactivity and airways inflammation^{3,7,8}. Our investigations on children attending indoor chlorinated swimming pools have shown that

trichloramine, together probably aerosolized hypochlorous acid and chloramines, can damage the lung epithelium and promote the development of asthma, particularly among children with higher concentrations of total serum IgE⁹⁻¹². These effects might be behind the strong ecological associations that were recently reported between childhood asthma prevalence and the availability of indoor chlorinated swimming pools in Europe¹³. Other researchers have confirmed these adverse effects of pool chlorine on the airways of recreational swimmers while providing further evidence that exposure to indoor chlorinated pools might contribute to the development of allergic diseases¹⁴⁻¹⁶. The chlorine compounds responsible for these respiratory effects are largely unknown. Currently, the most suspected culprit is trichloramine also called nitrogen trichloride, the gas that builds up in the air of indoor pools, giving them their distinctive chlorine smell. Trichloramine has been identified as a cause of asthma and respiratory problems in pool workers^{17,18}. Swimmers, however, are mainly exposed when they actively inhale the volatile and aerosolized chlorination products in the air just above pool surface. Another potential source of exposure for swimmers, especially the youngest learning to swim, is the direct contact of airways with chlorinated water that enters the upper respiratory tract and can be carried out more or less deeply in the lungs depending on the ventilation rate¹².

These uncertainties regarding the chlorine compounds responsible for the respiratory problems in swimmers attending indoor pools necessarily raise the question of the safety of open-air swimming pools. This question is especially important for countries with a warm climate where backyard pools are very common and can be attended by children and their family almost all over the year. In this epidemiological study focused on secondary school adolescents, we have explored the relationships between the attendance of outdoor pools, at

home or during holidays, and the prevalences of asthma, respiratory allergies and airways inflammation using whenever possible objective outcome measures.

MATERIALS AND METHODS

Study population

We recruited adolescents in three secondary schools located in the French speaking part of Belgium, in the cities of Louvain-la-Neuve (“Ecole Martin V”), Bastogne (“Institut Notre-Dame Séminaire”) and of Lessines (“Athénée Royal René Magritte”). Students of Louvain-la-Neuve had access to an indoor non-chlorinated swimming pool sanitized by the copper-silver method while students of the two other schools visited only indoor chlorinated swimming pools. Of the 1,200 adolescents who were contacted in these three schools, a total of 1,137 (94.8%) returned the questionnaire. Among them, 857 had the written agreement of their parents to participate to the study, giving an overall participation rate of 71.4%. There were little variations in participation rate between the three schools (Louvain-la-Neuve, 72.0%; Bastogne, 70.6%, Lessines, 72.1%), nor between girls (70.8%) and boys (72.2%). Our study included nevertheless more girls than boys largely because of the school population at Bastogne that included 66% of girls. We had to exclude eight adolescents who did not give blood and two others because of incomplete information provided in the questionnaire. Comparison of questionnaires from the participants (n=847) and the non-participants (n=280) did not reveal any significant difference in the prevalence of doctor-diagnosed asthma, nor in the proportions of adolescents having a backyard pool or having attended an outdoor pool during their holidays. The study protocol was approved by the Ethics Committee of the Faculty of Medicine of the Catholic University of Louvain and complied with all applicable requirements of the international regulations.

Questionnaire

Parents were asked to complete a questionnaire inquiring about the family history of allergic diseases, the health of their child and his exposure to a variety of environmental or lifestyle

factors likely to affect the studied outcomes. The questionnaire comprised also questions about the attendance of indoor or outdoor swimming pools with the school, as a recreational or sport activity, at home or during holidays. For each type of swimming pool, parents were asked to specify whether the pool was disinfected using chlorine or another disinfectant and to provide for each year an estimate of the number of hours per week and when appropriate (e.g. during holidays) of the number of weeks per year their child had attended the pool. Returned questionnaires were checked and completed by interviewing the adolescents during their examination in schools.

Examination of adolescents

Adolescents were examined in schools between March and May 2006. After the measurement of height and body weight and the collection of one blood sample on a dry tube (10 ml), we measured the concentration of nitric oxide (NO) in exhaled air with the NIOX™ analyzer (Aerocrine AB, Sweden) by following the guidelines of the American Thoracic Society¹⁹. Exercise-induced bronchoconstriction (EIB) was screened by measuring the fall in FEV₁ after a six-minutes indoors running with submaximal effort²⁰.

Serum analyses

We measured total and aeroallergen-specific IgE concentrations in serum using the Immulite IgE kit (Diagnostic Products Company, Los Angeles, CA, USA). We screened specific IgE against the following allergens: house-dust mite (*Dermatophagoides pteronyssinus*), cat epithelium, dog dander, moulds (*Penicilium notatum*, *Cladosporium herbarum*, *Aspergillus fumigata*, *Candida albicans*, *Alternaria tenui*), tree pollen mixture (*Alnus incana*, *Betula verrucosa*, *Corylus avellana*, *Quercus alba*, *Salix caprea*), grass pollen mixture (*Antoxanthum odoratum*, *Sacale cereale*, *Holcuns lanatus*, *Lolium perenne*, *Phleum pretense*) and

herbaceous pollen mixture (*Chenopodium album*, *Solidago virgaurea*, *Urtica dioica*, *Artemisia absinthium*, *Artemisia vulgaris*).

Study outcomes

We defined asthma either as “ever asthma” corresponding to asthma diagnosed by a physician at any time in life or as “current asthma” corresponding to a physician-diagnosed asthma that was under medication or associated with a positive exercise-induced bronchoconstriction test at the time of the study. The EIB test was considered positive when the exercise caused a fall of FEV₁ by 10% or more, which is the standard criterion for diagnosing exercise-induced asthma in athletes²¹. The exhaled NO test was considered positive when the concentration of NO in exhaled air was higher than 30 ppb. Sensitization against the specific aeroallergens was defined as a serum concentration of specific IgE higher than 0.35 kIU/l.

Statistical analysis

Continuous variables were described as median with interquartile range. We used the Mann-Whitney test for two-group comparisons and the Kruskal-Wallis non-parametric ANOVA test for comparing more than two groups. Categorical variables were compared by the chi-square test or by a chi-square test for trend for assessing the significance of exposure-response relationships. We used backyard logistic regression models to analyze associations between outcomes and swimming pool attendance. Backyard selection started with a model including all potential control variables and executing each step by deleting the least significant predictor until the model only contained variables with a $P < 0.20$. This level of significance was used as inclusion criterion to ensure that all important confounders end up in the model²². The following control variables were tested: age, sex, body mass index (BMI), ethnicity (white/non-white), birth weight, maternal smoking during pregnancy, breastfeeding, day

nursery attendance, maternal and/or paternal history of asthma or allergy, total IgE in serum (unit, 100 kIU/l), number of older siblings, socio-economic status based on mother's and father's educational level, house cleaning with bleach, parental smoking at home, active smoking, regular practice of a sport other than swimming and swimming pool attendance cumulated over lifetime or before the age of seven years. The attendance at swimming pool before the age of seven years was tested by adjusting for the cumulated attendance after seven years. For each type of pool, we categorized the lifetime cumulative attendance as low, average or high according to whether it varied between zero and 100 hours, 100 and 500 hours or was higher than 500 hours. We divided these cut points by two for creating the corresponding categories for the pool attendance before the age of seven years. We calculated the crude and adjusted odds ratios for these categories by using as reference level the occurrence of the outcome in adolescents having never attended the studied outdoor swimming pool. Independent variables were checked for the absence of multicollinearity by calculating the tolerance and variance inflation factors for each variable. In order to test interactions between cumulative pool attendance and atopy, we repeated these logistic regression analyses by stratifying adolescents according to their total or aeroallergens-specific IgE in serum. We selected as cut points for total serum IgE the concentrations of 25, 50 and 100 KIU/L that were derived from the median serum IgE concentration in the whole population (50.1 KIU/L). P values were two-sided and results were considered as statistically significant at P values below 0.05.

RESULTS

Table 1 displays the characteristics of the adolescents who participated to the study. Students from the three schools had on average the same age (15 years). Gender ratio was close to 1 except at Bastogne where the school population included more girls. The socio-economic status as evaluated on the basis of parental education was higher at Louvain-la-Neuve than in other schools and this was reflected in several lifestyle factors such as BMI, exposure to tobacco smoke, breastfeeding and day care attendance. Because they had access to an indoor copper-silver pool, students of Louvain-la-Neuve had spent much less time in indoor chlorinated pool than their peers of Bastogne and Lessines but their attendance of outdoor chlorinated pools was higher. There were, by contrast, no significant differences between the three schools regarding the prevalences of ever or current asthma or of parental asthma, or in the rate of sensitization to aeroallergens, at the exception of pollen. The rate of sensitization to pollen like also the mean values of total serum IgE and exhaled NO were indeed slightly higher at Louvain-la-Neuve than in the two other schools. The lifetime cumulative attendance of a residential or non-residential outdoor pool considered separately was not significantly different between adolescents with ever diagnosed asthma and those without asthma diagnosis ($P=0.61$ and 0.10 , respectively). The total lifetime attendance of outdoor pools, by contrast, was significantly greater among adolescents with ever asthma than in those who had no asthma diagnosis (median 348 versus 203 hours, $P=0.008$).

Table 2 shows that the cumulative attendance of a residential or of non-residential pool is associated with a rather similar pattern of asthma risks. Adolescents with the highest attendance (>500 hours) of either type of pool showed approximately a two- to three-fold increase in the risk of ever or current asthma, even though the odds for current asthma did not reach the level of statistical significance of 0.05. By contrast, the risk of elevated exhaled NO

was significantly increased only among adolescents with the highest attendance of a residential pool. Other significant predictors identified in these analyses were total serum IgE (ever or current asthma and eNO), parental asthma (ever or current asthma), house cleaning with bleach (ever or current asthma), gender (exhaled NO) and maternal smoking during pregnancy (ever asthma). Of note, none of the other variables that differed between the three schools (i.e. gender, BMI, parental education, active or passive smoking, breastfeeding, day care attendance) entered in the models, even at a $P < 0.20$. Interestingly, house cleaning with bleach was found to exert a protective effect against the risk of ever asthma (odds ratio, 0.48, 95% CI, 0.27-0.87, $P = 0.016$) and current asthma (odds ratio, 0.33, 95% CI, 0.14-0.76, $P = 0.01$). All these associations persisted with pool attendance indices cumulated from birth to the age of seven years (results not shown).

Since the attendance of a residential or a non-residential pool similarly increased asthma risks, we combined the attendance of both types of pools in order to increase the numbers of subjects in the different pool attendance categories. This allowed us to assess exposure-response relationships while stratifying adolescents according to their family history of asthma and their attendance of indoor chlorinated pools. Figure 1 shows that the prevalences of ever and current asthma increased in a dose-dependent manner with increasing lifetime outdoor pool attendance, both when considering all adolescents (Figure 1A) or only those without parental asthma (Figure 1B). The sequential exclusion of adolescents with increasing attendance of indoor pools noticeably strengthened these relationships, especially for ever asthma. For instance, among adolescents having attended an indoor pool for less than 250 hours, the prevalences of ever and current asthma were respectively 4 and 9 times higher in those with the highest outdoor pool attendance (>500 hours) as compared with their peers who had never swum in an outdoor pool (both $P < 0.001$) (Figure 1A). Quite remarkably,

among adolescents without parental asthma, the prevalence of current asthma increased almost linearly with the outdoor pool attendance by a factor of more than 10 (Figure 1B). Interesting also, the group of adolescents with the lowest exposure to pool chlorine – i.e. those who never swam in an outdoor chlorinated pool and had attended an indoor chlorinated pool for less than 100 hours - had a prevalence of current asthma that was four times lower than in the rest of the population (2/125 [1.6 %] vs 53/722 [7.4 %], P=0.02).

We studied the influence of atopic status on asthma risks associated with outdoor pool attendance by calculating the odds for ever and current asthma in adolescents stratified according to atopy defined on the basis of total IgE or aeroallergens-specific serum IgE. We performed this analysis by excluding adolescents with parental asthma (n=113) and those with an indoor pool attendance higher than 500 hours (n=214) in order to specifically assess the effects of outdoor pool attendance. When atopy was defined on the basis of total serum IgE, outdoor chlorinated pool attendance was associated with an increased risk of ever or current asthma only in children with total serum IgE above 25 kIU/l. Above this threshold, outdoor pool attendance and serum IgE level strongly interacted to cause a dose-dependent increase in asthma risk. From the odds ratios given in Table 3, we can estimate an overall 100 to 200% increase of ever or current asthma risk with each 100 hours-increase in outdoor pool attendance, depending on the level of serum IgE and of pool attendance. When atopy was defined on the basis of allergens-specific serum IgE, risks of ever and current asthma significantly increased with outdoor pool attendance only among sensitized subjects but the odds ratios were about twice lower than those observed in subjects with high concentrations of serum IgE (results not shown).

We ascertained that the interactions between outdoor pool attendance and total serum IgE were not specific of one school, in particular that of Louvain-la-Neuve whose students had mainly attended the copper-silver pool. As shown in Table 4, the interactions between outdoor pool attendance and total serum IgE persisted and even appeared stronger when students of Louvain-la-Neuve and those of Bastogne and Lessines were analyzed separately. The interaction was particularly remarkable at Louvain-la-Neuve, probably because the cumulated exposure of referents to indoor chlorinated pools was much lower than in the two other schools (median of 24 vs 256 hours).

There were no significant associations between the risks of sensitization to aeroallergens and the attendance of a residential or non-residential outdoor pool when cumulated over lifetime. However, when studying associations with pool attendance during early childhood, we found that adolescents who had regularly attended a residential pool before the age of seven years were more likely to be sensitized against to aeroallergens, and particularly to cat or house-dust mite allergens (Table 5). Risks of asthma and of elevated exhaled NO were particularly elevated among these adolescents. By contrast, attendance of a non-residential pool during early childhood was not associated with an increased risk of sensitization to aeroallergens.

When considering the whole population, no significant association emerged between asthma and attendance of indoor chlorinated pools, whether cumulated over lifetime or during the early childhood. However, when considering adolescents with a low exposure to outdoor pools (less than 100 hours), the highest indoor pool attendance (>500 hours) was associated with a significant increase in the risk of ever asthma (odds ratio, 5.7, 95% CI, 1.2-26.7, P=0.02) and a non-significant increase in the risk of current asthma (odds ratio 2.17, 95% CI 0.84-5.61, P=0.11). The attendance of indoor chlorinated pools did not influence the risks of

respiratory allergies. We also found no significant associations between any of the studied outcomes and the attendance of the copper-silver pool.

DISCUSSION

Our study shows that regular attendance of an outdoor chlorinated pool, at home or during holidays, is associated with an exposure-dependent increase in the risks of asthma. Adolescents having regularly attended a residential pool were also more likely to be positive in the exhaled NO test and, when attendance was during infancy, to be sensitized against cat or house dust mite allergens. These associations cannot be explained by differences in socio-economic level and related lifestyle factors such as BMI, breastfeeding or exposure to tobacco smoke. They are also unlikely to result from a reverse causation due to a greater propensity of adolescents to attend an outdoor pool because they had been diagnosed with asthma. The cumulative attendance of either type of outdoor pools was indeed not significantly different between adolescents diagnosed with asthma and their peers with no asthma. Furthermore, as there were no public outdoor pools in the studied centres, the hypothesis of a reverse causation would imply that parents would have been encouraged to install a backyard pool or to spend holidays in places having an outdoor pool by the fact that their child had asthma. Such a confounding appears especially improbable as asthmatics are advised to swim not in outdoor pools but in indoor pools whose warm and humid atmosphere is less conducive to trigger asthma symptoms²³.

As expected, total serum IgE and parental asthma ranked as the strongest predictors of ever and current asthma. Maternal smoking during pregnancy emerged as a significant predictor only for ever asthma while exposure to parental smoking at home had no influence. This is consistent with earlier studies showing that the associations between passive smoking and asthma risks are the strongest during early childhood and then disappear with increasing age²⁴. Interestingly, our study confirms that house cleaning with bleach protects against the risk of asthma probably by decreasing the exposure to indoor allergens and harmful microbial

agents such as fungal products or endotoxins^{25,26}. This protection afforded by bleach is not inconsistent with the increased asthma risks associated with swimming pools since children living in a house cleaned with bleach are not directly in contact with chlorination products as they are when playing or swimming in an chlorinated pool²⁵.

Our study reveals new insights into the pool factors responsible for respiratory problems in swimmers. We are now more in a position to exclude the possibility that asthma and allergy risks associated with swimming pool attendance are caused by swimming itself since none of studied outcomes showed a significant association with the attendance of the copper-silver pool. This conclusion is supported by the fact that some outcomes such as exhaled nitric oxide or sensitization to house-dust mite were already significantly associated with the outdoor pool attendance cumulated before the age of seven years i.e. when most adolescents could not really swim. We think that the cause of respiratory effects found in our study has to be sought among the chlorination products in pool water or building up at the surface of the pool. Trichloramine is unlikely to be responsible for these effects as this highly volatile gas is very quickly dispersed into the atmosphere, explaining why open-air swimming pools have not the characteristic “chlorine” smell of indoor pools. The most concentrated and reactive chlorine compound to which swimmers are exposed in outdoor pools is hypochlorous acid, i.e. the active chlorine itself. Hypochlorous is a well-known lung toxicant²⁷ and at concentrations used in pool water (1-3 ppm^{1,2}), this powerful oxidizing agent could quite conceivably affect the airways of swimmers when they inhale aerosols or small volumes of water. Pool water and the air just above the water surface also contain a variety of reactive chlorination by-products including chloramines, trihaloacetic acid or trihaloacetonitriles²⁸. Although being usually less concentrated in pool water than active chlorine, there is no doubt that these chemicals also contribute to the burden of oxidants or irritants inhaled by swimmers.

In addition to the increased risk of asthma, our study has identified associations between the attendance of a residential outdoor pool and the risks of respiratory allergies or airways inflammation measured by the exhaled NO test. The fact that we did not observe such associations with non-residential outdoor pools is interesting. This difference is indeed in accordance with the mechanism by which chlorine-based oxidants could promote allergic sensitization and that consists in a disruption of epithelial barriers, facilitating the delivery of antigens^{3,10}. To be induced, such a mechanism implies a certain coincidence between the exposure to allergens and the exposure to chlorination products, a coincidence that for indoor allergens is more likely to occur when the pool is at home than in a resort or in a summer house. This is particularly obvious for pets like cats that usually do not follow their owner on holidays. Exposure to house-dust mite allergen is probably also lower in places of holidays owing to the drier climate or to the more efficient destruction of allergens by professional laundering²⁹.

Although chlorine and hypochlorous acid are among the most powerful oxidants to be found, the possibility that these chemicals could adversely affect organs of the swimmers in contact with pool air or water has so far received little attention. Yet, these chlorine-based oxidants are known to cause oxidative damage to the epithelial and endothelial layers in contact with chlorinated water or aerosols³⁰⁻³³. While for any other air pollutant, such effects would be considered as unacceptable, most regulatory bodies regard them as simply a source of discomfort for swimmers². This lack of concern for the oxidant effects of these chemicals on swimmers is reflected by the current guidelines that allow concentrations of active chlorine up to 3 ppm and even higher (e.g. after a shock treatment). We believe that such high concentrations of active chlorine are not necessary and may even be hazardous to the

swimmers, particularly the youngest who can spend hours playing in outdoor pools. The experience with public indoor swimming pools teaches us indeed that in a well-designed and operated pool, an adequate disinfection can be achieved with active chlorine concentrations in the range 0.5 to 1.0 ppm and even with lower concentrations if one refers to the German standards (0.3 to 0.7 ppm)¹⁶. There are no reasons to think that concentrations of active chlorine in this range should not be sufficient also to disinfect outdoor pools, especially the residential pools where the infectious risks - primarily due to fecal contamination - are normally lower than in public pools.

The principal strength of our study lies in the use of robust outcome measures, which allowed us to considerably reduce the risk of recall or response bias. Although our study required a blood sampling in schools, we could achieve a relatively good response rate (71.4%) which further reduced the risks of selection bias. We have also taken advantage of the existence in Belgium of an indoor copper-silver pool that was in activity for more than 20 years. Since in industrialized countries swimming pool attendance has become a very popular and even a compulsory activity in schools, this was indeed a rather unique opportunity to recruit a control group with no or a minimal exposure to chlorination products while being well matched with the exposed group according to swimming practice.

Our assessment of exposure to pool chlorine was, however, more limited as we had no choice but to use the information provided by the questionnaire filled by the parents. We do not think, however, that the responses of the parents to the questions about outdoor attendance could have been biased by the health of their child or the perception they had of the benefits or risks of swimming in chlorinated pools. First, the parents were blinded to the tested hypothesis since outdoor pool attendance was only one of the many environmental or lifestyle

factors that were addressed by the questionnaire. Second, the hypothesis that outdoor pool attendance could cause adverse effects is probably very far from the belief that parents had when they offered their child the possibility to swim in an outdoor pool, at home or during the holidays. Third, even assuming a bias in the parental responses to the questionnaire, it is difficult to imagine that this bias could have distorted our analysis in proportion to the serum IgE level of adolescents, generating consistent relationships between asthma prevalence and cumulative pool attendance across the categories of increasing serum IgE.

In summary, our study shows that the attendance of outdoor chlorinated swimming pools, at home or during holidays, is associated with an exposure-dependent increase in the risk of asthma, especially among children with higher serum IgE levels. Attendance of a residential outdoor pool appears to increase also the risk of airways inflammation and of sensitization to some indoor aeroallergens. Since these associations were not found with the attendance of the copper-silver pool, they are most likely due to some airways damage caused by chlorine-based oxidants added to pool water or released at the surface of the pool as aerosols or gases. These findings may have important implications in countries where outdoor pools are very common.

References

1. CDC. Healthy swimming http://www.cdc.gov/healthyswimming/ph_chlorine.htm
2. World Health Organization. 2006. Guidelines for safe recreational waters. Volume 2. Swimming pools and similar recreational-water environments. Geneva, Switzerland.
3. Bernard A. Chlorination products: emerging links with allergic diseases. *Curr Med Chem.* 2007;14:1771-1782.
4. Haag JR, Giese RG. Effects of swimming pool water on the cornea. *JAMA.* 1983 13;249: 2507-2508.
5. Momas I, Brette F, Spinasse A, Squinazi F, Dab W, Festy B. Health effects of attending a public swimming pool: follow up of a cohort of pupils in Paris. *J Epidemiol Community Health.* 1993;47:464-468.
6. Pardo A, Nevo K, Vigiser D, Lazarov A. The effect of physical and chemical properties of swimming pool water and its close environment on the development of contact dermatitis in hydrotherapists. *Am J Ind Med.* 2007;50:122-126.
7. Helenius IJ, Tikkanen HO, Sarna S, Haahtela T. Asthma and increased bronchial responsiveness in elite athletes: atopy and sport event as risk factors. *J Allergy Clin Immunol.* 1998;101:646-652.

8. Helenius I, Ryttilä P, Sarna S, Lumme A, Helenius M, Remes V, Haahtela T. Effect of continuing or finishing high-level sports on airway inflammation, bronchial hyperresponsiveness, and asthma: a 5-year prospective follow-up study of 42 highly trained swimmers. *J Allergy Clin Immunol.* 2002;109:962-968
9. Carbonnelle S, Francaux M, Doyle I et al. Changes of serum pneumoproteins caused by short-term exposures to nitrogen trichloride in indoor chlorinated pools. *Biomarkers.* 2002;7:464-478.
10. Bernard A, Carbonnelle S, Michel O, et al. Lung hyperpermeability and asthma prevalence in schoolchildren: unexpected associations with the attendance of indoor chlorinated pools. *Occup Environ Med.* 2003;60:385-394.
11. Bernard A, Carbonnelle S, De Burbure C, Michel O, Nickmilder M. Chlorinated pool attendance, atopy and the risk of asthma during childhood. *Environ Health Perspect.* 2006; 114:1567-1573.
12. Bernard A, Carbonnelle S, Dumont X, Nickmilder M, Nickmilder M. Infant swimming, pulmonary epithelium integrity and the risk of allergic and respiratory diseases later in childhood. *Pediatrics.* 2007;119:1095-1103.
13. Nickmilder M, Bernard A. Ecological association between childhood asthma and availability of indoor chlorinated swimming pools in Europe. *Occup Environ Med.* 2007;64:37-46

14. Lagerkvist B, Bernard A, Blomberg A et al. Pulmonary Epithelial integrity in children - relationship to ambient ozone exposure and swimming pool attendance. *Environ Health Perspectives*. 2004;112:1767-1772.
15. Stav D, Stav M. Asthma and whirlpool baths. *N Engl J Med*. 2005;353:1635-1636.
16. Kohlhammer Y, Doring A, Schafer T, Wichmann HE, Heinrich J for the KORA study group. Swimming pool attendance and hay fever rates later in life. *Allergy*. 2006;61:1305-1309.
17. Thickett KM, McCoach JS, Gerber JM, Sadhra S, Burge PS. Occupational asthma caused by chloramines in indoor swimming pool air. *Eur Respir J*. 2002;19:827-832.
18. Jacobs JH, Spaan S, van Rooy GB, Meliefste C, Zaat VA, Rooyackers JM, Heederik D. Exposure to trichloramine and respiratory symptoms in indoor swimming pool workers. *Eur Resp J*. 2007;29:690-698.
19. American Thoracic Society. Recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children. *Am J Respir Crit Care Med*. 1999;160: 2104-17.
20. McFadden ER, Gilbert IA. Exercise-induced asthma. *N Engl J Med*. 1994;330:1362-1367.

21. Dickinson J W, Whyte G P, McConnell A K, Nevill A M, Harries M G. Mid-expiratory flow versus FEV₁ measurements in the diagnosis of exercise induced asthma in elite athletes. *Thorax*. 2006;61:111-121.
22. Greenland S. Modelling and variable selection in epidemiologic analysis. *Am J Public Health* 1999; 79:340-349.
23. Nemery B, Hoet PHM, Nowak D. Indoor swimming pools, water chlorination and respiratory health. *Eur Resp J*. 2002; 19:790-793.
24. Gilmour I, Jaakola M, London S, Nel A, Rogers C. How exposure to environmental tobacco smoke, outdoor air pollutants, and increased pollen burdens influences the incidence of asthma. *Environ Health Perspect* 2006 114:627-633.
25. Nickmilder M, Carbonnelle S, Bernard A. House cleaning with chlorine bleach and the risks of allergic and respiratory diseases in children. *Pediatr Allergy Immunol* 2007;18:27–35
26. Zock JP, Plana E, Jarvis D, Kromhout H, Olivieri M, Radon K, van Sprundel M, Torén K, Dahlman-Höglund A, Antó J M, Kogevinas M. Hygiene and allergy: the use of bleach at home and atopic sensitisation in adults. Annual meeting of the European Respiratory Society, Stockholm, 2007.
27. Hammerschmidt S, Büchler N, Wahn H. Tissue lipid peroxidation and reduced glutathione depletion in hypochlorite-induced lung injury. *Chest*. 2002;121:573-581.

28. Erdinger L, Kirsch F, Sonntag HG. Irritating effects of disinfection by-products in swimming pools. *Zentralbl hyg Umweltmed.* 1998;200, 491-503.
29. Arlian LG, Vyszynski-Moher DL, Morgan MS. Mite and mite allergen removal during machine-washing of laundry. *J Allergy Clin Immunol.* 2003;111:1269-1273.
30. Schraufstätter, I, Browne, K, Harris, A, Hyslop, P, Jackson, J. Mechanisms of hypochlorite injury to target cells. *J. Clin Invest.* 1990;85:554-562.
31. Tatsumi, T, Fliss, H. Hypochlorous acid and chloramines increase endothelial permeability: possible involvement of cellular zinc. *Am J Physiol.* 1994;267:H1597-607.
32. Maldonado MJ. Corneal epithelial alterations resulting from use of chlorine-disinfected contact tonometer after myopic photorefractive keratectomy. *Ophthalmology.* 1998;105:1546-1549.
33. Maurer JK, Molai A, Parker RD, Li L, Carr GJ, Petroll WM, Cavanagh HD, Jester JV. Pathology of ocular irritation with bleaching agents in the rabbit low-volume eye test. *Toxicol Pathol.* 2001;29:308-319.

Legend of Figure

Prevalences of ever asthma and current asthma in all adolescents (Panel A) and in those without parental asthma (Panel B) according to their lifetime attendance an outdoor chlorinated swimming pool, considering either all subjects or subjects with cumulative indoor pool attendance lower than 100, 250 and 500 hours. The numbers of subjects in these four categories were respectively 847, 235, 410 and 633 in Panel A and 734, 211, 357 and 547 in Panel B. P values correspond to the chi square test for trend.

Table 1 Characteristics of adolescents

Characteristics	Louvain-la-Neuve n = 357	Bastogne n = 349	Lessines n =141	P
Boys	167 (46.8%)	130 (37.2%)	72 (51.1%)	0.006
Age, mean (SD), years*	15.4 (0.81)	15.5 (0.83)	15.5 (0.87)	0.54
BMI, mean (SD), kg/m ² *	20.1 (2.3)	21.0 (3.3)	21.0 (3.2)	<0.001
Number of older siblings mean (SD)*	0.93 (0.98)	0.98 (1.02)	0.85 (0.99)	0.43
Parental education, N° (%)†	278 (77.9)	98 (28.1)	28 (19.9)	<0.0001
Smokers, N° (%)†	20 (5.6)	14 (4.0)	16 (11.3)	0.007
Maternal smoking during pregnancy, N° (%)†	25 (7.0)	55 (15.8)	25 (17.7)	0.0002
Parental smoking at home, N° (%)†	20 (5.6)	14 (4.0)	16 (11.3)	0.007
Breastfeeding, N° (%)†	305 (85.4)	191 (54.7)	76 (53.9)	<0.0001
House cleaning with bleach, N° (%)†	77 (21.6)	91 (26.1)	60 (42.6)	<0.0001
Mould on bedroom walls, N° (%)†	30 (8.4)	19 (5.5)	10 (7.1)	0.30
Day care attendance, N° (%)†	241 (67.5)	111 (31.8)	43 (30.5)	<0.0001
<u>Swimming pool attendance</u>				
Indoor copper-silver sanitized pool N° (%)†	339 (95.0)	4 (1.2)	0 (0)	<0.0001
CPA, median (IQR), hours‡	225 (108-434)	84 (46-210)	0 (0-0)	<0.0001
Outdoor chlorinated pool, residential N° (%)†	76 (21.3)	65 (18.6)	14 (9.9)	0.01
CPA, median (IQR), hours‡	275 (88-848)	154 (48-412)	232 (72-336)	0.07
Outdoor chlorinated pool, non residential N° (%)†	244 (68.3)	142 (40.7)	53 (37.6)	<0.0001
CPA, median (IQR), hours‡	168 (49-336)	126 (56-273)	306 (108-599)	0.0005
Outdoor chlorinated pool, total N° (%)†	270 (75.6)	183 (52.4)	61 (43.3)	<0.0001
CPA, median (IQR), hours‡	229 (70-477)	147 (57-336)	308 (134-599)	<0.0001
Indoor chlorinated pool, public N° (%)†	243 (68.1)	348 (99.7)	138 (97.9)	<0.0001
CPA, median (IQR), hours‡	126 (48-286)	400 (255-657)	407 (217-724)	<0.0001
<u>Asthma</u>				
Parental asthma, N° (%)†	49 (13.7)	39 (11.2)	25 (17.7)	0.15
Ever asthma ever, N° (%)†	38 (10.6)	36 (10.3)	14 (9.9)	0.97
Current asthma, N° (%)†	21 (5.9)	21 (6.0)	10 (7.1)	0.87
<u>Aeroallergen-specific serum IgE</u>				
House dust mite, N° (%)†	103 (28.9)	90 (25.8)	32 (22.7)	0.34
Dog, N° (%)†	15 (4.2)	19 (5.4)	10 (7.1)	0.40
Cat, N° (%)†	49 (13.7)	44 (12.6)	14 (9.9)	0.50
Pollen, N° (%)†	65 (18.2)	92 (26.4)	19 (13.5)	0.002
Mould, N° (%)†	11 (3.1)	6 (1.7)	7 (5.0)	0.14
At least one aeroallergen, N° (%)†	136 (38.1)	136 (39.0)	43 (30.5)	0.19
<u>Total serum IgE</u>				
Median (IQR), kIU/l‡	60.0 (22.1-173)	42.0 (16.1-127)	44.9 (13.8.-147)	0.004
<u>Exhaled nitric oxide (exhaled NO)</u>				
Median (IQR), ppb‡	13.4 (10.0-21.5)	13.0 (9.5 -20.6)	12 (8.3-17.3)	0.03
Exhaled NO >30 ppb, N° (%)†	61 (17.1)	49 (14.0)	13 (9.2)	0.07

Abbreviations: SD, standard deviation; IQR, interquartile range; CPA, lifetime cumulative pool attendance. * By two-sided unpaired *t* test ; † By two-sided χ^2 test; ‡ By two-sided Mann-Whitney *U* test

Table 2 Risks of ever or current asthma and of elevated exhaled nitric oxide (eNO) in adolescents according to their lifetime attendance of an outdoor chlorinated swimming pool at home (residential) or during the holidays (non-residential).

Indicator	Residential outdoor chlorinated pool				Non-residential outdoor chlorinated pool				
	attendance (hours)	Crude OR (95% CI)	P	Adjusted OR (95% CI)*	Crude OR (95% CI)	P	Adjusted OR (95% CI)*	P	
Ever asthma	0	1.0 (1.0-1.0)		1.0 (1.0-1.0)	1.0 (1.0-1.0)		1.0 (1.0-1.0)		
	>0-100	0.52 (0.16-1.72)	0.29	0.43 (0.12-1.51)	0.19	0.68 (0.34-1.36)	0.28	0.60 (0.29-1.23)	0.16
	>100-500	1.11 (0.49-2.53)	0.80	1.30 (0.56-3.07)	0.54	1.29 (0.76-2.20)	0.35	1.17 (0.67-2.05)	0.59
	>500	2.37 (1.05-5.37)	0.04	2.44 (1.01-5.90)	0.05	2.28 (1.14-4.54)	0.02	2.09 (0.99-4.41)	0.05
Current asthma	0	1.0 (1.0-1.0)		1.0 (1.0-1.0)		1.0 (1.0-1.0)		1.0 (1.0-1.0)	
	>0-100	0.28 (0.04-2.06)	0.21	0.24 (0.03-1.86)	0.17	0.97 (0.44-2.15)	0.94	0.81 (0.35-1.92)	0.64
	>100-500	1.00 (0.35-2.88)	1.00	1.26 (0.42-3.73)	0.68	1.40 (0.72-2.70)	0.32	1.19 (0.59-2.42)	0.62
	>500	2.76 (1.10-6.96)	0.03	2.50 (0.88-7.12)	0.09	1.96 (0.80-4.75)	0.14	1.67 (0.64-4.36)	0.29
Exhaled NO>30 ppb	0	1.0 (1.0-1.0)		1.0 (1.0-1.0)		1.0 (1.0-1.0)		1.0 (1.0-1.0)	
	>0-100	1.44 (0.70-2.97)	0.38	1.37 (0.66-3.02)	0.42	1.04 (0.62-1.74)	0.88	0.96 (0.55-1.65)	0.87
	>100-500	0.54 (0.21-1.37)	0.19	0.54 (0.20-1.38)	0.21	1.02 (0.64-1.64)	0.98	0.94 (0.57-1.56)	0.82
	>500	2.87 (1.40-5.87)	0.004	2.88 (1.35-6.12)	0.006	1.19 (0.59-2.40)	0.99	0.99 (0.47-2.09)	0.98

*ORs for ever asthma were adjusted for total serum IgE, parental asthma, gender, maternal smoking during pregnancy, house cleaning with bleach, the presence of mould on bedroom walls, the number of older siblings and the attendance at the other type of outdoor pool. ORs for current asthma were adjusted for total serum IgE, parental asthma, gender, maternal smoking during pregnancy, house cleaning with bleach and the attendance at the other type of outdoor pool. ORs for exhaled NO were adjusted for total serum IgE, parental allergy, gender and house cleaning with bleach. The numbers of subjects among the referents and the three pool attendance categories were as follows: residential pools, 692, 54, 63, 38; non-residential pools 408, 164, 208, 67.

Table 3 Risks of ever and asthma among adolescents without parental asthma and having attended an indoor pool for less than 500 hours according to their lifetime outdoor pool attendance and their total serum IgE level.

Outdoor pool attendance (hours)		Total serum IgE (kIU/l)										
		All adolescents n=547		< 25 kIU/l n=175		> 25 kIU/l n=372		> 50 kIU/l n=281		> 100 kIU/l n=184		
Range	Median*	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	
Ever	0	1.0 (1.0-1.0)		1.0 (1.0-1.0)		1.0 (1.0-1.0)		1.0 (1.0-1.0)		1.0 (1.0-1.0)		
asthma	>0-100	42	0.74 (0.24-2.28)	0.69	0.43 (0.05-3.88)	0.43	1.21 (0.33-4.47)	0.88	1.42 (0.36-5.56)	0.61	1.61 (0.38-6.86)	0.55
	>100-500	240	2.21 (0.97-5.02)	0.06	0.96 (0.22-4.27)	0.96	3.58 (1.29-9.91)	0.01	3.82 (1.29-11.3)	0.02	5.44 (1.64-18.2)	0.006
	>500	784	3.96 (1.60-9.86)	0.003	2.42 (0.51-11.5)	0.27	4.67 (1.50-12.9)	0.008	4.98 (1.51-16.5)	0.009	9.50 (2.46-36.1)	0.001
Current	0	0	1.0 (1.0-1.0)		1.0 (1.0-1.0)		1.0 (1.0-1.0)		1.0 (1.0-1.0)		1.0 (1.0-1.0)	
asthma	>0-100	42	1.19 (0.26-7.57)	0.40	2.18 (0.13-36)	0.58	1.67 (0.33-8.46)	0.54	1.72 (0.34-8.81)	0.52	1.54 (0.30-8.00)	0.61
	>100-500	240	3.10 (0.92-10.5)	0.07	0.0 (0 -ND)	0.98	4.45 (1.17-16.9)	0.03	4.14 (1.08-15.8)	0.04	4.40 (1.13-17.2)	0.03
	>500	784	6.38 (1.78-22.8)	0.004	3.60 (0.22-60.1)	0.37	6.14 (1.47-25.6)	0.01	5.77 (1.37-24.3)	0.02	7.88 (1.77-35.0)	0.007

*These values correspond to the whole population included in this analysis (n=547). The numbers of subjects among referents (OR, 1.00) and the three pool attendance categories were as follows: total population, 211, 118, 147, 71; serum IgE < 25 kIU /l, 73, 34, 47, 21 ; serum IgE > 25 kIU/l, 138, 84, 100 and 50; serum IgE > 50 kIU/l, 101, 60, 80, 40; serum IgE > 100 kIU/l, 66, 44, 52, 22; ORs for ever or current asthma calculated on all adolescents were adjusted for total serum IgE with also the presence of mould on bedroom wall in case of ever asthma. After stratification for serum IgE concentration, ORs for ever asthma were adjusted for the presence of mould on bedrooms wall, the only predictor remaining in the model at p<0.20 (no adjustment was made for serum IgE that was the stratification criterion).

Table 4 Risks of ever asthma among adolescents from the schools of Louvain-la-Neuve and Bastogne-Lessines without parental asthma and having attended an indoor pool for less than 500 hours according to their lifetime outdoor pool attendance and their total serum IgE level

		Outdoor pool attendance (hours)						Total serum IgE					
		< 25 kIU/l			> 25 kIU/l			<>50 kIU/l			> 100 kIU/l		
Range	Median*	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Louvain-la-Neuve (n=285)	0	1.0 (1.0-1.0)	1.0	1.0 (1.0-1.0)	1.0	1.0 (1.0-1.0)	1.0	1.0 (1.0-1.0)	1.0	1.0 (1.0-1.0)	1.0	1.0 (1.0-1.0)	1.0
>0-100	35	1.77 (0.14-22.5)	0.86	0.69 (0.03-14.3)	0.81	0.74 (0.04-14.8)	0.85	0.85 (0.05-15.5)	0.91				
>100-500	280	0.80 (0.06-10.5)	0.84	11.7 (1.11-124)	0.04	10.9 (1.07-110)	0.54	10.9 (1.13-106)	0.04				
>500	860	0.81 (0.11-6.0)	0.20	17.7 (1.51-207)	0.02	16.1 (1.41-184)	0.03	17.7 (1.60-196)	0.02				
Bastogne and Lessines (n=262)	0	1.0 (1.0-1.0)	1.0	1.0 (1.0-1.0)	1.0	1.0 (1.0-1.0)	1.0	1.0 (1.0-1.0)	1.0	1.0 (1.0-1.0)	1.0	1.0 (1.0-1.0)	1.0
>0-100	45	0.0 (0-ND)	1.0	1.77 (0.38-8.20)	0.46	2.51 (0.48-13.2)	0.28	3.00 (0.50-18.4)	0.22				
>100-500	200	0.0 (0-ND)	1.0	2.10 (0.56-7.9)	0.27	2.58 (0.61-10.9)	0.04	3.67 (0.76-17.9)	0.11				
>500	725	0.0 (0-ND)	1.0	3.9 (0.82-18.6)	0.09	4.76 (0.87-26.0)	0.07	12.8 (1.69-97.8)	0.01				

*These values correspond to the adolescents without parental asthma and with a cumulated indoor chlorinated pool attendance lower than 500 hours. The numbers of subjects among referents (OR, 1.00) and the three pool attendance categories were as follows: Louvain-la-Neuve, total population, 78, 72, 90, 45; serum IgE < 25 kIU/l, 25, 15, 33, 11; serum IgE > 25 kIU/l, 53, 57, 57 and 34; serum IgE > 50 kIU/l, 40, 42, 46 and 27; serum IgE > 100 kIU/l, 27, 29, 29 and 16 serum; Bastogne-Lessines, total population, 133, 46, 57, 26; serum IgE < 25 kIU/l, 48, 19, 14, 10; serum IgE > 25 kIU/l, 85, 27, 43 and 16; serum IgE > 50 kIU/l, 61, 18, 34 and 13; serum IgE > 100 kIU/l, 39, 15, 23 and 6 serum. ORs were adjusted for the presence of mould on bedroom walls, the only predictor entering in the model at p<0.20 (no adjustment was made for serum IgE that was the stratification criterion).

Table 5 Risks of asthma, increased exhaled NO and of sensitization to aeroallergens associated with the attendance at a residential outdoor chlorinated swimming pool before the age of seven years during a cumulative time of more than 50 hours

	Residential outdoor pool					
	attendance before the age of seven years		OR (95% confidence interval)*			
	no (n=804)	yes (n=43)	Unadjusted	P	Adjusted	P
Any aeroallergen IgE (N, %)	292 (36.4)	23 (53.5)	2.01 (1.08-3.72)	0.03	2.20 (1.14-4.22)	0.02
House-dust mite IgE (N, %)	207 (25.9)	18 (41.9)	2.08 (1.11-3.88)	0.02	2.42 (1.26-4.64)	0.008
Cat IgE (N, %)	97 (12.1)	10 (23.3)	2.20 (1.05-4.61)	0.04	2.57 (1.21-5.47)	0.014
Dog IgE (N, %)	42 (5.2)	2 (4.7)	0.89 (0.21-3.78)	0.87	1.13 (0.26-4.92)	0.87
Mould IgE (N, %)	1 (2.3)	23 (2.9)	0.81 (0.11-6.1)	0.84	1.09 (0.14-8.46)	0.93
Pollen IgE (N, %)	169 (21.0)	7 (16.3)	0.73 (0.32-1.67)	0.46	0.82 (0.35-1.89)	0.63
Ever asthma (N, %)	77 (9.6)	11 (25.6)	3.25 (1.57-6.70)	0.001	3.49 (1.61-7.57)	0.002
Current asthma (N, %)	48 (6.0)	7 (16.3)	3.06 (1.30-7.24)	0.01	2.98 (1.15-7.73)	0.025
Exhaled NO>30 ppb (N, %)	111 (13.8)	12 (27.9)	2.41 (1.20-4.84)	0.01	2.67 (1.28-5.55)	0.009

NO, nitric oxide. The median pool attendance of the 43 adolescents having attended a residential pool before the age of seven was 300 hours (IQR, 148-480). * OR for ever asthma was adjusted for total serum IgE, parental asthma, gender, maternal smoking during pregnancy, house cleaning with bleach, mould on bedroom walls, number of older siblings and the attendance at non-residential outdoor pool before the age of 7 years; OR for current asthma was adjusted for total serum IgE, parental asthma, gender, maternal smoking during pregnancy and house cleaning with bleach. OR for NO>30 ppb was adjusted for total serum IgE, parental allergy, gender and house cleaning with bleach while ORs for sensitization to aeroallergens were adjusted for total serum IgE, parental allergy and gender (house dust mite, any aeroallergen), total serum IgE and parental allergy (cat, dog) or for total serum IgE and gender (mould and pollen).

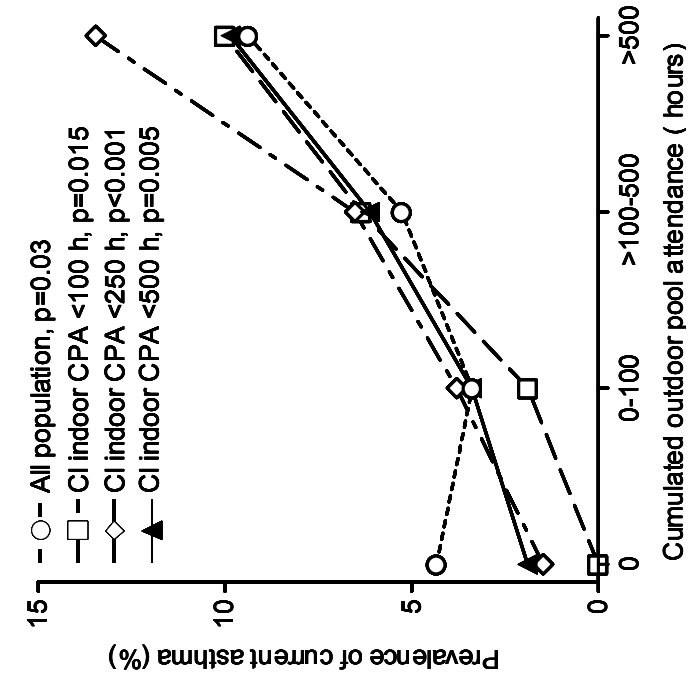
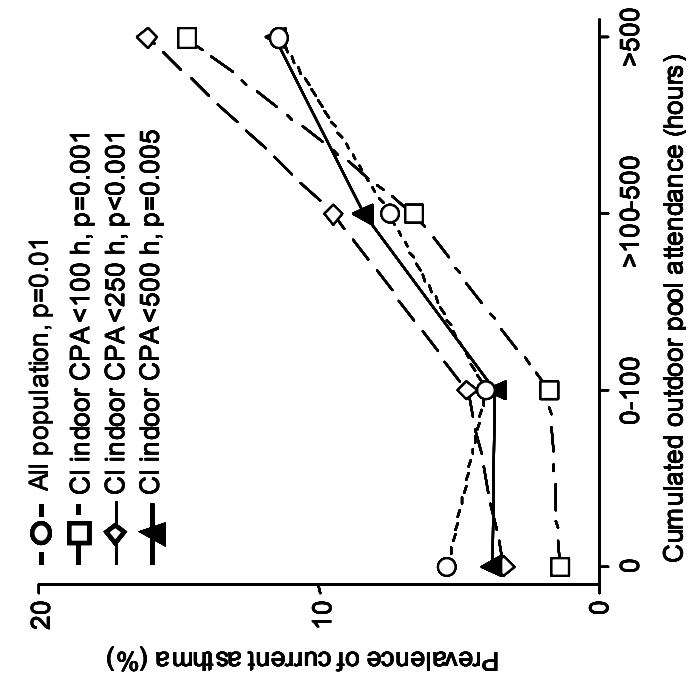
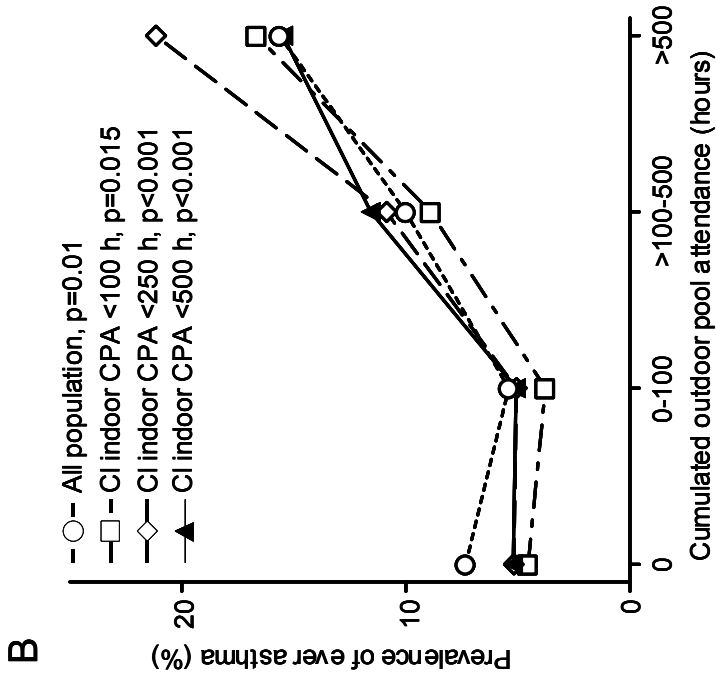
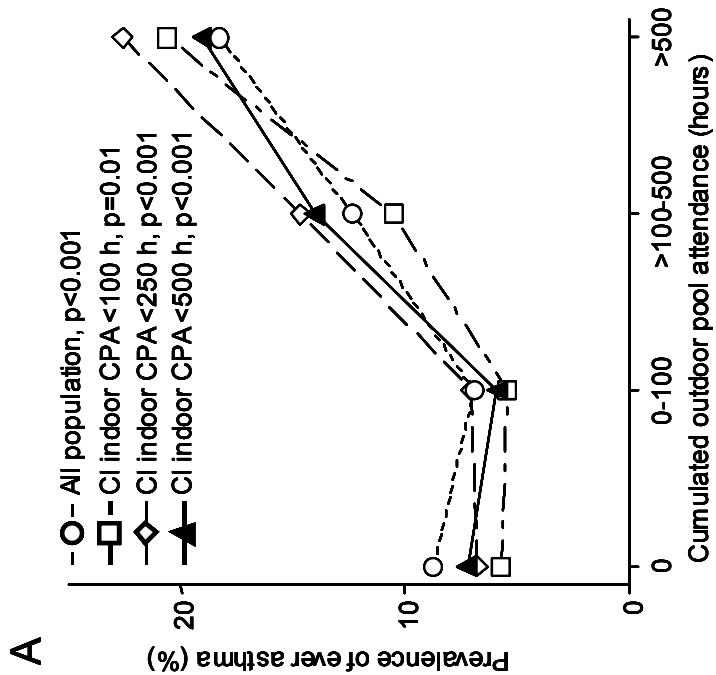


Figure 1