

House cleaning with chlorine bleach and the risks of allergic and respiratory diseases in children

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.

© 2006 The Authors

Journal compilation © 2006 Blackwell Munksgaard

Marc Nickmilder, Sylviane Carbonnelle and Alfred Bernard

Department of Public Health, Catholic University of Louvain, Brussels, Belgium

Chlorine bleach or sodium hypochlorite can inactivate common indoor allergens. In this cross-sectional study we evaluated to what extent regular house cleaning with bleach can influence the risks of respiratory and allergic diseases in children. We studied a group of 234 school-children aged 10–13 yr among whom 78 children were living in a house cleaned with bleach at least once per week. Children examination included a questionnaire, an exercise-induced bronchoconstriction test and the measurement of exhaled nitric oxide (NO) and of serum total and aeroallergen-specific immunoglobulin (Ig)E, Clara cell protein (CC16) and surfactant-associated protein D (SP-D). Children living in a house regularly cleaned with bleach were less likely to have asthma (OR, 0.10; CI, 0.02–0.51), eczema (OR, 0.22; CI, 0.06–0.79) and of being sensitized to indoor aeroallergens (OR, 0.53; CI, 0.27–1.02), especially house dust mite (OR, 0.43; CI, 0.19–0.99). These protective effects were independent of gender, ethnicity, previous respiratory infections, total serum IgE level and of family history of allergic diseases. They were however abolished by parental smoking, which also interacted with the use of bleach to increase the risk of recurrent bronchitis (OR, 2.03; CI, 1.12–3.66). House cleaning with bleach had effect neither on the sensitization to pollen allergens, nor on the levels of exhaled NO and of serum CC16 and SP-D. House cleaning with chlorine bleach appears to protect children from the risks of asthma and of sensitization to indoor allergens while increasing the risk of recurrent bronchitis through apparently an interaction with parental smoking. As chlorine bleach is one of the most effective cleaning agent to be found, these observations argue against the idea conveyed by the hygiene hypothesis that cleanliness *per se* increases the risk of asthma and allergy.

Key words: chlorine; bleach; hypochlorite; trichloramine; nitrogen trichloride; asthma; allergy; hygiene hypothesis; day care; swimming pool; lung biomarkers

Alfred Bernard, Department of Public Health, Université Catholique de Louvain, 30.54 Clos Chapelle-aux-Champs, B-1200 Brussels, Belgium
Tel.: +32 2 764 5334
Fax: +32 2 764 5338
E-mail: bernard@toxi.ucl.ac.be

Accepted 12 September 2006

Chlorine bleach or sodium hypochlorite is the most commonly used disinfecting and cleaning agent in the developed world. First introduced in the late 19th century to treat sepsis, chlorine bleach is now used in daily life for a variety of applications such as water and food disinfection and cleaning of surfaces in public and private buildings. Advantages of bleach include low cost, easy use, residual protection, deodorizing and a strong germicide activity against a wide spectrum of microorganisms (1). Recently, chlorine bleach

has also been found to be effective in the inactivation of cat (Fed1) and house dust mite (Der p1) allergens (2, 3), suggesting that it might perhaps offer some protection against allergic diseases.

However, chlorine bleach is a unstable and highly reactive chemical that must be used with caution. When mixed with other cleaning agents or when reacting with organic matter or some metals, chlorine bleach can release chlorine or trichloramine, two gases which are strong irritant

to the eyes and the respiratory tract (4–6). Chlorine gas is released when hypochlorite is mixed with an acid or dissociates as a result of exposure to UV or of contact with some metallic surfaces. Trichloramine, by contrast, is formed when hypochlorite reacts with nitrogenous substances such as ammonia or urea. As opposed to chlorine gas, trichloramine is a water-insoluble gas that is not retained by the upper respiratory tract and thus exerts its toxic action predominantly on the deep lung (7). Both chlorine and trichloramine gases have caused many cases of acute lung injury among professional cleaners, housewives and other people doing cleaning tasks (8, 9).

While the acute toxicity of gases released when using bleach has been known for over one century, the possibility that these gases can cause chronic lung damage has been acknowledged only in recent studies. Our investigations on children attending indoor chlorinated swimming pools have shown that trichloramine, the gas giving swimming pools their characteristic smell, can damage the lung epithelium and promote the development of atopic asthma (10–12). Recently also, trichloramine has been found to induce asthma in lifeguards working in indoor swimming pools (13). Other studies focusing on occupational risks have demonstrated that women employed in domestic cleaning and using chlorine bleach have an elevated risk of respiratory diseases such as asthma and chronic bronchitis (14–16).

To our knowledge no study has attempted to evaluate the potential health impact of house cleaning with bleach, not for persons using this chemical but for those living in places regularly cleaned with it. In this cross-sectional study, we compared the respiratory health of children living in a house regularly cleaned with bleach with that of children living in homes not cleaned with this disinfectant.

Methods

Study population

The ethics committee of the Faculty of Medicine of the Catholic University of Louvain approved the study protocol. Children were selected from a population of schoolchildren recruited in Brussels from the fifth and sixth grades of 10 primary schools. They were examined after their parents had given written informed consent. Of the 341 children initially enrolled in the study, we excluded 107 children who had a backyard swimming pool, had swum when they were baby or were

members of a swimming club. As revealed by recent studies (10–12), exposure to volatile chlorination products when regularly attending swimming pools during early life or as part of a sport activity, are risk factors of childhood asthma likely to confound or to mask the possible effects of bleach use at home.

Questionnaire

Parents of children were asked to complete a questionnaire that included a total of 38 questions inquiring, among other items, about family history of allergic diseases (asthma, hay fever or eczema), recurrent infectious diseases (doctor-diagnosed bronchitis, otitis or pneumonia ever), asthma and allergic diseases (doctor-diagnosed asthma, eczema or hay fever ever), respiratory symptoms (wheezing, cough, chest tightness and shortness of breath during the last 12 months) and some risks factors during early life and infancy (smoking of mother during pregnancy, birth weight, breastfeeding, day nursery attendance before the age of 2 yr). There were also several questions about lifestyle or environmental factors likely to be involved in the development of respiratory or allergic diseases such as number of siblings, housing density (persons/room), sporting activity, living with pets at home (since birth or from <2 yr), exposure to environmental tobacco, home cleaning with chlorine bleach (at least once per week), house with double-glazed windows, mould on child's bedroom walls and living in a rural or urban area. The questionnaire also included 12 questions specifically developed to calculate for each year since birth the total time each child spent weekly in an indoor chlorinated pool with the school (a compulsory activity in Belgium), with their parents or as a sporting activity with a club. Parents were also interrogated about the existence of an outdoor or indoor pool at home and on whether their child had regularly attended a pool before the age of 2 yr (swimming baby).

Examination of children

All children were examined in their school between 28 March and 29 May 2002, thus outside main periods of pollination in Belgium. Examinations took place in the morning between approximately 9:00 and 13:00 hours in order to minimize circadian variations. Examination started with the measurement of height and weight and the collection of one blood sample after application of an anaesthetic cream (Emla, Astra-Zeneca, Sweden). A volume of 7.5 ml of

venous blood was taken by venipuncture and collected on a dry tube. Blood samples were allowed to clot for a minimum of 12 h at 4°C. They were then centrifuged at 2000 g for 10 min and serum was decanted and stored at -18°C until protein analyses.

Exhaled nitric oxide, lung function and exercise-induced bronchoconstriction test

The nitric oxide (NO) concentration in exhaled breath was measured on-line by chemiluminescence using the NIOXTM analyser (Aerocrine AB, Solna, Sweden). The test was performed in compliance with the guidelines of the American Thoracic Society (17). The forced vital capacity and the forced expiratory volume in 1 s (FEV₁) were then measured with a Vitalograph-Compact (Vitalograph Ltd, Buckingham, UK) according to the American Thoracic Society standards (18). Exercise-induced bronchospasm (EIB) was attested by a decrease in FEV₁ ≤ 20% (FEV₂₀) from baseline after a 6-min runabout with submaximal effort (heart beats > 180/min). The heart rate was monitored continuously with a Polar Electro OY (Kempele, Finland). The test was performed indoors to avoid confounding by weather conditions. At least five measurements were taken before the exercise and three measurements 5 and 10 min after the exercise, until a minimum of two values differing by < 5% were obtained. At each time we used the highest value. The difference between the baseline value and the more negative value obtained after exercise was retained to appreciate EIB. Total asthma prevalence was then calculated as the prevalence of children positive in the EIB test plus the prevalence of children negative in this test but with a doctor-diagnosed asthma reported in the questionnaire (ever doctor-diagnosed asthma).

Serum pneumoproteins and total and specific immunoglobulin E

Clara cell protein (CC16) was determined by latex immunoassay using a rabbit anti-CC16 antibody (Dakopatts, Glostrup, Denmark) and as standards CC16 purified in our laboratory (19). All samples were run in duplicate at two different dilutions. This assay has been validated by comparison with a monoclonal antibody-based enzyme-linked immunosorbent assay (19). The between- and within-run coefficients of variation range from 5% to 10%. Serum concentration of surfactant-associated protein D (SP-D) were measured by SP-D EIA kit of Yamasa Corporation® (Tokyo, Japan). Total serum immunoglobulin (Ig)E was measured by Immulite® Total IgE

kit DPC (Los Angeles, CA, USA). Specific IgE to common airborne allergens were measured by DPC Immulite AlaTOP® Allergy Screen immunoassay containing a balanced mixture of the top 12 allergens most commonly associated with inhalant allergy: *Dermatophagoides pteronyssinus*, Cat Epithelium, Dog Dander, Bermuda Grass, Timothy Grass, *Penicillium notatum*, *Alternaria tenuis*, Birch, Japanese Cedar, Common Ragweed, English Plantain and *Parietaria officinalis*. IgE against six specific allergens were also measured separately by DPC Immulite Allergen-specific IgE® kits (*Dermatophagoides pteronyssinus*, Cat Epithelium, Dog Dander, Timothy Grass, Birch and *Artemisia vulgaris*).

Statistical analyses

All statistical tests were applied after checking the normality of variables that were normalized by log transformation when necessary. We used the Student's t-test (two-sided), the chi-squared test or the Fisher's exact test to compare mean values or prevalences between children living in a house cleaned with bleach and the other children. The Mann-Whitney test was used to compare the cumulated indoor swimming pool attendance between the two groups. The predictors of outcomes were identified by multiple logistic regression models testing the following independent variables: age, gender (0, girl; 1, boy), body mass index, ethnic origin (0, non-white; 1, white), serum IgE (kIU/l), family size (siblings), housing density (persons/room), cumulated chlorinated pool attendance (unit = 100 h), sporting activity other than swimming, daycare attendance in infancy, mould on the wall of the sleeping room, house with double-glazed windows, parental history of allergic diseases (mother or father with asthma, eczema or hay fever), environmental tobacco smoke, mother who had smoked during pregnancy, child breast-fed in infancy, pets since < 2 yr, pets since birth and house cleaned with chlorine bleach at least once per week. Best-fit models were built with stepwise approach by backward elimination until all retained variables had reached a $p < 0.10$. Factors influencing NO concentration in exhaled air and concentrations of CC16 and SP-D in serum were identified by stepwise multiple regression analysis by testing the same independent variables as in logistic regression analysis. The statistical package StatView® 5 (Release 5.0.1, 3rd edn, a business unit of SAS; SAS Institute Inc., Cary, NC, USA, 2001) was used for all analyses. The level of statistical significance was assigned at $p < 0.05$.

Results

A total of 78 children were living in a house that was cleaned with chlorine bleach at least once per week. As shown in Table 1, these children did not differ from the others with respect to age, gender, number of siblings, cumulated swimming pool attendance, living with pets, breastfeeding or family history of atopic diseases. The proportion of children exposed to parental smoking was, however, noticeably greater among children living in a house cleaned with bleach. Housing density, ethnicity, proportion of children regularly practising a sport other than swimming or of children living in a house with double-glazed windows were also significantly greater among children of the chlorine bleach group (Table 1).

The respiratory health, allergic status and lung epithelium integrity of children living in a house cleaned with bleach and of their peers are compared in Table 2. There were no significant differences in the respiratory symptoms between the two groups with the exception perhaps of the prevalence of wheezing that was less frequently reported by the bleach group (3.8% vs. 10.9%, $p = 0.07$). The two groups also did not differ

Table 1. Characteristics of children

	House cleaning with chlorine bleach		p-value
	Yes	No	
n	78	156	
Gender (male)	43 (55.1)	81 (51.9)	0.64
Age (yr)*	11.6 (0.8)	11.5 (0.7)	0.38
BMI (kg/m ²)*	19.0 (2.9)	18.3 (2.5)	0.07
Ethnicity (no. white)	50 (64.1)	128 (82.1)	0.002
Birth weight (kg)*	3.229 (0.531)	3.36 (0.56)	0.10
Breastfeeding	52 (66.7)	114 (73.1)	0.36
Mother smoking during pregnancy	11 (14.1)	23 (14.7)	0.90
Daycare attendance	31 (39.7)	83 (53.2)	0.05
Number of siblings*	2.7 (1.3)	2.6 (1.3)	0.47
Housing density (persons/room)*	0.89 (0.40)	0.76 (0.23)	0.003
Parental asthma	14 (17.9)	29 (18.6)	0.91
Parental eczema	11 (14.1)	18 (11.5)	0.58
Parental hay fever	15 (19.2)	47 (30.1)	0.08
Parental smoking	27 (34.6)	30 (19.2)	0.01
Pets from <2 yr	29 (37.2)	66 (42.3)	0.45
Pets since birth	10 (12.8)	28 (17.9)	0.32
Mould on bedroom walls	5 (6.4)	14 (9.0)	0.50
House with double-glazed windows	51 (65.4)	123 (78.8)	0.03
Cumulated swimming pool attendance (h)†	72.0 (0.0–892)	69.0 (0.0–716)	0.53
Sporting practice (other than swimming)	33 (42.3)	100 (64.1)	0.002

Values in parentheses are expressed in percentage.

BMI, body mass index.

*Values given are mean (s.d.).

†Values given are median (min–max).

Table 2. Respiratory symptoms, spirometric tests, lung biomarkers and immunological characteristics of children

	Use of chlorine bleach (n = 78)	No use of chlorine bleach (n = 156)	p-value
Symptoms			
Wheezing	3 (3.8)	17 (10.9)	0.07
Chest tightness	3 (3.8)	7 (4.5)	0.99
Shortness of breath	2 (2.6)	11 (7.1)	0.23
Cough	11 (14.1)	26 (16.7)	0.61
Spirometry			
FVC (pred %)*	97.8 (12.2)	93.3 (12.4)	0.17
FEV ₁ (pred %)*	94.5 (12.3)	91.2 (14.2)	0.08
FEV ₁ /FVC (%)	87.9 (5.0)	87.9 (6.3)	0.99
Lung biomarkers			
Exhaled NO (p.p.b.)†	10.7 (4.4–55.7)	10.3 (2.8–101)	0.69
Serum CC16 (μg/l)†	9.9 (1.4–25.5)	9.5 (1.6–25.6)	0.46
Serum SP-D (μg/l)†	81.9 (19–218)	93.3 (13–312)	0.07
Allergy			
Serum total IgE (kIU/l)†	69.8 (5–3545)	68.4 (4–3204)	0.92
Aeroallergen-specific IgE	17 (21.8)	54 (34.6)	0.04
Indoor aeroallergens IgE	11 (14.1)	36 (23.4)	0.09
House mite-specific IgE	10 (12.8)	33 (21.3)	0.11
Cat-specific IgE	3 (3.8)	10 (6.5)	0.55
Dog-specific IgE	2 (2.6)	6 (3.9)	0.72
Pollen-specific IgE	7 (9.0)	15 (9.7)	0.85
Diseases			
Exercise-induced asthma (FEV ₂₀)	1 (1.3)	7 (4.7)	0.20
Asthma diagnosed by a physician	2 (2.6)	14 (9.0)	0.07
Total asthma (diagnosed and/or FEV ₂₀)	3 (3.8)	21 (13.5)	0.02
Recurrent bronchitis	36 (46.2)	53 (34.0)	0.07
Recurrent cold	29 (37.2)	40 (25.6)	0.07
Recurrent sinusitis	9 (11.5)	11 (7.1)	0.25
Doctor-diagnosed hay fever	11 (14.1)	13 (8.3)	0.17
Doctor-diagnosed eczema	3 (3.8)	22 (14.1)	0.02

Values in parentheses are expressed in percentage.

FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 s; FEV₂₀, FEV₁ ≤ 20%; NO, nitric oxide; IgE, immunoglobulin E; CC16, Clara cell protein; SP-D, surfactant-associated protein D.

*Values given are mean (s.d.).

†Values given are geometric mean (min–max).

significantly with respect to the lung function parameters which, if anything, were slightly higher in the bleach group. The rate of sensitization to pollen-specific IgE and the mean levels of exhaled NO, serum CC16 and total serum IgE were also very similar between the two groups of children. By contrast, children of the bleach group showed a lower prevalence of asthma, either diagnosed by a doctor or screened with the EIB test, a difference that reached the level of statistical significance when combining these two indicators. Prevalences of doctor-diagnosed eczema and sensitization rate to indoor aeroallergens were also significantly reduced in these children whereas the prevalences of recurrent

bronchitis and of frequent cold tended on the contrary to rise ($p = 0.07$, Table 2).

We further analysed these data by logistic or multiple regression analyses, both to detect

possible interactions with other risk factors and to check that our observations were not due to the dependence of the use bleach on another risk factor. As illustrated in Fig. 1, house cleaning

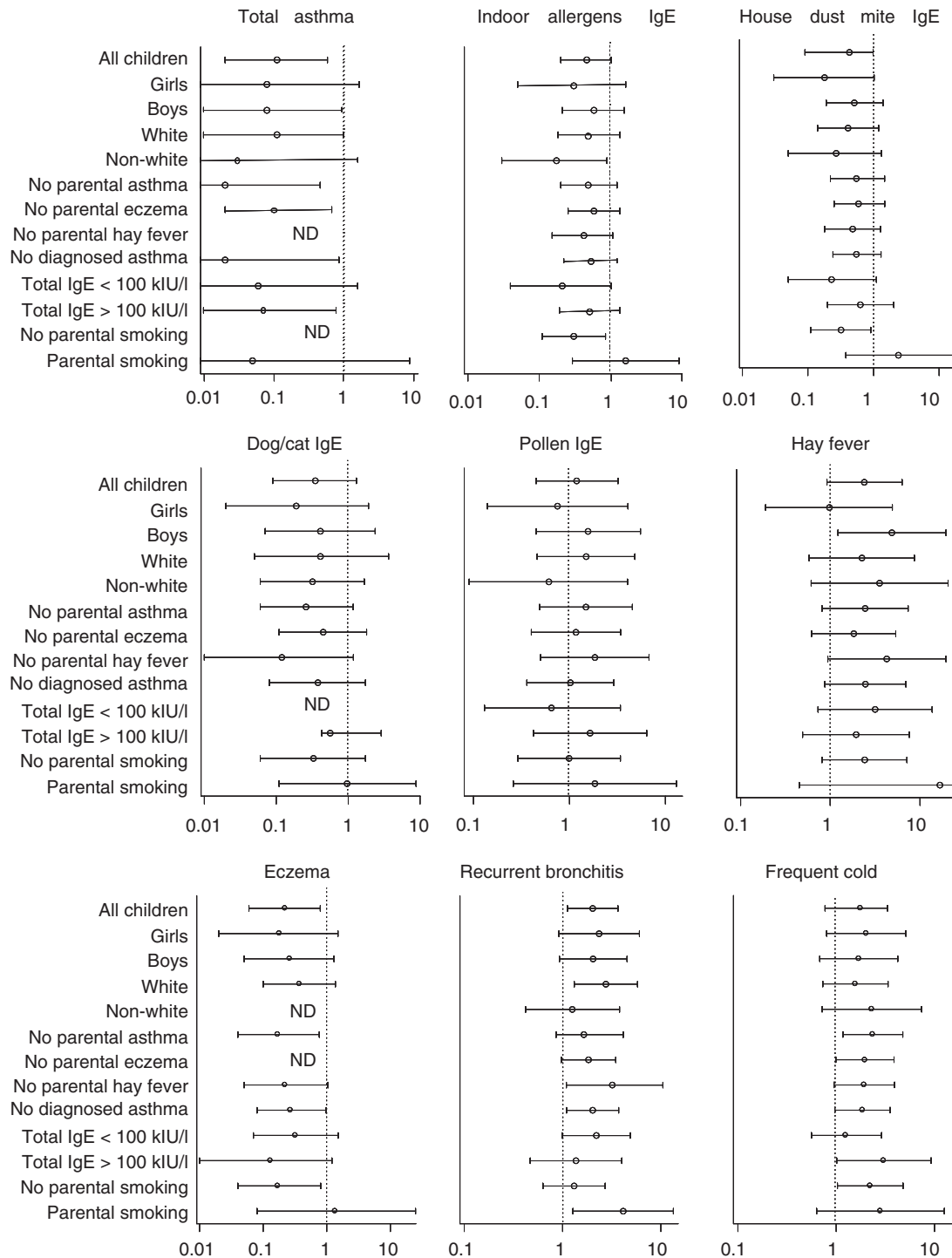


Fig. 1. Adjusted odds ratios for the risks of respiratory and allergic diseases in children living in a house regularly cleaned with chlorine bleach when considering all children or when separating them according to gender, ethnicity, parental allergic diseases, total serum immunoglobulin E levels and exposure to environment. ND, odds ratio could not be determined because there was no positive case for the studied endpoint among children living in a house cleaned with bleach. Total asthma refers to asthma diagnosed by a doctor and/or screened with the exercise-induced bronchospasm test.

with bleach reduced the risk of developing asthma (doctor-diagnosed and/or screened with the EIB test) to about the same extent regardless of the gender, ethnicity, total serum IgE level and the allergic status of parents. This reduction even persisted when excluding children with a medical diagnosis of asthma retaining only the EIB test as asthma indicator. The risk of having eczema or of being sensitized to indoor aeroallergens, in particular to house dust mite and to a less extent to pets, was also reduced by the use of bleach. The decrease in the rate of sensitization to indoor allergens was however lower than that observed with asthma. Children of all categories were protected by cleaning with bleach, at the exception of those exposed to parental smoking. When cleaning with bleach was associated with parental smoking, children were no more protected against the risk of sensitization to pollen-specific IgE and there was even a tendency for the risk of hay fever to increase. By contrast, children in the bleach group were more frequently affected by recurrent bronchitis, an effect that largely stemmed from an interaction with parental smoking. This interaction also emerged in the odds ratio for recurrent bronchitis associated with passive smoking, which was significant than 1.0 in children of the bleach group (OR, 2.86, 95% CI, 1.09–7.52, $p = 0.03$) but not in other children (OR, 0.97, 95% CI, 0.42–2.24, $p = 0.93$).

Other predictors of asthma (doctor-diagnosed and/or screened with the EIB test) identified included parental hay fever (OR, 5.74; 95% CI, 2.18–15.2, $p = 0.0004$), total serum IgE (OR for each 100 kUI/l increase, 1.12; 95% CI, 1.04–1.21, $p = 0.004$) and housing density (OR for person/room, 5.67; 95% CI, 1.23–26.2, $p = 0.03$). Of note, although we excluded children the most exposed to chlorine of swimming pools, the asthma risk showed still an increase with cumulated swimming pool attendance (OR, 1.33; 95% CI, 0.97–1.84, $p = 0.08$), especially in children with an elevated total serum IgE ($n = 82$, OR, 1.60; 95% CI 1.03–2.5, $p = 0.04$). The risk of sensitization to indoor allergens increased with male sex (OR, 2.53; 95% CI, 1.21–5.28, $p = 0.01$), number of siblings (OR, 1.33; 95% CI, 1.02–1.74, $p = 0.04$) and with the presence of mould on the walls of the child's bedroom (OR, 4.81; 95% CI, 1.68–13.8, $p = 0.004$). Interestingly, the probability of being sensitized to indoor aeroallergens was decreased by daycare attendance (OR, 0.46; 95% CI, 0.22–0.95, $p = 0.01$).

The protection conferred by cleaning with bleach was independent of the possible influence

of recurrent bronchitis and frequent cold, two conditions that were more frequently reported in the bleach group. When tested in logistic regression analysis together with other predictors, recurrent bronchitis and frequent cold did not emerge as risk factors for asthma ($p = 0.46$ and 0.48 respectively), which remained strongly diminished by the use of chlorine bleach (OR, 0.13, 95% CI, 0.026–0.67, $p = 0.02$). The protective effect of chlorine bleach against sensitization to indoor aeroallergens also persisted (OR, 0.37; 95% CI, 0.16–0.87, $p = 0.02$). Interesting, while the recurrent bronchitis had no influence ($p = 0.77$), frequent cold appeared as a very significant risk factor for sensitization against indoor allergens (OR, 3.2; 95% CI, 1.49–6.89, $p = 0.003$). There was also no difference in the protection offered by bleach against the risk of asthma between children who reported recurrent bronchitis (OR, 0.049; 95% CI, 0.002–1.22, $p = 0.07$) and those who did not (OR, 0.12; 95% CI, 0.013–1.066, $p = 0.057$).

Regarding other pulmonary tests, multiple regression analyses confirmed that cleaning with chlorine bleach had neither influence on the lung function, nor on the levels of exhaled NO and serum CC16 (results not shown). The tendency for serum SP-D to decrease in the chlorine bleach group completely disappeared after adjustment for other determinants (age and ethnicity).

Discussion

Our findings show that children living in a house regularly cleaned with chlorine bleach have a significantly lower risk of developing asthma and of being sensitized to indoor allergens, especially house dust mite. These protective effects of chlorine bleach were observed in all categories of children regardless of gender, ethnicity, previous respiratory infections, parental history of allergic diseases and even of their total serum IgE level. The only factor interfering with these effects was parental smoking, which abolished the protection given by the use of bleach against the risk of sensitization to indoor allergens while increasing the risk of recurrent bronchitis through apparently an interaction with the use of bleach.

Confounding by parental behaviour is the first issue to consider when discovering a situation that appears to protect children against the risks of asthma or allergy (20). This is especially true in the case of chlorine bleach, a cleaning agent that parents may decide to use or not to use depending on their perception of the risks and benefits of chlorine. Parents with asthma or

having children with respiratory problems might indeed fear that chlorine would aggravate their conditions and therefore they might avoid exposing their children or themselves to this irritant. If chlorine toxicity is not a matter of concern for them, parents on the contrary might be encouraged to use chlorine bleach for a more effective removal of household allergens. However, we think that it is unlikely that parental behaviour biased our findings. Three observations indeed argue against this possibility. First, the proportions of children whose parents had asthma, eczema or hay fever were not significantly different between children who were living in a house cleaned with bleach and the other children, suggesting that our results have not been confounded by the allergic status of the parents. Secondly, the mean concentrations of total IgE in serum were very similar between these two groups of children, also suggesting that these children were not different with regard to their inherited propensity to develop allergic diseases. Thirdly, and this is probably the strongest argument, the protection given by chlorine bleach was not abolished by the exclusion of children who had a medical diagnosis of asthma and who were thus protected by bleach against the risk of having a positive EIB test or of being sensitized to indoor allergens.

Protection offered by cleaning with chlorine bleach against the risk of sensitization to indoor allergens is most probably the consequence of a lower exposure to these allergens as the rate of sensitization to indoor allergens has been found to correlate to the levels of allergens in homes (21, 22). This lower rate of atopic sensitization in turn might account for the reduced risk of asthma, the two outcomes being frequently associated, especially among children sensitized to house dust mite. This explanation ties in with previous studies showing that measures reducing the levels of indoor allergens such as smooth floor covering, daily cleaning and use of mite allergen-impermeable mattress encasings are effective strategies to prevent allergic diseases (23, 24). The decreased exposure to indoor allergens associated with the use of bleach could largely result from the inactivation of domestic allergens by hypochlorite, an explanation suggested by recent *in vitro* studies (2, 3). But other factors correlating with the use of bleach might also contribute to reduce the exposure to indoor allergens. If parents clean their home with bleach, it is presumably because they are particularly concerned by the cleanliness, thus meaning that they might clean their home more frequently or more carefully than parents who do not use

bleach. In such homes, even if they are not cleaned with bleach, carpets, curtains and soft furnishing might thus be also cleaner and contain less allergens and microbial contaminants than in households not using bleach. Cleaning with a liquid and aggressive cleaner such as chlorine bleach also implies housing conditions and materials that are resistant to this agent and therefore less likely to trap allergens, such as smooth soil or surfaces made of marble, porcelain or ceramic tiles.

If one assumes that house cleaning with bleach protects children from allergic diseases by decreasing their exposure to indoor allergens, logically this protective effect should be greater on the rate of sensitization to indoor allergens than on the rate of asthma and not be just the inverse as observed in our study. As recurrent bronchitis was more frequently reported in the bleach group, this discrepancy could be explained by a differential reporting as in this age group symptoms consistent with a diagnosis of recurrent infection or bronchitis may be due to underlying asthma. However, the protection offered by bleach towards asthma risk did not rest solely upon observations made with doctor-diagnosed asthma. This protection also emerges when considering as asthma outcome a positive EIB test without a medical diagnosis of asthma or the current wheezing reported by questionnaire, even if the protection observed with the latter indicator did not reach the level of statistical significance ($p = 0.07$). We think that the explanation for the stronger protective effect of bleach towards asthma than towards sensitization to indoor allergens has to be sought in the high cleanliness achieved with bleach whose disinfecting action indeed does not limit to the inactivation of allergens. Cleaning with a strong biocide such as bleach should necessarily reduce also the exposure to other harmful microbial agents such as fungal products or endotoxins that have been associated with an increase in asthma severity and symptoms (25–28). Thus, the strong reduction in asthma risk observed in children living in a house cleaned with bleach might be explained by their lower domestic exposure not only to allergens but also to microbial agents such as endotoxin that are known to be important risk factors for the development of childhood asthma.

The apparent beneficial effects of chlorine used for indoor cleaning should, however, be weighed against the possible adverse effects of irritating gases released when using bleach. The acute respiratory toxicity of these gases is well documented especially among domestic cleaners who

have accidentally mixed bleach with ammonia or hydrochloric acid (8, 9). Acute lung injury developing under these conditions is usually characterized by a sudden onset of asthma-like symptoms, which is followed by a form of persistent non-immunological asthma called reactive airways dysfunction syndrome. By contrast, the chronic effects of gases released during normal use of hypochlorite have been identified only recently, first, in swimming pool attendees (10–12) and more recently in domestic cleaners (15). These chronic risks are not really surprising when one considers the airborne concentrations that can be reached when volatile chlorination by-products build up in poorly ventilated areas. During house cleaning, concentrations of chlorine gas can peak at values up to 0.5 p.p.m. (15) and it is likely that trichloramine reach concentrations of the same magnitude when chlorine reacts with ammonia- or nitrogen-containing substances. *A priori* these respiratory risks should not concern children who are directly not involved in cleaning tasks. The increased risk of recurrent bronchitis in children who were living in a house cleaned with bleach and with parents who are smoking was a quite unexpected finding. This phenomenon is clearly the consequence of an interaction since, taken alone, neither parental smoking, nor the use of bleach, did increase the risk of recurrent bronchitis in children. The explanation for this interaction is still unclear. For instance, it might be the consequence of chlorine gas or trichloramine residues persisting in some poorly ventilated areas of the home that would potentiate the effects of environmental tobacco smoke. Another possibility would be the formation of more toxic pollutants when volatile chlorination by-products react with some components of environmental tobacco smoke. The lung biomarkers we used did not reveal any particular epithelial damage in children exposed to bleach or to environmental tobacco smoke but it should be noted that these biomarkers reflect chiefly the integrity of the deep lung epithelium. They are thus unsuitable to detect damage affecting the upper airways where the water-soluble chlorine gas mostly deposits.

Our findings do not support the hygiene hypothesis when the latter postulates that the increase in childhood asthma and allergy can be ascribed to cleanliness *per se*. There is no more effective cleaning product than chlorine bleach, whose strong oxidizing properties allow to destroy or inactivate most biological agents including common household allergens. The finding that the use of such a powerful cleaner is associated with markedly decreased risks of asthma and

atopic sensitization is thus in contradiction with the hygiene hypothesis. Several other findings in our study do not plead in favour of the hygiene hypothesis. For instance, we found no evidence of a protective effect of recurrent respiratory infections on the asthma or allergy risks, an observation that concords with recent studies (29–31). The number of siblings and the housing density, which in the hygiene hypothesis are used as purported indicators of microbial exposure (32), were also found to give no protection but on the contrary to increase the risks of asthma and of sensitization to indoor allergens. The only evidence of a protective effect, consistent with the hygiene hypothesis, came from the attendance of a daycare centre during infancy, a factor reducing indeed the risk of sensitization to indoor allergens. This protective effect of daycare attendance has been reported by other studies (33, 34) which attributed it to the higher risks of cross-infections in this environment. However, and this is a point that has been overlooked so far, daycare centres are places that require a daily disinfection precisely because of this higher microbial exposure. In all daycare centres of Belgium, like probably in most industrialized countries, all objects and surfaces the children can be in contact with are daily disinfected with chlorine bleach. This practice probably explains why the levels of indoor allergens, especially of house dust mite, are usually lower in daycare centres when compared with private houses (35, 36). Paradoxically thus, the protective effect of daycare attendance during infancy against allergic diseases might be the consequence, not of the higher risks of infectious diseases in those places but on the contrary, of the high level of hygiene achieved to cope with these risks.

In conclusion, children living in a house regularly cleaned with bleach appear to have a lower risk of developing asthma and of being sensitized against indoor allergens, a protective effect that might result from a more effective destruction of indoor allergens and microbial agents (e.g. endotoxins or fungal products). These children, however, appear to have a higher risk of recurrent bronchitis when they are exposed to environmental tobacco smoke. These findings do not support the hygiene hypothesis when it proposes that the rise of childhood asthma is linked to the increased cleanliness. They also provide further evidence that chlorine bleach used for indoor cleaning or disinfection releases volatile by-products that can cause detrimental effects on the respiratory tract of children.

Acknowledgments

This study was supported by the Government of the Brussels Capital Region. Alfred Bernard is Research Director of the National Fund for Scientific Research, Belgium.

References

1. RUTALA WA, WEBER D. Uses of inorganic hypochlorite (bleach) in health care facilities. *Clin Microbiol Rev* 1997; 10: 597–610.
2. CHEN P, EGGLESTON PA. Allergenic proteins are fragmented in low concentrations of sodium hypochlorite. *Clin Exp Allergy* 2001; 31: 1086–93.
3. MATSUI E, KAGEY-SOBOTKA A, CHICHESTER K, EGGLESTON PA. Allergic potency of recombinant Fel d 1 is reduced by low concentrations of chlorine bleach. *J Allergy Clin Immunol* 2003; 111: 396–401.
4. DAS R, BLANC PD. Chlorine and the lung: a review. *Toxicol Ind Health* 1993; 9: 439–55.
5. TANEN DA, GRAEME KA, RASCHKE R. Severe lung injury after exposure to chloramine gas from household cleaners. *N Engl J Med* 1999; 341: 848–9.
6. GAGNAIRE F, AZIM S, BONNET P, et al. Comparison of the sensory irritation response in mice to chlorine and nitrogen trichloride. *J Appl Toxicol* 1994; 14: 405–9.
7. CARBONNELLE S, FRANCAUX M, DOYLE I, et al. Changes in serum pneumoproteins caused by short-term exposures to nitrogen trichloride in indoor chlorinated swimming pools. *Biomarkers* 2002; 7: 464–78.
8. RACIOPPI F, DASKALEROS PA, BESBELLI N, et al. Household bleaches based on sodium hypochlorite: review of acute toxicology and poison center experience. *Food Chem Toxicol* 1994; 32: 845–61.
9. GORGUNER M, ASLAN S, INANDI T, CAKIR Z. Reactive airways dysfunction syndrome in housewives due to a bleach-hydrochloric acid mixture. *Inhal Toxicol* 2004; 16: 87–91.
10. BERNARD A, CARBONNELLE S, MICHEL O, et al. Lung hyperpermeability and asthma prevalence in school-children: unexpected associations with the attendance at indoor chlorinated swimming pools. *Occup Environ Med* 2003; 60: 385–94.
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: 1567–73.
12. BERNARD A, NICKMILDER M. Respiratory health and baby swimming. *Arch Dis Child* 2006; 91: 620–1.
13. 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: 827–32.
14. ZOCK JP, KOGEVINAS M, SUNYER M, et al. Asthma risk, cleaning activities and uses of specific cleaning products in Spanish indoor cleaners. *Scand J Work Environ Health* 2001; 27: 76–81.
15. MEDINA-RAMON M, ZOCK JP, KOGEVINAS M, et al. Asthma symptoms in women employed in domestic cleaning: a community-based study. *Thorax* 2003; 58: 950–4.
16. MEDINA-RAMON M, ZOCK JP, KOGEVINAS M, et al. Asthma, chronic bronchitis, and exposure to irritant agents in occupational domestic cleaning: a nested case-control study. *Occup Environ Med* 2005; 58: 1750–4.
17. SLUTZKY AS, DRAZEN JM, SILKO PE, et al. Recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children – 1999. *Am J Respir Crit Care Med* 1999; 160: 2104–17.
18. ATS. Standardization of spirometry – 1994 update. *Am J Respir Crit Care Med* 1995; 152: 1107–36.
19. HERMANS C, ALY O, NYBERG BI, PETERSON C, BERNARD A. Determinants of Clara cell protein (CC16) concentration in serum: a reassessment with two different immunoassays. *Clin Chim Acta* 1998; 272: 101–10.
20. VAN SCHYCK CP, KNOTTNERUS JA. Can the hygiene hypothesis be explained by confounding by behavior? *J Clin Epidemiol* 2004; 57: 435–7.
21. LAU S, FALKENHORST G, WEBER A, et al. High Mite-allergen exposure increases the risk of sensitization in atopic children and young adults. *J Allergy Clin Immunol* 1989; 84: 718–25.
22. MUNIR AK, KJELLMAN NI, BJORKSTEN B. Exposure to indoor allergens in early infancy and sensitization. *J Allergy Clin Immunol* 1997; 100: 177–81.
23. CUSTOVIC A, SIMPSON A, CHAPMAN MD, WOODCOCK A. Allergen avoidance in the treatment of asthma and atopic disorders. *Thorax* 1998; 53: 63–72.
24. HALMERBAUER G, GARTNER C, SCHIER M, et al. Study on the prevention of allergy in children in Europe (SPACE): allergic sensitization in children at 1 yr of age in a controlled trial of allergen avoidance from birth. *Pediatr Allergy Immunol* 2002; 13 (Suppl. 15): 47–54.
25. SHEIKH A, SMEETH L, HUBBARD R. There is no evidence of an inverse relationship between Th2-mediated atopy and Th1 autoimmune disorders: lack of support for the hygiene hypothesis. *J Allergy Clin Immunol* 2003; 111: 131–5.
26. VERHOE AP, BURGE HA. Health risk assessment of fungi in home environments. *Ann Allergy Asthma Immunol* 2004; 78: 544–54.
27. MICHEL O, KIPS J, DUCHATEAU J, et al. Severity of asthma is related to endotoxin in house dust. *Am J Respir Crit Care Med* 1996; 154: 1641–6.
28. RIZZO MC, NASPITZ CK, FERNANDEZ-CALDAS E, LOCKEY RF, MIMICA I, SOLE D. Endotoxin exposure and symptoms in asthmatic children. *Paediatr Allergy Immunol* 1997; 8: 121–6.
29. REED JC, MILTON DK. Endotoxin-stimulated innate immunity: a contributing factor for asthma. *J Allergy Clin Immunol* 2001; 108: 157–66.
30. KRAMER MS, GUO T, PLATT RW, et al. Does previous infection protect against atopic eczema and recurrent wheeze in infancy? *Clin Exp Allergy* 2004; 34: 753–6.
31. UMETSU DT. Flu strikes the hygiene hypothesis. *Nat Med* 2004; 10: 232–4.
32. STRACHAN DP. Family size, infection and atopy: the first decade of the hygiene hypothesis. *Thorax* 2000; 55: S2–10.
33. KRAMER U, HEINRICH J, WJST M, WICHMANN HE. Age of entry to day nursery and allergy in later childhood. *Lancet* 1998; 352: 450–4.
34. BALL T, CASTRO-RODRIGUEZ J, GRITH K, HOLBERG C, MARTINEZ F, WRIGHT A. Siblings, day-care attendance, and the risk of asthma and wheezing during childhood. *N Engl J Med* 2000; 343: 538–43.
35. MAMOON H, HENRY R, STUART J, GIBSON P. House dust mite allergen levels in carpeted sleeping accommodation are higher in private houses than public places. *J Paediatr Child Health* 2002; 38: 568–70.
36. ENGELHART S, BIEBER T, EXNER M. House dust mite allergen levels in German day-care centers. *Int J Hyg Environ Health* 2002; 205: 453–7.