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Relationships between molds and asthma suggesting non-allergic mechanisms. A rural-urban comparison

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Abstract

Background: A fungal index, based on specific microbial volatile organic compounds (MVOCs) emission, was employed and related to asthma in children from rural and urban dwellings after stratification on the children atopic status.

Methods: A nested case-control design was used to draw, from 2 cross-sectional surveys, 20 asthmatics and 26 controls living in urban areas, and 24 asthmatics and 25 controls in rural areas. MVOCs levels were assessed in the living-room during one week; during that week, children performed clinical tests and their parents were invited to fill in a questionnaire on respiratory health.

Results: According to the objective fungal index, 70.5% of cases and 49.0% of controls were exposed to molds. More children with current asthma had experienced mold exposure in their homes (OR=3.38, 95% CI (1.16; 9.90)), especially amongst children living in rural areas. Atopic status modified this association: exposure to molds was found to be related to current asthma only in non-atopic children (OR=10.42, 95% CI (2.42; 44.81)). Among urban -dwelling children that could be screened at hospital, asthmatic children living in contaminated dwellings had a higher proportion of blood neutrophils and a lower FEV_1 (forced expiratory volume in 1 second) than non-exposed ones.

Conclusion: Our findings based on an objective assessment of MVOCs suggest adverse respiratory effects of molds. Our results suggest that when looking at the aetiology of non-atopic asthma, mold exposure should be systematically assessed.

In a recent meta-analysis, an increased risk of wheeze, cough, and asthma has been found in relation to exposure to molds or dampness (1). This was first attributed to an allergic pathway, but recent data have suggested that non-IgE mechanisms are also related to fungal components (2–4).

In most studies investigating respiratory effects of molds, individuals were considered as exposed on the basis of mold detection by dwellers or technicians or through spore counts. These assessments present serious limitations (poor reproducibility, bias toward certain species, and non-detection of dead molds), and they did not enable the detection of 'hidden' contamination (development of molds behind wall paper or in ventilation filters) or recent contamination by molds. To overcome these limits, other methods have been developed, based on objective measurements of mold markers or fungal products such as mycotoxins (5) or microbial volatile organic compounds (MVOC) overall in buildings that display a high level of contamination (6, 7). More recently, to take the limitations of previous measurements into account, a chemical index of fungal contamination was developed (8). This index, calculated as a function of the source and specificity of each VOC tracer emitted by molds, has allowed a better understanding of the dependence of some of these molecules on the mold-material combination observed by various authors (6, 7).

The aim of this study was to explore the relationship between mold exposure, objectively assessed, and asthma. These relationships were investigated by focusing on potential mechanisms involved in childhood asthma.

Methods

Study samples and protocols

Children were drawn from two cross-sectional studies conducted in the city of Clermont-Ferrand (Auvergne, France) in the framework of the French Six Cities Study (FSCS) (9) and in

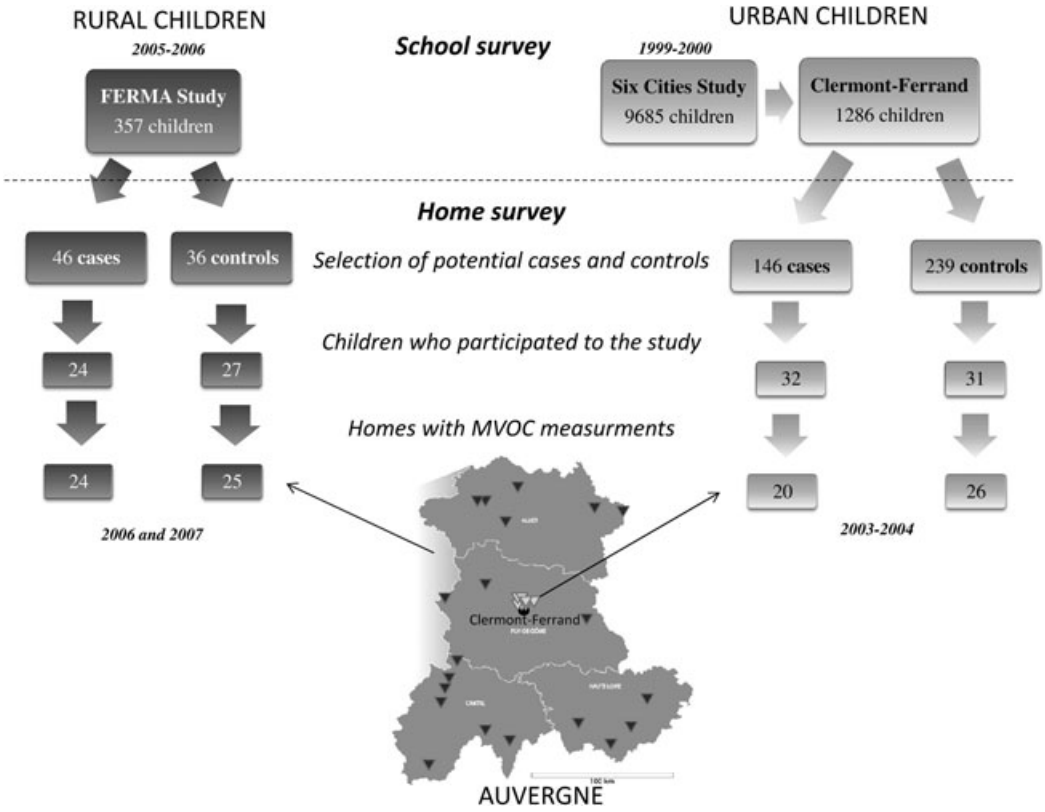

Figure 1 Presentation and localization of the urban and rural populations. In dark are presented the rural children and in gray the urban ones.

the surrounding rural areas in the framework of the FERMA study (10). As part of these studies, two nested case (asthmatic)-control studies, including, respectively, 63 children living in the city and 51 children living in the surrounding rural areas, were conducted (Figure 1). Asthmatic cases were identified according to affirmative responses to questions on lifetime asthma and wheezing in the chest or asthma medications in the last 12 months (10), namely 'Has your child ever had asthma?', 'Has your child had wheezing in the chest in the past 12 months?', or 'Has your child ever taken medications against asthma attacks?'. Among cases, some children presented current asthma defined as any report of symptoms or medications during the last 12 months. Controls had to answer 'no' to all the three questions.

The school survey protocol includes a standardized questionnaire and a clinical examination according to an enriched version of the ISAAC standardized protocol (11). Skin prick tests (SPT) to common allergens (mixed grass and tree pollen, house dust mites, cat and cockroach, *Alternaria alternata*, cod, egg, and peanut) were performed to define atopic status (9). All elements of the studies protocol obtained approval of the medical ethics committee, and written informed consent was provided by the parents. The home survey protocol includes:

1 a specific questionnaire on respiratory and allergic symptoms, mainly during the last 12 months. This questionnaire was based on the school survey questionnaire.
2 A medical examination for urban children.
3 Pollution measurements at home.

As the home survey was carried out several months after the school survey, the home questionnaire on respiratory and allergic diseases was used for recent asthmatic symptoms.

Further details can be found in the online data supplement.

Medical examination in urban children

Urban children were invited for a hospital visit including blood sample and lung function. Baseline lung function was measured by qualified and trained technicians with a portable Spirobank G spirometer. Pulmonary function test was performed according to ERS guidelines (12). At least three acceptable forced expiratory volume in one-second (FEV1) were recorded.

Due to logistic reason, this protocol was not carried out among rural population.

Mold exposure assessment

Mold exposure was assessed through a technician visit at home. These visits took place between November 2003 and July 2004 for the urban environment and during 2 periods (May and July 2006 and April and June 2007) for the rural. Home exposure to molds was evaluated by visual detection and through a binary fungal index developed and validated by one

of our collaborator, the French Scientific and Technical Centre for Building (CSTB) (8, 13). The engineer who performed all analyses was blinded to asthma status.

VOCs were assessed, in the living room, during 1 wk by passive diffusion samplers (Radiello). The detailed characteristics of this analytical system are described in the study by Moularat et al. (13). As VOCs can originate from non-fungal sources as well as fungal ones, laboratory experimentation was performed to identify the nature of VOCs emitted during fungal growth on construction materials (8). VOCs were classified as a function of their synthetic origin and a list of 19 pertinent chemical markers defined. A fungal contamination index (FI) was then developed, using the data supplied regarding the presence or absence of identified tracers (8). This index allowed identifying with certainty whether or not mold was present in a dwelling.

Statistical analyses

A generalized estimating equation (GEE) approach with an independent working correlation structure was used to estimate the associations between mold exposure and asthma while counteracting the potential lack of independence of the data, which was obtained from children living in the same neighborhood. Schools attended by children during the first phase of the studies were used as a proxy for living area (neighborhood or villages). Interaction terms between exposure and location, and exposure and atopy were tested. As mold exposure was more representative of recent periods at least, sensitivity analyses restricted to children with current asthma were also performed. Among lifetime asthmatics, specific analyses, using marginal models, were carried out to better evaluation potential adverse respiratory effects of molds. Therefore, cases who declared asthma but not in the previous 12 months were excluded.

Models were adjusted for sex, atopy as defined by SPT positivity, parental history of allergy (mother and/or father), exposure to passive smoking during childhood (defined as any current exposure to cigarettes, pipes or cigars at home, *in utero* or during the first year of life) and living area (rural or urban). Due to the low number of urban asthmatics (n = 20), models on this restricted population were only adjusted on personal factor (sex and atopy).

Statistical analysis was carried out using SAS for Windows (V 9.1, Cary, NC, USA). Statistical significance was determined using a critical p-value of 0.05.

Results

Population characteristics

Due to delays in the recruitment, canceled appointments and other scheduling conflicts, MVOC was not assessed in all 19 homes. The final sample included 95 children living either in urban or rural areas and for whom mold assessments were available (Figure 1). Comparison between participants and non-participants did not show significant differences in terms of socio-demographic characteristics. In our sample, only two children moved in the last 12 months.

Table 1 Demographic characteristics of the study population (N = 95)

Characteristics	Cases		Controls
	Lifetime asthmatics	Current asthmatics*	
Sample size			
Urban	20	12	26
Rural	24	16	25
All	44	28	51
Age (years), means ± SD			
Urban	13.4 ± 0.6	13.4 ± 0.5	14.3 ± 0.6
Rural	10.9 ± 0.9	11.0 ± 0.9	10.7 ± 0.8
All	12.0 ± 1.5	12.0 ± 1.4	12.5 ± 1.9
Girls (%)			
Urban	50.0	50.0	53.9
Rural	50.0	37.5	50.0
All	50.0	42.9	52.0
Family history of allergy (%)			
Urban	30.0	50.0	42.3
Rural	30.4	25.0	16.7†
All	30.2	35.7	30.0
ETS during childhood (%)			
Urban	60.0	58.3	53.9
Rural	39.1	31.3	37.5
All	48.9	42.9	46.0
Atopy (%)			
Urban	57.9	63.6	37.5
Rural	43.5‡	53.3‡	0.0†
All	50.0‡	57.7‡	18.4

*Cases restricted to current asthmatics only.
†Differences between urban and rural children ($p < 0.05$).
‡Differences between cases and controls ($p < 0.05$).

Characteristics of the final sample are shown in Table 1. In our population, 46.5% of urban children were atopic vs. 20.8% in rural areas ($p = 0.0093$). The highest prevalence of atopy was observed for indoor allergens (Figure 2). In our study, only one child had a positive SPT to *Alternaria alternata*.

Exposure to molds at home and asthma

Sixty percent of dwellings were classified as containing molds according to our index. Overall, 75% of cases were exposed to molds compared with 49% of controls ($p = 0.0342$; Table 2). A significant odds ratio was observed when restricting the definition of asthma to current asthma in the analysis (OR = 3.38, 95% CI [1.16; 9.90]; Table 2).

Differences of exposure according to the type of location were observed (Table 2), with a slightly higher proportion of rural homes contaminated (67.4% vs. 50.0% $p = 0.0859$). Children living in contaminated dwellings in rural areas presented a higher risk of asthma, whereas no significant association was found in urban areas ($p = 0.1077$ for interaction terms).

To focus on potential mechanisms associated with mold exposure, the effect of atopy on the association between asthma and fungal contamination was evaluated (Figure 3).

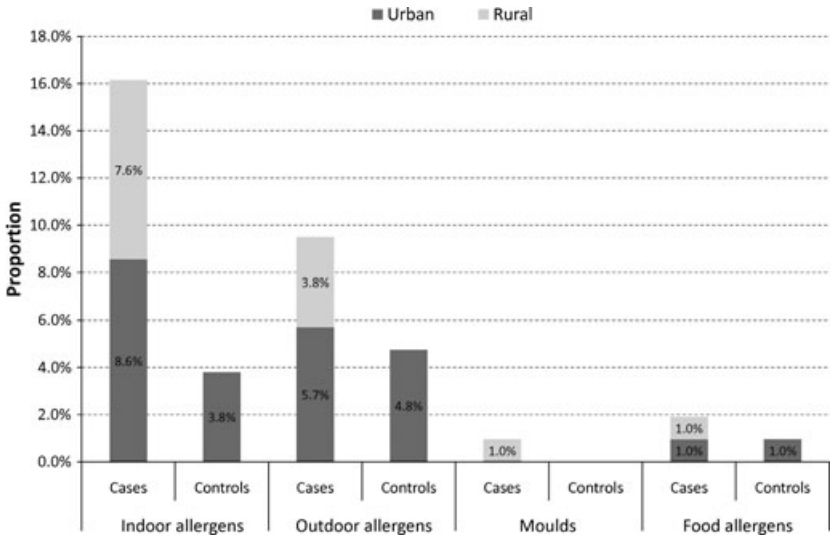

Figure 2 Proportion of allergic sensitization to aeroallergens in the study sample (N = 95)

Table 2 Exposure to molds according to the fungal index based on MVOC at home in controls (N = 51) and cases, defined as lifetime asthmatics (N = 44) or current asthmatics (N = 28) and adjusted odds ratio*

	Lifetime asthma			Current asthma		
	Exposure (%)		Adjusted Odds Ratio	Exposure (%)		Adjusted Odds Ratio
	Cases	Controls	OR [CI 95%]	Cases	Controls	OR [CI 95%]
All population	70.5	49.0‡	1.92 [0.75; 4.96]	75.0	49.0‡	3.38 [1.16; 9.90]
Urban	55.0	46.2	0.95 [0.23; 4.0]	58.3	46.2	1.70 [0.29; 9.85]
Rural	83.3†	52.0‡	4.37 [1.38; 13.79]	87.5†	52.0‡	7.54 [1.64; 34.72]

*Odds Ratio and confidence interval were obtained with a GEE model adjusted for age, sex, family history of allergy, exposure to passive smoking during childhood, atopy, and location (urban or rural).
†Differences between urban and rural children ($p < 0.05$).
‡Differences between cases and controls ($p < 0.05$).

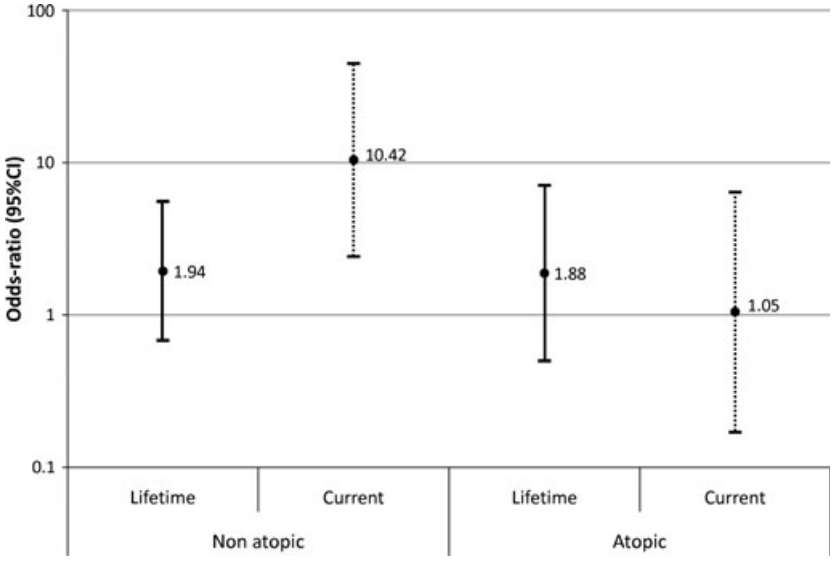

Figure 3 Associations between asthma (lifetime and current) and exposure to molds (fungal index) according to atopic status (N = 95). Odds Ratio and confidence interval were obtained with a generalized estimating equation (GEE) model adjusted for age, sex, family history of allergy, exposure to passive smoking during childhood, atopy, and location.

When restricted to cases with current asthma, the analysis showed a higher association between mold exposure and asthma in non-atopic children than in atopic ones ($p = 0.0581$ for interaction terms). A tenfold increase risk of asthma was observed among non-atopic children, in relation with fungal index (OR = 10.42, 95% CI [2.42; 44.81]).

Table 3 Associations between lung function and white blood cell counts with exposure to molds according to the fungal index based on MVOCs in urban lifetime asthmatic children

	Mean ± SD		Adjusted associations*
	Exposed	Non-exposed	β [CI 95%]
White blood cell counts (N = 20)			
Neutrophils (G/L)	3.53 ± 0.99	2.95 ± 0.91	0.73 [0.18; 1.27]
Eosinophils (G/L)	0.23 ± 0.07	0.26 ± 0.18	−0.03 [−0.12; 0.06]
Basophils (G/L)	0.034 ± 0.011	0.029 ± 0.008	0.005 [−0.002; 0.013]
Lymphocytes (G/L)	2.39 ± 0.50	2.42 ± 0.63	−0.1 [−0.58; 0.38]
Monocytes (G/L)	0.52 ± 0.14	0.55 ± 0.17	−0.05 [−0.17; 0.07]
Lung function (N = 13)			
FEV (%)	95.0 ± 13.3	112.1 ± 13.5	−17.8 [−22.3; −13.3]
FVC (%)	84.1 ± 14.8	98.6 ± 19.5	−16.3 [−23.6; −8.9]
FEV/FVC (%)	104.3 ± 8.5	103.0 ± 9.6	2.5 [−1.9; 6.9]
$FEF_{25–75}$ (%)	87.0 ± 22.0	106.9 ± 20.6	−14.5 [−25.7; −3.4]
PEF (%)	94.6 ± 2.2	94.2 ± 11.4	3.7 [−4.6; 11.9]

β is the regression coefficient.

*Adjusted associations and confidence interval were obtained with a GEE model adjusted for sex and atopy.

Exposure to molds and health effects in asthmatics

Among the 20 asthmatic children who agreed to blood sampling in Clermont-Ferrand, a higher neutrophil count was observed in those exposed to molds (Table 3). After adjustment, asthmatic children living in contaminated houses had 0.73 G/l more neutrophils than non-exposed ones (p = 0.0086). No significant association was observed for eosinophilia.

Among the 20 asthmatic children who had performed lung function tests (Table 3), seven were excluded because they did not succeed in realizing correct tests. Exposed children had a lower FEV_1, corresponding to a decrease of 17.8% in FEV_1, after adjustment for confounders. A negative association was also found between FVC and $FEF_{25–75}$, but the FEV_1/FVC ratio did not change significantly according to mold exposure.

Discussion

Our data obtained in two population-based samples show a potential association between mold exposure and childhood asthma, especially in the rural environment. Atopic status modified this association, as an increased risk of current asthma was found among non-atopic children but not in atopic ones.

Most of studies on mold exposure and asthma assessed exposure trough questionnaires, and the reliability of such methods has been questioned (1). In our population, while only 19.2% of homes had visible molds, 58.5% had some sort of fungal development according to the FI based on MVOCs (14). Such proportion is higher than what can be found in other part of France (15): 35.3% in a representative sample of dwellings and using the same objective FI. However, when considering populations with high proportion of farmers, similar high percentage can observed (16, 17), suggesting transmission of fungi from cow barns to farm houses (17). Therefore, it suggests that the choice of exposure assessment can lead to important classification bias. Only few studies have based their exposure assessment on objective measurements generally by involving spore counts, which present several drawbacks (5). These studies reported positive relationship of mold spore counts and asthma-like symptoms (18). Our results, based on objective assessment, confirm a higher risk of asthma in children living in dwellings contaminated by mold.

There is increasing evidence that inflammatory mechanisms other than eosinophilic inflammation may be involved in asthma (19). Experimental (4, 20) as well as epidemiological studies suggest that some mold compounds, such as MVOCs (3, 21, 22) or β-glucans (4), could have respiratory effect through inflammation and irritation leading to respiratory effect through non-allergic pathways. In our study, associations between asthma and molds were observed among non-atopic population. However, it could be argued that children could have been sensitized to other molds than the species tested in our study, that is, *Alternaria alternate*, even if allergic sensitization to *Alternaria* is one of the most common sensitization to molds (23). It would have been interesting to have SPT with other molds such as *Aspergillus, Cladosporium, Pennicilum* to dismiss this hypothesis. But in Northern Europe, prevalence of sensitization to molds is low (23, 24). The differential diagnosis of bronchopulmonary aspergillosis could also be discussed, but it is very rare in French children, except in association with cystic fibrosis (25). In our study, we know that no child suffered from cystic fibrosis, because the parents were asked to report chronic conditions of their children. Therefore, this supports the hypothesis that mold-related respiratory effects could also be driven through a non-allergic response pathway, as suggested in some epidemiological studies (26–28). Moreover, the observation of a significantly higher number of neutrophil, asthmatic children exposed to molds (without any increase in eosinophils), corroborate our hypothesis. However, the number of asthmatic children included in our study is not sufficient to conclude to a link between mold exposure and neutrophilic counts. It would be of interest to explore the effect of molds on asthmatic population using clinical markers (through lung function or measurement

in blood samples for example) to better understanding the underlying mechanisms.

A significant relationship between asthma and exposure to molds was found among rural children in our study. The farm environment has been shown to present a higher exposure to biocontaminants, in particular to endotoxins but also molds (29). Mold exposure has been associated with a reduced risk of atopic wheezing among rural children in the PARSIFAL study (2). In our rural population where 20.8% of children were atopic and only one child was sensitized to molds, a positive association was found between exposure to molds and asthma. According to the so-called 'agricultural asthma paradox' (30), there is no antinomy between a reduced risk of allergic asthma and an increase risk of non-atopic asthma in farm populations due to environmental exposures found in this setting (31).

Several limits of our study have to be discussed. Firstly, a lack of power in our study prevented us from highlighting some relationships or confirming some results that were close to the significant level. Prevalence of asthma in our initial population and participation rate can explain the low number of homes included, while the long period of time separating the two phases of recruitment can explain the low participation rate (24%). However, comparison of socio-demographic factors between non-participants and participants did not reveal significant differences. Moreover, lung function and cell counts have not been performed among rural population. As homes were too far from hospitals, it was difficult for rural parents to go there. In addition, proximity to a hospital was set as a necessary condition by the Ethical Committee for the conduction of the study. Secondly, exposure was based on assessments of MVOCs during a single week and may therefore not be representative of usual levels. However, data collection was avoided during vacations and a representative week was chosen, and analyses restricted to current asthmatics allowed avoiding misclassifications of exposure. Moreover, their use as an indicator of mold exposure has been questioned (3) based on the non-specificity of MVOCs toward molds and their low emission levels. However, our FI has been constructed to overcome these limitations: selection of several specific MVOCs selected to obtain a signature for the presence of fungal development (8, 13). In addition, mold assessments were performed mostly in spring in the rural area, whereas they were conducted during the cold season and spring in the urban area, which could have led to exposure misclassification. This could not be controlled for in our study due to the reduced population size. However, spring assessment of molds led to exposure underestimation and thus to underestimation of the relationship between mold exposure and asthma, which even so was statistically significant. Indeed, during summertime, natural ventilation is important and individuals spend more time outdoors thus reducing individual exposure to air pollutants. Lastly, assessment of other microbial exposures, such as endotoxin, would have been very informative to determine whether the associations found in our study were due to molds or others biocontaminants.

Conclusions

In conclusion, our study confirms previous results on the potential impact of molds – objectively assessed through MVOCs – on asthma and supports the hypothesis that molds could act also through non-allergic mechanisms. The higher effect of mold exposure found in the rural environment could be due to a higher susceptibility of rural individual to develop non-atopic asthma due to their excessive exposure. However, further studies are needed to understand the mechanisms behind mold exposure respiratory effects and the impacts of MVOCs on respiratory health.

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Conflict of interest

No conflict of interest for any authors.

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