

Gaseous air pollution and atopy

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Summary

Background Photochemical air pollutants are commonly thought to be implicated in the gradual increase in the prevalence of atopy. However, no epidemiological data are available.

Methods To clarify this issue, we performed a cross-sectional epidemiological survey in 2604 primary school children, 10 and 11 years old, living in seven communities among which some have the highest photochemical exposure in France. The mean levels of the main gaseous air pollutants (SO₂, NO₂ and O₃) were measured during a 2-month period in 1993. The protocol included a standardized questionnaire, skin prick tests to common aeroallergens and in the atopic children, collection of a sample of mattress dust to measure group 1 mite allergens. Atopy was only defined on the basis of the skin prick tests.

Results Percentage of positive skin tests and the number of positive skin tests were similar in the different communities looked at. The distribution of dust samples with a group 1 allergen level greater than 2 µg/g dust, was also similar. Logistic regression analysis including potential confounding factors, as well as the mean level of air pollutants, did not demonstrate any association between atopy and mean SO₂, NO₂ and O₃ levels.

Conclusion The increase in photochemical air pollutants is unlikely to be a major determinant for the recent increase in the prevalence of atopy.

Keywords: air pollution, asthma, atopy, epidemiology, occurrence

Clinical and Experimental Allergy, Vol. 29, pp. 1474–1480. Submitted 21 April 1998; revised 24 March 1999; accepted 24 April 1999.

Introduction

There is evidence for an increase in atopic diseases in developed countries [1]. Animal experiments suggest that gaseous air pollutants such as SO₂, NO₂ and O₃, may increase the permeability of the mucous membrane in the airways, which may favour the penetration of allergens and the development of allergic reactions [2,3]. Thus it has often been speculated that chronic exposure to air pollutants may play a role in the increase in the prevalence of allergic diseases. Recently, several East–West comparisons of the prevalence of atopic diseases in school children, have demonstrated a higher prevalence of hay fever and a higher percentage of positive skin tests to common allergens in school children living in Western countries, than in their Eastern counterparts [4,5].

These observations have led to the hypothesis that living conditions in Eastern countries, especially early childhood infections, could protect against the subsequent development of allergic disease by activation of the TH1 lymphocytes, so preventing proliferation of the TH2 cell population [6,7]. An alternative hypothesis may be that living conditions in the Western countries, in particular photochemical air pollution from automobile exhausts and a high exposure rate to indoor allergens, may be responsible for an increased prevalence of atopy.

Atopy is a well-known risk factor for asthma and asthmatic symptoms. Since chronic exposure to air pollutants can be responsible for an increased prevalence of non-specific symptoms, such as chronic cough and wheezing, we decided not to include asthmatic symptoms or the diagnosis of asthma in our definition of atopy [8], that is ‘that form of immunological reactivity in subjects in whom a reaginic antibody, now identifiable as the IgE antibody, is readily

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produced in response to ordinary exposure to common allergens in the subject's environment'. As Pepys pointed out, this definition does not imply the presence of symptoms, but is a description of the immunological reactivity of the subjects [8]. In this survey we compared the prevalence of atopy in school children, living in seven communities with different exposure to air pollutants, but the same way of life, and we controlled for exposure to the main indoor allergen, the mite allergen. Atopy was considered as skin reactivity to one or more common aeroallergens.

Materials and methods

Rationale and geographical location

The survey was performed in the 'Etang de Berre' area, because of repeated complaints from the population and associations, and was sponsored by the local authorities, as well as the Ministry of the Environment and the Ministry of Health. The 'Etang de Berre' area is located in south-east France, along the Mediterranean coast. Its size is approximately 30 km long and 10 km large. It includes several small towns. Air pollution in this area originates mainly from the petrochemical industry, which was set up in the mid-1970s. Automobile exhausts also contribute to air pollution, because there are several highways and an international airport in the district. The area is also very sunny and thus it is known as the part of France with the highest photochemical air pollution [9]. The towns of Arles and Salon de Provence are located further inland, and are not exposed to any major industrial plants.

Measurement of air pollutants

Measurement of the major air pollutants (SO₂, NO₂ and O₃) is routinely performed in the communities located around the lake (Etang de Berre) (AIRFOBEP network, Ministry of Industry). SO₂ is measured using an UV fluorescence technique, NO₂ by a chemiluminescence technique and O₃ by UV photometry at 253.7 nm. In Salon de Provence, air pollution had not previously been measured. Thus during the survey, which took place in January and February 1993,

we set up one monitoring station measuring SO₂, NO₂ and O₃ in Arles and Salon de Provence. In the results section, we used the mean level of each pollutant (SO₂, NO₂, O₃), measured in January and February 1993, as an index of the mean air pollution in the area (Table 1). Air pollutant measurements were performed routinely between 1989 and 1993, in the communities located around the lake. The hierarchy of the air pollution was similar for measurements from between 1989 and 1993, when compared with those from the study.

Study group

The study group included all school children from the 4th and 5th grades (10 and 11 years old), who were enrolled in state-run and private schools, and were living in these towns, which had had an air pollution monitoring system, for at least 3 years. All the schools were located within 3 km of the monitoring stations. The main characteristics of the study group are listed in Table 2.

Protocol

The questionnaire was derived from the 1978-ATS questionnaire for children [10], and was filled out by the parents. It included additional questions about potential confounding factors: the socioeconomic status (three categories: low, medium or high) and passive smoking at home (three categories: none, less than 10 cigarettes smoked daily while the children at home, or more than 10 cigarettes daily).

Skin prick tests

Skin prick tests were performed using a multitest device (Stallerkit[®], Laboratoire des Stallergènes, Paris, France). This device has been shown to be valid, when compared with standard skin prick tests [11]. Moreover, it is more suitable for epidemiological studies, because it allows serial examinations to be made in a shorter time and induces less pain. Allergens were chosen according to a previous study [12] performed on 1000 outpatients visiting our clinic,

Table 1. Mean ($\pm$ SD) expressed in $\mu\text{g}/\text{m}^3$ of daily measurements performed during the survey (January and February 1993)

	Arles	Istres	Port de Bouc	Rognac-Velaux	Salon de Provence	Sausset	Vitrolles
SO ₂ (24 h)	29.7 $\pm$ 15.5	23.8 $\pm$ 12.7	32.3 $\pm$ 24.5	39.5 $\pm$ 21.8	17.3 $\pm$ 11.6	29.0 $\pm$ 28.7	57.4 $\pm$ 32.0
NO ₂ (24 h)	40.1 $\pm$ 16.2	50.0 $\pm$ 18.1	38.3 $\pm$ 17.5	51.3 $\pm$ 24.4	20.3 $\pm$ 4.4	27.8 $\pm$ 11.2	50.5 $\pm$ 19.1
O ₃ (8 h)	30.2 $\pm$ 16.7	39.6 $\pm$ 18.4	46.1 $\pm$ 14.3	40.1 $\pm$ 15.7	35.3 $\pm$ 13.7	52.1 $\pm$ 15.0	51.6 $\pm$ 18.4
O ₃ (24 h)	20.1 $\pm$ 13.2	27.8 $\pm$ 14.2	35.8 $\pm$ 11.8	27.6 $\pm$ 10.9	26.1 $\pm$ 11.7	42.1 $\pm$ 13.4	39.8 $\pm$ 14.8

Table 2. Number of children enrolled, skin tested and their mean ($\pm$ SD) age and sex-ratio

	Arles	Istres	Port de Bouc	Rognac-Velaux	Salon de Provence	Sausset	Vitrolles	Total
Number of children enrolled	448	587	427	170	296	88	588	2604
Number of children skin tested	348	478	320	150	207	73	497	2073
Mean ($\pm$ SD) age	10.7 $\pm$ 0.8	10.8 $\pm$ 0.8	11.0 $\pm$ 0.9	11.0 $\pm$ 0.7	10.9 $\pm$ 0.9	10.7 $\pm$ 0.7	10.8 $\pm$ 0.8	
Male/female ratio	1.18	0.88	1.02	1.26	1.09	0.97	1.01	

which demonstrated that in our area, the most relevant allergens were as follows: house dust mite, cat danders, grass pollen, cypress pollen and *Alternaria*. The Stallerkit includes the five allergens (listed above), a negative control (Coca solution) and two positive controls (9% codeine phosphate), located at the opposite corner of the device. The allergenic potency of the extracts is expressed in terms of reactivity units per mL of solution. The tests were performed on the volar surface of the forearm, by pressing the device gently onto the skin. The results were measured 20 min later using a ruler, by working out the mean of the largest diameter and its orthogonal diameter. If the parents refused permission for skin testing, they were asked for the reason. If they mentioned ongoing desensitization, treatment or previous knowledge of positive skin tests as reasons, the child was considered to be atopic. Several criteria have been used in the literature to define a skin test as positive: a weal induced by an allergen larger than the weal from the negative control test [13], a weal whose diameter is 3 mm or more larger than that induced by the negative control test [14], a weal larger than 3 mm [15], or a weal equal to or larger than half of the weal induced by the positive control test and larger than the negative test [16]. We used all of these criteria in the analysis. Apart from that, we also used the number of positive skin tests in the analysis, as an index of the severity of atopy.

Measurement of the mite allergen levels

Those children in whom the diameter of the weal induced for at least one allergen was equal to or larger than half of the diameter of the weal induced by codeine phosphate and larger than the weal induced by the negative control tests, were considered as atopic and were asked to bring back to school a sample of their mattress dust. Dust collection was performed according to international recommendations [17]. Disposable paper bags were collected and stored at 4°C until processing [17]. The fine dust was collected and weighed. Samples with less than 100 mg of fine dust were

discarded. The mite allergen content was determined by ELISA, using monoclonal antibodies [18].

The assay was performed with two dilutions (in most cases 1/3 and 1/30) of the extracted sample, each time using a double control method. The antigen concentration used in the statistical analysis was the arithmetical mean of the values in the linear section of the standard curve. The results were expressed in micrograms (μ g) of mite group 1 per gram of house dust.

Statistical analysis

In the questionnaire, the parents were first asked if their child had been living in the same area for at least 3 years. Only these children were included in the analysis ($n = 2604$). We will present the distribution of skin test reactivity in the various towns and then the results of logistic regression analysis (BMDP Software, University of Berkeley, CA, USA) using atopy as the predicted variable. We have included items, which could act as confounding factors in this relationship, as predictive variables, namely passive smoking and socioeconomic status (low, medium, or high). As mite allergen levels were only measured in the atopic children, they were not included as a predictive variable in the logistic regression analysis. Because air pollution was measured on a town basis, not on an individual (child) basis, use of logistic regression analysis may be questioned. Thus we also performed a simple regression analysis plotting, for each town, mean levels of each of the three gaseous air pollutants in abscissa and percentage of sensitization to each of the aeroallergens tested, or percentage of atopic children in ordinate.

Logistics

The parents had to fill out the questionnaire, and were also asked for written informed consent to allow skin testing to be performed on their child. The study received approval from the Ethics Committee. Skin testing was performed at

Table 3. Percentage of atopic children (at least one positive skin test), according to various criteria, in the different towns

	Arles	Istres	Port de Bouc	Rognac/Velaux	Salon de Provence	Sausset	Vitrolles	Total
% Weal > 0	109 (31.4%)	203 (42.5%)	108 (35.0%)	59 (40.1%)	78 (37.9%)	34 (47.9%)	215 (43.4%)	806 (38.9%)
% Weal >1/2 pos. control & > neg. control	90 (28.1%)	166 (38.4%)	68 (25.5%)	52 (37.4%)	68 (34.9%)	22 (33.3%)	170 (36.6%)	636 (33.8%)
Weal $\geq$ 3 mm larger than neg. control	86 (25.1%)	131 (28.1%)	78 (26.2%)	35 (24.0%)	56 (27.5%)	20 (24.3%)	118 (24.3%)	524 (26.0%)

school, and its results were immediately given to the parents and to the family doctor. The atopic children were given a form asking their parents to provide a sample of their mattress dust.

Results

The questionnaire was answered by 2421 out of 2604 (93%) of the parents or guardians and 2073 (79.6%) granted permission to perform skin tests. The percentage of tests to the negative control which produced a weal diameter larger than 0 mm, varied from 18.3 to 25.1% in the various towns (a non-significant difference). Similarly, the percentage of tests to codeine phosphate less than or equal to 1 mm, ranged from 18.8 to 30.6%, depending on the town (a non-significant difference). The percentage of refusal to give permission for skin testing was no different in the various towns. Additionally, out of the 685 atopic children, 308 (45%) provided us with house dust samples, although 57 samples did not contain enough dust, so in accordance with international recommendations [17], they were discarded.

Thus 251 samples were processed and included in the statistical analysis. Interestingly, the prevalence of hay fever, perennial rhinitis and asthma was similar in the children whose parents gave permission for skin testing (20.0, 10.9 and 9.0%, respectively) and in those whose parents refused it (20.4, 12.8 and 10.4%, respectively). Similarly, the prevalence of allergic symptoms was similar in the atopic children who provided a sample of house dust (31.5, 15.4 and 16.7%, respectively) and in those who did not (32.8, 14.9 and 16.3%, respectively).

Table 3 shows the percentage of children with at least one positive skin test, according to the various criteria. Which-ever criteria were chosen, the figures were similar in all the towns. Table 4 shows the percentage of children in each town with 1, 2, 3, 4 or 5 (all) positive skin tests. The percentage of children with several positive skin tests was similar in all of the towns. The percentage of mattress dust samples with a group 1 allergen level greater than 2 $\mu\text{g/g}$ dust, the level considered as the threshold for sensitization [17], was not statistically different in the various towns, ranging from 83.5 to 100%.

Logistic regression analysis comparing atopic status (yes/no) with several independent variables: age, sex, passive smoking, socioeconomic status and the mean level of the air pollutants showed, after taking into account potential confounding factors, that the mean level of each of the three pollutants measured was not linked to the prevalence of atopy in the children. In this analysis, each air pollutant was taken separately in three different logistic regression analyses. Simple regression analyses, plotting for each town, mean levels of each of the three gaseous air pollutants

Table 4. Percentage of children with one to five positive skin tests, in the different towns. Arles and Salon-de-Provence are the communities with low-pollution

	Arles	Istres	Port de Bouc	Rognac/Velaux	Salon	Sausset	Vitrolles	Total
Number of tests performed and meaningful	343	465	295	146	204	70	485	2008
Number (percentage) of positive* tests								
1	56 (16.3%)	89 (19.1%)	48 (16.3%)	26 (17.8%)	35 (17.2%)	15 (21.4%)	81 (16.7%)	350 (17.4%)
2	19 (5.5%)	25 (5.4%)	19 (6.4%)	5 (3.4%)	14 (6.9%)	5 (7.1%)	25 (5.2%)	112 (5.6%)
3	9 (2.6%)	11 (2.4%)	6 (2.0%)	2 (1.4%)	6 (2.9%)	0 (0%)	8 (1.6%)	42 (2.1%)
4	1 (0.3%)	3 (0.6%)	2 (0.7%)	2 (1.4%)	1 (0.5%)	0 (0%)	3 (0.6%)	12 (0.6%)
5	1 (0.3%)	1 (0.2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.2%)	3 (0.1%)

*Weal $\geq$ 3 mm larger than the negative control test.

and percentage of skin sensitization, did not provide any significant association.

Discussion

This large cross-sectional epidemiological study did not demonstrate any association between air pollution, in an area where some communities with high photochemical exposure, and the atopic status of the children living in the various communities. Since the target population included all the 4th and 5th grade children and since the participation rate was high, we do not believe that any selection bias occurred. The skin tests were performed using a valid technique and the allergens used had been standardized using an *in vivo* method. However, some misclassification of the exposure to the air pollutants is likely to have occurred, because children may move from one area to another for leisure activities. However, since we selected only those children who had been living in their town for at least 3 years and since primary school-children usually do not move much, we believe that there should not be so much misclassification as far as air pollution is concerned. Also for technical reasons, in the analysis we used the mean air pollutant levels measured over a 2-month period. Nevertheless, there was a strong correlation in the more polluted areas, between these levels and the mean annual levels measured over the 5 previous years. Another limitation in the evaluation of the total individual exposure to air pollution lies in the fact that we did not perform any measurement of indoor pollution. We used as a surrogate of total exposure outdoor exposure which might be different from the former when there are important indoor sources of pollutants, for example NO₂ from gas appliances. A last limitation, as far as air pollution is concerned, is that we did not consider short-term variation in air pollutant levels. Their repetition could lead to chronic respiratory effects. The level of mite allergens in the mattresses was only measured on one occasion, but since mattresses are a reservoir for allergens, the level of allergens measured can be considered to be a reflection of the cumulative exposure. The percentage of dust samples with a mite allergen level higher than 2 µg/g dust was very high, and similar in the various towns. Thus mite allergen exposure is not likely to act as a confounding factor in the relationship between exposure to air pollutants and atopy.

This negative result varies from other *in vitro* studies, which suggested that the allergenicity of pollen collected in urban areas has increased, compared with pollen collected in areas with low pollution [19]. Also, animal experiments demonstrated an interaction between air pollution and allergen exposure, causing allergic sensitization [20,21]. So, is there epidemiological evidence for the relationship between chronic exposure to air pollutants and atopy? A few

studies have compared the prevalence of atopy in populations living in urban and rural areas. The results from the Second National Health and Nutrition Examination Survey [22] demonstrated a higher prevalence of skin test reactivity in urban than in rural areas, but the difference was only statistically significant for whites. Popp *et al.* performed the Phadiatop® test in a group of 222 urban and 129 rural probands, who had undergone routine venipuncture, to exclude venereal disease [23]. A positive Phadiatop test was found in 43.7% of urban and 32.6% of the rural probands, a difference which was statistically significant. In our area, we performed a cross-sectional comparative study in a sample of the general adult population, living in a large city (Marseille) and a small town with low pollution (Trets). The percentage of positive skin tests to grass pollen was 10% in the urban dwellers and 8% in the rural dwellers, a non-significant difference [24]. In Sweden, Braback and Kalvesten demonstrated that 31% of urban, but only 19.6% of rural school children were atopic [25]. The role of an 'urban factor' in the genesis of allergic sensitization is possible, but still unclear because other confounding factors, such as exposure to irritants and allergens, could also explain these differences. Since 1990, several 'East-West' comparisons have shed new light on this issue. Following von Mutius' comparative study involving school children from Leipzig and Munich [26], other population surveys performed in Poland and Estonia [27] have demonstrated convincingly that the prevalence of atopic sensitization is much lower in children living in former communist Eastern-block countries, which are characterized among other things, by higher industrial air pollution (SO₂ and particulates). These surveys strongly suggest that industrial air pollution is not a risk factor for atopic sensitization. However, domestic allergen exposure has not been measured in any of these studies. A low allergenic level in the former Eastern-block countries might be anticipated, because there were few domestic animals and because living conditions (no carpets and a dry, cold climate) are not favourable to mite development. An alternative explanation to account for this 'East-West' difference could be that the air pollution prevailing in Western countries (photochemical air pollution from automobile exhausts) could be a risk factor for atopic sensitization. However, there are no data supporting this hypothesis to date. In our area, we have shown that there is no association between air pollution, especially ozone levels which are quite high, and skin test reactivity. In a survey comparing small groups of children living in two communities with a large difference in ozone exposure, Zwick *et al.* were unable to demonstrate a difference in sensitization to aeroallergens [28]. In Switzerland, large national collaborative studies, performed in randomly selected groups of children (SCARPOL) and adults (SAPALDIA Study [29]), did not show any relationship

between mean air pollution and the prevalence of allergic sensitization or allergic diseases.

In conclusion, this cross-sectional epidemiological survey performed on a large group of school children, living in an area with contrasting exposure to air pollutants, was not able to demonstrate a relationship between chronic air pollution and the rate of allergic sensitization. These results, which differ from *in vitro* and animal experiments, probably mean that the levels of air pollutants measured in developed countries do not induce such an effect. It can also be concluded that changes in air pollution levels are not likely to be a major determinant for the recent increase in the prevalence of allergic diseases.

Acknowledgements

Funding: Ministère de la Santé, des Affaires Sociales et de la Solidarité, Ministère de l'Environnement, ADEME agency, Réseau national de Santé Publique, Conseil Général des Bouches du Rhône, Townships and main local industries.

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