

Epidemic Asthma in Barcelona: An Evaluation of New Strategies for the Control of Soybean Dust Emission

María-José Rodrigo^a María-Jesús Cruz^a María-del-Mar García^b
Josep-María Antó^c Teresa Genover^d Ferran Morell^a

^aUnitat de Recerca en Neumologia i Unitat Immunologia, Servei de Pneumologia, Hospital Universitari Vall d'Hebrón, Universitat Autònoma de Barcelona, ^bDivisió de Medicina Preventiva i Salut Pública, Facultat de Medicina, ^cDepartament d'Epidemiologia i Salut Pública, Institut Municipal d'Investigacions Mèdiques i Universitat Pompeu Fabra, i ^dCentre d'Atenció Primària Turó, Barcelona, Espanya

Key Words

ELISA-inhibition · Soybean · Aeroallergen

Abstract

Background: Asthma attacks and mortality due to inhalation of soybean antigens in Barcelona have been well documented. Strict protective measures in the unloading process were established in 1998 to avoid the release of soybean dust into the atmosphere. The present study was undertaken to assess the effectiveness of these latest environmental measures, and, if effective, to recommend their implementation in the many harbours where soybean is unloaded. **Methods:** Levels of soybean aeroallergen were analysed daily during a period of 5 years and 2 months in a total of 1,854 samples, 125 from the pre-intervention period and 1,729 from the postintervention period. Additionally, the number of asthma admissions to the emergency rooms of the city's three largest hospitals was recorded. Asthma patients attended at home by the public home emergency service and judicial autopsies registering asthma deaths were also investi-

gated. **Results:** The mean concentration of soybean aeroallergen was 159 U/m³ in the pre-intervention period and 39 U/m³ in the postintervention period ($p < 0.0001$). Significant differences in postintervention aeroallergen concentrations were found between days of soybean unloading (42 U/m³) and days of no unloading (33 U/m³), with $p < 0.0001$. No significant relationship was found between concentrations of environmental soybean aeroallergens and the number of emergency room admissions for asthma. **Conclusions:** Implementation of stricter protective measures in silos for the soybean unloading process has reduced the concentration of soybean dust in the atmosphere and evidences the effectiveness of the measures adopted.

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The first two authors have contributed equally to the design of the study and the writing of the manuscript.

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Correspondence to: Dr. M^a José Rodrigo
Unitat de Recerca en Neumologia i Unitat Immunologia
Hospital General Vall d'Hebrón, Passeig Vall d'Hebrón, 119–129
ES–08035 Barcelona (Spain)
Tel./Fax +34 93 4894049, E-Mail mjrodrigo@vhebron.net

Introduction

From 1981 to 1987, 26 asthma outbreaks occurred in Barcelona. During that time, acute asthma accounted for 958 emergency hospital admissions, mostly due to unusually severe asthma exacerbation, as well as a high rate of admissions to the intensive-care unit and 20 deaths. Investigation into these epidemics revealed that the asthma outbreaks were caused by soybean dust generated by soybean unloading in the harbour when certain wind conditions carried the soybean dust over the city [1–3].

In 1987, measures were taken to avoid emission of large amounts of soybean dust into the atmosphere during unloading. These measures, based on emission channeling and the use of bag filters for unloading, drastically reduced the spread of dust into the atmosphere and, for many years, prevented new outbreaks [4]. In 1994 and 1996, however, new asthma outbreaks were recorded, even though no changes had been made in port or industrial facilities. Furthermore, evidence of occasional increases in environmental allergen levels, documented persistence of soybean sensitivity in affected patients and sporadic admissions of sensitised patients with acute asthma supported the hypothesis that significant leakage of soybean dust into the atmosphere still occurred during unloading [5, 6].

Consequently, in 1998, new environmental measures were adopted based on the recommendations of the Catalan health authorities. These involved fitting the silos with greater particle-retention capacity bag filters in order to avoid the release of soybean dust into the atmosphere and daily soybean monitoring in a district close to the harbour using a large-volume automated air sample, as a means of determining exposure risk in the asthmatic population.

Soybean is loaded and unloaded in many harbours around the world, and epidemic asthma has also been documented in the ports of Saint-Nazaire (France) [7], New Orleans (USA) [8], Naples (Italy) [9] and other cities in Spain [10–12], but there are no published studies on the use of daily soybean allergen monitoring as a means of prevention in these or other cities. As a result of this daily monitoring in Barcelona, an ample registry of aeroallergen concentration data has been compiled for the first time and has formed the basis of an environmental model that may serve as a reference for atmospheric studies elsewhere. Taking into account these considerations, the aims of the present study were: (1) to analyse the effect of the latest protective measures on the atmospheric soybean aeroallergen concentration and (2) to study the consequences of current concentrations of soy aeroallergens on

the number of asthma admissions in the city's hospitals. The results of this study could encourage other cities to control their atmospheric levels of soybean allergen and, if these levels are elevated, to initiate preventive measures to avoid exposure of the soybean-sensitised population to allergen levels that might provoke asthma symptoms and even induce severe asthma attacks.

Material and Methods

Intervention Measures

The protective measures adopted in the port of Barcelona consisted of the installation of micropore size bag filters (polyester with inox fibres, BHA Purifilter, SL) in the silos for the unloading process and sleeve rupture detectors on all filters, in order to minimise channelled emissions.

Air Sampling

As described in previous studies [13], a large-volume automated air sampler (CAV-A/HF, MCV, SA, Barcelona) containing 1- μ m pore size glass microfibre filters (Whatman International Ltd., UK) was installed at 0.5 km from the harbour area. The filter was changed daily. The measurements were obtained on successive days over the study period, and there was one measurement per day. A 55 m³/h air volume was used for dust collection, and the sampler was active 24 h a day. Over a period of 5 years and 2 months, 1,854 samples were analysed, including 125 initial samples collected before protective measures were implemented (pre-intervention period, up to March 1998), and 1,729 samples collected after (postintervention period, April 1998 to December 2002). Soybean unloading data in the harbour were recorded during these periods. Daily average temperature, relative humidity, rainfall, barometric pressure and wind conditions were recorded daily from the Barcelona harbour weather station.

ELISA Inhibition Assay

Soybean aeroallergens were extracted from the filters in 5 ml PBS/0.2% BSA/0.5% Tween 20, overnight at 4°C, after which the filter was discarded and the eluates were analysed.

Soybean concentration was determined by an amplified ELISA inhibition assay based on a method described previously [14], using a serum pool with specific IgE antibodies of subjects allergic to soybean from the city of Barcelona. In the new batches of the human pool of sera a level of specific IgE to soybean of 19 kU/l (UniCAP 100, Pharmacia AB, Uppsala, Sweden) was maintained. A low-molecular-mass soybean allergen (soybean-LMM) standard, containing proteins with a molecular weight below 10,000 daltons, was used [15]. The following studies were conducted to assess the properties of the new batches of soybean-LMM: (1) protein concentration measurement (BCA method, Pierce, Rockford, Ill., USA), (2) protein composition (electrophoresis of the extract), (3) specificity of the reference preparation (samples from asthmatic patients with a known concentration of IgE to soybean were assayed by ELISA using the soybean-LMM allergen as antigen) [15].

Asthma Admissions and Home-Attended Acute Asthma Exacerbations

The number of admissions for asthma in the emergency rooms and intensive-care units of the three largest urban hospitals during 68 days (November 2001 to February 2002) of the postintervention period were recorded and compared with results obtained in previous intervention studies. Asthma patients attended at home during the same period by the public home emergency service were also recorded. Judicial autopsies during these days were studied to detect asthma deaths.

Patients who went to the emergency rooms were classified with respect to their asthma symptoms into 4 groups according to the GINA guidelines [16]: mild, moderate, severe and near fatal asthma.

Sensitisation to Soybean Allergens

A subgroup of patients agreed to visit a central office where a blood sample was taken to determine specific IgE to soybean by a previously described ELISA method [17]. Results were expressed as U A_{450 nm}. Values above the mean +2 standard deviations of the results obtained in a control population of 30 healthy individuals previously studied in our laboratory were considered positive. This cut-off was established at 0.330 U A_{450 nm}.

Contact with the patients was made as soon as possible after their admission (no more than 1 month after) with the aim of reliably collecting clinical data and obtaining the blood sample without implicating the time factor.

Statistical Analysis

Mean and standard deviation were used as descriptive measures to describe the aeroallergen series in the pre- and postintervention periods as well as unloading in the postintervention series.

The soybean aeroallergen level is a time-ordered sequence of observations. This characteristic determines that its values are not independent, i.e. they are autocorrelated. Many traditional statistical techniques may not be adequate for the analysis in this context. Hence, a time series model according to Box-Jenkins methodology was proposed to explain the evolution of aeroallergen levels [18]. With this approach the time series is assumed to be a linear model known as an autoregressive integrated moving average (ARIMA) [19–21]. This statistical method is considered to be more suitable than treating the time series with techniques in which independence of the observations is assumed [22–24]. The model can incorporate the statistical effects of variables such as protective measures or unloading. The analysis and interpretation of changes in the model structure due to the protective measures or unloading is similar to the use of dummy variables in regression analyses. The variable that accounted for the effect of the protective measures (Ft) had a value of zero prior to their implementation (in March 1998) and a value of 1 after the measures had been adopted. In the case of unloading, we defined the indicator variable (Ut) as having a value of 1 on unloading days and a value of zero for no unloading. The effect of unloading was assessed only in the postintervention period due to the low number of no-unloading days in the pre-intervention period. The correlation coefficients between weather recordings, as well as asthma events, and the concentration of the allergen were computed over a range of time lags. The SPSS 8.0 computer program for Windows was used for the analysis.

Table 1. Soybean aeroallergen levels (U/m³) in the 1,854 days studied

	Pre-intervention period (n = 125 days)	Postintervention period (n = 1,729 days)
Mean ± SD	159 ± 114*	39 ± 48*
Median	134	23
Range	10–561	10–636
Number of unloading days	121 (97%)	1,137 (66%)
Mean ± SD on unloading days	162 ± 119*	42 ± 50*
Mean ± SD on no-unloading days	–	33 ± 44**
Level >165 U/m ³	42 (34%)*	40 (3%)*

* p < 0.0001, ** p < 0.001, mean differences between unloading and no-unloading days in the postintervention period; level: number of days with levels higher than 165 U/m³.

Results

Soybean Aeroallergen Levels

The daily levels of soybean aeroallergen obtained in the 5 years and 2 months studied (n = 1,854, daily record) are shown in table 1. The mean soybean aeroallergen levels in the pre-intervention period were significantly higher (159 U/m³) than in the postintervention period (39 U/m³; p < 0.0001). Moreover, the pre-intervention period showed a more unstable pattern of aeroallergen concentration values than the postintervention period (fig. 1). With regard to unloading days exclusively, significant differences were also found in mean soybean aeroallergen levels between the pre-intervention (162 U/m³) and postintervention (42 U/m³) periods (p < 0.0001). In the postintervention period, significant differences were also found between unloading and no-unloading days (mean ± SD = 42 ± 50 and 33 ± 44 U/m³, respectively; p < 0.0001). In the pre-intervention period, only 4 days without unloading were observed. Using 165 U/m³ as the cut-off (highest value recorded in 22 unloading days in a previous study during which no asthma episodes were observed) [4], we found a significant decrease in the number of peaks (over the cut-off value) in the postintervention period (3% of the total daily levels) as compared with the number in the pre-intervention period (34%; p < 0.0001; table 1).

According to time series analysis, the best model proposed for the series was an integrated mixed model, whose notation was ARIMA (1, 1, 1). Indicators of the protective measures adopted according to the ARIMA (1, 1, 1)

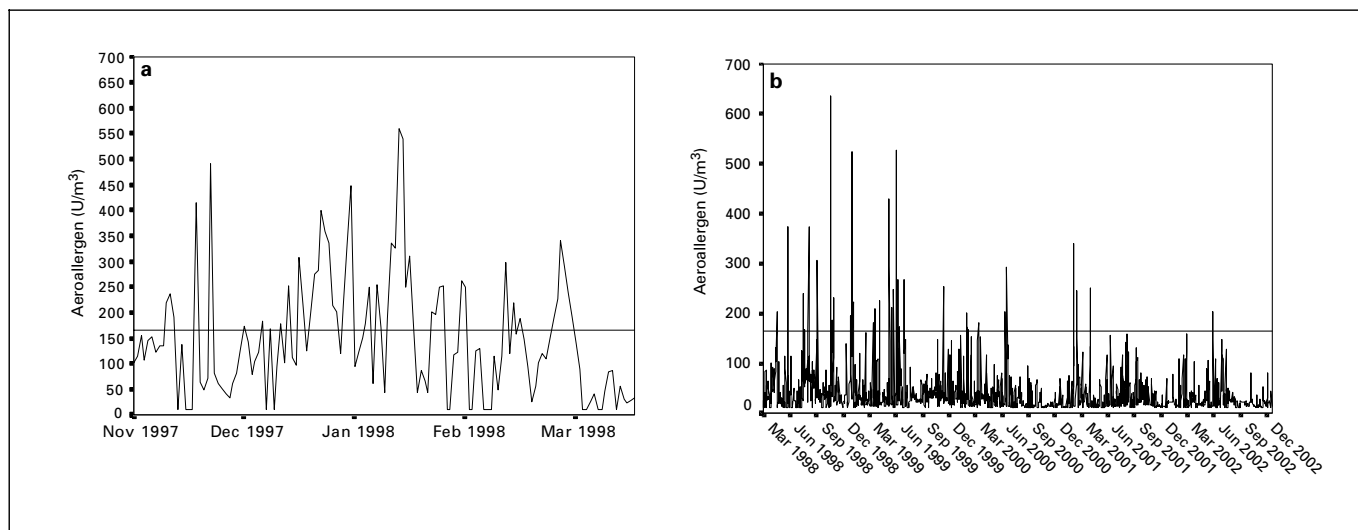


Fig. 1. Values of soybean aeroallergen concentrations obtained in the period studied. **a** Pre-intervention series (daily data from November 1997 to March 1998). **b** Postintervention series (daily data from April 1998 to December 2002). The baseline level is 165 U/m³.

Table 2. Effect of the protective measures adopted on the complete series and effect of the unloading on the postintervention period

	Effect on soybean level	Standard error	p
Complete series			
ARIMA components (1, 1, 1)			
Protective measures (F_t)	-1.25 ¹	0.22	<0.0001
Postintervention period			
ARIMA components (1, 0, 1) (1, 0, 0)			
Unloading (U_t)	-0.20 ²	0.04	<0.0001

ARIMA (1, 1, 1): integrated and first-order autoregressive and moving average component; ARIMA (1, 0, 1) (1, 0, 0): first-order autoregressive and moving average component and first-order seasonal autoregressive component; protective measures: variable that accounts for the effect of the greater capacity retention bag filter; unloading: variable that accounts for the effect of unloading.

¹ Decrease in 1.25 U/m³ in logarithmic scale.

² Increase in 0.20 U/m³ in logarithmic scale.

model are summarised in table 2. The model showed that the protective measures are effective, as evidenced by the decreased mean soybean aeroallergen level in the postintervention series.

The complete series presented no type of periodicity or cyclic variations. However, assessment of the changes in the series before and after adoption of the protective measures disclosed different behaviour. The postintervention series showed a seasonal first-order autoregressive com-

ponent, whose notation was ARIMA (1, 0, 1) (1, 0, 0). This seasonal component showed a 7- to 8-day periodicity, which may be interpreted as weekly behaviour. Moreover, the unloading variable proved significant as shown by an increasing effect on the mean level of soybean aeroallergen concentration in the series (table 2). No significant differences were observed in soybean aeroallergen concentration according to the meteorological variables recorded.

Asthma Admissions and Home-Attended Acute Asthma Exacerbations

A total of 279 cases of asthma were attended in the emergency services of the three hospitals during the 68 days studied, giving a mean of 4.1 patients per day. In the group that agreed to visit a central office in order to take a blood sample ($n = 106$), the distribution of the 4 groups with respect to their asthma symptoms were: mild (36%), moderate (40%), severe (21%) and near fatal asthma (3%). In the group that did not visit this central office, the percentage was: mild (45%), moderate (39%), severe (14%) and near fatal asthma (2%). No significant differences were observed between the 2 groups of patients with respect to their asthma symptoms.

Additionally, the public home emergency service attended 116 patients during this time, giving a mean of 1.7 patients per day. Two non-sensitised asthma patients had to be attended in the intensive-care units of one of the hospitals studied, and no asthma deaths were recorded in the hospitals or judicial autopsy registries during the days studied. No significant relationship was found between concentrations of environmental soybean aeroallergens and the number of emergency room admissions for asthma and the number of home emergency services attended.

Sensitisation to Soybean Allergens

After discharge from the emergency service, 106 patients agreed to participate in a determination of soybean-specific IgE. The mean of values in this group was 0.204 (range 0.034–0.719) U $A_{450\text{ nm}}$. Among the patients analysed, 9 presented positive IgE values above the previously established cut-off value (8%).

Discussion

The present study demonstrates that the latest protective measures adopted for the unloading of soybean in the harbour have been effective in decreasing the mean environmental soybean aeroallergen concentrations in our area.

Soybean is an important source of proteins used in the food industry for humans and for enriching animal feed. World-wide soybean production is around 120 million tons per year. Leading producers are the USA, Brazil and Argentina, and from these countries soybeans are shipped to, and unloaded in, many harbours around the world. As in other harbours, two milling plants located in the Barcelona facilities produce close to 80% of the soybean con-

sumed in the area. Owing to successive asthma epidemics caused by soybean in the city of Barcelona since the 1980s resulting from the unloading of this legume in the port, soybean aeroallergen emission levels within the city have been evaluated in several studies [4, 13, 14, 25]. In the 1980s, high aeroallergen values ranging from 1,600 to 10,600 U/m³ were recorded on epidemic days. These levels contrast with the low values observed on non-epidemic days (0.3–320 U/m³) [13]. In response to these findings, measures directed towards reducing the emission of soy dust were adopted in 1987, and a subsequent study demonstrated their efficacy in drastically reducing the levels of soy dust in the environment and in avoiding a large number of admissions for asthma in the emergency services [4]. Nevertheless, a new asthma outbreak was detected in 1994, in which environmental soybean aeroallergen levels reached 1,122 U/m³ [6], contrasting with 165 U/m³ recorded in the last study on days with no asthma episodes [4]. As a consequence of that latest outbreak, soybean aeroallergen levels were measured daily in our laboratory and high levels were still observed on certain days. Thus, to assure that asthmatic sensitised patients were not exposed to elevated levels of aeroallergen, new protective measures were recommended by the health authorities in 1998. These consisted of bag filters with greater retention capacity installed in the harbour facilities for the unloading process.

The latest protective measures adopted for the unloading of soybean demonstrated a decrease in mean environmental soybean aeroallergen concentrations on unloading days to a level of 42 U/m³, which contrasts with the 162 U/m³ obtained in the pre-intervention period (table 1). The protective measures adopted have led to a significant decrease in the number of values above the established cut-off (3 vs. 34% in the pre-intervention period). Additionally, the mean number of asthmatic patients admitted per day in the present study, 4.1 patients/day [4], is similar to the number obtained during non-epidemic days in pre-intervention studies (6 patients/day) [1], taking into account that in the present study only 3 of the 4 hospitals checked in previous studies were considered. The hospital which is not included in this study receives a reduced number of casualty admissions daily, therefore it is unlikely that the possible admissions to this centre would increase the overall number of daily admissions to more than 6. Moreover, only 2 of the patients had to be attended in intensive-care units, in contrast to the situation before these measures were adopted [4]. A case-control study performed during the pre-intervention period [3] showed that soybean-specific IgE in asthmatic patients who at-

tended in the emergency rooms on non-epidemic days was present in 6%, a value similar to the 8% found in the population studied in the present work. On the basis of the results, recommendations for maintaining environmental control on the concentration of this allergen by detecting levels exceeding certain amounts can be established, and this will facilitate prevention policies by the public health authorities of Barcelona and other cities.

The ultimate goal in Barcelona is not only to eliminate severe asthma attacks due to soybean inhalation, but also to eliminate asthma symptoms and bronchial inflammation from this cause. Unfortunately, establishment of an exposure limit is difficult since an adequately standardised assay for quantifying exposure and consequent symptoms is not yet available [26–29]. The lower limit of 165 U/m³, obtained on 22 unloading non-epidemic days in a previous work has been assumed provisionally as the desirable limit under which soybean allergen should be maintained [4]. Nevertheless, this threshold may have to be changed in the future in view of the results of the bronchial provocation test studies currently under way in our laboratory and the data from other clinical studies. Though differences between unloading and no unloading days in the postintervention period were found in the present study, these were not clinically significant, as evidenced by the fact that there were no increases in asthma hospital admissions or home emergencies. Nevertheless, we do not know whether this level might produce some degree of bronchial mucosa inflammation that would not require a visit to the emergency room.

In any case, the most important consequence of these new adopted measures is the reduction in the average aeroallergen concentration in the postintervention period and in the number of peaks during this period. Moreover, despite the fact that there are differences in the postintervention period in the unloading and non-unloading days (table 1), the level of soybean aeroallergen concentration is lower than in the pre-intervention period, so this are important reasons to recommend these measures to other cities where soybean is unloaded.

Taking advantage of the large number of atmospheric determinations performed, we used time series analysis with application of the ARIMA model to evaluate the results. This is the most suitable statistical test in intervention studies that evaluate the impact of a specific action [30]. The ARIMA model showed that the latest protective measures significantly decreased the mean atmospheric soybean level of the postintervention series, which corroborates the protective effect of the measures taken. The reasons for the weekly cyclic variation in the

postintervention series are likely to be complex and probably include an interplay of related factors such as air pollution, soybean unloading processes and meteorological variables. With respect to these meteorological variables, no relationship with concentration of the allergen was found in the present study, probably because these variables correspond to the mean of a 24-hour period. Future investigations should be aimed at carrying out measurements during shorter sampling periods of both soybean aeroallergen concentrations and meteorological conditions to be able to detect possible specific increases in concentrations of the allergen and their possible relationship with certain atmospheric conditions, and thus be able to construct a predictive model of the concentration of the allergen.

In conclusion, this is the first study that has used time series analysis to describe the changes in soybean environmental concentrations, in this case recorded in the city of Barcelona over a period of 5 years and 2 months. The results show the importance of adopting stricter protective measures during soybean unloading for decreasing the atmospheric levels of soybean allergen and asthma admissions in emergency room services. This policy can be recommended in all the cities where soybean is loaded or unloaded in order to prevent soybean-related bronchial mucosa inflammation and attacks of severe acute asthma in patients sensitised to this antigen.

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References

- 1 Antó JM, Sunyer J, Rodriguez-Roisin, et al: Asthma epidemics associated with soybean dust relevant during harbour activities. *N Engl J Med* 1989;320:1097-1102.
- 2 Rodrigo MJ, Morell F, Helm RM, et al: Identification and partial characterization of the soybean-dust allergens involved in the Barcelona asthma epidemic. *J Allergy Clin Immunol* 1990;85:778-784.
- 3 Sunyer J, Antó JM, Rodrigo MJ, et al: Case-control study of serum immunoglobulin-E antibodies reactive with soybean in epidemic asthma. *Lancet* 1989;1:179-182.
- 4 Antó JM, Sunyer J, Reed CE, et al: Preventing asthma epidemics due to soybean by dust-control measures. *N Engl J Med* 1993;329:1760-1763.
- 5 Manzanera R, Armengol R, Villalbí JR, et al: Public Health and Soybean-Related Asthma in Barcelona. Barcelona, Ajuntament de Barcelona, 1999.
- 6 Soriano JB, Antó JM, Plasència A, et al: Repeaters count: A sentinel method for asthma outbreaks. *Thorax* 1995;50:1101-1103.
- 7 Albert R: A propos de douze cas de sensibilisation au groupe des légumineuses comestibles. *Rev Fr Allergol* 1973;13:399-410.
- 8 White MC, Etzel RA, Olson DR, et al: Re-examination of epidemic asthma in New Orleans, Louisiana, in relation to the presence of soy at the harbour. *Am J Epidemiol* 1997;145:1-7.
- 9 Cocco G, Schiano M, Sacerdoti G: Functional characteristics in soybean asthma (abstract). *Am J Respir Crit Care Med* 1995;152:469.
- 10 Navarro C, Marquez M, Hernando L, et al: Epidemic asthma in Cartagena, Spain, and its association with soybean sensitivity. *Epidemiology* 1993;4:76-79.
- 11 Garcia-Ortega P, Rovira E, Mora E: Epidemias de asma alérgica a semilla de soja en ciudades pequeñas. *Med Clin (Barc)* 1997;108:677.
- 12 Ballester F, Soriano JB, Otero I, et al: Asthma visits to emergency rooms and soybean unloading in the harbours of Valencia and A Coruña, Spain. *Am J Epidemiol* 1999;149:315-322.
- 13 Aceves M, Grimalt JO, Sunyer J, et al: Identification of soybean dust as an epidemic asthma agent in urban areas by molecular marker and RAST analysis of aerosols. *J Allergy Clin Immunol* 1991;88:124-134.
- 14 Cruz MJ, Rodrigo MJ, Antó JM, et al: An amplified ELISA inhibition method for the measurement of airborne soybean allergens. *Int Arch Allergy Immunol* 2000;122:42-48.
- 15 Swanson MC, Li J, Wentz-Murtha PE, Trudeau WL, et al: Source of the aeroallergen of soybean dust: A low molecular mass from the soybean tela. *J Allergy Clin Immunol* 1991;87:783-788.
- 16 Global Initiative for Asthma: Global Strategy for Asthma Management and Prevention NHLBI/WHO Workshop Report. NIH Publ No 96-3659B. Bethesda, Md., National Institute of Health, National Heart, Lung and Blood Institute, 1998.
- 17 Morell F, Codina R, Rodrigo MJ, et al: Diagnosis of soybean-induced asthma. *J Allergy Clin Immunol* 1995;96:320-324.
- 18 Box GE, Jenkins GM: *Time Series Analysis: Forecasting and Control*. San Francisco, Holden-Day, 1976.
- 19 Diggle P: *Time Series: A Biostatistical Introduction*. New York, Oxford Science Publications, Oxford University Press, 1992.
- 20 Chatfield C: *The Analysis of Time Series: An Introduction*, ed 5. London, Chapman & Hall, 1996.
- 21 Murillo C: *Métodos estadísticos de series temporales. Aplicaciones sanitarias*. Barcelona, SG Editores SA, 1994.
- 22 Schnell D, Zaidi A, Reynolds G: A time series analysis of gonorrhea surveillance data. *Stat Med* 1989;8:343-352.
- 23 Helfenstein U, Ackermann-Liebrich U, Braun-Fahrlander C, et al: The environmental accident at 'Schweizerhalle' and respiratory diseases in children: A time series analysis. *Stat Med* 1991;10:1481-1492.
- 24 Fernandez-Perez C, Tejada J, Carrasco M: Multivariate time series analysis in nosocomial infection surveillance system: A case study. *Int J Epidemiol* 1998;27:282-288.
- 25 Antó JM, Soriano JB, Sunyer J, et al: Soybean sensitization in epidemic asthma patients 8 years after an intervention. *Am J Respir Crit Care Med* 1996;153:A515.
- 26 Platts-Mills TAE, de Weck AL (chairmen): Dust mite allergens and asthma: A worldwide problem. *J Allergy Clin Immunol* 1989;83:416-427.
- 27 Platts-Mills TAE, Thomas WR, Aalberse RC, et al: Dust mite allergens and asthma: Report of a 2nd international workshop. *J Allergy Clin Immunol* 1992;89:1046-1060.
- 28 Hollander A, Gordon S, Renström A, et al: Comparison of methods to assess airborne rat and mouse allergen levels. I. Analysis of air samples. *Allergy* 1999;54:142-149.
- 29 Renström A, Gordon S, Hollander A, et al: Comparison of methods to assess airborne rat and mouse allergen levels. II. Factors influencing antigen detection. *Allergy* 1999;54:150-157.
- 30 Antó JM, Murillo C: Aplicaciones sanitarias del análisis de series temporales (editorial). *Gac Sanit* 1991;22:3-4.