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Identification of soybean dust as an epidemic asthma agent in urban areas by molecular marker and RAST analysis of aerosols

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From 1981 to 1987, 26 outbreaks of asthma have occurred in the city of Barcelona, Spain, affecting a total of 687 subjects and causing 1155 emergency room admissions. Assays of urban aerosols collected with high-volume samplers between October 1986 and May 1989 have indicated that soybean dust originating from harbor activities (and not traffic or industrial pollution) is the causal agent for these epidemics. Soybean particulates in the filters have been characterized from the composition of the alcohol fraction, namely, campesterol, stigmasterol, β -sitosterol, n-triacontan-1-ol, and n-dotriacontan-1-ol, constituting a series of aerosol components correlated with the epidemiologic data. This result has also been confirmed by immunochemical assay of specific soybean allergens. The concentration of these sterols in the air corresponds to a 24-hour average level of soybean dust in the order of $25 \mu\text{g}/\text{m}^3$ on epidemic days. These results suggest the advisability of monitoring soybean dust in air particulates of populated areas surrounding soybean storage or processing plants. The techniques presented here afford a simple way for the recognition of soybean dust in aerosols containing high concentrations of organic pollutants of traffic or industrial origin. (J ALLERGY CLIN IMMUNOL 1991;88:124-34.)

Key words: Epidemic asthma, soybean, molecular markers, β -sitosterol, stigmasterol, n-triacontan-1-ol, RAST, urban aerosol

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Despite improved medical care, asthma mortality has not been significantly reduced.¹ Whether or not this is due to changes in the background frequency of the diseases or to the appearance of new etiologic factors in the community is not clear. The prevalence of asthma in developed countries has been attributed to environmental factors associated to the urban way of life: traffic fumes, industrial and domestic pollutants, tobacco smoke, natural allergens, etc. The irritant effect of some of these agents has been proved,² but their relative significance as asthma inducers in

urban populations has still to be elucidated. The identification of these causal factors is especially important in cases of epidemic asthma. However, although the occurrence of asthma outbreaks in large cities has been documented,³⁻⁷ their direct causal agents have rarely been established.

The asthma outbreaks identified in Barcelona were characterized for their clinical severity.⁸⁻¹⁰ Increased concentration of nitrogen oxide was initially suspected as the asthma inducer,⁹ but later epidemiologic data demonstrated that the outbreak was a point-source epidemic.¹⁰ Further epidemiologic studies led us to conclude that these outbreaks of asthma were caused by the inhalation of soybean dust released during the unloading of soybeans at the city harbor.¹¹ These results were consistent with a serologic case-control study encompassing the reaction of soybean antigen extracts with IgE Abs obtained from asthma-epidemic patients¹² and with the characterization of a low-weight protein of the soybean dust reactive against asthma epidemic sera.¹³

Paralleling these medical investigations in October 1986, two control stations of atmospheric particulates were established in highly populated areas of the city in which many epidemic cases were identified (Fig. 1). This research was undertaken with two main objectives: first, to obtain direct evidence of the presence of the agent responsible for these episodes in urban air particulates, and second, to develop a method for the regular monitoring of this allergen in heavily polluted atmospheres.

Accordingly, the urban aerosol collected at these two stations was regularly analyzed for organic and inorganic compounds. The study included a detailed survey of the solvent extractable organic matter, metals, and the development of RAST-inhibition tests with extracts of selected aerosol samples. The results that compare asthma epidemic and nonepidemic days are presented here. Particular attention is devoted to data allowing the recognition of soybean dust in atmospheric samples as well as data providing direct evidence of the allergenic properties of soybean-rich aerosols.

MATERIAL AND METHODS

Identification of asthma-epidemic days

Epidemiologic data were obtained from the clinical records of the four largest hospitals of Barcelona, which account for approximately 90% of the emergency care in the city. An ER admission for asthma was defined as a visit during which any asthma-related diagnosis was recorded. To avoid differences in terminology, a list of expressions for inclusions as asthma was established by a panel of chest physicians.¹⁰ In all participating hospitals, asthma admissions were registered by a trained physician. All records

Abbreviations used

PAH:	Polycyclic aromatic hydrocarbon
TSP:	Total suspended particle
F1:	Fractions of aliphatic hydrocarbons
F2:	Fractions of aromatic hydrocarbons
F3:	Fractions of alcohols and other polar compounds
GC:	Gas chromatography
MS:	Mass spectrometry
ER:	Emergency room
Ab:	Antibody

were later reviewed, and their completeness was checked. Only patients older than 14 years were included in the study because no abnormal increases in cases of acute severe asthma in childhood were observed during asthma outbreaks.

An *asthma-epidemic day* was defined as a day fulfilling two conditions: (1) The number of ER visits was so high that the probability that such a number or a higher number was the result of chance was 0.025 or less. This probability was calculated by assuming a Poisson distribution with the 15-day moving average representing the number of cases expected.¹⁴ (2) This high number of ER admissions was clustered on an hourly basis. The "hourly cluster" was defined as the occurrence in one 4-hour period of so many ER visits for asthma that the probability that such a high number of visits was the result of chance was 0.05 or less. The 4-hour periods were selected on the basis of the distribution of cases during asthma outbreaks. This probability was calculated according to the Knox and Lancashire¹⁵ approximation to the scan method.¹⁶

Aerosol sampling

Samples (1200 m³ and 50 m³/hr) were collected with high-volume suction pumps (CAV-P; MCV, Collbato, Spain) with 20.3 × 25.4 cm of glass microfiber filters (catalog No. 1820866, Whatman International Ltd., Maidstone, England). These were cleaned by kiln firing to 400° C before use (12 hours). The filters were weighed before and after aerosol collection for determination of total suspended particles. Filters were then cut in four equivalent portions, and each portion was devoted to a different analysis (organics or metals) or immunochemical assay.

Material for organic analysis

All organic solvents were distilled on a 1 m packed (Rashig) column equivalent to 12 theoretical plates, with a reflux ratio of 12:1. The purity was checked by GC analysis of solvent concentrates (100 ml to 10 µl under vacuum). Blank requirements were as follows: splitless injection of 2.5 µl should result in chromatograms with peaks representing <1 ng in terms of their flame ionization detector response. This threshold, under the above dilution factor, is equivalent to a level of artifacts <15 pg/m³ when the

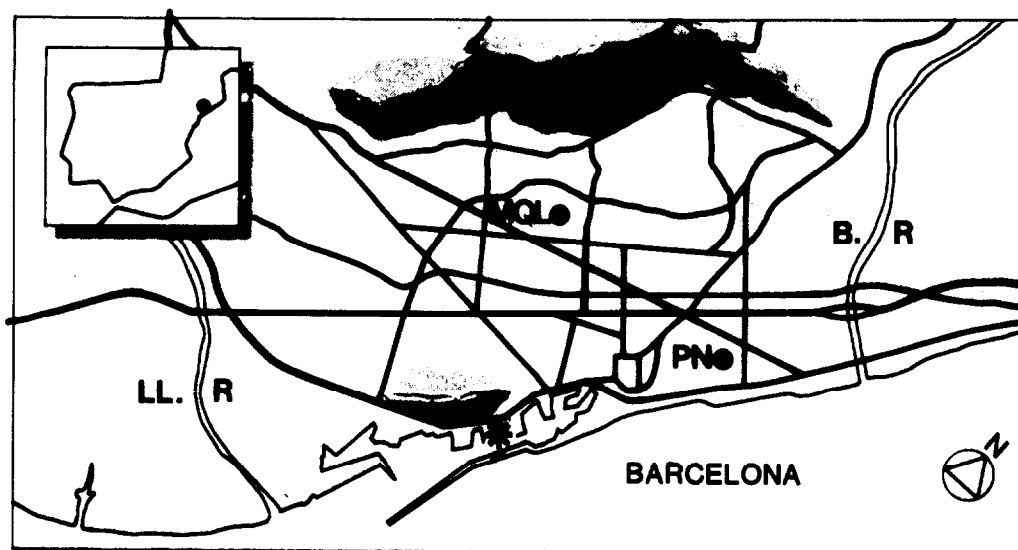


FIG. 1. Map of the city of Barcelona illustrating the location of the soybean processing plants (*) and the control stations, MOL and PN, respectively, situated at 4 and 4.4 km from the discharge site. LL. R, Llobregat River; B. R, Besos River. Thick continuous lines, main streets.

threshold is referred to the average air volume corresponding to the filter portions analyzed separately (300 m³).

Silica gel (70 to 230 mesh, Merck, Darmstadt, Germany) and alumina (70 to 230 mesh, Merck) were extracted with methylene chloride-methanol (2:1) in a Soxhlet apparatus for 24 hours. After solvent evaporation, these adsorbents were heated at 120°C and 350°C, respectively, during 12 hours. A total 5% (wt/wt) of Milli-Q-grade water (Millipore Corp., Bedford, Mass.) was then added for deactivation.

Extraction and isolation of hydrocarbons and alcohols

The filters were torn into approximately 1 cm² pieces with precleaned tweezers. They were then extracted by Soxhlet with 150 ml of methylene chloride for 30 hours. The extract was vacuum evaporated to 0.5 ml and fractionated by column chromatography according to previously established methods.¹⁷ A column filled with 1 gm of both 5% water-deactivated alumina (top) and silica (bottom) was used. Three fractions were collected: 6 ml of *n*-hexane (F1), 6 ml of 20% methylene chloride in *n*-hexane (F2), and 6 ml of 20% methanol in methylene chloride (F3). Before instrumental analysis, fractions F1 and F2 were vacuum evaporated to small volumes (≈100 μl). Fraction F3 was evaporated to dryness under nitrogen, redissolved in 100 μl of isooctane, and silylated with 100 μl of bis(trimethylsilyl)trifluoroacetamide (80°C for 30 minutes).

Instrumental analysis

GC analysis was performed with a Carlo Erba (Carlo Erba Instruments, Hofheim, Germany) HRGC 5300 instrument equipped with a flame ionization detector and a splitless injector. A column of 25 × 0.25 mm inside diameter,

coated with CP-Sil 8 CB (Chrompack International BV, Middleburg, The Netherlands) was used (film thickness, 0.13 μm). Hydrogen was the carrier gas (50 cm · s⁻¹). Sweep and split flows were 2 and 50 ml · min⁻¹, respectively. Nitrogen was used as makeup gas (30 ml · min⁻¹). Injector and detector temperatures were, respectively, 300 and 330°C. Detector gas flows were hydrogen, 30 ml · min⁻¹, and air, 300 ml · min⁻¹. The injection was in the splitless mode (solvent, isooctane; hot needle technique) keeping the split valve closed for 35 seconds. The oven temperature was programmed from 60° to 300° C at 4° C/min for the analysis of hydrocarbons and from 60° to 150° C at 10°/min, 150° to 250° C at 6° C/min, and 250° to 300° C at 2° C/min for the analysis of alcohols and sterols.

Combined GC-MS analysis was performed with a Hewlett-Packard 5995 (Hewlett-Packard Co., Cupertino, Calif.) instrument coupled to an HP 300 data system.

The chromatographic conditions were the same as described above except that helium was the carrier gas, and no make-up gas was used. MS temperatures were transfer line, 300° C; ion source, 200° C; and analyzer, 230° C. Data were acquired in the electron impact mode (70 eV). Scanning range was 50 to 650 m/z at 1 sec per decade. An NBS library was used for mass spectral identification, which was confirmed by coinjection with authentic standards.

Quantitation of hydrocarbons and alcohols

Quantitation was performed from the GC traces by comparison with adequate external standard mixtures: *n*-tetradecane, *n*-docosane, *n*-dotriacontane, and *n*-hexatriacontane for F1, phenanthrene, chrysene, benzo(a)pyrene, and benzo(ghi)perylene for F2, and cam-

pesterol, β -sitosterol, and *n*-triacontan-1-ol for F3. Samples and standards were repeatedly injected until <5% dispersion was observed in the measured areas.

Immunochemical assay of allergens

Allergens were eluted from the fiberglass filter sheets and assayed by RAST inhibition as described according to the method of Agarwal et al.¹⁸ Reference soybean husk allergens and sera from epidemic cases containing IgE Abs were prepared as previously described.¹² The major allergen in soybean dust is a glycoprotein with a molecular weight of approximately 10,000 daltons and is located predominantly in the husk. We are as yet unable to express the allergen concentration in mass units, and we assigned an arbitrary value of 10^6 units to the reference soybean husk extract. One unit contains approximately 1 ng of allergen. The allergen appears in the pod and husk of growing soybeans before the bean is mature and reaches highest concentration in the husk at harvest time.¹⁹ Extensive studies reveal no cross-reactivity with extracts of pollens, molds, mites, insects, or other legumes. There is some cross-reactivity from corn and wheat dust but not from rapeseed.

RESULTS

Aerosol sampling

Adequate sampling is the most difficult aspect for the resolution of this type of problems. The origin of the outbreaks was unknown at the time that aerosol collection was initiated for this study. No information was available on products or concentration levels to be analyzed, nor was it even the certitude that the allergen occurred in association with air particles. Therefore, reference data on dates of collection, sample volume, etc., were unknown. In fact, the only "a priori" information was the characteristic "hourly cluster" of the outbreaks (see MATERIAL AND METHODS), a trend that was likely reflecting the atmospheric distribution pattern of the allergen during the asthma episodes. This trend pointed to intervals of 24 hours as reasonable sampling time. Longer periods could involve an excessive dilution effect of the allergen products, becoming minor components among other filter materials. Shorter collection times had the risk of missing the hours of higher air allergen concentration because outbreak forecasting was not possible.

The samples included in this study can be classified in three groups. The first group of samples (Nos. 1 to 22, November 1986 to September 1987, Table I) corresponds to a period in which the origin of the outbreaks was unknown, and the samples were collected in epidemic and nonepidemic days under protocolized conditions. In some cases, that is, Nov. 25, 1986, No. 4 filters were changed after outbreak detection for a closer study of the air variations associated with the episodes. The second group of samples

corresponds to a period of time starting in October 1987 after identification of a direct relationship between soybean unloading in the harbor and asthma episodes that resulted in the installation of bag filters in the harbor silo. This installation drastically reduced the emission of dust during the soybean unloading processes (filters 23 to 38, October 1987 to August 1988). An additional group of samples is constituted by two samples corresponding to a day in which a breakdown in the harbor silo caused a rise to an unusually high soybean-dust emission.

The organic composition of aerosols from epidemic and nonepidemic days

An extensive study of the solvent extractable organic matter present in the aerosols of Barcelona has been performed in the framework of the present study. The first column chromatography fraction (MATERIAL AND METHODS) is dominated by a modal distribution of C17-C33 *n*-alkanes maximized at *n*-tricosane that occurs along with a complex distribution of steranes, and hopanes of the 17 α ,21 β (H) series. These hydrocarbons are of petrogenic origin, likely released from the exhausts of the motor vehicles.²⁰⁻²² The aromatic hydrocarbons of F2 are predominated by parent compounds, such as fluoranthene, pyrene, benzo(ghi)fluoranthene, cyclopenta(cd)pyrene, chrysene plus triphenylene, benzo(a)fluoranthene, benzopyrenes, benzo(ghi)perylene, and indeno(1,2,3-cd)pyrene (Fig. 2), which reflect pyrolytic sources.²¹ The uniformity of aliphatic and aromatic hydrocarbon profiles, irrespective of sampling station and date of collection, is consistent with the epidemiologic observation that air pollution was not related to asthma outbreaks.²³

The composition of F3 is not as uniform as that of F1 or F2. In nonepidemic days, distributions of even carbon number predominated C22 to C32 *n*-alkan-1-ols, maximized at the C24 or C26 homolog, are usually found. In quantitative terms, these compounds only represent minor constituents of the aerosol samples. These features strongly contrast with the composition of F3 in epidemic days. As illustrated in Fig. 2, β -sitosterol is the predominant alcohol in these cases, occurring along with stigmasterol and campesterol, as well as with an increased amount of *n*-triacontan-1-ol and dotriacontan-1-ol. All these compounds are of biogenic origin and are pointing to a biogenic source for the compositional differences between aerosols collected in epidemic and nonepidemic days. Conversely, the uniformity of F1 and F2 indicates that asthma epidemics occurred irrespective of the levels of traffic or pyrolytic pollution in the city.

TABLE I. Alcohol and sterol composition of aerosol samples collected in epidemic and nonepidemic days

Sample number	Location	Date (mo/d/yr)	Activity harbor	Asthma outbreak	Pollution parameters			
					TSP	Pb	ALK	PAH
1	MOL	11/11/86	U	Y	210	1.1	60	87
2	PN	11/12/86	R	AE	240	1.5	80	60
3	MOL	11/25/86d	U	Y	150	1.1	630	180
4	MOL	11/25/86n	U	AE	120	0.8	420	90
5	PN	11/25/86	U	Y	260	0.6	320	110
6	MOL	11/26/86	R	AE	260	0.5	210	70
7	MOL	1/31/87	R	N	370	3.8	570	140
8	PN	1/31/87	R	N	470	2.3	620	180
9	MOL	2/8/87	U	Y	270	4.7	740	210
10	PN	2/8/87	U	Y	250	3.2	500	140
11	PN	2/9/87	R	Y	180	2.1	130	170
12	PN	3/4/87	U	Y	320	0.9	97	37
13	MOL	3/20/87	U	N	71	0.9	110	32
14	PN	3/20/87	U	N	160	0.6	280	21
15	MOL	3/28/87	R	N	128	1.1	58	27
16	PN	3/28/87	R	N	75	0.3	44	5.7
17	MOL	4/5/87	U	N	160	1.8	200	50
18	PN	4/5/87	U	N	230	1.9	130	48
19	MOL	6/8/87	U	N	130	0.7	80	18
20	PN	6/8/87	U	N	320	0.4	170	9.1
21	MOL	9/4/87	U	Y	150	0.9	130	18
22	PN	9/4/87	U	Y	210	1.2	140	27
23	MOL	10/14/87	R	N	150	1.4	210	49
24	PN	2/5/88	CU	N	240	3.2	480	160
25	PN	2/8/88	CU	N	250	3.0	500	180
26	PN	3/6/88	R	N	320	0.92	96	38
27	PN	3/22/88	R	N	160	0.57	170	23
28	MOL	4/18/88	CU	N	190	1.2	220	13
29	PN	4/18/88	CU	N	340	1.5	84	50
30	MOL	5/6/88	R	N	130	1.2	210	120
31	PN	5/6/88	R	N	330	1.7	200	100
32	MOL	6/8/88	CU	N	130	1.7	77	20
33	PN	6/8/88	CU	N	320	0.37	170	91
34	MOL	7/20/88	R	N	71	0.63	90	14
35	PN	7/20/88	R	N	110	0.17	47	13
36	MOL	8/19/88	CU	N	170	0.7	90	24
37	PN	8/19/88	CU	N	160	0.51	91	31
38	MOL	8/27/88		N	170	0.72	140	42
39	MOL	2/17/89	U	*	130	1.5	170	39
40	PN	2/17/89	U	*	190	1.6	110	41

d, Day; n, night; U, soybean unloading; CU, controlled soybean unloading; R, not unloading; Y, yes; AE, after episode; N, no; ALK, petrogenic *n*-alkanes; Pb, lead; A, *n*-octacosan-1-ol; B, campesterol; C, stigmasterol; D, β -sitosterol; E, *n*-triacontan-1-ol; F, *n*-dotriacontan-1-ol; ND, not determined.

Representative parameters indicating the levels of traffic and industrial pollution are also included.

TSP and Pb in micrograms per cubic meter; ALK, PAH, alcohols, and sterols in nanograms per cubic meter.

*Days with a breakdown in the harbor silo; asthma occurrence not yet epidemiologically studied.

†<0.5 ng/m³.

Quantitation

According to these features, diverse parameters related to urban pollution (traffic and combustion) and the above discussed alcohols have been quantitated.

The values corresponding to the samples included in this study are listed in Table I. The activity in the harbor related to soybean unloading, as well as the occurrence of asthma outbreaks in the day of collec-

Alcohols and sterols						Allergen values
A	B	C	D	E	F	
12	1.8	13	79	28	3.0	ND
3.7	—†	6.5	32	11	0.9	ND
12	2.3	8.9	89	30	3.0	ND
2.2	0.7	2.4	17	5.8	0.55	ND
11	3.0	10	96	33	2.9	ND
4.8	1.3	5.2	27	9.3	1.0	ND
1.7	0.7	2.7	4.5	1.5	—	ND
2.3	—	1.9	3.0	1.3	—	54.1
9.7	16	41	55	12	1.6	ND
23	16	33	77	35	7.7	9248
21	7.0	14	33	29	4.2	ND
23	2.2	8.2	45	19	2.9	10590
6.2	1.4	4.7	12	3.7	1.9	ND
14	1.2	2.6	9.8	9.4	2.6	ND
93	—	2.8	9.3	9.0	0.9	ND
75	2.2	—	4.0	8.1	—	15.3
19	0.81	6.5	9.8	3.6	0.8	ND
22	3.5	8.0	23	7.1	2.7	319
18	—	1.6	3.7	15	3.2	ND
32	—	—	7.8	13	2.6	82.8
7.0	2.7	24	190	14	1.8	ND
17	2.8	19	110	19	4.7	1555
8.8	—	—	2.3	5.6	2.9	ND
1.6	—	0.88	6.0	1.4	0.5	163
5	1	2.7	10	4.7	1.5	5.0
2.5	0.5	1	5.8	0.75	3.	18.0
3.0	0.4	0.8	5.0	1.0	2.0	99.2
14	—	1.0	6.3	3.7	—	ND
7.0	—	1.4	10	5.2	1.2	0.3
—	—	15	1.6	9.4	1.5	ND
—	—	13	1.4	8.8	1.4	0.9
—	—	—	—	—	—	ND
—	—	—	—	—	—	0.2
5.2	—	1.9	9	5.2	—	ND
9.0	—	3.0	9	6.5	—	41.9
—	—	—	—	—	—	ND
8.2	—	4	8.7	4.5	—	15.3
6.4	—	1.5	8.8	4.9	1	ND
67	15	40	68	34	11	ND
83	7	17	60	45	15	ND

tion, are also indicated. Epidemic days were defined from the number of ER admissions for asthma according to the statistical criteria established in the MATERIAL AND METHODS section. Urban pollution is represented by two organic parameters: total *n*-alkanes and total parent PAHs. These are complemented with the airborne lead measurements corresponding to the studied days. TSPs are also considered.

The average values of these parameters for epidemic and nonepidemic days are presented in Table

II. TSP matter has about the same mean for both populations, but the average traffic and combustion parameters are higher in epidemic than in nonepidemic days. This reflects the air stagnation conditions usually observed in association with the asthma episodes.^{9, 10} However, the significance of the differences of means between the two populations are not high if the standard deviations are considered (Student's *t* test in Table II). In epidemic days, air stagnation can also involve the increase in concentration of some biogenic compounds, such as *n*-octacosan-1-ol.

This finding strongly contrasts with the important increase of campesterol, stigmasterol, β -sitosterol, *n*-triacontan-1-ol and *n*-dotriacontan-1-ol in outbreak days. High confidence intervals are found when the hypothesis of different means is tested for these components (i.e., > 0.99 for β -sitosterol and *n*-triacontan-1-ol, Table II).

Soybean alcohol/sterols in epidemic days

The results indicated above strongly suggest that the occurrence of asthma outbreaks in Barcelona was related with rises in airborne concentration of campesterol, stigmasterol, β -sitosterol, *n*-triacontan-1-ol, and, to a lesser extent, *n*-dotriacontan-1-ol, a set of compounds that must reflect the presence of the allergen in the aerosols of the city.

In Fig. 3, the alcohol composition of urban aerosols from epidemic days is compared with that of soybean dust from the filters of the harbor silos and airborne dust collected in the harbor during soybean unloading (high-volume pumping and filtration). The concentration of these compounds in diverse soybean dusts and husks is presented in Table III. The similarity between the profiles of epidemic urban aerosol and harbor dust during soybean unloading is striking. This alcohol/sterol composition appears to be intermediate between that of dust collected at soybean processing and dehushing silo filters.

These results provide additional evidence that soybean dust was effectively present in the atmosphere of Barcelona during the epidemic days.¹¹ However, the possibility that other biogenic sources also contributed to these distributions of alcohols/sterols must be considered. As illustrated in Fig. 2, some level of *n*-triacontan-1-ol and dotriacontan-1-ol is generally present in the atmosphere of the city in nonepidemic days. As indicated above, in these cases, these alcohols are minor homolog members of a distribution of C22-C32 *n*-alkan-1-ols maximized at C24 or C26. In contrast, soybean dust is characterized by a high predominance of *n*-triacontan-1-ol among the acyclic alcohols (Fig. 3), which represents an important distinct feature usually observed in most aerosol samples from epidemic days. In any case, the average values

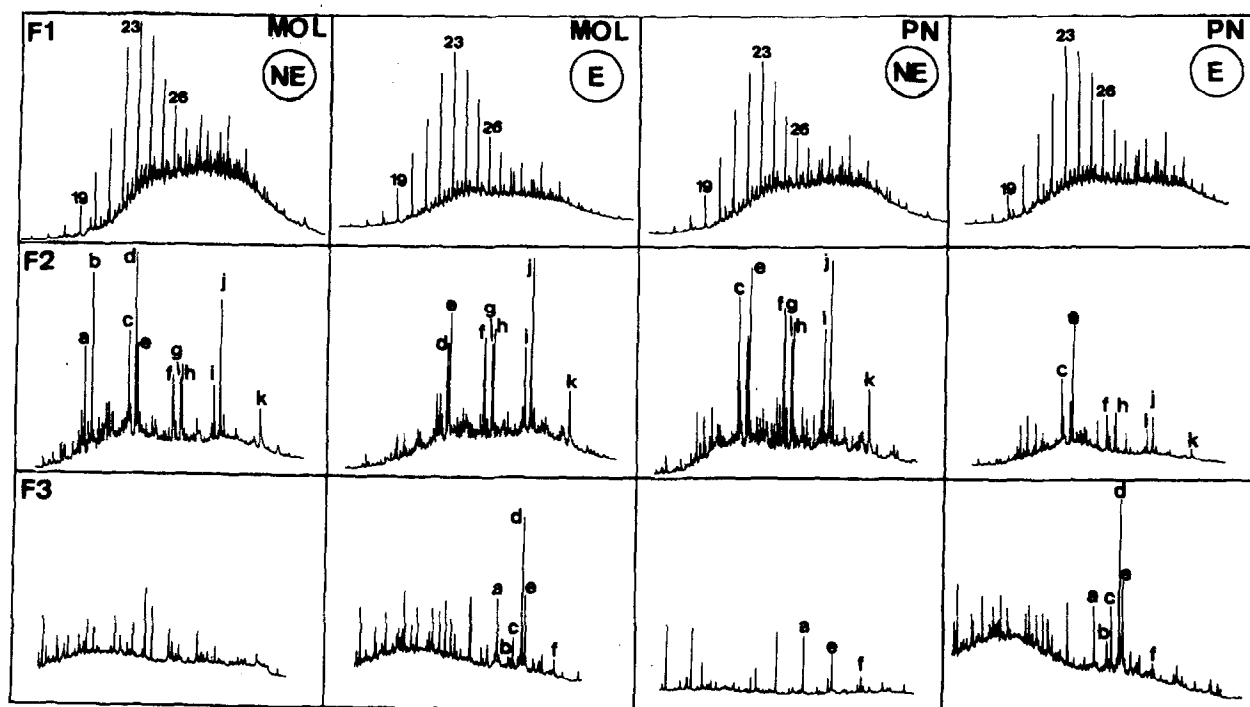


FIG. 2. Representative chromatograms of the three lipid fractions corresponding to the filter extracts collected in epidemic (*E*) and nonepidemic (*NE*) days at Molina (*MOL*) and Poble Nou (*PN*) stations. Peak identification is as follows: *F1*, numbers refer to chain length of *n*-alkanes; *F2*: *a*, fluoranthene; *b*, pyrene; *c*, benzo(ghi)fluoranthene; *d*, cyclopenta(cd)pyrene; *e*, chrysene plus triphenylene; *f*, benzo(ghi)fluoranthene; *g*, benzo(e)pyrene; *h*, benzo(a)pyrene; *i*, indeno(1,2,3-cd)pyrene; *j*, benzo(ghi)perylene; *k*, coronene; *F3*: *a*, *n*-octacosan-1-ol; *b*, campesterol; *c*, stigmasterol; *d*, β -sitosterol; *e*, *n*-triacontan-1-ol; *f*, *n*-dotriacontan-1-ol.

TABLE II. Average concentrations of alcohols, sterols, allergen, and pollution parameters of samples collected in asthma epidemic and nonepidemic days (individual values listed in Table I)

	Asthma epidemic		Nonepidemic		Significance of the differences of means	
	Mean	SD	Mean	SD	Estimated <i>t</i> value	Confidence interval*
TSP†	211	59	206	105	0.18	0.10
Lead†	1.7	1.2	1.3	0.94	0.98	0.60
Petrogenic <i>n</i> -alkanes‡	275	240	200	160	0.95	0.60
PAH‡	96	70	58	55	1.6	0.80
<i>n</i> -Octacosan-1-ol‡	26	25	14	22	1.4	0.80
Campesterol‡	6.9	5.9	0.45	0.84	3.6	0.99
Stigmasterol‡	21	12	3.0	3.8	4.9	0.99
β -Sitosterol‡	82	42	6.6	4.9	5.9	0.999
<i>n</i> -Triacontan-1-ol‡	27	10	5.1	4.1	7.0	0.999
<i>n</i> -Dotriacontan-1-ol‡	5.3	4.3	1.1	1.1	3.2	0.98
Soybean allergen§	7100	4900	63	91	4.8	0.99

The mean differences corresponding to the two groups of samples are evaluated with the Student's *t* test for populations of significantly different standard deviations.³³

*Confidence interval of the nearest tabulated *t* value lower than the estimation.

†In micrograms per cubic meter.

‡In nanograms per cubic meter.

§Units of allergen.

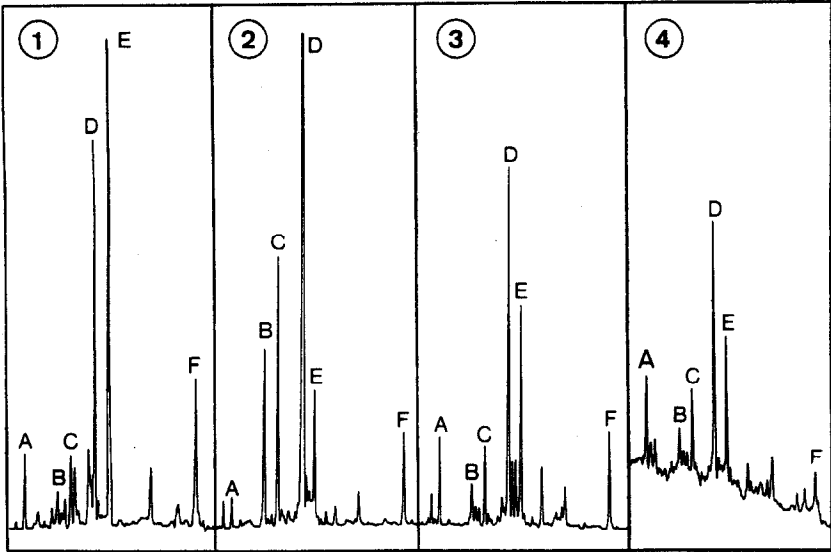


FIG. 3. Representative gas chromatograms of the alcohol/sterol fraction (F3) of (1) filter dust at soybean processing, (2) soybean husk, (3) harbor dust during soybean unloading and husk removal, and (4) air particulates collected in an epidemic day. Peak identification the same as in Table I.

TABLE III. Main alcohols and sterols of dust generated during soybean unloading and processing in the harbor plants

	<i>n</i> -Octacosan-1-ol	Campesterol	Stigmasterol	β -Sitosterol	<i>n</i> -Triacontan-1-ol	<i>n</i> -Dotriacontan-1-ol
Husk-1*	18	130	140	610	84	60
Husk-2†	32	140	160	760	96	56
Husk-3†	11	75	130	520	94	75
Filter dust at discharge*	110	74	160	360	380	220
Filter dust at processing*	280	180	300	700	1100	560
Husk dust	290	540	700	2800	420	290
Harbour dust‡	44	24	51	260	150	74

Grain husk composition for diverse soybean shipments is also provided (concentration, micrograms per gram).

*Husk and filter dusts collected during controlled soybean processing.

†Grain husks corresponding to soybean shipments unloading of which was coincident with asthma outbreaks (September 4, 1987).

‡Collected by air filtration during controlled unloading and treatment (concentration, nanograms per cubic meter. ng/m³).

of epidemic and nonepidemic days listed in Table II indicate that this "background level" of *n*-triacontan-1-ol or dotriacontan-1-ol has a minimal masking effect of the characteristic increased concentrations of outbreak days.

In contrast, biogenic sources, such as pollen, may also contribute to the presence of β -sitosterol or other soybean-related sterols.²⁴⁻²⁸ However, they generally reveal other alcohol/sterol patterns allowing a differentiation with respect to soybean inputs. In any case, the organic fraction of the aerosols of Barcelona is

dominated by compounds related with traffic and pyrolytic pollution; contributions from pollen are small. In this respect, the sterol composition of the samples collected during the period of highest pollination (late February to April²⁹) in nonepidemic days demonstrate sterol concentrations well below concentrations of the aerosols sampled during the outbreaks. The lack of significant interferences is also illustrated by the mean values of Table II. The probability that a simple random effect is responsible for the strong differences between epidemic and nonepidemic days is in the

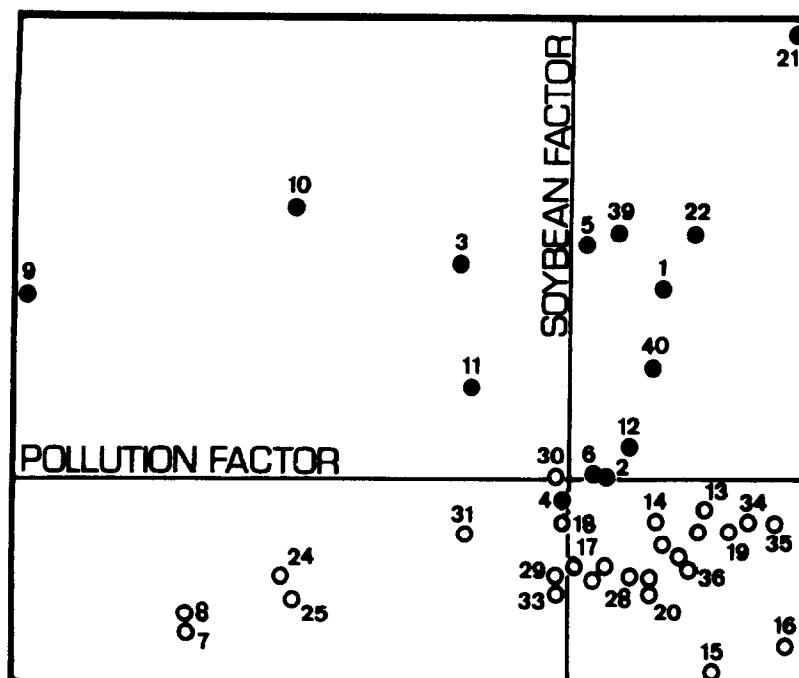


FIG. 4. Factor analysis scores of the correlation matrix corresponding to the parameters of Table I. Numbers correspond to Table I; epidemic days (●) and non-epidemic days (○). The pollution factor was significantly loaded by TSP, lead, total *n*-alkanes, and PAH. The soybean factor was significantly loaded by campesterol, stigmasterol, β -sitosterol, *n*-triacontan-1-ol, and *n*-dotriacontan-1-ol.

order of 0.001 for β -sitosterol and 0.01 for stigmasterol and campesterol. The airborne concentration of these sterols in non-epidemic days has a "background level" effect limiting the capacity of detection of small amounts of soybean dust. This effect is not important in the atmosphere of Barcelona. However, it represents one of the first parameters to be determined for the application of this molecular-marker approach to other urban areas.

Soybean allergens in epidemic days

Serum IgE Abs were obtained from patients affected by asthma epidemics. A strong association between these Abs and soybean antigens has been demonstrated in previous studies.¹² In the present study, these Abs were used as reagents to measure allergens from extracts of portions of the same filters used for alcohol/sterol analysis.

The results are also included in Table I. High allergen values are obtained in epidemic days (1600 to 10,600 units) that contrast with the low values observed in non-epidemic samples (0.3 to 320 units). As expected, the strong contrast between the allergen content of filter particulates collected in epidemic and non-epidemic days is reflected in a significant difference between the means of the two populations (Table II). In this respect, the higher estimated *t* values of

the soybean alcohol/sterol markers likely reflect the larger number of samples analyzed.

These results are consistent with the observation that subjects affected in the epidemics have IgE Abs to soybean-dust allergens. Furthermore, these results provide strong evidence for soybean dust and atmospheric transport as, respectively, source and transport agent of the asthma episodes. These results also suggest the need for the regular monitoring of soybean air particulates in areas surrounding soybean-treatment plants.

Factor analysis

Additional assessment into the representativity of these soybean markers and allergen values has been obtained from factor analysis of the correlation matrix corresponding to the parameters included in Table I. One main factor is loaded by lead, PAH, total *n*-alkanes, and TSP, the pollution parameters, and another is loaded by β -sitosterol, stigmasterol, *n*-triacontan-1-ol, campesterol, and *n*-dotriacontan-1-ol, the soybean alcohol/sterol markers. These two factors indicate that urban pollution and soybean material are two main independent sources determining the variance of results in Table I. Furthermore, these two factors afford the differentiation between samples collected in epidemic and non-epidemic days (Fig. 4).

This observation is consistent with the results reported above, but since it is based on the correlation matrix corresponding to the whole Table I, it indicates that these previous observations represent a major trend within the data set selected for study.

The concentration of airborne soybean dust in epidemic days

The TSP results of Tables I and II indicate that the asthma episodes did not involve significant increments of dust particulates in urban atmosphere. Nevertheless, the average level of airborne dust in epidemic days can be estimated by comparison of the concentrations of soybean markers in the urban aerosol and in the dust collected at the harbor silos. Two different aerosol concentrations have been selected for this comparison: (1) the average alcohol/sterol composition corresponding to all epidemic days studied (Table II) and (2) the composition of the samples that, according to factor analysis, lie at the boundary between epidemic and nonepidemic days (i.e., sample No. 4, Fig. 4). Concentrations of 25 and 5 $\mu\text{g}/\text{m}^3$ are obtained for the first and second approach, respectively. These two values elicit an estimate of the 24-hour mean concentration of atmospheric dust in the city for (1) the average of all epidemic days studied and for (2) the case of lowest soybean concentration related with an outbreak.

DISCUSSION

Alcohol/sterol analysis of aerosols collected in Barcelona during asthma epidemic and nonepidemic days has demonstrated that airborne soybean dust released from the harbor silos was effectively present in the urban atmosphere during these episodes. The soybean markers have been identified within aerosol extracts largely dominated by petrogenic and pyrolytic hydrocarbons. The strong association between airborne soybean dust and asthma outbreaks is reinforced by the allergen results demonstrating high values in the epidemic days that contrast with the low values of nonepidemic samples. However, the concentration of soybean dust in the urban atmosphere always represented a minor amount of the total suspended particulates, even during epidemic days. In this respect, factor analysis has confirmed the lack of relationship between asthma outbreaks and traffic or combustion pollution.

The quantitation of soybean markers at which asthma epidemics occurred is of great interest, although it deserves further discussion. In this respect, the concentrations of soybean dust must be considered as very tentative estimates. First, the soybean particles released to the atmosphere in the harbor were mixtures of dusts from diverse stages of silo processing. As

presented in Table III, the composition of dust collected at each stage filter was variable, which implies that any reference alcohol/sterol content considered as characteristic of "soybean dust" is approximate. Second, and more important, the aerosols analyzed correspond to 24 hours of sampling, whereas the asthma outbreaks, and likely the presence of airborne allergen, cluster in periods of 4 hours. A significant dilution effect is therefore produced. Asthma outbreaks are probably provoked by undetermined peak concentrations larger than these 24-hour averages. Third, sampling sites were located next to the places where most of the epidemic asthma cases occurred.

These concentrations can also be compared with concentrations of soybean allergen that can be estimated from the immunochemical assays. The allergens in filters corresponding to epidemic days range between 1600 and 11,000 units (approximately 1 to 10 ng/m^3), which are in the same order of magnitude as allergens found in some studies of occupational asthma.³⁰⁻³²

Most of the population exposed to soybean dust ($\approx 1,700,000$) was not affected by epidemic asthma, but for some individuals (≈ 1000), soybean dust constituted a very hazardous substance. Probably, these acute effects have not been previously identified because they did not become apparent when smaller populations were exposed or simply because they have not been systematically searched. The asthma outbreaks of Barcelona indicate the need for regular monitoring of soybean air particulates in populated areas located near soybean plants. The methodology presented here is adequate to fulfill this objective even in cases in which the aerosol composition is dominated by urban pollution.

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