

Daily Asthma Severity in Relation to Personal Ozone Exposure and Outdoor Fungal Spores

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Epidemiologic investigations of ambient ozone (O_3) effects on daily asthma status have not used personal O_3 exposures and have often lacked well-characterized allergen exposures. To address this, we studied 12 asthmatic subjects aged 9 to 16 yr, who recorded daily asthma symptoms (functional levels 0 to 5) and as-needed inhaler use during September and October 1993 in San Diego, California. Outdoor aeroallergens, O_3 , and fine particle concentrations were measured at a central outdoor site, and personal 12-h daytime exposures to O_3 were measured daily. Personal O_3 differed greatly between subjects and was 27% of mean outdoor O_3 . In random-effects autoregression models controlling for weekend days and fungal spores, personal O_3 was associated with asthma severity: for a 90th percentile increase in O_3 (25 ppb), symptom scores increased by 25% (95% CI: 0 to 49%) and inhaler use increased by 26% (95% CI: 3 to 48%) over their averages. Outdoor 12-h O_3 , but not 1-h maximum O_3 , was associated with inhaler use ($p < 0.03$). Fungal spores were significantly associated with symptoms (scores increased by 0.1 to 0.3/1,000 spores/ m^3) and inhaler use (0.1 to 0.4 puffs/1,000 spores/ m^3) across speciated groups. Pollen and fine particles (low levels) were not associated with any outcomes. These findings illustrate that the epidemiologic importance of O_3 and allergenic cofactors can be underestimated by failure to account for personal O_3 and fungal exposures. **Delfino RJ, Coate BD, Zeiger RS, Seltzer JM, Street DH, Koutrakis P. Daily asthma severity in relation to personal ozone exposure and outdoor fungal spores.**

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The relevance of allergens to respiratory symptomatology, particularly as triggers of asthma, has been well-documented (1). Moreover, there is evidence that the morbidity and mortality associated with asthma may be worse in individuals with atopic than in those with nonatopic asthma, particularly during the warm season (2). However, only recently has evidence been found that air pollution may potentiate the effects of aeroallergens on asthma. In an experimental study of seven subjects with mild atopic asthma, Molfino and colleagues (3) found that ozone (O_3) concentrations of 120 ppb led to a significant increase in bronchial responsiveness to ragweed or grass pollen ($p < 0.05$).

The main objective of the present panel study was to investigate the potential interactive and independent effects of O_3 and aeroallergens (pollens and molds) on daily asthma status. The study used time-series analysis of daily data for a panel of asthmatic subjects with a defined allergic status with respect to aeroallergen sensitivities as an approach to detecting the relative clin-

ical importance of exposures to different pollens and fungi. The analysis of aeroallergen effects on daily asthma severity was stratified by different fungal spore types to which each subject did or did not demonstrate allergic sensitivities according to epicutaneous (skin prick) allergen tests (SPT). This approach was intended to clarify the variability in asthma that was either unexplained by current epidemiologic methods or misspecified as effects of weather or other environmental factors. Additionally, the study utilized measurements of personal daily exposure to O_3 in order to decrease the magnitude of expected exposure misclassification in studies that have relied only upon O_3 measurements from outdoor stationary-site monitors to represent exposure to O_3 .

METHODS

Population and Outcomes

This asthma panel study was conducted in an area of San Diego near the Pacific ocean, having a flat topography and comprising about 160 km^2 , and referred to as the Mesa region. The study protocols were approved by the institutional review boards of San Diego State University and the Kaiser Permanente Medical Group. Informed written consent was obtained from all subjects and one of their legal guardians. Eligibility criteria for entry into the study included: (1) physician-diagnosed asthma; (2) a history of asthma for at least the preceding year; (3) asthma exacerbations during several months of the warm seasons in San Diego (March to October) on at least 2 d per week and which required the use of as-needed oral or inhaled bronchodilator(s) (excluding medication

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used to prevent exercise-induced bronchoconstriction); (4) age from 9 to 18 yr; (5) a home and school address in the Mesa region of study; and (6) no patient, parental, or indoor household smoking. The study began with nine boys and six girls, three of whom dropped out of the study after the first week, primarily because of aversion to wearing the personal ozone sampler used in the study (two boys and one girl). The remaining 12 subjects were followed with daily measurements for up to 6 wk from September 20 through October 31, 1993. In contrast to inclusion criterion (3), five subjects were not frequently symptomatic and did not require as-needed bronchodilators during the study.

Subjects were classified according to the presence or absence of positive SPTs for several pollens and molds common to the area of study. A positive test was defined as a skin wheal diameter of either 3 mm or greater or more than 50% of a histamine control, in the presence of a negative saline control. Allergen tests (Bayer Corp., Allergy Products, Spokane, WA) were classifiable according to eight categories of tree, grass, and weed pollens common to the area, and to the following fungal spore types: (1) *Alternaria tenuis*; (2) *Hormodendrum cladosporioides* (*Cladosporium cladosporioides*); (3) *Helminthosporium interseminatum*; (4) *Aspergillus* species mix; and (5) *Penicillium* species mix.

Participants were given a background interview in accompaniment with their parents and instructed about the study procedures within 2 wk prior to the start of the study. After the start of the study, follow-up at the patients' homes was made on a weekly basis to collect personal O₃ samplers and to discuss any questions concerning the study procedures. Outcomes included daily diary responses of asthma severity, time spent outdoors, and time spent at moderate to strenuous activity. A composite of asthma symptoms (wheeze, cough, sputum production, shortness of breath, and chest tightness) were rated by subjects at six levels of severity: 0, no asthma symptoms today; 1, asthma symptom(s) present, but not causing discomfort; 2, asthma symptom(s) causing discomfort, but not interfering with daily activities or sleep; 3, asthma symptom(s) interfering somewhat with daily activities or sleep; 4, asthma symptom(s) interfering with most activities (and which may have required bed rest, school absence, or telephoning a physician's office); and 5, asthma symptom(s) requiring a physician or emergency clinic visit or hospital admission. Subjects were instructed to record the daily number of puffs they required from as-needed β -agonist inhalers. It was discovered at the end of the study that one subject was inappropriately using cromolyn as an as-needed inhaled medication for asthma symptoms instead of his albuterol. Given that this use could be interpreted as an indication of asthma exacerbations, the subject's inhaler data were retained for analysis. Subjects also reported the presence or absence of any upper or lower respiratory tract infections. The asthma symptom score, extent of inhaler use, and other variables were recorded at the end of each day, and subjects were instructed to not fill in items on the diary if they had forgotten to enter them on previous days.

Environmental Variables

Twenty-four-hour samples of particulate matter aerodynamic diameter < 2.5 μ m (PM_{2.5}), sulfate and nitrate fractions of PM_{2.5}, and gas-phase acidity (HNO₃, HNO₂) were measured with the Harvard annular denuder filter pack system (4), using an 8-d automated sequential sampling system. The concentration of particle phase acidity (H⁺) was predicted from the charge balance of sulfate, nitrate, and ammonium ions measured by ion chromatography.

The aeroallergens were measured with a Burkard 7-d recording volumetric pollen and fungal spore collector (Burkard Manufacturing Co. Ltd., Rickmansworth, UK) placed at 10 m above ground. Fungal spore and pollen genera were identified and counted on 24-h tape segments under a compound microscope at $\times 500$ and $\times 1,000$. Raw counts were converted to particles per cubic meter of air and associated with specific time periods and dates. Concentrations were summed to total fungal spore or total pollen concentrations and to groups related to genera represented by positive SPTs, in order to provide exposure levels relevant to the specific sensitivities of each subject.

Personal O₃ exposures were measured with the Harvard passive sampler, which was worn by each subject every day over a 12-h period between approximately 8:00 A.M. and 8:00 P.M. This time period is relevant because it coincides with peak outdoor O₃ exposures among children. The personal O₃ sampler is a small device that is clipped at chest level to the clothing of the subject. The samplers were prepared with a commercially available passive sampling device (Ogawa and Co., Pampano

Beach, FL) and two glass-fiber filters coated with potassium carbonate and sodium nitrite. The nitrite ion is oxidized by O₃ to a nitrate ion (5). The concentration of O₃ is determined by the measurement of nitrate through ion chromatography. Excellent agreement was found between the levels of O₃ determined from a standard continuous monitor and those measured with the passive sampler; however, problems of face velocity and O₃ depletion at body surfaces remained (5, 6). The depletion at body surfaces was minimized by using a method found to be effective in previous field tests (6). This method employs a badge on a 10 $\times$ 10-cm plastic base plate attached to clothing, which has an extension that keeps the sampling device 5 cm away from the body surface. Continuous outdoor stationary-site measurements of O₃ were made at a single central site by the San Diego Air Pollution Control District, using ultraviolet (UV) photometry.

Crude prediction models for personal O₃ exposure were tested with the following model: $C_p = C_i F_i + C_o F_o$ (1) where C_p is the 12-h average personal O₃ level (from about 8:00 A.M. to 8:00 P.M.); C_o is the corresponding 12-h concentration of outdoor O₃ measured at the central regional stationary site; C_i is the indoor concentration of O₃, estimated as $0.3 \times C_o$ from data in a recently published study using the Harvard personal sampler in Toronto (7); and F_i and F_o are the fractions of time spent indoors and outdoors, respectively.

As compared with adults, children are expected to receive a greater internal thoracic dose of O₃ for equivalent conditions of exposure (8) and are expected to spend more time outdoors at higher levels of physical activity. These factors are likely to lead to intrinsic differences in potential dose across individuals. To account for this, personal O₃ exposure was adjusted for estimated ventilation rates to yield an estimate of the delivered dose, and relative effects of the adjusted and unadjusted expressions of O₃ exposure were compared. Subjects recorded data on the number of quarter-hours they spent at moderate to strenuous activity, as well as on the number of quarter hours they spent outdoors. The subjects' minute ventilation rates ($\dot{V}_E$) were estimated from published average values at four levels of physical activity among older children and adolescents (8). The $\dot{V}_E$ for rest to light activity was estimated to be 9 L/min, and that for moderate to strenuous activity was estimated at 31 L/min. The dosimetric models for estimating the 12-h delivered dose of O₃ in μ g were adapted as follows from Kleinman and colleagues (8):

$$D_{Ti} = \sum_{a=1}^2 \sum_{i=1}^{12} (R_{a,i}) (\dot{V}_{Ea,i}) (t_a) ([O_3]_{Ti}) \quad (2)$$

where T = 12-hour day of study; i = individual subject; R = retention fraction of O₃ in the deep lung at ventilation rate $\dot{V}_E$ at the two levels of a, rest or light activity, and moderate to strenuous activity; $\dot{V}_E$ = minute ventilation rate in L/min; t_a = duration of exposure at activity level a; and $[O_3]$ = 12-h mean daytime concentration of personal O₃, μ g/m³.

Statistical Analysis

The data were first examined with descriptive statistics, including time plots to examine possible confounding trends in the data, and exposure correlation matrixes to determine the degree of intercorrelation that could suggest confounding or collinearity effects in regression analyses.

Regression analyses utilized the general linear mixed model (both fixed and random effects) for longitudinal data analysis (9), which is available as the SAS MIXED procedure and uses a ridge-stabilized Newton-Raphson algorithm to optimize a restricted-maximum-likelihood (REML) function (10, 11). Modeling a variable as a random effect accounts for the heterogeneity from one cluster of observations to another cluster. Repeated measurements over time in each research subject constitute such a group of clusters, and accordingly, random effects attributable to each subject were modeled in this analysis. The model should accommodate, as a random effect, the degree to which certain medications are effective in blocking or counteracting an individual's asthmatic response to an airborne irritant. The random-effects model can treat observations relating to an individual as a sample of intercepts and slopes (regression coefficients) from some underlying population distribution, and is essentially a set of separate regression models of the response data for each individual. This approach applies to the variables in this study, given the known heterogeneity in the periodicity of attacks and etiology of asthma among asthmatic subjects. The analysis therefore

allows each subject to act as his or her own control with repeated measurements over time, and allows for the evaluation of time-dependent covariates that are the fixed effects of interest, and which in the present study included the aeroallergen, air pollutant and meteorologic variables. A common regression slope was estimated for each model, and fixed effects were considered significant if the two-sided *t* statistic for the parameter estimate was $p < 0.05$. To account for delays in responses to increased levels of environmental factors, respiratory outcomes were regressed on exposures lagged 1 d prior to the day on which the diary was completed, in addition to regressions involving same-day levels of exposures to these factors.

The mixed model is an advancement of the general linear model in that it allows for the dependence of repeated measurements made on the same individual, and for correlated responses. Since the asthmatic responses of a particular individual, as well as the levels of environmental agents, are likely to be correlated from one day to the next, an adjustment must be made for this correlation in order to avoid a bias in the estimation of statistical significance. Akaike's Information Criterion (REML log likelihood minus the number of covariance parameters) was used to assess model fit for models with the same fixed effects but different variance structures (specifically, with or without modelling autocorrelation of repeated measurements). Accordingly, a significant autocorrelation of residual errors was found for repeated measures for each individual, which was best modelled as a first-order autoregressive process (the current day's observation was dependent on the previous day's observation). This longitudinal modelling approach can be applied to time series with unequal numbers of observations in different individuals and with missing observations.

Although the ordinal outcome variable of symptom scores and as-needed inhaler doses were not strictly Gaussian in their distributions, residual errors from the regression models for these variables had means of 0 and were approximately normal as indicated by normal probability plots. Therefore, parametric tests of hypotheses from regression analyses were unlikely to have led to spurious results. Influential observations were found for the last days of the study, when local brush fires required a separate analysis excluding this terminal period (as discussed subsequently). Controls for maximum temperature and average relative humidity measured at the monitoring site were applied when this was needed. Day-of-week trends in the outcome and environmental variables can lead to bias in the estimation of effects, and tests for them were therefore used in the regression models.

RESULTS

Outcomes

A total of 462 person-days of observation were collected from

completed daily diaries, of an anticipated 482 person-days; this excludes late starts (20 person-days). Table 1 shows the characteristics of the 12 subjects in the asthma panel. Subjects were on a variety of as-needed and daily prescribed medications, with six subjects taking medications prescribed for daily use. Five subjects did not use available as-needed inhalers during the study, and only seven participants therefore contributed data to the analysis of inhaler use (276 person-days of observation). Subjects not using as-needed inhalers were among those with the lowest average symptom scores (all < 0.7). Two subjects tested negatively for all five fungal extracts, but all subjects tested positively for a variety of pollens.

Mean symptom scores were significantly lower on Friday, Saturday, and Sunday than during the rest of the week (0.65 versus 0.92, $p < 0.005$). A significantly lower level of moderate to strenuous activity occurred on Sundays than during the rest of the week (0.9 h versus 1.3 h, respectively; $p < 0.04$).

Exposures

On 3 of 42 days, the UV photometer at the central monitoring site was not operational. Subjects demonstrated variable compliance with O₃ badge wearing, with four of 12 subjects not wearing badges toward the last 1 to 2 wk of study (41 samples), as indicated by O₃ levels that dropped to 0 and stayed at 0 until the end of the study despite the absence of a notable change in these subjects' outdoor activity patterns. An additional 48 samples among the remaining potential of 441 personal O₃ samples were invalidated, primarily as a result of badges not being worn (as noted by subjects in their sample logs), as well as because of other field errors by subjects or analytical problems. This left 393 samples for analysis (Table 2). The limit of detection (LOD) of O₃, defined as 3 times the standard deviation (SD) of the average blank value (from unexposed samplers), was 8.67 ppb. Partly as a result of subjects spending most of their daytime periods indoors (mean: 80%), 47% of the personal O₃ samples were below the LOD.

Mean levels of personal O₃ were significantly lower on Friday, Saturday, and Sunday than during the rest of the week (10 ppb versus 13 ppb, respectively; $p < 0.03$). Interestingly, the day-of-week trend in the stationary-site outdoor O₃ measurement was opposite that of personal O₃. This outdoor O₃ trend was probably due to transport episodes from Los Angeles and to lower lev-

TABLE 1
CHARACTERISTICS OF PARTICIPANTS IN THE MESA PANEL STUDY

Subject	Gender	Age (yr)	Medications*	Positive Fungal Spore Skin Tests	Symptom Score Mean (SD)	As-needed Inhaler Puffs Mean (SD)
1	Male	14	Al	A, H	0.6 (0.9)	0
2	Female	9	Al, Cr [†] , Becl [‡]	none	0.02 (0.1)	0
3	Female	12	Al [†] , Az [†]	A, C, H, P	0.06 (0.2)	0
4	Male	16	Al, Az [†]	A, C, H, As, P	0.4 (0.8)	0
5	Female	14	Al [†] , Becl [‡]	A, C, H, As, P	1.5 (0.7)	0.7 (1.2)
6	Male	11	Cr [§]	A	2.0 (1.2)	0.4 (0.8)
7	Male	13	Al	A, C, H, As, P	1.5 (1.1)	1.5 (0.8)
8	Male	9	Al, T	none	0.6 (0.8)	1.4 (0.5)
9	Female	16	Al, Becl [‡]	A, C, H, As, P	1.0 (0.5)	6.0 (0.5)
10	Female	14	Al [†] , Cr [†] , Becl [‡]	A, C, H, As, P	0.7 (0.8)	0.5 (1.0)
11	Male	9	Al	A, H	0.2 (0.6)	0
12	Male	14	Al	A	0.8 (1.0)	1.9 (0.8)

Definition of abbreviations: Al = albuterol; Az = triamcinolone acetonide; Becl = beclomethasone dipropionate; Cr = cromolyn sodium; T = theophylline; A = *Alternaria*; C = *Hormodendrum cladosporoides*; H = *Helminthosporium interseminatum*; As = *Aspergillus*; P = *Penicillium*.

* Medications taken during the study were as needed only, except that:

[†] medication was prescribed for daily use, rather than as needed;

[‡] medication was used as both daily and as-needed metered-dose inhalers.

[§] This subject inappropriately used only cromolyn sodium as needed for symptoms.

TABLE 2
DESCRIPTIVE STATISTICS FOR DAILY AIR POLLUTION
AND WEATHER MEASUREMENTS, SEPTEMBER 20 TO
OCTOBER 31, 1993, MESA AREA OF SAN DIEGO

Exposure	n	Mean (SD)	Minimum/ Maximum	90th Percentile
O ₃ outdoor site 1-h max, ppb*	39	68 (30)	26/146	120
O ₃ outdoor site 12-h day, ppb	39	43 (17)	14/87	68
O ₃ personal 12-h day, ppb	393	11.6 (11.2)	0.0/84.8	25.0
PM _{2.5} 24-h, µg/m ³	42	24.8 (11.1)	6.5/66.5	36.5
SO ₄ ²⁻ 24-h, nmoles/m ³	42	45.8 (24.6)	7.4/96.5	80.3
Predicted H ⁺ 24-h, nmoles/m ³	42	17.9 (8.7)	1.5/43.8	28.9
HNO ₃ 24-h, ppb	42	1.8 (1.1)	0.0/5.2	2.8
Temperature maximum, °F	42	80 (7)	70/97	91
Relative humidity mean, %	42	62 (17)	25/81	77
Fungal spores, /m ³	42	2,461 (1,307)	648/7,512	3,897
Pollen grains, /m ³	42	34 (25)	4/115	58

* The outdoor site stationary monitor was at the Kearny Mesa site operated by the San Diego Air Pollution Control District.

els of NO from local traffic on the weekends, since NO reduces O₃. The day-of-week trend in personal O₃ was not explained by the number of hours spent outdoors, which did not significantly differ by day of week (2.3 to 2.6 h/d, $p < 0.8$). Given the non-compliance noted earlier with regard to wearing personal samplers, noncompliance during the weekends could have led to this trend in personal O₃.

Large interindividual differences in daytime 12-h personal O₃ exposure were found, with a low average correlation between any two individuals (average interindividual Pearson's $R = 0.41$; SD of R values = 0.21). Also, for 367 comparisons between the 12-h average O₃ concentration measured at the outdoor stationary site and personal O₃ measurements, there was a low level of correlation (overall $R = 0.45$; range: 0.36 to 0.69 by individual), and the O₃ measured at the stationary site was 31 ppb greater on average than the personal O₃ levels. These findings illustrate a great potential for exposure misclassification in an epidemiologic study relying solely on stationary-site outdoor O₃ measurements (Figure 1). Both predictor variables (C₁F₁ and C₀F₀) were strongly associated with personal O₃ ($p < 0.0001$), and the variance explained (R^2) by the prediction model (I) was 0.25.

Levels of PM_{2.5}, the SO₄²⁻ fraction of PM_{2.5}, predicted aerosol H⁺, and HNO₃ were low (Table 2). Levels of fungi were moder-

ately high, but pollen was low, as expected for the fall season in the region of the study (Table 2). Pollen levels were dominated by two pollen types, *Artemisia* (mean: 14 grains/m³) and *Ambrosia* (mean: 4 grains/m³), to which only three subjects had positive SPTs. The most common spore type was *Cladosporium* (mean: 1,278 spores/m³). A correlation matrix for the environmental variables is presented in Table 3. A lower correlation between O₃ and the weather variables was noted for personal O₃ exposure than for stationary-site outdoor O₃ concentration. There were also several modest, yet significant, correlations of the air pollutants (PM_{2.5} and O₃) with the aeroallergens (total pollen and total fungal spores) examined in the study. The inverse correlation between O₃ and fungal spore concentrations was found across all fungal genera (data not shown).

Regression Models

Asthma symptom scores were positively associated with personal O₃ levels ($p < 0.05$), with an increase of 0.8 in the score per increase of 100 ppb O₃ in the personal level of O₃ exposure (Table 4, Model A). A time-series plot of mean symptom scores versus mean personal O₃ levels illustrates this relationship, but it should be noted that the regression analysis was based on individual time series data rather than aggregate data (Figure 2). However, this association was temporally confounded by a day-of-week effect (Table 4, Model B).

Estimated O₃ dose remained significantly associated with symptoms when the weekend indicator variable was included (Table 4, Models C and D). The effect at the 90th percentile of the O₃ distributions (25 ppb for personal O₃ and 360 µg for estimated O₃ delivered dose) was calculated in order to allow the two O₃ variables to be compared regardless of their units of measurement or concentration ranges. The 90th percentile effect is calculated as the concentration of a pollutant at the 90th percentile of its distribution, multiplied by the pollutant's regression parameter, with the result divided by the mean level of the dependent variable, and all multiplied by 100%. The 90th-percentile effect, when controlling for weekends and fungi, was similar for personal O₃ exposure and estimated O₃ delivered dose, since symptom scores increased by 25% (95% CI: 0 to 49%) and 26% (95% CI: 5 to 49%), respectively. Asthma symptom scores were not associated with outdoor stationary-site measurements of either 12-h daytime average O₃ or hourly maximum O₃ levels.

Personal O₃ exposure was also significantly associated with as-needed inhaler use, but was not confounded by day of week

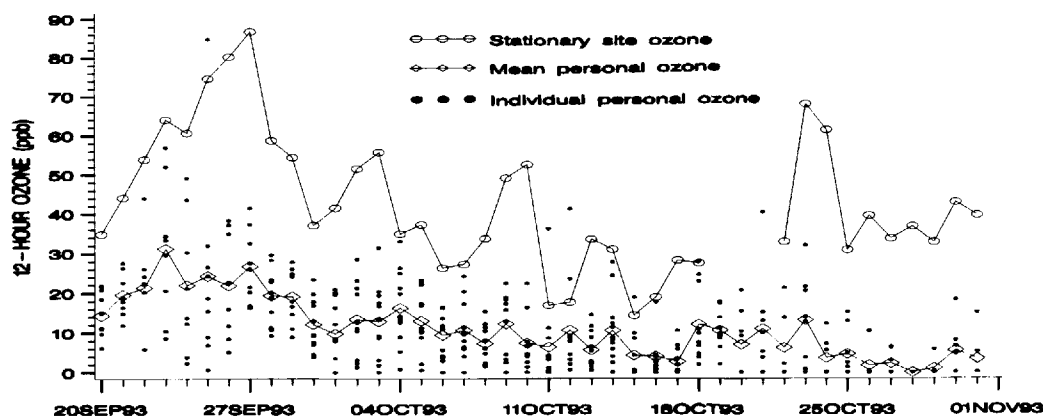


Figure 1. Time plot of stationary-site outdoor ozone versus personal ozone levels, Mesa area of San Diego. The sampling period was 12 h during the daytime (8:00 A.M. to 8:00 P.M.) for the stationary outdoor site and the same approximate 12-h period for the personal passive ozone samplers. Individual personal ozone sample levels are given as a scatterplot of points around the mean personal levels.

TABLE 3
PEARSON'S R CORRELATION MATRIX OF EXPOSURE LEVELS, MESA AREA OF
SAN DIEGO, SEPTEMBER 20 TO OCTOBER 31, 1993*

	PM _{2.5}	Personal O ₃ (12 h)	Stationary Site O ₃ (12 h)	Fungal Spores	Pollen	Maximum Temperature	Mean Relative Humidity
PM _{2.5}	1.00 (42)	0.03 (393)	0.27 [§] (42)	0.14 [‡] (42)	0.36 [§] (42)	0.18 [§] (42)	-0.28 [§] (42)
Personal O ₃ , 12 h		1.00 (393)	0.45 [§] (367)	-0.28 [§] (393)	0.02 (393)	0.20 [§] (393)	-0.07 (393)
Stationary-site O ₃ , 12 h			1.00 (39)	-0.11 [†] (39)	0.15 [‡] (39)	0.52 [§] (39)	-0.34 [§] (39)
Fungal spores				1.00 (42)	0.29 [§] (42)	0.15 [‡] (42)	-0.21 [§] (42)
Pollen					1.00 (42)	0.10 [†] (42)	-0.16 [§] (42)
Maximum temperature						1.00 (42)	-0.78 [§] (42)
Mean relative humidity							1.00 (42)

* Sample sizes given within the parentheses.

† $p < 0.05$.

‡ $p < 0.01$.

§ $p < 0.001$.

(Table 4). Controlling for fungal spores, inhaler use for a 90th-percentile increase in 12-h mean O₃ (25 ppb) increased by 26% (95% CI: 3 to 48%). Estimates of O₃ dose did not improve the estimate of effect on inhaler use over the unadjusted O₃ exposure model (Table 4, Models J versus I). Inhaler use was associated with stationary-site measurements of 12-h daytime average O₃ (inhaler puffs increased by 1.1/100 ppb O₃; $p < 0.03$), but was not associated with hourly maximum O₃ levels.

Lagged daily mean fungal spore concentrations (measured approximately 12 h prior to the recording of daily symptom severity)

were positively associated with symptom scores: scores increased by 0.12/1,000 spores/m³ (Table 4, Model E). This effect was not confounded by day of week. A time-series plot of mean symptom scores versus total fungal spore concentrations illustrates this strong relationship (Figure 3). The effect of fungal spores appeared to be less in simultaneous regressions with O₃. However, this was due to missing personal O₃ data and not to confounding by O₃. In the coregressions of O₃ and fungal spores, O₃ had a stronger effect on symptoms than it did in regression models of O₃ alone (Table 4, Models G versus D, and H versus B, respectively).

Total fungal spore counts were also significantly associated with as-needed inhaler use (Table 4, Model K), and this effect was not confounded by O₃ (inhaler puffs increased by 0.14/1,000 spores/m³).

The analysis was repeated for the 10 of 12 subjects who demonstrated positive SPTs with at least one of the five fungal genera tested, of whom six subjects utilized as-needed inhalers. There was a wide range of fungal spore effects on symptom severity when the analysis was stratified across speciated groups (Table 5; symptom scores increased by 0.1 to 0.3/1,000 spores/m³), with the greatest effects being seen for fungal spore types classified either as unidentified under the microscope or not included in the SPT test battery (Table 5, Model C). These non-test genera accounted for 41% of the total average spore count. Effects were actually smallest and only borderline significant for the variable that was the sum of positive SPT fungal types specific to each subject (Table 5, Model B). For the six fungal-allergic subjects with positive SPTs for *Cladosporium* (88% of SPT fungi concentrations), a regression model involving the personal fungal allergy variable was statistically significant (asthma symptom scores increased by 0.14/1,000 spores/m³; $p < 0.04$).

It is particularly notable that in contrast to the results for asthma symptom scores, which were least affected by the fungi causing positive SPTs, the effect on inhaler use was greater for positive SPT than for total fungal spore concentrations (Table 5, Models E versus D, respectively). However, the largest parameter estimate and most statistically significant association was between as-needed inhaler use and the spores not represented in SPTs or not identified (Table 5, Model F), resembling the case with the relative effects on asthma symptom score (Table 5, Model C).

TABLE 4
SELECTED REGRESSION MODELS FOR ASTHMA SYMPTOM SEVERITY
SCORE AND AS-NEEDED METERED DOSE INHALER USE*

Model	Variables [†]	Estimated Coefficient	Standard Error [‡]	p Value
Symptom Severity Score				
A	Personal O ₃	0.7686	0.3888	0.05
B	Personal O ₃	0.6055	0.3916	0.12
	Weekend	-0.2082	0.0829	0.012
C	O ₃ dose	0.0587	0.0242	0.02
D	O ₃ dose	0.0506	0.0242	0.04
	Weekend	-0.2030	0.0826	0.02
E	Fungal spores	0.0122	0.0030	0.00005
F	Fungal spores	0.0096	0.0034	0.005
	O ₃ dose	-0.0641	0.0236	0.007
G	Fungal spores	0.0088	0.0034	0.01
	O ₃ dose	0.0568	0.0238	0.02
	Weekend	-0.1633	0.0807	0.04
H	Fungal spores	0.0086	0.0034	0.012
	Personal O ₃	0.7411	0.3835	0.054
	Weekend	-0.1668	0.0811	0.04
As-needed Inhaler Use				
I	Personal O ₃	1.52	0.75	0.04
J	O ₃ dose	0.0880	0.0460	0.06
K	Fungal spores	0.0141	0.0065	0.03

* Twelve subjects were analyzed for symptom scores and seven subjects for inhaler use.

† Personal ozone (O₃) = 12-h personal samples in ppb; ozone dose = personal O₃ adjusted for ventilation rate, and units are in µg delivered dose; total fungal spore units are in particles/m³; and Weekend 1 = Friday through Sunday, 0 = Monday through Thursday.

‡ Regression coefficients and SE values are ×100, except for the weekend variable, which is binary.

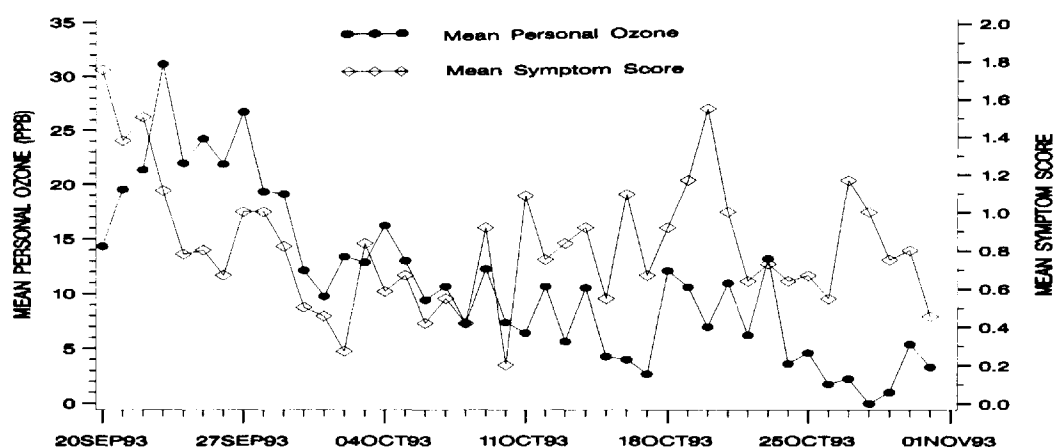


Figure 2. Time plot of mean symptom score and mean personal ozone exposure. The date refers to the evening of the day ending the 24-h symptom log and the evening ending the 12-h daytime sampling period for the personal passive ozone samplers. Each written date is a Monday.

A further analysis of genera not included in the SPT battery revealed that the fungal group of Basidiomycetes (including the genera *Ganoderma*, *Gastromycetes*, and *Coprinus*) and Ascomycetes (including the genera *Chaetomium*, *Leptosphaerulina*, *Pleospora*, and *Chlorophyllum*) constituted 26% of the non-SPT group of fungi (daily mean of 89 and 169 spores/m³, respectively). The bulk of the remaining fungi not definitively included in the SPTs were spores that could not be identified under the microscope (mean: 435 spores/m³), hyphal fragments (mean: 152 spores/m³), and *Periconia* species (mean: 144 spores/m³). Regression models involving three fungal groups not skin-tested were examined (regression data not in tables). The Basidiospores (maximum: 261 spores/m³) and Ascospores (maximum: 1,452 spores/m³) had notable effects on symptom levels (symptom scores increased by 0.25/1,000 Ascospores/m³; $p < 0.03$ and by 1.2/1,000 Basidiospores/m³; $p < 0.07$). The remaining non-SPT group of fungal spores also had a significant effect on symptoms (symptom scores increased by 0.19/1,000 spores/m³; $p < 0.01$).

In contrast to the findings for asthma symptoms, regression models of Basidiospore and Ascospore counts versus inhaler use were not significant ($p < 0.4$), whereas a regression model of non-SPT fungal spores other than Basidiospores and Ascospores was significant (inhaler puffs increased by 0.4/1,000 spores/m³; $p < 0.02$).

The low atmospheric levels of PM_{2.5}, the SO₄²⁻ fraction of PM_{2.5}, predicted aerosol H⁺, HNO₃, and pollen were not associated with either asthma symptom scores or as-needed inhaler use. Also, no statistical interactions between O₃ and fungi could be found for this panel, since there were only 11 of 393 person-days of observation in which both personal O₃ and fungal spore levels exceeded the 75th percentiles of their distributions, and only one subject showed increased asthma symptoms, on 5 of those days.

Asthma symptom scores were 0.5 higher when subjects reported respiratory tract infections ($p < 0.003$), but no change in inhaler use was observed. The presence of respiratory tract in-

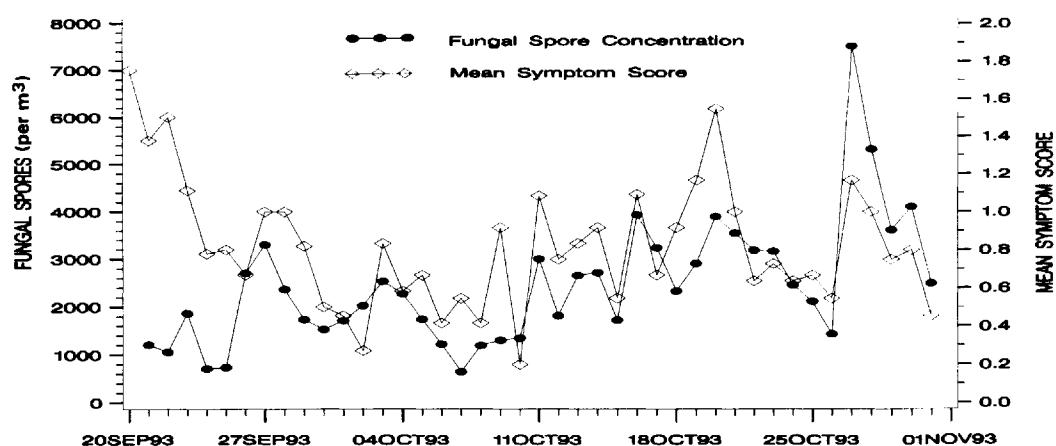


Figure 3. Time plot of mean symptom score and total fungal spore concentration lagged 12 h prior to the recording of symptoms. The date refers to the evening of the day ending the 24-h symptom log and the morning ending the 24-h period for the Burkard spore-trap collection. Each written date is a Monday.

TABLE 5
REGRESSION MODELS FOR ASTHMA SYMPTOM
SEVERITY SCORE AND AS-NEEDED METERED DOSE INHALER USE:
COMPARISON OF THE EFFECT OF FUNGAL SPORE
TYPE AMONG FUNGAL-ALLERGIC SUBJECTS

Model	Variables*	Estimated Coefficient	Standard Error†	p Value
Symptom severity score				
A	Total fungal spores	0.0142	0.0034	0.00005
B	Positive SPT fungi‡	0.0128	0.0075	0.09
C	Non-test fungi§	0.0274	0.0068	0.0001
As-needed inhaler use				
D	Total fungal spores	0.0143	0.0065	0.03
E	Positive SPT fungi‡	0.0336	0.0145	0.02
F	Non-test fungi§	0.0372	0.0130	0.005

* SPT = skin prick test.

† Regression coefficients and standard errors are $\times 100$; fungal spore units are in spores/m³.

‡ Variable = fungal spore concentration of those five genera that were skin tested (*Alternaria*, *Helminthosporium*, *Cladosporium*, *Aspergillus*, and *Penicillium*) and to which each subject showed a positive SPT (10 of 12 subjects for the symptom variable, and six of seven subjects for the inhaler-use variable).

§ Variable = total fungal spore concentration minus all five genera skin tested.

fections did not confound the effects of either O₃ or fungal spores on asthma symptoms. Maximum daily temperature and average relative humidity were not significantly associated with either symptoms or inhaler use and did not confound the effects of either fungal spores or O₃.

Major firestorms in southern California produced smoke in the study area during the last 5 d of the study. Therefore, the analysis was repeated without this period in order to control for the potential confounding effect of the firestorm smoke. All regression parameters and standard errors for models excluding the firestorm period were similar to those in the analysis including the firestorm period.

DISCUSSION

Overview

To our knowledge, this is the first asthma panel study utilizing longitudinal data analysis to show that daily ambient levels of fungal spores have an adverse effect on daily asthma status as evidenced by symptom reports and as-needed inhaler use. An independent effect on asthma status of personal O₃ exposure was considerably stronger than effects from outdoor stationary-site O₃ levels. This is the first epidemiologic study utilizing daily personal O₃ measurements to directly demonstrate the clinical importance of O₃ exposure misclassification in assessing the respiratory health effects of O₃.

Ozone Effects

Positive associations of O₃ to symptoms and to as-needed inhaler use were found in the present study despite levels of personal O₃ exposure below those noted to be important in controlled human exposure studies. Among the lowest dose effects found in such studies were deficits in FVC and FEV₁ in normal subjects given 6.6 h of exposure to 80 ppb O₃ accompanied by 5 h of moderate exercise (12). This still represents an exposure-dose distribution estimated to be higher than the maximum value found in the present study (1,325 μ g delivered O₃).

Although it is conceivable that adverse effects on lung function of low O₃ exposures could occur among individuals with underlying lung disease and baseline lung function deficits, few differences in symptoms or spirometry have been found between normal healthy subjects and asthmatic subjects in controlled exposure studies (13). However, some specific effects of O₃ suggest that O₃ could enhance the severity of underlying and active re-

spiratory-tract illness (13). In contrast to experimental exposure studies a panel study by Krzyzanowski and colleagues (14) showed that adult and pediatric asthmatic subjects had greater decreases in peak expiratory flow rate (PEFR) in relation to increased O₃ concentrations than did nonasthmatic subjects, with 1-h maximum O₃ levels never exceeding 92 ppb (lower outdoor levels than in the present study). Differences between panel-study findings and controlled-exposure studies may be due to differences in exposure profiles (15) and to the fact that many of the asthmatic subjects in panel studies are frequently symptomatic, in contrast to experimental studies, which typically use subjects with milder asthma who are asymptomatic at the time of controlled O₃ exposure.

A day-of-week trend in symptom scores, with lower scores on weekends, was found in the present study, with a decrease in the parameter estimate and a loss of statistical significance for the effect of personal O₃ exposure on symptoms when a weekend indicator variable was added to the regression model. This could have been due to lower levels of physical activity and/or personal exposure on weekends, and consequently to lower pulmonary doses of airborne contaminants. This view is consistent with the present finding that adjusting personal exposures for approximate ventilation (which was lower on weekends), in order to give an estimate of 12-h delivered dose, improved the precision of the effect estimate for O₃ and asthma symptoms. The estimate of an O₃ dose-effect remained statistically significant when coregressed with the weekend variable. However, concerns have been expressed about the internal validity of self-reported data on exertion levels (16). Also, as a means of improving diary compliance, exertion levels in the present study were expressed at two levels instead of a more optimal three or four levels.

In epidemiologic studies similar to the present one, the accuracy of the exposure estimate for individuals (the degree to which this estimate is representative of the true exposure value) is reduced by the use of outdoor stationary-site monitors to represent the true exposure of people who are mobile and who probably spend a large amount of time in indoor environments. The consequence of this could be an underestimation of pollutant effects. In the present study, no effect on asthma symptom level was found for either the 1-h maximum or 12-h average O₃ level, both measured at the stationary outdoor site by a continuous UV photometer that is considerably more accurate than the personal passive sampler (5). An isolated effect of 12-h average O₃ on inhaler use was found, although the parameter estimate was less than that for the personal O₃ variable (an increase of 1.1 versus 1.5 inhaler puffs per 100 ppb, respectively).

The use of large sample sizes has been the standard epidemiologic approach to overcoming inherent exposure misclassification caused by the use of regional monitors to represent personal exposure (i.e., statistical power overwhelms statistical inaccuracy). Panel studies that have found significant effects of O₃ have had considerably larger sample sizes than the present investigation. For instance, one of the smallest asthma panel studies, conducted by Lebowitz and colleagues (17), involved 22 asthmatic subjects living in Tucson, Arizona, who contributed about 2,000 person-days of observation in each of four panel periods (compared with 393 person-days for personal O₃ in the present panel). Lebowitz and colleagues found significant associations of increased O₃ with worsening symptoms and PEFR deficits in three of four warm-season periods, despite reliance on outdoor stationary-site monitors. Other daily panel studies involving asthmatic subjects have found similar effects with larger sample sizes and with outdoor O₃ measurements as the estimate of individual exposure (14, 18, 19).

Fungal Spore Effects

The present investigation found that asthma severity was positively associated with fungal spore levels recorded during a period

of 0 to 12 h prior to the 24-h period encompassed by the diary responses. This is consistent with evidence that aeroallergens, including fungi, can elicit both immediate and late-phase bronchospastic reactions (4 to 6 h after exposure) induced by an initial IgE-mediated response (20, 21). Unfortunately, there are no data for the threshold concentration needed for inducing an asthmatic response by any fungal species, although this is likely to vary with individual sensitivity and fungal species. Aside from evidence suggesting very adverse effects of outdoor fungal spores on some asthmatic individuals (2), we lack sufficient information to determine the overall direct importance of fungal spores in IgE-mediated responses among persons with allergic asthma.

Some cohort studies have suggested acute adverse effects of outdoor airborne fungi on daily asthma status (22, 23). However, no asthma panel studies have been reported in the literature that have both utilized a formal time-series analysis to determine the presence of such effects and also utilized impaction samplers like the Burkard sampler, which are efficient for the size range of most fungal spores (2 to 15 μm in their smallest cross-sectional diameters) (24). Because of their small size, many spore types can penetrate deep into the distal airways and alveoli of the lungs.

The finding in the present study that outdoor fungal spores have an adverse effect on asthma status may not be relevant to patients who experience their symptoms during periods outside the fungal aeroallergen season. Also, the classification of asthmatic subjects in this study with respect to their fungal allergic status is relative only to the fungal types included in the SPTs. The regression analyses of various fungal groups, on symptom severity and inhaler use, including concentrations of allergy-specific fungi for each subject, yielded markedly different estimates of effect (Table 5). These differences should be interpreted with caution for the following reasons: (1) the number of fungi tested may have been insufficient to adequately classify fungal-allergic status, particularly with respect to the Ascomycetes and Basidiomycetes (*see the subsequent DISCUSSION*); (2) 18% of the total spore concentration consisted of unidentified genera; and (3) false-negative results are possible with SPTs, particularly without radioallergosorbent testing (RAST) or intradermal testing for negative SPTs.

The first two issues are supported by our finding that concentrations of genera not tested for allergenicity, such as the Basidiomycetes and Ascomycetes, as well as unidentified spores, produced some of the greatest adverse effects on both asthma symptom scores and inhaler use. The allergenicity of Basidiomycete species (25), and their ability to induce bronchoconstriction among asthmatic subjects allergic to Basidiospores (26), have been previously demonstrated. However, these fungi are not generally tested for allergenicity. Additional evidence for the adverse effects of outdoor concentrations of Basidiomycetes on asthmatic individuals has come from three studies of aggregate databases for hospitalizations and emergency room visits for asthma (27–29). Another study demonstrated a temporal association between Ascomycete counts and asthma deaths (30). None of these four correlational studies, however, was a formal time-series analysis, and none controlled for possible temporal confounding.

Reported dampness or evidence of mold in homes has been associated with the risk of asthma and prevalence of lower respiratory symptoms in two large cross-sectional studies (31, 32). Although indoor bioaerosols are highly correlated with outdoor concentrations in buildings that are not air conditioned, indoor sources of mold are expected to contribute substantially to personal daily exposure to fungi (33). The importance of indoor fungal spore levels was not assessed in the present study, which may have led to a misclassification of exposure, and consequently a misspecification of health effects. Another bias could have oc-

curred if there were an unmeasured confounding variable, such as another allergen or air pollutant, whose levels also increased during times of fungal sporulation as a result of shared meteorologic determinants. One possibility in this regard is an increase in dust mite antigen levels in the fall and a coinciding seasonal increase in airway hyperresponsiveness (34). Eleven of 12 subjects in the present study had positive SPTs for dust mites.

Conclusions

In this study, personal O_3 exposure was positively associated with both asthma symptom severity and medication use, which was unexpected in view of the low levels of exposure. In addition, when only outdoor O_3 levels measured at a stationary site were used as the index of exposure, the associations were weakened considerably or became nonsignificant. These results suggest that ambient exposures of symptomatic asthmatic subjects to O_3 may lead to greater respiratory effects than are predicted from experimental results in subjects who are asymptomatic at baseline and who are exposed for relatively short periods. Such effects may also be underestimated or missed entirely if estimates of exposure do not take into account microenvironmental determinants or potential differences in dose based on cumulative VE .

This is the first asthma panel study using formal time-series analytic approaches to show that outdoor fungal spore concentrations are associated with asthma symptom severity and medication use and that this effect varies with the spore type. Surprisingly, the greatest effects were caused by the group of spores for which allergy skin tests were not performed, such as the Basidiomycetes. To confirm the present findings, further investigations are needed in other geographic regions in order to relate asthmatic responses to both ambient fungal concentrations and the individual's spectrum of fungal sensitivities. Given the relevance of a fungal spore effect to perennial asthma occurring in the warm seasons, accurate fungal aeroallergen exposures should also be taken into account in epidemiologic investigations of the effect of photochemical air pollutants on asthmatic individuals.

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