Acute Effects of Ozone on Mortality from the "Air Pollution and Health: A European Approach" Project

Alexandros Gryparis, Bertil Forsberg, Klea Katsouyanni, Antonis Analitis, Giota Touloumi, Joel Schwartz, Evangelia Samoli, Sylvia Medina, H. Ross Anderson, Emilia Maria Niciu, H.-Erich Wichmann, Bohumir Kriz, Mitja Kosnik, Jiri Skorkovsky, Judith M. Vonk, and Zeynep Dörtbudak

Department of Hygiene and Epidemiology, University of Athens Medical School, Athens, Greece; Department of Biostatistics, Department of Public Health and Clinical Medicine, and Department of Environmental Health, Harvard School of Public Health, Boston, Massachusetts; Umeå University, Umeå, Sweden; Institut de Veille Sanitaire, Paris, France; St. George's Hospital Medical School, University of London, London, United Kingdom; Department of Environmental Health, Institute of Public Health, Bucharest, Romania; GSF, National Research Center for Environment and Health, Munich, Germany; Charles University, Prague; Institute of Hygiene, Teplice, Czech Republic; Institute of Public Health, Ljubljana, Slovenia; Department of Epidemiology and Statistics, University of Groningen, Groningen, The Netherlands; and Koç University, School of Health Sciences, Istanbul, Turkey

In the Air Pollution and Health: A European Approach (APHEA2) project, the effects of ambient ozone concentrations on mortality were investigated. Data were collected on daily ozone concentrations, the daily number of deaths, confounders, and potential effect modifiers from 23 cities/areas for at least 3 years since 1990. Effect estimates were obtained for each city with city-specific models and were combined using second-stage regression models. No significant effects were observed during the cold half of the year. For the warm season, an increase in the 1-hour ozone concentration by 10 $\mu g/m^3$ was associated with a 0.33% (95% confidence interval [CI], 0.17–0.52) increase in the total daily number of deaths, 0.45% (95% CI, 0.22–0.69) in the number of cardiovascular deaths, and 1.13% (95% CI, 0.62–1.48) in the number of respiratory deaths. The corresponding figures for the 8-hour ozone were similar. The associations with total mortality were independent of SO_2 and particulate matter with aerodynamic diameter less than 10 μm (PM_{10}) but were somewhat confounded by NO_2 and CO. Individual city estimates were heterogeneous for total (a higher standardized mortality rate was associated with larger effects) and cardiovascular mortality (larger effects were observed in southern cities). The dose–response curve of ozone effects on total mortality during the summer did not deviate significantly from linearity.

Keywords: cardiovascular mortality; respiratory mortality; time series

A large body of scientific results consistently indicates that air pollution levels currently observed have short-term effects on mortality and morbidity (1–3). Most of the evidence comes from studies of ambient particles concentrations. However, in Europe and elsewhere, the air pollution profile has gradually changed toward a more pronounced photochemical component. Ozone is one of the most toxic components of the photochemical air pollution mixture. Several epidemiologic studies (4–13) have suggested significant effects of exposure to O_3 on human health. Controlled studies, mostly in healthy adult subjects (as well as animal studies), suggest that exposure to elevated concentrations of ozone results in inflammatory response (14, 15) and lung epithelial damage (16), including decreases in mucociliary clearance (17). These effects can cause susceptibility to bacterial respiratory infections. Recent results indicate that oxidant pollutants can amplify the generation of proinflammatory cytokines by rhinovirus 16 (RV16)-infected cells and suggest that virus-induced inflammation in upper and lower airways may be exacerbated by concurrent exposure to ambient levels of oxidants commonly encountered in the indoor and outdoor environments (18). Furthermore, ozone induces decrements in pulmonary function, characterized by alterations in lung volumes and flow and increased airway responsiveness and resistance. Exposure studies indicate that lung function responses attenuate after several days of exposure (19, 20), but this is not true for inflammation (21, 22). In addition, there are large individual differences in the responsiveness to ozone, and the responsiveness dependence on disease status and other exposures is poorly understood (23). Also, much is unknown about the synergistic effects of ozone and the complex mix of pollutants found in the ambient air.

An important development in the investigation of the health effects of air pollution has came from multicity studies that used a predefined, standardized protocol for the city level data analysis and a second-stage analysis procedure to obtain combined effect estimates. Examples of such studies are the Air Pollution and Health: A European Approach (APHEA) project in Europe (24, 25), the National Mortality, Morbidity, and Air Pollution Study (26), the Harvard Six Cities in the United States (27). The impact of their results has been important in the revisions of air quality standards in the United States and Europe (28, 29).

This article presents the results of the APHEA2 project on short-term effects of ozone, both annual and seasonal, on daily mortality (total natural, respiratory, and cardiovascular), adjusting for the possible confounding effects of other pollutants and an estimation of the dose–response curve. APHEA2 uses an extended database that includes 23 cities and more extensive exposure data than the first APHEA project (APHEA1, which only included four cities with ozone data) (30). Preliminary results on the same issue have previously been reported in the form of an abstract (31).

METHODS

Data on mortality, with deaths categorized according to the International Classification of Diseases, 9th edition (ICD9), were, by daily number: total natural, less than 800; cardiovascular, 390–459; and respi-

ratory, 460–519. Data were obtained from 23 cities/areas spread throughout Europe, with a population of more than 50 million. Daily air pollutant measurements were provided by the monitoring network established in each town. All urban monitoring sites, provided that they fulfilled certain completeness criteria (24), were used for all pollutants; especially for ozone, suburban monitoring sites were also included if available. Average daily concentrations of each pollutant were calculated from all monitors included. The time series used were practically complete. The very few remaining missing values were replaced according to a procedure described in Katsouyanni and colleagues (3). The maximum daily 1- and 8-hour ozone concentrations (average of lags 0 and 1) were chosen to represent daily exposures.

A hierarchical modeling approach was used for data analysis. Generalized additive models (32) were fitted in each city separately to allow specific control for seasonal effects, weather, and other potential confounders. The results from the individual city analysis were used in a second-stage analysis to provide combined overall estimates.

We analyzed the data for each city separately following a predefined standardized method, which resulted in a city-specific model (33). In summary, we applied generalized additive models extending Poisson regression to model the nonlinear effects of the covariates, using a local nonparametric LOESS (34) smoother to control for seasonal patterns and long term trends, allowing for overdispersion. Control for seasonality and meteorology is crucial in analyses of ozone effects on mortality (35). We controlled for potential confounding effects of meteorologic variables (mean daily temperature and mean daily relative humidity) and respiratory epidemics, adjusted for day of the week, and took into account local characteristics such as national and school holidays and unusual events (such as heat waves and strikes) if necessary. We decided *a priori* to use the average of lags 0 and 1 for air pollutant measurements, based on previous evidence (36). This approach avoids potential bias, which could result from selectively reporting the most significant lags. If serial correlation remained in the residuals of the final models, autoregressive terms were added based on the method presented in Brumback and colleagues (37). To evaluate the association of ozone concentrations and daily mortality in the warm season (April–September) and the cold season (October–March), respectively, we used the previous models with two additional terms: the interaction term between ozone and season and a dummy variable denoting the winter period. Additionally, we fitted two-pollutant models to adjust for the possible confounding effects of SO_2, NO_2, particulate matter with aerodynamic diameter less than 10 μm (PM_{10}), and CO, by including the other pollutants alternatively in the models.

We applied a second-stage analysis to provide a quantitative summary of all individual city results and attempt to explain the observed heterogeneity between city estimates by exploring the role of potential effect modifiers. Several potential effect modifiers were considered. A list of these variables may be found in Katsouyanni and colleagues (3). Only those effect modifiers explaining more than 10% of the heterogeneity are reported.

In the second-stage analysis, we assumed the city-specific effect estimates to be normally distributed around an overall estimate, assuming heterogeneity. We estimated fixed effects pooled regression coefficients by weighted regression of city-specific estimates on potential effect modifiers (at city level) with weights inversely proportional to their city-specific variances. If substantial heterogeneity among city results, beyond the variation associated with the effect modifiers, remained, random effects regression models were applied. In these models, it was assumed that the individual coefficients are a sample of independent observations from the normal distribution with mean equal to the random effects pooled estimate and variance equal to the between-cities variance. We estimated the between-cities variance from the data, using the maximum likelihood method described by Berkey and colleagues (38), and this was added to the city-specific variances. In contrast to the usual univariate second-stage regression, in which results from each pollutant are analyzed separately, we used multivariate second-stage regression models (applying the method described by Berkey and colleagues) (39), which provide more accurate estimates because they incorporate the correlation among pollutants within each city.

This approach assumes that the dose–response relationship is linear. To examine further the shape of the dose–response relationship between ozone and mortality, we used a method introduced by Schwartz and Zanobetti called meta-smoothing (40), which combines smooth curves across cities in a hierarchical model to obtain the benefits of the meta-analysis and the nonparametric regression.

All analyses were done using S-PLUS (41) with more strict convergence criteria, as suggested by Dominici and colleagues (42). To avoid the underestimation of parameters' variances, we used nonparametric bootstrap estimates (43).

RESULTS

Table 1 shows descriptive data on study period, outcome, and exposure from the 23 cities/areas included in the analysis. The Netherlands is considered as one urban area because of its relative small size and dense population. For most of the cities, the study period was longer than 5 years. The total population was over 50,000,000. The mean daily number of total natural, cardiovascular, and respiratory deaths ranged from 6 to 347, 2 to 143, and 0 to 31, respectively. The percentage of cardiovascular and respiratory deaths over the total number of deaths ranged from 31–58% and 0–17%, respectively.

The maximum daily 1- and 8-hour ozone concentrations are highly correlated (the correlation coefficient ranges from 0.91 to 0.99, with a median of 0.98). Summer ozone concentrations are much higher than winter ones. The value of the 90th percentile for 8-hour summer ozone is close or above 120 μg/m³, the value of the World Health Organization Air Quality Guideline for ozone (30), in nine cities. In the winter, the highest value for the 90th percentile of 8-hour ozone is 78. The seasonal pattern of ozone is in contrast to the annual cycle of daily mortality, which usually peaks in the winter. Turin, Prague, Budapest, and Athens had the highest median ozone concentrations, whereas Tel-Aviv, London, and Paris had the lowest. The winter ratio of 1-hour ozone to 8-hour ozone is somewhat larger than the summer ratio, indicating that the number of hours with elevated ozone concentrations is smaller during the winter. The summer ratio is similar among cities and ranges between 1.1 and 1.4, with two exceptions (Ljubljana and Rome), where it is approximately 1.95. In the various cities, there is generally a negative correlation between ozone and CO, but positive associations are between ozone and the 1-hour maximum NO_2. Between ozone and SO_2 as well as PM_{10}, there is a negative or no correlation during the winter, but there is a positive correlation during the summer. There is a substantial variability among cities in the levels of all pollutants in mean daily temperature and humidity. Additional details on the seasonal ratio of ozone measurements, its correlation with other pollutants, and the levels of other pollutants in the cities analyzed may be found in the online supplement.

Figure 1 shows the percentage increase in the daily total mortality associated with an increase of 10 μg/m³ in the levels of daily maximum 1- and 8-hour ozone (upper and lower points, respectively) for each city, as well as the pooled estimates. Only eight cities had positive effect estimates for both 1- and 8-hour ozone, which were both significant in five. There was substantial heterogeneity in the city-specific estimates. The pooled estimates of the increase in total mortality were weak and not significant: for an increase in 1-hour ozone by 10 μg/m³, it was 0.10% (95% confidence interval [CI], −0.11%, 0.26%) and the corresponding figure for 8-hour ozone was 0.03% (95% CI, −0.18%, 0.21%). Figure 2 shows the percentage increase in the daily number of deaths associated with an increase of 10 μg/m³ in 1- and 8-hour ozone concentrations (upper and lower points, respectively) for each city, as well as the combined overall estimates, for the summer period only. There was substantial heterogeneity in the estimates. In four cities, both the estimated effects were negative, ranging from −1 to 0% (none of them significant), whereas in 19, they were positive, ranging from 0.1 to 1%. The pooled estimates under the random effects model were 0.33% (95% CI, 0.17, 0.52%) for 1-hour ozone and 0.31% (95% CI, 0.17, 0.52%)

TABLE 1. DESCRIPTIVE DATA ON THE STUDY PERIOD, POPULATION, OUTCOME (TOTAL, CARDIOVASCULAR DISEASE, RESPIRATORY DAILY NUMBER OF DEATHS), AND EXPOSURE (SEASONAL MAXIMUM DAILY 1- AND 8-HOUR OZONE)

						Ozone ($\mu g/m^3$)							
						Summer Period				Winter Period			
						Median		90th Percentile		Median		90th Percentile	
City	Study Period	Population × 1,000	Mean No. of Total Deaths per Day	Mean No. of CVD Deaths per Day	Mean No. of Respiratory Deaths per Day	1 Hour	8 Hour	1 Hour	8 Hour	1 Hour	8 Hour	1 Hour	8 Hour
Athens	1/92–12/96	3,073	73	36	5	109	90	150	118	57	44	88	69
Barcelona	1/91–12/96	1,644	40	16	3	90	75	121	100	49	33	79	60
Basel	1/90–12/95	360	9	4	1	87	74	135	118	35	24	68	58
Birmingham	1/92–12/96	2,300	61	28	9	66	52	93	78	47	36	69	61
Budapest	1/92–12/95	1,931	80	40	3	108	97	144	131	56	49	88	78
Erfurt	1/91–12/95	216	6	—	—	94	—	152	—	48	—	77	—
Geneva	1/90–12/95	317	6	2	0	92	78	141	120	35	24	66	50
Helsinki	1/93–12/96	828	18	9	1	66	58	90	81	50	44	68	62
Ljubljana	1/92–12/96	322	7	3	0	107	54	166	90	39	12	84	39
London	1/92–12/96	6,905	169	71	29	53	41	88	72	32	21	55	44
Lyon	1/93–12/97	416	9	3	1	87	65	140	110	40	19	63	42
Madrid	1/92–12/95	3,012	61	22	6	75	59	111	91	31	20	58	42
Milan	1/90–12/96	1,343	29	11	2	85	66	143	119	11	8	40	26
Netherlands	1/90–12/95	16,000	347	143	31	83	74	140	121	48	38	71	62
Paris	1/91–12/96	6,700	124	38	9	56	44	107	91	22	13	43	31
Prague	2/92–12/96	1,213	38	22	1	107	91	168	147	57	41	85	71
Rome	1/92–12/96	2,775	56	23	3	59	30	110	57	24	11	48	25
Stockholm	1/90–12/96	1,126	30	15	3	75	69	100	92	53	48	69	64
Tel-Aviv	1/91–12/96	1,141	27	12	2	44	—	62	—	26	—	44	—
Teplice	1/90–12/97	625	18	8	1	75	66	119	106	31	24	66	53
Torino	1/90–12/96	926	21	9	1	117	99	173	154	34	23	84	63
Valencia	1/94–12/96	753	16	6	2	72	58	95	77	44	31	65	49
Zurich	1/90–12/95	540	13	6	1	92	79	141	124	37	24	66	54

Definition of abbreviation: CVD = cardiovascular disease.

for the 8-hour ozone. It should be noted that for comparability, the 1-hour pooled estimates shown in the figures do not include two cities (Tel-Aviv and Erfurt), which did not have 8-hour ozone measurements available. We also calculated pooled 1-hour ozone effects with these two cities. The estimate became 0.03% (95% CI, −0.11, 0.25) for the annual effects and 0.32% (95% CI, 0.14, 0.50) for the summer season only. The results for the association between ozone concentrations and daily mortality, for the winter period, were considerably lower and not statistically significant.

Table 2 shows the results from two pollutant models, adjusting in turn for the confounding effects of SO_2, NO_2, PM_{10}, and CO. The number of cities that were included in this part of the analysis was reduced, as we did not have data for all the previously mentioned pollutants from each city (*see* additional data in the online supplement). Ozone associations with mortality during the summer period do not appear to be substantially confounded by SO_2 and PM_{10}. In contrast, the estimated combined increase in mortality for 10-$\mu g/m^3$ increase in 1 or 8-hour ozone decreased by 26–27% when adjusting for the daily 1-hour maximum NO_2 and increased by 30–40% when adjusting for 8-hour maximum CO, indicating possible confounding. In all of the multivariate second-stage models results, there remained substantial heterogeneity in the other pollutant-adjusted coefficients for ozone, except for those adjusting for CO. The winter effects of ozone were practically unchanged when adjusting for NO_2 but were increased when adjusting for PM_{10}, SO_2, and CO, becoming statistically significant under the fixed effects model and, in the latter case, remaining significant under the random effects model.

Table 3 shows the pooled estimates for the percent increase in total, respiratory, and cardiovascular mortality associated with an increase of 10 $\mu g/m^3$ in the levels of daily ozone by season. The effects are all statistically significant in summer, whereas the associations in winter are nonsignificant. No further analysis was done for the winter period. Respiratory mortality is more strongly associated with ozone exposure than total or cardiovascular. For CVD mortality estimates, there is significant heterogeneity for summer period estimates but not for respiratory mortality.

Figure 3 shows the pooled over all cities estimated dose–response curve for the summer period. The relationship between ozone and mortality does not seem to deviate significantly from linearity. This finding allows the use of metaregression to examine factors that influence the exposure–response relationship and to investigate the observed heterogeneity in the effect estimates of ozone (even after adjusting for other pollutants) during the summer period.

For the winter period and also for respiratory mortality in both seasons, we did not investigate effect modification, as ozone did not generally have any effect on daily mortality or there was no heterogeneity in the estimates. For the association between ozone and daily total and cardiovascular disease (CVD) mortality in the warm period, we examined several variables as potential modifiers of the ozone effect. For the effects on total mortality, only the standardized mortality rate explained more than 10% of the heterogeneity (16%). The estimated effect was larger in cities with a higher standardized mortality rate (a 0.48% increase associated with a change of 10 $\mu g/m^3$ in ozone concentration in the cities with standardized mortality rate at the 75th percentile of its distribution) compared with those with lower (a corresponding increase of 0.18% for those cities with standardized mortality rate at the 25th percentile). For CVD mortality, only geographic differences were observed. The effect was higher in Southern European cities (0.80% increase in the daily number of CVD deaths per 10 $\mu g/m^3$ increase in ozone) compared with northwestern (0.54%) and with central eastern (0.19%). No
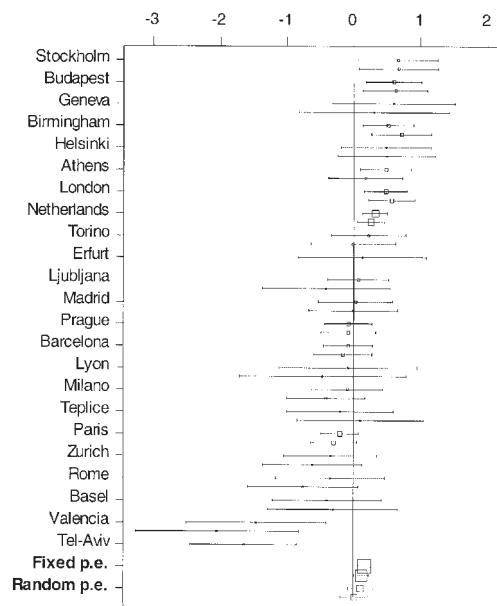
Figure 1. Percentage increase in the total daily number of deaths (excluding deaths from external causes) and their 95% confidence interval associated with an increase of 10 μg/m³ in the levels of 1-hour (upper point) and 8-hour (lower point) ozone (data are for the whole period). The size of the point representing each increase is inversely proportional to its variance.
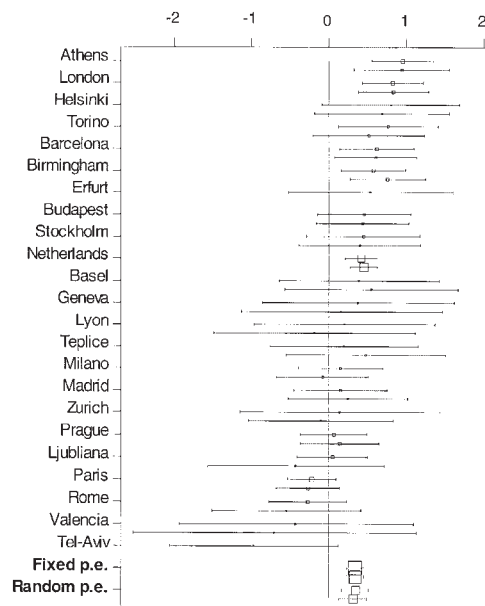

Figure 2. Percentage increase in the total daily number of deaths (excluding deaths from external causes) and their 95% confidence interval associated with an increase of 10 μg/m³ in the levels of 1-hour (upper point) and 8-hour (lower point) ozone, only for the summer period. The size of the point representing each increase is inversely proportional to its variance.

other effect modifier explained more than 10% of the heterogeneity in CVD mortality.

DISCUSSION

This study presents the results on short-term effects of ozone exposure on daily total and cause-specific mortality from the 23 cities/areas, widely distributed across Europe, which contributed data on ozone in the APHEA2 project. The main findings of this study may be summarized as follows: (*1*) Ozone effects on mortality (total and cause specific) are mainly seen during the warm period of the year. (*2*) The effects on respiratory mortality are higher, followed by those on cardiovascular. (*3*) The effects on total mortality remain statistically significant and of comparable magnitude when other pollutants are adjusted for in two pollutant models, and (*4*) the dose response curve for total mortality effects does not deviate significantly from linearity within the range of ozone concentrations commonly observed in European cities.

The choice of analytic method for time series studies has recently been the object of scientific discussion. Dominici and colleagues (42) identified a problem with generalized additive model convergence in the U.S. multicity National Mortality, Morbidity, and Air Pollution Study project models, which resulted in overestimating the PM effects and slightly underestimating the ozone effects. At the same time, further questions were raised on the choice of the optimal smoothing method, contrasting parametric and nonparametric methods. Ramsey and colleagues (44) also found that a programming routine used in the S+ software resulted in underestimating the true standard errors of the effect estimates in city-specific models. The U.S. Environmental Protection Agency requested several groups of researchers who had published results using the previously mentioned or similar modeling strategies to do sensitivity analyses using various prespecified modeling approaches. The results

were published in a Special Report of the Health Effects Institute, which includes five reanalyses of APHEA2 data assessing PM effects (45). The APHEA2 models were shown to be robust when convergence criteria were set to more stringent values. On the other hand, the use of a parametric smoothing method (natural splines) compared with the nonparametric LOESS smoothers decreased the PM_{10} total mortality effects by approximately 30%, left the PM_{10} effects on respiratory hospital admissions unchanged, and led to an increase by approximately 50% of the PM_{10} effects on cardiac admissions. Although it is clear that there may be some sensitivity in the results depending on the smoothing method, the effects remain statistically significant under all models applied. In the concluding statements of the Health Effects Institute Report (45), it is stated that at this stage no optimal method may be recommended. In this analysis, we chose to present results applying nonparametric smoothers with the more stringent convergence criteria set to the values proposed by Dominici and colleagues (42) and requested by the Environmental Protection Agency (45). Caution should be exercised in interpreting the exact quantitative estimate from this or indeed any similar analysis or using it for health impact assessment.

The other method problem, the underestimation of standard errors when using the generalized additive model algorithm in S+, was addressed here by applying the bootstrap for the estimation of the correct standard errors. It has been shown that in a multicity project, when the city-specific effect estimates are combined using a random effects model, the standard errors of the combined estimates are not underestimated, as the decreased within-city variance is compensated by increased between-city variance (46). However, the underestimation of the standard errors for city-specific estimates will lead to increased heterogeneity of the between city estimated effects and can affect our ability to detect effect modifiers. We therefore chose to apply

TABLE 2. POOLED*ESTIMATES FOR THE INCREASE IN THE TOTAL DAILY NUMBER OF DEATHS ASSOCIATED WITH 1- AND 8-HOUR OZONE INCREASES OF 10-μg/m^3 (AVERAGE OF LAGS 0 AND 1) ADJUSTING ALTERNATIVELY FOR OTHER POLLUTANTS IN TWO POLLUTANT MODELS

Other Pollutant (Number of Cities)[†]	Ozone (h)	Summer Period Increase % (95% CI)		Winter Period Increase % (95% CI)	
		FE Model	RE Model	FE Model	RE Model
None (21)	1	0.33 (0.24, 0.45)	0.33 (0.17, 0.52)	0.08 (−0.09, 0.18)	0.09 (−0.25, 0.28)
(21)	8	0.34 (0.27, 0.50)	0.31 (0.17, 0.52)	0.13 (−0.03, 0.29)	0.12 (−0.12, 0.37)
SO$_2$ (21)	1	0.34 (0.26, 0.46)	0.33 (0.18, 0.50)	0.22 (0.07, 0.36)	0.23 (−0.02, 0.47)
(21)	8	0.33 (0.22, 0.47)	0.31 (0.13, 0.51)	0.28 (0.10, 0.45)	0.29 (−0.01, 0.55)
NO$_2$ (21)	1	0.22 (0.14, 0.35)	0.24 (0.09, 0.40)	0.09 (−0.09, 0.20)	0.07 (−0.19, 0.30)
(21)	8	0.23 (0.12, 0.38)	0.23 (0.07, 0.41)	0.12 (−0.04, 0.29)	0.07 (−0.20, 0.39)
PM$_{10}$ (19)	1	0.25 (0.16, 0.40)	0.27 (0.10, 0.47)	0.27 (0.09, 0.41)	0.21 (−0.07, 0.48)
(19)	8	0.25 (0.13, 0.40)	0.27 (0.08, 0.49)	0.35 (0.12, 0.51)	0.22 (−0.08, 0.51)
CO (19)	1	0.44 (0.33, 0.56)	0.43 (0.30, 0.58)	0.22 (0.06, 0.38)	0.25 (0.02, 0.48)
(19)	8	0.44 (0.30, 0.57)	0.44 (0.29, 0.59)	0.28 (0.08, 0.46)	0.34 (0.04, 0.59)

Definition of abbreviations: CI = confidence interval; FE = fixed effects; PM$_{10}$ = particulate matter with aerodynamic diameter less than 10 μm; RE = random effects.

* The combined estimates were calculated using a multivariate second-stage regression program.

[†] Without Erfurt and Tel-Aviv for ozone 1 hour.

the bootstrap for the estimation of standard errors, which yields unbiased estimates.

For the daily ozone exposures, we used the maximum daily 1 and 8-hour averages. The 1 hour was used to yield results comparable to those of APHEA1 and other projects and makes full use of available data. Preliminary results concerning 1-hour ozone concentrations and total mortality have been presented before (30). The 8 hour was used because it is the averaging time recommended by the World Health Organization for reflecting the most health-relevant exposure to ozone (47). The two metrics are highly correlated, and the ratio of the mean of maximum ozone 1-hour average to the average of 8 hours for the 21 cities with both has a median of approximately 1.2 for both seasons. Others have found a similar (1.3) relationship (48).

The magnitude of ozone effects according to the causes of mortality indicates that a major part of the excess mortality related to elevated ozone levels is classified as cardiovascular and respiratory deaths. These cases probably occur among cardiopulmonary-compromised individuals. There are several potential underlying mechanisms: inflammation of pulmonary tissues, which can induce a spectrum of mediators that also may alter cardiac functions, or irritant receptor-mediated stimulation of parasympathetic pathways (49). De Leon and colleagues (50) found that there is increased risk of pollution-associated mortality in individuals with co-existing respiratory disorders.

The APHEA2 project is a continuation of APHEA1, which was based on a more limited database. In the APHEA1 project based on only four cities (London, Athens, Barcelona, and Paris), an annual ozone 1-hour effect was found to be equal to 2.9% (95% CI, 1.0, 4.9) increase in the daily total number of deaths associated with an increase in ozone concentration of 50 μg/m^3. This corresponds to 0.6% (0.2, 1.0) per 10 μg/m^3 of ozone increase, which is larger than the one reported in this article for the warm season only (30). If we investigate the city-specific effects for the cities included in the APHEA1 results, we see that the annual effect estimates for Barcelona and Paris are now smaller, whereas for the other two cities, they are practically identical. The order of magnitude of the effects per city is preserved. The differences may be attributed to the more recent data sets used in this article and to the different modeling strategies used in the two stages of APHEA. Thus, in APHEA1, the reported associations were based on the best model found for lags 0–5, whereas in APHEA2, we decided *a priori* to use the average of lags 0 and 1. Also, the adjustment for seasonal patterns is more flexible in APHEA2, where nonparametric instead of parametric adjustment was used, and this may have led to better control of seasonal confounding. In APHEA1, no seasonal analysis results were reported, but it was noted that summer estimates were higher (30). From the APHEA1 project, annual effects on cardiovascular and respiratory mortality were also statistically significant but smaller compared with those of total mortality (0.2% and 0.4% per 50 μg/m^3 increase in ozone concentrations).

TABLE 3. POOLED*ESTIMATES FOR THE INCREASE IN THE TOTAL, RESPIRATORY, AND CARDIOVASCULAR MORTALITY ASSOCIATED WITH 1- AND 8-HOUR OZONE INCREASES OF 10 μg/m^3 (AVERAGE OF LAGS 0 AND 1)

Mortality (Number of cities)[†]	Ozone (h)	Summer Period Increase % (95% CI)		Winter Period Increase % (95% CI)	
		FE Model	RE Model	FE Model	RE Model
Total (21)	1	0.33 (0.24, 0.45)	0.33 (0.17, 0.52)	0.08 (−0.09, 0.18)	0.09 (−0.25, 0.28)
(21)	8	0.34 (0.27, 0.50)	0.31 (0.17, 0.52)	0.13 (−0.03, 0.29)	0.12 (−0.12, 0.37)
Respiratory (21)	1	0.76 (0.67, 1.36)	1.13 (0.62, 1.48)	−0.31 (−0.53, 0.39)	−0.16 (−0.67, 0.52)
(21)	8	1.08 (0.74, 1.51)	1.13 (0.74, 1.51)	0.20 (−0.42, 0.65)	0.26 (−0.50, 0.84)
Cardiovascular (21)	1	0.49 (0.34, 0.64)	0.45 (0.22, 0.69)	0.01 (−0.22, 0.17)	0.02 (−0.28, 0.30)
(21)	8	0.50 (0.33, 0.69)	0.46 (0.22, 0.73)	0.11 (−0.15, 0.32)	0.07 (−0.28, 0.41)

For definition of abbreviations, *see* Table 2.

* The combined estimates were calculated using a multivariate second-stage regression program.

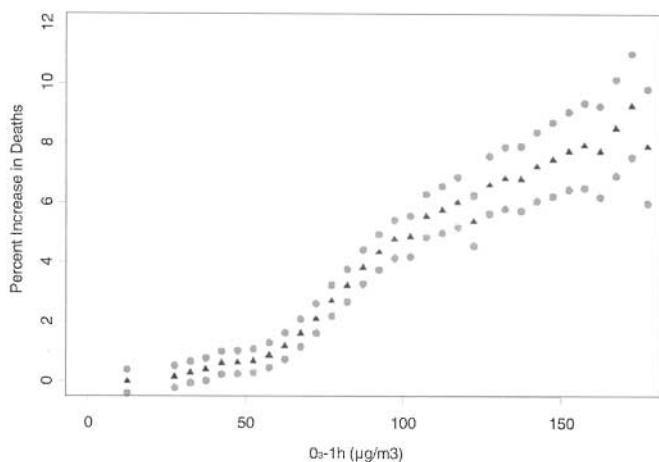[†] Without Erfurt and Tel-Aviv for ozone 1 hour.

Figure 3. Dose–response of ozone 1 hour (average of lags 0 and 1) and daily number of deaths during the summer season.

In APHEA1, days with concentrations above 200 $\mu g/m^3$ were excluded from the analysis. In APHEA2, no days were excluded. The days above 200 $\mu g/m^3$ were very few (only 0.26% of the total), and the dose response for these cities did not display a markedly nonlinear pattern.

In a recent synthesis of studies from the nineties Thurston and Ito (35) estimated the ozone effect on mortality to 0.36% for an increase in the daily 1-hour maximum of 10 ppb, or approximately 20 $\mu g/m^3$, and 0.56% for seven of these studies, including nonlinear temperature terms and adjustment for particle concentration. This latter figure is very close to our summer estimate. In the National Mortality, Morbidity, and Air Pollution Study using the 20 largest U.S. cities, Samet and colleagues (26) found after adjustment for PM_{10} a general ozone effect on mortality only in the three hottest summer months, equal to 0.41% for an increase in the daily 1-hour maximum of 10 ppb, or approximately 20 $\mu g/m^3$. In the Health Effects Institute Report, the effect presented after reanalysis, based on 90 cities, is 0.50% increase in total mortality (45). The results on the seasonality of the effect are consistent to ours. The magnitude of the estimated effect is somewhat lower, but this may be due to the use of a 1-day exposure in National Mortality, Morbidity, and Air Pollution Study, whereas we use the average of 2 days.

Ozone concentrations are higher during the summer. Furthermore, ozone is very reactive, and its concentrations indoors are much lower than those outdoors. Therefore, it is natural that people are exposed to ozone outdoors. The finding that ozone effects are mainly seen during the summer may be due to the higher concentration levels but may also be a result of longer time spent outdoors, probably exercising, during the summer, which results in higher exposures. The lack of an effect during the winter in this and other studies may be an indication of the existence of a threshold in the ozone–mortality association. Alternatively, it may be a result of different seasonal intercorrelations of ozone with other pollutants. It should be noted that the ozone effect estimates for the winter period increase when adjustment is made for some other pollutants. This may be an indication of negative confounding effects.

In 19 of the 23 cities in this project, ozone was found to be associated with an increase in mortality (in many cases nonsignificant) during the warmer season, whereas in four cities (namely Rome, Paris, Valencia, and Tel-Aviv), ozone was found to be associated with a nonsignificant decrease in mortality. Ozone concentration in the atmosphere is related to the concen-tration of NO_2, as ozone is a secondary pollutant that is created from photochemical reactions in the atmosphere in which NO_2 participates (51). However, emissions of nitric oxide, primarily from cars, reduce the concentration of ozone in a local and short-term scale. This leads to low levels of ozone in the center of town where there is an abundance of primary pollutants from traffic. In such cases, a high ozone level may indicate good dispersion conditions (wind) and low levels of traffic exhausts, which may decrease daily mortality. In contrast, ozone is trans-ported to the city outskirts, where it remains at high levels, in the absence of primary pollutants. Consequently, the level of ozone reported depends on the placement of the monitors. On the other hand, the daily maximum ozone concentrations in all monitors in a city or region correlate positively, and thus, the estimated effects on mortality must reflect the same (positive or negative) association. The average population exposure, how-ever, may be underestimated when center of town sites are mainly used, especially close to large traffic emissions and in a badly ventilated area and overestimated when sites at the out-skirts are only used. In these situations, the effect maybe overesti-mated or underestimated respectively. In the APHEA2 project, we used the maximum possible number of monitors. There was no selection according to the placement.

In most of the cities, the annual correlation between the maximum daily 1-hour average of ozone and the maximum daily 1-hour average of NO_2 was found to be positive (*see* online supplement). The same correlation for the warm period was posi-tive for all cities, except Valencia, which, as shown in Figure 2, has the larger negative association between ozone concentration and increase in mortality. That might imply particularities in the production, destruction, or placement of measurement sites of ozone in this city and in its association with NO_2. To address the questions that other air pollutants may confound the associations between ozone levels and daily mortality, we used two pollutant models where we adjusted in turn for the confounding effects of SO_2, NO_2, PM_{10}, and CO. Because not all cities provided data on SO_2, NO_2, PM_{10}, and CO, the number of the cities that participated in the multivariate second-stage regressions differs for each pollutant (Table 3). Although ozone associations with mortality in the warm period do not appear to be confounded by SO_2 and PM_{10}, the same is not true for NO_2 and CO. When the daily 1-hour maximum NO_2 is adjusted for, ozone effect estimates become lower (although they remain significant), whereas when the daily 8-hour maximum CO is adjusted for, they become higher.

The effect modification pattern in this analysis does not pro-vide an adequate explanation for the observed heterogeneity in the effects on total and CVD mortality, in contrast to the APHEA2 results on PM_{10} effects on mortality (3). For total mortality the only effect modifier identified, the standardized mortality rate, concerned the health of the population. It appears that in populations where life expectancy is shorter, the ozone effects are larger. For cardiovascular mortality, we have not been able to identify meaningful effect modifiers, other than geographic differences. The ozone effects are larger on average in southern European cities, where the concentrations are higher. We did not have available data to investigate possible effect modification by the dietary intake of antioxidant vitamins that has been found by Romieu and colleagues (52) in a study of Mexican children with asthma.

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