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Assessment of Recent Health Studies of Long-Term Exposure to Ozone

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Assessment of Recent Health Studies of Long-Term Exposure to Ozone

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ABSTRACT

This report summarises the assessment of the policy relevant long-term health studies published since the last update of the ozone Air Quality Limit Value (AQLV). This project was undertaken in preparation for the European Union 2013 Year of Air discussions on the Air Quality Directives, and the impact of new health science on the AQLV for key pollutants, including ozone. The types of studies reviewed in this assessment included chronic mortality and morbidity air pollution epidemiology studies and repeat-dose toxicology and mechanistic studies.

For each study, a summary of the findings as reported was prepared along with a critical review identifying the strengths and weaknesses of the study. In total thirteen chronic mortality studies, nine respiratory morbidity studies, nine epidemiology studies evaluating long-term exposure to ozone and pulmonary function and nine repeat dose animal inhalation studies were reviewed. Reliability scores were provided for both the epidemiology and toxicology studies, and a weight-of-evidence approach was implemented to determine causality.

In summary, for chronic mortality, the data were considered not sufficient to draw a causal conclusion between long-term exposure to ozone and mortality. The available toxicology and mechanistic data did not support the mortality hypothesis at current ambient ozone levels since much higher levels (500 ppb) were required to produce serious effects. For chronic morbidity, the data were considered insufficient to establish a causal relationship between long-term exposure to ozone and new onset asthma. In addition, the data do not indicate that long-term exposure to ozone at current ambient levels causes reductions in lung function development. Short-term exposure to higher levels of ozone can cause transient change in lung function which, if accompanied with symptoms, could be considered as adverse. The serious effects in animals as a result of repeated high level (500 ppb) exposure are not expected to occur in humans exposed to ambient levels.

In summary, the quality of the evidence to evaluate the association between chronic exposure to ozone and mortality is highly unreliable, and information to support an objectively-based Air Quality Target Values (AQTV) is lacking.

KEYWORDS

Ozone, mortality, morbidity, epidemiology, pulmonary function, asthma, air quality target values

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CONTENTS		Page
SUMMARY		IV
1.	INTRODUCTION	1
2.	METHODS	2
3.	RESULTS	3
3.1.	SUMMARY OF RESULTS	3
4.	CONCLUSIONS	5
5.	GLOSSARY	6
6.	REFERENCES	7
APPENDIX 1	ASSESSMENT OF RECENT HEALTH STUDIES OF LONG-TERM EXPOSURE TO OZONE	8

SUMMARY

An assessment was conducted of the policy-relevant long-term health studies published since the last update of the ozone Air Quality Limit Value (AQLV). This project was undertaken in preparation for the European Union 2013 Year of Air discussions on the Air Quality Directives, and the impact of new health science on the AQLV for key pollutants, including ozone. The types of studies reviewed in this assessment included chronic mortality and morbidity air pollution epidemiology studies and repeat-dose toxicology and mechanistic studies. This assessment focused on health effects such as mortality, respiratory morbidity, and lung function changes associated with long-term exposure to ozone.

For each study, a summary of the findings as reported was prepared along with a critical review identifying the strengths and weaknesses of the study. In total thirteen chronic mortality studies, nine respiratory morbidity studies, nine epidemiology studies evaluating long-term exposure to ozone and pulmonary function and nine repeat dose animal inhalation studies were reviewed. Reliability scores were provided for both the epidemiology and toxicology studies, and a weight-of-evidence approach was implemented to determine causality.

In summary:

- For chronic mortality, the data on ozone were considered not sufficient to draw a causal conclusion between long-term exposure to ozone and mortality. The available toxicology and mechanistic data did not support the mortality hypothesis at current ambient ozone levels since much higher levels (500 ppb) were required to produce serious effects.
- For chronic morbidity, the data on ozone were considered insufficient to establish a causal relationship between long-term exposure to ozone and new onset asthma. In addition, the data do not indicate that long-term exposure to ozone at current ambient levels causes reductions in lung function development. Short-term exposure to higher levels of ozone can cause transient change in lung function which, if accompanied with symptoms, could be considered as adverse. The serious effects in animals as a result of repeated high level (500 ppb) exposure are not expected to occur in humans exposed to ambient levels.
- The quality of the evidence to evaluate the association between chronic exposure to ozone and mortality is highly unreliable, and information to support an objectively-based Air Quality Target Values (AQTV) is lacking.

1. INTRODUCTION

In preparation for the European Union “2013 Year of Air” when the EU would review their air quality directives, CONCAWE initiated a project to review and assess the recent science to be able to provide input on scientific discussions on key pollutants, including ozone.

The objective of this project was to identify and critically review new policy-relevant science on the health effects of long-term exposure to ozone and to determine the potential impact of the new science on current and future Air Quality Target Values (AQTV). The current AQTV for ozone is 120 µg/m³ as an 8-hour mean was promulgated in 2002 (OJ L 67, 9.3.2002).

Chronic ozone respiratory mortality is emerging as a new significant health event of concern in air pollution policy worldwide. Therefore, it was important to understand the recent literature to be able to provide input into the 2013 AQ directives.

2. METHODS

This project focused primarily on studies that were published after promulgation of the AQTV in 2002. However, to ensure that studies published before or close to the previous review were not missed, this review included studies published in the 1998-2002 timeframe. The health effects evaluated were identified from a review of the scientific literature and various regulatory-based reviews of ozone. These health effects included mortality, respiratory morbidity, and lung function changes associated with long-term exposure to ozone.

Relevant studies for this assessment were identified using the following criteria:

- Studies published since the last update of the ozone AQLV or not considered in the last review
- Studies focused on chronic mortality, chronic morbidity and repeat-dose toxicology/mechanistic studies
- Studies that could be used to by policy makers to support the ambient air quality criteria by the WHO, USEPA, Health Canada and other regulatory bodies.
- Studies identified by examining regulatory based documents and through targeted literature search

A summary of each study was prepared along with a critical review identifying strengths and weakness.

A weight-of-evidence (WoE) assessment was performed using the ECETOC human relevance framework as a guideline.

Further details on the methods can be found in **Appendix 1**.

3. RESULTS

3.1 SUMMARY OF RESULTS

Type of Study	Number of Studies	Conclusions
Epidemiology		
Mortality Studies	13	In summary the quality of the evidence to evaluate the association between chronic exposure to ozone and mortality is highly unreliable, and information to support an objectively-based AQTV is lacking.
Respiratory Morbidity	9	In summary, evidence for or against causal relationships in the field of morbidity studies of long-term ambient ozone exposure is not persuasive. Attempts to fashion a scientifically defensible ambient ozone standard from this set of studies would be ineffectual, as the body of literature is unreliable. In addition, these studies <i>in toto</i> lack a concentration-response function critical to regulatory decisions.
Long-term exposure and pulmonary function	9	Overall, this field of studies is moderately reliable but the findings were inconsistent, sometimes generating more questions than answers. Regarding positive findings (respiratory function decrement) even if those results were statistically significant, clinical judgment is necessary to assess the clinical relevance of the decrement. On weight, these studies of respiratory function do not support a causal inference between ambient levels of ozone and diminished respiratory function.

Toxicology		
Inhalation (rodents)	6	<p>In general, the studies indicated that ozone is not tumorigenic but may be a contributing factor to lung remodelling. The data indicate that chronic exposure to ozone at levels far above the existing air quality standards results in significant airway modifications which are of unknown biological impact at lower ambient levels. Since mortality is not observed at relatively high ozone levels in animal studies, the findings from the animal data do not support the hypothesis that current ambient levels of ozone cause chronic mortality.</p>
Inhalation (primates)	3	

Further details on the results can be found in **Appendix 1**.

4. CONCLUSIONS

The main conclusions are summarised below. Further details on this assessment are provided in Appendix 1.

1. Does ozone cause chronic mortality?

A single model result from a single observational study (Jerrett et al), combined with a large body of negative evidence, is not adequate to establish causality at current ambient levels.

2. Does long term ozone exposure cause increased morbidity?

The data are insufficient to establish a causal relationship between long-term exposure to ozone and new onset asthma. The data are too limited to allow an evaluation of bronchiolitis or cardiac morbidity. The association for lung cancer is considered not causal.

3. Does long-term ozone exposure cause decrements in pulmonary function?

The available data do not indicate that long-term exposure to ozone at current ambient levels causes decrements in lung function development. Short-term exposure to higher levels of ozone can cause transient changes in lung function which, if when accompanied with symptoms could be considered as adverse. The serious effects in animals resulting from repeated high level exposure are not expected to occur in humans exposed to ambient levels.

5. GLOSSARY

Acronym	Definition
ACS	American Cancer Society
AQLV	Air Quality Limit Value
AQTV	Air Quality Target Value
C-R-F	Concentration Response Function
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
EMBSI	ExxonMobil Biomedical Sciences, Inc.
LUR	Land Use Regression
MRRR	Minimally Reliable Relative Risk
O ₃	Ozone
PM	Particulate Matter
PM ₁₀	Particulate Matter of 10 microns
PM _{2.5}	Particulate Matter of 2.5 microns
ppb	Parts per billion
RR	Relative Risk
USEPA	United States Environmental Protection Agency
WHO	World Health Organisation
WoE	Weight of Evidence

6. REFERENCES

Refer to Appendix 1

APPENDIX 1

Assessment of Recent Health Studies of Long- Term Exposure to Ozone

Prepared by
ExxonMobil Biomedical Sciences, Inc. (EMBSI)
under contract with CONCAWE
9/28/2012

Table of Contents

1. Introduction	10
2. Methods.....	10
3. Epidemiology Studies.....	11
3.1 Mortality Studies.....	11
Table 1. Key studies of the effects of chronic ozone exposure on mortality	16
3.2 Morbidity Studies.....	21
Table 2. Key studies of the effects of chronic ozone exposure on respiratory morbidity....	24
3.3 Pulmonary Function.....	27
Table 3. Key studies of the effects of chronic ozone exposure on respiratory function.	28
4. Toxicology and Mechanistic Studies	31
4.1 Studies in Rodents	31
4.2 Studies in Primates	34
Table 4. Key studies of the effects of chronic ozone exposure on respiratory function	36
Table 5. Key primate studies of the effects of chronic ozone exposure on respiratory function.....	38
4.3 Data Evaluation Considerations.....	39
4.4 Summary of Conclusions.....	40
5. Weight of Evidence Assessment.....	41
5.1 Chronic mortality and morbidity.....	41
5.2 Pulmonary Function.....	46
Annex 1	48
Critical Review of Individual Epidemiology Studies	48
Mortality Studies	48
Morbidity Studies.....	80
Lung Function Studies.....	98
REFERENCES.....	115

1. Introduction

The objective of this work was to identify and critically review new policy relevant science on the health effects of long-term exposure to ozone and to determine the potential impact of the new science on current and future Air Quality Target Values (AQTVs). The current AQTV for ozone of 120 $\mu\text{g}/\text{m}^3$ as an 8-hour mean was promulgated in 2002 (OJ L 67, 9.3.2002). This review focused primarily on studies that were published after promulgation of the AQTV in 2002. However, to ensure that studies published before close of the previous review were not missed, we also included studies published in the 1998-2002 timeframe. The health effects evaluated were identified from a review of the scientific literature and various regulatory-based reviews of ozone. These health effects included mortality, respiratory morbidity, and lung function changes associated with long-term exposure.

2. Methods

A series of approaches were used to identify new long-term health studies on ozone. EMBSI reviewed the ozone health effects literature captured in various regulatory-based summary documents including the United States Environmental Protection Agency (USEPA) 2006 Air Quality Criteria for Ozone and Related Photochemical Oxidants (EPA 600/R-05/004aF), and the September 2011 EPA draft Integrated Science Assessment for Ozone and Related Photochemical Oxidants. Additionally, EMBSI performed a systematic search of the literature. Finally, new studies were also identified through EMBSI's ongoing monthly surveillance of the new literature on the health effects of ozone.

As described in the EMBSI proposal for work, policy relevant studies were defined as a study of sufficient weight and relevance that could be or has been important in establishing Air Quality Criteria by recognized authoritative bodies including the World Health Organization, USEPA, and Health Canada. For each policy-relevant epidemiology study, a summary of the findings of the study, as reported, was provided. This was followed by a critical assessment of the content of the study, including strengths and weaknesses and any effect or no effect levels that were discernible. Not included in this assessment are epidemiologic studies for which the primary indicator of effect was a potential genetic or molecular biomarker. Rather, we focused on studies incorporating diagnoses, certified causes of death, and direct measurements of respiratory function.

For each long-term health endpoint EMBSI performed a weight-of-evidence (WoE) evaluation to determine whether or not the health effect was anticipated to be caused by exposure to ambient ozone across the range of concentrations considered in the literature. To help structure the WoE evaluation, EMBSI used the Framework for the Integration of Human and Animals Data in Chemical Risk Assessment outlined in the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) Report Number 104 and Lavelle et al. (2011). Briefly, using this framework, once the key human data have been gathered and evaluated, the causality criteria developed by Bradford Hill are considered to help draw potential causal inferences in general associations between exposure and a health effect. The intrinsic quality of the human data is categorized as high, good, compromised, or poor quality, or not valid information using the criteria described by ECETOC. The intrinsic quality of the animal data is categorized according to the approach described by Klimisch et al. (1997). The mode of action

(MoA) by which a chemical causes a specific health effect is also considered. Both the human and animal data are considered and integrated for purposes of determining whether or not exposure to the agent under consideration causes a particular health effect at the exposure levels under consideration.

3. Epidemiology Studies

3.1 Mortality Studies

We identified 13 studies that evaluated the association between long-term exposure to ozone and mortality. These studies used a number of well-known cohorts including the American Cancer Society (ACS), Harvard Six Cities, Veterans Affairs, and California Smog study cohorts. Studies using a number of new cohorts in the United States and Australia were also identified. A summary of the results of these studies is summarized in Table 1 along with the study quality categories for each study. A detailed critical review of each study appears in Annex 1.

Many of the studies report weak and/or non-statistically significant associations between long-term exposure to ambient concentrations of ozone and mortality. The two updates of the ACS study of air pollution typified this pattern (Pope et al., 2002; Krewski et al., 2009). The Krewski et al. (2009) extended follow-up and spatial analysis of that cohort delivered the most convincing statistics, yet the statistically significant effect estimates were below 1.03 for each of the 3 mortality categories. The Health Review Committee of the Health Effects Institute (HEI) noted that some influential ecologic covariates (e.g., temperature, population change) that were identified in previous cohort analyses were not included, raising the prospects of residual confounding by the omitted variables (Krewski et al., 2009, Health Review Committee commentary). Also missing from the model were co-pollutants, particularly PM_{2.5}. This potential bias adds to the already high potential for these weak associations to have been the result of confounding by those variables that were measured.

Using data from the ACS cohort, Jerrett et al. (2009) reported an association between chronic exposure to ozone and respiratory mortality, but not for all-cause, cardiopulmonary, cardiovascular and ischemic heart disease in two-pollutant models with PM_{2.5}. Based largely on the results of this ozone-specific study, the U.S.EPA (2011) concluded that there is casual relationship between chronic exposure to ozone and respiratory mortality. Given the importance of this study, we summarize its limitations below.

To examine the potential association between ozone and mortality, Jerrett et al. (2009) incorporated a number of enhanced socioeconomic factors that were not included in previous studies. These factors included individual covariates for lifestyle, dietary, demographic, occupation and education variables, and eight ecologic variables to control for contextual neighborhood confounding that considered income, income inequality, education, population size, racial composition and unemployment. Jerrett et al. (2009) also used land use regression (LUR) in their exposure assessment methodology, a potential advancement over the common use of ambient monitor measurements alone.

Jerrett et al. (2009) incorporated 23 years of ozone air quality (1977 to 2000) into the study. In contrast, they obtained only 2 years of PM_{2.5} data (1999-2000) due to limitations in data

availability. In the U.S., the levels of both pollutants decreased significantly over the years 1977-2000. Consequently, the exposure values for ozone included higher levels observed in the past whereas PM_{2.5} values included much lower levels observed in the 1999-2000 timeframe, setting up situation in which confounding by PM_{2.5} was not adequately controlled. Secondly, while the approach to examine the association for ozone focused on daily maximum hourly levels in the summer, the exposure metric used to assess potential confounding by PM_{2.5} was the annual average. Overall, this uneven analytic approach maximized the potential to observe an association between ozone and mortality and minimized the potential for PM_{2.5} to confound the ozone association. The authors appear to recognize this fault in the discussion section where they state “it is likely that we have underestimated the effect of PM_{2.5} in our analysis.” Finally, Jerrett et al. (2009) did not consider confounding by SO₂, a pollutant that had previously demonstrated a stronger mortality association than PM_{2.5} in the ACS cohort (Krewski, Burnett et al. 2000). Thus, Jerrett et al. (2009) has not demonstrated that the ozone-mortality association they report is independent of other pollutants.

Jerrett et al. (2009) did not provide an adequate explanation for the full spectrum of results they reported and why long-term ozone exposure produced a seemingly ‘protective’ effect on cardiovascular mortality in two-pollutant models with PM_{2.5}. While making a claim for a protective effects from ozone exposure would be illogical, risks for death from ischemic heart disease, cardiovascular disease, and all causes are, in fact, less than 1.00 and statistically significant. While these authors encourage readers to accept on face that the lone positive, albeit weak, association for respiratory mortality as being reliable, the author ignores the negative associations. If one takes the risk of accepting all of the estimates at face value—and not just the one positive association—the overall impact of chronic ozone exposure results in a net decrease in mortality, i.e., a slight increase in respiratory mortality, a slight decrease in various indicators of cardiovascular mortality, and a slight decrease in all causes of mortality.

Based on single-pollutant models, Jerrett et al. (2009) reported a prominent, yet unexplained, degree of regional variability in the risk estimates. Such heterogeneity of effect argues against making a strong causal determination from the results. Only the Southeast and Southwest U.S. had clearly elevated (i.e., statistically significant) effect estimates. The other 5 regions showed little to no increase in risk attributable to a 10 ppb change in ozone. Firm inferences, however, remain elusive due to the statistical imprecision regarding the effect estimates on those regions with fewer study subjects (essentially regions west of the Mississippi River). The above variability cannot be explained by chemistry differences as is often used to explain the heterogeneity reported in observational studies of particulate matter. Also, there is a general trend towards higher risk in regions with higher summertime temperatures, a well-known independent risk factor for mortality that was not accounted for in the analysis. The accuracy of the estimates for cooler areas of the world is questionable. Nevertheless, the results of Jerrett et al. (2009) are used for risk assessments, for example, to project EU-wide or world-wide estimates of increased chronic respiratory mortality from exposure to ozone. Likewise, mortality estimates from warmer regions are likely to be less than reliable because it will not be clear if the estimates are due to ambient ozone alone.

Zanobetti and Schwartz (2011) also reported a positive association between ozone and various types of cardio-pulmonary mortality in a U.S. Medicare cohort. However, potential confounding by exposure to ambient PM was not evaluated. Therefore, it is not possible to discern an independent association for ozone in this study.

In the original study of the Harvard Six City prospective cohort study, Dockery et al. (1993) reported no association whatsoever between long-term exposure to ozone and mortality, although that analysis was constrained due to the low exposure contrast between cities. Specifically, Krewski et al. (2000) reported the relative risk estimate for long-term ozone concentrations comparing the highest and lowest concentrations in the cities (a difference of 8.3 ppb) was negative (relative risk = 0.87, 95% confidence interval (CI) 0.76,1.00).

Two Adventist Health and Smog Study cohort-based studies reported no statistically significant association between long-term exposure to ozone and mortality (Abbey et al., 1999; Chen et al., 2005). Another study from that cohort (Beeson et al., 1998), reported statistically significant moderate elevations in relative risk in lung cancer incidence noted only in males, an observation attributed to gender differences in outdoor exposure time. These investigations were based on a cohort living in California, which has very high levels of ambient ozone.

In an ecological study in Brisbane Australia, Wang et al. (2009) evaluated the association between long-term exposure to various gaseous pollutants, including ozone, and cardio-respiratory mortality in Brisbane, Australia. The authors reported no association between ozone and mortality. Likewise, Lipfert et al. (2006) reported no association between chronic exposure to ozone and mortality in the latest update of the Veterans Affairs cohort air pollution study.

In total, 13 separate studies evaluated the association between long-term ozone exposure and mortality, 12 of which had clearly interpretable risk estimates. Of those 12 studies -- including the most recent update of the ACS cohort (Krewski et al., 2009) the original Harvard Six Cities Study (Dockery et al. 1993), two updates of the Seventh Day Adventists (Abbey et al., 1999; Chen et al., 2005), two studies using the Veterans Affairs cohort (Lipfert et al., 2006a; Lipfert et al., 2006b), the California Teachers Study (Lipsett et al., 2011) and one study using the Brisbane Australia cohort (Wang et al., 2008) -- fail to offer a convincing argument for a causal association between long-term ozone and mortality. These studies reported either weak effects which are easily influenced by various types of measurement errors, or no effects (see Table 1 for details). Two studies suggest otherwise, although far from compellingly. Jerrett et al. (2009) reports a positive association using one endpoint model but no association using two other such models. Another study (Zanobetti and Schwartz, 2011) reported a positive association for various types of cardiovascular mortality, but these results are from a single-pollutant model likely to have produced biased results due to the exclusion of important co-exposures.

Cardio-pulmonary mortality showed relatively more convincing results than did the other categories of mortality with effect estimates of 1.08 for males and 0.97 for females in Abbey et al. (2009), 0.99 in Jerrett et al. (2009), 1.027 in Krewski et al. (2009) and around 1.10 in Pope et al. (2002). These statistical associations are obviously weak, thus prone to bias from confounding. This will be discussed below.

Intrinsic Quality Evaluation of the Epidemiology Data

Overall, the reliability is low for the field of mortality studies. The primary reason is the consistently weak statistical associations, potentially overwhelmed by potential confounders that are either not controlled or are partially controlled. Indeed, all studies had such limitations. As a result, the weak statistical associations observed in the mortality studies might easily be the result of the additive effects of measurement and confounding biases, particularly from

factors that were not considered in the statistical models that could be risk factors for morbidity and mortality (Gamble, 1998). Many notable epidemiologists have reliability thresholds for observational studies that are even more stringent, typically 3.0 or 4.0 (Taubes & Mann, 1995). Using a rule-of-thumb that effect estimates above even 2.0 are at the threshold of true excess risk, none of the mortality studies approach that level.

Additionally, effect estimates in mortality studies are biased to an unknown extent and direction due to ever-pervasive exposure measurement error and exposure misclassification (i.e., classic instrumentation measurement error, assignment of ecologic ambient values to individuals, and not taking indoor versus outdoor time into account). This typically biases the effect estimates towards the null, although this is not a certainty. Despite these concerns, there are indications that weak effects might occur for respiratory/cardiopulmonary mortality, as the results from the few stratified analyses make sense (e.g., older people are more susceptible than younger people). The observed gender differences are also expected, as men typically spend more time outdoors than women and are therefore more highly exposed to ozone. Ambient ozone measurements would thus be more indicative of actual individual-level exposure for men than for women. While such measurements may be *relatively* more accurate for men, a low correlation between the monitoring station concentrations and residential concentrations still exists. As a result, a threat to validity remains, even in men. Despite results that make sense for a particular sub-group, such findings seem not to be reliable for entire populations.

The field of mortality papers is comprised of studies mainly of the 'semi-individual' cohort design, i.e., individual data are collected on some individual-level confounders, but ozone exposure was measured at the city level along with other ecological factors such as weather. Cause of death information vacillated between individual and ecologic measurement. This design offers the potential for controlling individual-level confounding, but cross-city analyses are still susceptible to bias and confounding. Cities differ not only with respect to ozone concentrations; they also tend to differ on a constellation of factors that share the same distribution as the pollutant (Elliott and Savitz, 2008) and contribute to the same health outcome being measured (Jerrett and Finkelstein, 2005). This constitutes a phenomenon known as compositional clustering from contextual variables, a form of confounding. Location may, then, simply be a surrogate marker for several exposures or risk factors--not just ozone--contributing to mortality. Unfortunately, many potential confounders of this genre are unmeasured. Empirical estimates of the risks imposed by contextual effects center around odds ratios of 2.0, a much stronger effect than that observed for specific constituents in most air pollution studies (Jerrett and Finkelstein, 2005), including these. Given the low RRs in this field of studies, confounding is the most likely explanation for the observed statistical associations.

Jerrett et al. (2009) provided the only concentration-response function for mortality (namely, respiratory), and a threshold somewhere between 60-70 ppb is compatible with the data. The analyses from other studies did not explore non-linearity, resulting in effect estimates based solely on the assumption of straight lines in which the slope is largely determined by one or two data points representing higher effect estimates at the highest concentrations, i.e., influential data points. Consequently, we have only the Jerrett et al. (2009) paper on which to visualize at what ozone concentration the effects are observed and, again, just for respiratory mortality. The endeavor of setting an AQTV based on mortality is, accordingly, challenging.

In summary, the quality of the evidence to evaluate the association between chronic exposure to ozone and mortality is highly unreliable, and information to support an objectively-based AQTV is lacking.

Table 1. Key studies of the effects of chronic ozone exposure on mortality

Study/Design	Study Population	Cause of death	Association?	Effect estimate	Ozone metric/conc	Co-pollutants in model(s)	Confounding control	Exposure Assessment	Study Reliability	Comments
Abbey et al (1999) Cohort study, 1977-1992	Seventh-Day Adventists (AHSMOG Study) N = 6,338	All-cause: M	Yes	1.09 (0.95-1.25)	Mean O ₃ conc (ppb) across locations: 26.1; range: 43.9; IQR: 12	None	Good for individual risk factors, e.g., smoking, but not updated except for location (residence & work); no ecologic control	Area monitors linked to zip code centroids; no adjustment for time spent in/outdoors	Low	* Non-malignant respiratory mortality. RRs per 12 ppb O ₃ increase. Effects also reported for hrs per year in excess of 100 ppb (IQR = 551 h/yr); RRs ~2x higher for lung cancer; gender differences still noted across all causes of death. Used zip code centroids vs census tracts (a weakness).
		All-cause: F	No	0.95 (0.85-1.06)						
		Cardio-pul: M	Yes	1.08 (0.91-1.29)						
		Cardio-pul: F	No	0.97 (0.84-1.12)						
		Respir*: M	Yes	1.12 (0.85-1.47)						
		Respir*: F	Yes	1.05 (0.82-1.35)						
Chen (2005) Cohort study, 1976-2000	AHSMOG Study Cohort N = 3,239	Lung ca: M	Yes	2.10 (0.99-4.44)	Mean O ₃ conc 26.2 (+/- 7.3) ppb	None	Good for individual factors; limited for ecological factors	Area monitors linked to zip code centroids; no adjustment for time spent in/outdoors	Low	Ozone also modeled as a PM _{2.5} co-exposure, strengthening the effect estimate for PM _{2.5} . Reportedly, the first study to observe gender differences in cardiopulmonary mortality. Used zip code centroids vs census tracts (a weakness).
		Lung ca: F	No	0.77 (0.37-1.61)						
		CHD: Male	No	0.89 (0.60-1.30)						
		CHD:Female	No	0.97 (0.68-1.38)						
Dockery (1993) Cohort study, 1975-1989	6 U.S. cities N = 8,111; range: 1,239-1,631	All natural causes	No	*	Range city avg ambient O ₃ : 27.6-28.0	None	Excellent for individ factors but lacking for cross-city confounders	Area monitors (ecological) w/in cities; no in/outdoor adjustment	Low	* No effect estimate since no concentration-response linearity. Ozone exposure contrast/range very narrow, rendered analysis practically useless.

Study/Design	Study Population	Cause of death	Association?	Effect estimate	Ozone metric/conc	Co-pollutants in model(s)	Confounding control	Exposure Assessment	Study Reliability	Comments
Jerrett et al (2009) Cohort study, 1982-2000	American Cancer Society (ACS) volunteers, friends, family N = 450,000	Any cause	No	0.99 (0.98-1.00)	Range, city avgs Apr-Sep: 33-104 ppb	PM _{2.5}	Limited: Individ, ecol, residence data not updated; PM _{2.5} data limited in terms of yrs	Area/city monitors (ecological); no in/outdoor adjustment	Fair	Results shown are from 2-pollutant model with PM _{2.5} per 10 ppb increase in ozone. * denotes effects (also) found in single pollutant model. Only 2 yrs of PM _{2.5} data vs 23 yrs for ozone. Exposure misclassification potential high. Cohort may not be representative of overall U.S. population. C-R for resp mortality appear to be somewhere between 60-70 ppb.
		Cardio-pulmon	No*	0.99 (0.98-1.00)						
		Cardiovascular	No*	0.98 (0.97-0.99)						
		Ischemic HD	No*	0.97 (0.96-0.99)						
		Respiratory	Yes*	1.04 (1.01-1.07)						
Krewski et al (2009) Cohort study, 1982-2000	ACS volunteers, friends, family N = 1.2 M	All-cause (nat)	Yes*	1.014 (1.006-1.023)	MSA average, summer: 22.9 ppb	None	Inconsistent: ambient temp missing but good geospatial; many data elements old	Area monitors (ecological) w/in counties; no in/outdoor adjustment	Fair	* Based on ZCA model with ecologic covariates; point estimates from MSA model were lower ** Based on model with no geospatial parameters or ecologic covariates Cohort not representative of overall US population. High O ₃ -PM correlation; difficult to separate effects.
		Cardio-pulmonary	Yes*	1.027 (1.014-1.040)						
		Ischemic HD	Yes**	1.01 (0.99-1.02)						

Study/ Design	Study Population	Cause of death	Association?	Effect estimate	Ozone metric/ conc	Co-pollutants in model(s)	Confounding control	Exposure Assessment	Study Reliability	Comments
Lipfert et al (2002) Serial cross-sectional study - 5 periods during 1960-1997	U.S. population	All natural causes	Yes	See Comments	Peak O ₃ , each monitoring location	None	Limited: No individual-level data collected; ecological proxies for potential risk factors used instead	Area monitors (ecological) throughout the U.S.	Low	Effect estimation from attributable risks (incremental mortality risk associated with the presence of ozone and period, based on the mean concentration for the largest datasets for each pollutant and period). Difficult to interpret. Results vary by age group and period. Statistically significant increased risk between 3%-9% for persons >age 65.
Lipfert et al (2006a) Cohort study, 1976-2001	U.S. military veterans with hypertension N = 75,000	All natural causes	Yes	1.030 (0.911-1.166)*	Peak range: 140 ppb in '76-'81 to 84 ppb in '97-'01	Traffic density, PM _{2.5}	Co-exposures only	Area monitors (ecological) w/in counties; no in/outdoor adjustment	Fair	Traffic density was focus. * Point estimate from 3-pollutant model (ozone, [log]traffic density, PM _{2.5}), counties with NO ₂ monitoring only; ozone-only model RR = 1.035. RR higher for all-county model. RRs per IQR = 38 ppb. Methods poorly described.
Lipfert et al (2006b) Cohort study, 1997-2001	U.S. military veterans with hypertension N = 75,000	All natural causes	No	With elemental C: 1.005 With Fe: 1.006	Peak O ₃	Several: ozone paired with other pollutants	Limited: individ biometrics appear old; fair for ecol factors	Area monitors (ecological) w/in counties; no in/outdoor adjustment	Unclear	Effect estimates represent maximum fractional changes in mortality that might be expected as a result of extreme controls measures, i.e., "achievable" effect. Weaker and non-significant effects in models with other co-pollutants, e.g., traffic density, PM _{2.5} .

Study/ Design	Study Population	Cause of death	Association?	Effect estimate	Ozone metric/ conc	Co-pollutants in model(s)	Confounding control	Exposure Assessment	Study Reliability	Comments
Lipsett et al (2011) Cohort study, 1997-2005	California Teachers N = 124,614	All-cause	No	0.97 (0.94-1.01)*	Monthly avg ambient levels, all-year & summer-only Range: 25-83 ppb Mean = 48.1 ppb	None	Good	Static monitors with assigned radial coverage area; pollutant surface concentrations from interpolations**	Fair	Effect estimates are hazard ratios from Cox proportional hazard models; no test of model assumptions. Insufficient follow-up time to assess lung cancer risk. * Results based on summer-only model which had slightly higher effect estimates than the full-year model; HR per IQR increase = 23 ppb **Monthly avg concentrations at residences developed with inverse distance-weighted interpolation. O ₃ concentrations higher than in most of the U.S.
		Cardiovascular	Possibly	1.02 (0.96-1.07)*						
		Non-malignant respiratory	Yes	1.09 (0.97-1.21)*						
		Lung cancer	No	0.95 (0.82-1.10)*						
		Ischemic HD	Yes	1.09 (1.01-1.19)*						
		Cerebrovascular	No	0.99 (0.88-1.10)*						
Medina-Ramon & Schwartz (2007) Case-crossover study, 1989-2000	48 U.S. cities N = 2.7M deaths	All-cause* All ages	Yes**	0.65% (0.38-0.93%)	Median daily summer O ₃ level; range: 6-60 ppb	None	Good	Area monitors, averaged within cities	Fair	* Natural causes only. ** Effects = Percent change in ozone-related mortality per 10 ppb increase. Use of administrative data introduces misclassification of underlying & contributing causes of death.
		All-cause Age >65 yrs	Yes**	1.10% (0.44-1.77%)						

Study/Design	Study Population	Cause of death	Association?	Effect estimate	Ozone metric/conc	Co-pollutants in model(s)	Confounding control	Exposure Assessment	Study Reliability	Comments
Pope et al (2002) Cohort study, 1982-1998	ACS volunteers, friends, family N = 1.2 M	All-cause	Yes	~1.06	Daily 1-hr max; 3rd quarter and full-year	None	Limited: indiv data on health behaviors, residential location not updated; enhanced spatial controls	Area monitors, location-specific	Low	Associations estimated from Figure 5 and are based on 3 rd quarter (Jul-Sep) readings; full- year associations were weaker. Only cardiopulmonary approached statistical significance. Exposure misclassification potential high. Biased sampling. Study strength: enhanced control of spatial correlation; however, the effects of these controls not shown for ozone. Study focus was on fine particulates.
		Cardio-pulmon	Yes	~1.10						
		Lung cancer	No	~0.95						
		All other causes	No	~1.00						
Wang et al (2008) Ecological study, 1996-2004	Brisbane, Australia N (avg) = 900,000	Cardio- respiratory	No*	0.999 (0.986-1.012)*	Range, avg O ₃ : 26.6-34.0 ppb	SO ₂ NO ₂	Good for ecologic factors; individual-level control via surrogates	Area monitors (ecological); no in/outdoor adjustment	Fair	* Results from multi-pollutant model with other gases. Ozone- only model RR = 1.002 (0.989-1.015) per 1 ppb O ₃ increase. Indications that surrogates for indiv confounders were competent.
		COPD	Yes	1.07 (1.05-1.10)	Range, May- Sep city avgs: 15-71 ppb	None	Limited: Ecological/ inter-city only.	Area/city monitors (ecological); no in/outdoor adjustment	Fair	Effect estimates are for May-Sept; lower (1.02-1.04) in spring/fall; based on 5 ppb increment, not 10 ppb or IQR. No sampling bias, but high potential exposure measurement error / misclassification. Used admin data. Cohort not representative of general population.
Zanobetti & Schwartz (2011) Cohort study, 1985-2006	U.S. Medicare cohort (65+ yrs) w/prediisposin g conditions N = 8,895	Chronic heart fail	Yes	1.06 (1.03-1.08)						
		Myocard Infarct	Yes	1.09 (1.06-1.12)						
		Diabetes	Yes	1.07 (1.05-1.10)						

3.2 Morbidity Studies

We identified 9 key studies that evaluated the association between long-term exposure to ozone and respiratory morbidity (see Table 2). Six of these studies focused on the association between ozone exposure and childhood asthma, with five showing a positive statistical association. These studies included only disease-free children at the beginning of each study, thus their results assess the potential for triggering or causing asthma, not exacerbating or aggravating preexisting asthma.

Strength of these positive associations ranged from weak to low-moderate, with the higher end of that spectrum being typified by imprecision that manifested as wide confidence intervals that conferred low reliability. Four studies in this group (McConnell et al., 1999; McConnell et al., 2002; Millstein et al., 2004; Peters et al., 1999) relied upon parent-completed questionnaires to document a doctor's diagnosis during the study period, i.e., 'doctor-told' to parents, a mixture of incidence (first diagnosis) or prevalence (history of asthma) either during the study period or ever. The effect estimates from these questionnaire-based studies were notably higher than the two studies that used objective data (Islam et al., 2007; Lin et al., 2008). This suggests potential recall bias in which parents of asthmatic children living in perceived or known high-ozone areas may have been more prone to report a doctor-told diagnosis (actual or speculated) than parents living in areas with lower ozone concentrations.

McConnell et al. (2002) assessed the joint effects of exercise and ozone, finding that ozone increased the risk of doctor-told asthma in children, but only in communities with high concentrations of ozone. Two of the six asthma studies (Islam et al., 2007; Lin et al., 2008) used objective data such as hospital discharge diagnoses, the latter showing weak associations and the former showing no correlation. Both had limited control for potential confounders.

Two studies (Millstein et al., 2004; Peters et al., 1999) assessed wheezing via parent questionnaires, generating the most impressive (but highly imprecise) effect estimates, relative risks of 2.87 and 1.30 respectively. Since one of the symptoms of childhood asthma is wheezing, these findings add some degree of credibility to the results discussed above.

One of the 9 morbidity studies (Karr et al., 2007) assessed acute infant bronchiolitis based on hospital discharge data and found no association. Like asthma, this illness is also characterized by wheezing. Given that asthma diagnosis is difficult in children less than 2 years old, bronchiolitis may be an early and competent predictor for development of asthma later in childhood. In a recent longitudinal cohort study (Lin and Lin, 2012) in Taiwan, children less than 2 years of age with bronchiolitis were 13.6 (95% CI 8.9-20.7) times more likely to develop asthma within 2 years than a control group of children without bronchiolitis. The Karr et al. (2007) study included the appropriate age group of children, i.e., those who developed bronchiolitis within their first year of life. Those findings from objective medical data detract from an ozone - asthma causal hypothesis.

The majority of asthma-related studies reported positive statistical associations (Table 2). However, the overall reliability of this group of studies to make a reasoned causal statement

is low. The potential for exposure measurement error and misclassification is high given the well-documented poor correlation between individual exposures and readings from area monitors. Adding to the inaccuracy is the array of potential confounders either not included or only partially accounted for, both pre- and post-natal. The field of asthma studies had numerous covariates in their models, resulting in fair to moderate control for confounding in general. But, the list of suspected or known triggers of asthma is vast (and growing), and no single study could possibly control for all potential confounders, e.g., genetics, indoor and outdoor multi-source aeroallergens, weather, indoor/outdoor activity patterns, environmental tobacco smoke, pollutant co-exposures. As a result, no study's results are particularly reliable.

Tzivian (2011) reviewed the then-most recent articles ($n = 25$) published on outdoor air pollution and asthma, concluding that outdoor pollutants affect the appearance and exacerbation of asthma in children, but this was not a causal indictment of ozone or any other pollutant. This review (Tzivian, 2011) suggested that the complexity of studying asthma—particularly dealing with confounding factors—precludes a causal inference from the field of studies reviewed as well as from previous reviews that Tzivian examined. Tzivian's final conclusion was "Although these findings are of great interest, the limitations of noted works make future investigations of the effect of air pollution on asthma in children essential."

In other morbidity studies, (Peters et al. (1999) studied bronchitis incidence among school-age children, finding a weak and statistically insignificant association with ambient ozone. Lipsett et al. (2011) examined adulthood chronic disease morbidity among over 124,000 California teachers. Incidence of both myocardial infarctions and strokes were weakly associated with ambient ozone, even at the high concentrations in that area of the country. These effect estimates were also based on an interquartile range (IQR) of 23 ppb, a significantly larger increment than that of most ozone studies. As a result, there is negligible support for a hypothesis that long-term ambient ozone levels cause these particular chronic diseases.

These studies are summarized in Table 2 along with the reliability indicators for each study. A critical review of each study appears in Annex 1.

Intrinsic Quality Evaluation of the Epidemiology Data

The overall reliability of these findings is questionable for all forms of morbidity examined. With one exception in which lung cancer was the focus (Beeson et al., 1998) the strength of the statistical associations was low. The relative risk in Beeson et al. (1998) was 3.56 for males, but the wide confidence interval impeded a cogent inference, i.e., the association/effect could have been either very weak or very strong. Associations were below 2.0 for the asthma studies based on objective medical/administrative data. Although higher for questionnaire-based asthma studies, the strength of those associations was still moderately low, ranging from 1.15 for ever asthma in Peters et al. (1999) to 2.35 for medication use in Millstein et al. (2004) in which the 95% confidence intervals around that effect estimate were extremely wide (0.92-6.05). Regarding the 2 wheezing studies, the Millstein et al. (2004) paper showed a relatively impressive relative risk of 2.87 but that

estimate was again too imprecise to impart reliability. Additionally, its cross-sectional design detracted from these findings. The remainder of the field of morbidity studies would be considered largely uninformative under the supposition that evidence for true risk begins to accrue at $OR > 2.0$ for observational studies, as many epidemiologists believe (Taubes & Mann, 1995).

A strong counter-argument against disregarding studies in which effect estimates are below some threshold is that, if these estimates are biased, then why is the bias consistently in the same direction—that is, away from the null (most studies had ORs greater than 1.0)? One could assert that such consistency supports the hypothesis that a true positive relationship exists between ozone exposure and disease risk. An additional argument could be made that the exposure misclassification errors present in these studies is most likely to be non-directional, which actually biases the effect estimate *towards* the null (i.e., the observed statistical associations are lower than they would be in the absence of the bias). Finally, a causal relation between ozone and some forms of respiratory morbidity, particularly asthma, seems biologically plausible. Despite these rational arguments equating this consistency with validation, the probability is low that the observed weak statistical effects represent the true effects of ozone given the likelihood that the effects of confounding and other biases could easily overwhelm the weak statistical associations. Another potential explanation for the consistently positive associations is publication bias, i.e., the preferential publishing of positive studies over studies with null findings. This form of bias is well established in the biomedical sciences, and no compelling reason exists for presuming that it failed to materialize here. Also, the possibility exists of a systematic directional bias associated with using stationary monitors to represent true individual exposure. Lastly, regarding asthma, positive associations may to some extent reflect exacerbation or aggravation of pre-existing morbidity rather than the *causal* trigger.

Setting an AQTV—finding the statistical ‘bright line’, a possible biological threshold—is not feasible without reliable concentration-response (C-R) analyses. Unfortunately, C-R functions are not available in this field of studies. Only one study (Lin et al., 2008) explored the potential for a non-linear function, in a semi-quantitative way. The default assumption in these studies was linearity, i.e., a specified incremental increase in ambient ozone increased risk by some estimated amount. Linear models do not facilitate C-R analyses unless non-linearity has been convincingly disproven. Consequently, this field of studies does not enable the determination of an AQTV.

In summary, evidence for or against causal relationships in the field of morbidity studies of long-term ambient ozone exposure is not persuasive. Attempts to fashion a scientifically defensible ambient ozone standard from this set of studies would be ineffectual, as the body of literature is unreliable. In addition, these studies *in toto* lack a concentration-response function critical to regulatory decisions.

Table 2. Key studies of the effects of chronic ozone exposure on respiratory morbidity

Study/ Design	Study Population	Health Endpoint	Association?	Effect estimate	Ozone metric/ conc	Co-exp in model(s)	Other Confound control	Exposure Assessment	Study Reliability	Comments
Beeson et al. (1998) Cohort study, 1977-1992	Non-smoking California adults N = 6,338	Lung cancer	Yes	Relative risk: 3.56 (1.35-9.4) M 0.94 (0.41-2 .16) F	Hours of O ₃ exposure in excess of 100 ppb (main analysis)	None	Good	Area monitors	Low	Low precision makes it difficult to gauge degree of increased risk in males. Primary metric: annual avg no. of hrs in excess of 100 ppb, selected because this filtered out lower background levels & showed strongest association with respiratory cancer in previous analyses; 3-yr lag pd imposed.
Islam et al. (2007) Cohort study, 1993-2001	Subset of cohort from Calif Children's Health Study (disease-free 4th-graders) N = 2,057	Asthma onset (physician-diagnosed)	No	Coefficient of correlation (R ²) = 0.08	Long-term (1994-2003) avg concentrations; approximate range: 30 ppb - 65 ppb	None	Limited	Air monitors; placed in communities specifically for this study	Low	Study aim was to assess effect modification by levels of pollutants on the protective effects of better lung function on new asthma incidence. Community-specific hazard ratios based on the 10th-90th percentile range of FEV ₂₅₋₇₅ . Small sample size limited precision & reliability.
Lin et al (2008) Cohort study, 1995-2000	1995-99 birth cohort from NY State; N = 1.2 M	Asthma incidence (1st hospitalization)	Yes	Odds ratios: 1.16 (F/U pd)* 1.22 (season)** 1.68 (>70ppb)***	Mean concentrations during follow-up, ozone season, and % of days >70 ppb	None	Limited: socio-demographics only	Static regional air monitors covering wide expanses	Low	* Mean O ₃ during follow-up, per 1 ppb incr; ** Mean O ₃ in season, per 1 ppb incr; *** Exceedence proportion (%) >70 ppb, per IQR increase. Maternal & post-birth smoking probably underreported. Potential for exp misclassification high.

Study/ Design	Study Population	Health Endpoint	Association?	Effect estimate	Ozone metric/ conc	Co-exp in model(s)	Other Confound control	Exposure Assessment	Study Reliability	Comments
Lipsett et al (2011) Cohort study, 1997-2005	California Teachers Study N = 124,614	Myocardial infarction incidence	Yes	Hazard ratio: 1.04 (0.96-1.12)	Monthly avg ambient concentrations, all-year and summer-only Range: 25.4-82.6 ppb; Mean = 48.1 ppb	None	Good	Static monitors with assigned radial coverage area; pollutant surface concentrations from interpolations	Fair	See Table 1 (Mortality) for details.
		Stroke incidence	Maybe	Hazard ratio: 1.02 (0.95-1.09)						
McConnell et al (1999) Cohort study, 1977-1992	Seventh Day Adventists in CA; N = 3,091	Asthma (doctor-told, parent questionnaire)	Yes*	Relative risk: 2.09 (1.03-4.16)	Hours of O ₃ exposure in excess of 100 ppb (main analysis)	PM ₁₀ SO ₄ NO ₂ SO ₂	Limited: asthma risk factor data not collected	Static air monitors	Low	* Observed in males only; RR based on 27 ppb increase in O ₃ . RR = 4.44 & 4.01 in 2 highest O ₃ tertiles.
McConnell et al (2002) Cohort study, 1993-1998	S. Calif Children's Health Study; N = 3,535	Asthma (doctor-told, parent questionnaire)	Yes*	Joint effects noted; see Comments	Max 1-hr annual mean ¹⁰⁰⁰⁻¹⁸⁰⁰ (lo-hi: 40-60 ppb) 24-hr mean ¹⁰⁰⁰⁻¹⁸⁰⁰ (lo-hi: 25-39 ppb)	None	Good	Air monitors; placed in communities specifically for this study	Moderate	* Effects = O ₃ is effect modifier of exercise-asthma association, in high O ₃ communities only. Asthma risk alone not greater in high exposure communities; 2 of 3 O ₃ metric RRs <1.0, other was 1.1.
Millstein et al (2004) Cross-sectional study, 1995	Subset of cohort from Calif Children's Health Study N = 2,034	Asthma (Rx use, via questionnaire)	Yes*	2.35 (0.92-6.05) Mar-Aug	Monthly O ₃ mean ¹⁰⁰⁰⁻¹⁸⁰⁰	None	Good	Air monitors, placed in study communities	Low	* ORs per IQR = 27.8 ppb. Effects = asthma Rx use or wheezing within most recent 12 months (via parent questionnaire). Weaker Rx effects Sep-Feb (OR=1.31). Wheeze effects greatest when spent outdoors > median, OR = 3.07 (1.61-5.86).
		Wheeze (via questionnaire)	Yes*	2.87 (0.65-12.6) Mar-Aug						

Study/Design	Study Population	Health Endpoint	Association?	Effect estimate	Ozone metric/conc	Co-exp in model(s)	Other Confound control	Exposure Assessment	Study Reliability	Comments
Karr et al (2007) Case-control study (nested), 1995-2000	Infants born in 1995-2000, S. Coast Calif Air Basin N = 18.5K cases, 169K controls	Bronchiolitis, acute (from hospital discharge data)	No	1.00*	Mean of daily 8-hr max avg from birth; Range: 2-96 ppb; Mean: 23 ppb	PM _{2.5} CO NO ₂	Good	Static air monitors; residences manually assigned to most relevant monitor	Moderate	*Results for chronic exposure model which adjusted for PM _{2.5} , CO, and NO ₂ . Subchronic model showed weaker effects. ORs based on 14 ppb increment in O ₃ . Post-birth outpatient dx/tx not included in study dataset.
Peters et al (1999) Cohort study	Cohort of school-children, grades 4, 7 or 10 in 1993 from 12 communities N = 3,676	Ever asthma*	Yes**	1.15 (0.91-1.44)*	Avg daily 1-hr max Range: 36-98 ppb Mean: 64.5 ppb (24-h avg O ₃ also measured; mean = 32 ppb)	None	Good	Static air monitors	Low	* Doctor-told/diagnosed as reported via parent questionnaire ** Effects observed in males only; based on 1986-1990 ambient measurements; ORs based on 40 ppb increment. ORs based on 1994 data essentially the same.
		Current asthma*	No	0.95 (0.70-1.29)						
		Bronchitis*	Yes	1.18 (0.85-1.54)						
		Cough*	No	0.98 (0.82-1.17)						
		Wheeze*	Yes*	1.30 (0.89-1.89)*						

3.3 PULMONARY FUNCTION

We identified nine key studies that evaluated the association between long-term exposure to ozone and various indicators of pulmonary function. These studies are summarized in Table 3 along with quality categories for each study. A critical review of each study appears in Annex 1.

Most of these observational studies do not report a positive association between ozone and various indicators of pulmonary function. (See Table 3). While the respiratory function endpoints under study are objectively and, presumably, accurately measured, exposure assessment and classification/ assignment were still ecologically based. Some of the exposure estimates were based on spatial interpolations, which at least provide a residential-level approximation of ozone concentrations. Individual-level exposures were not estimated for the most part. Only 4 of the 9 studies above accounted for time spent indoors vs outdoors, and 3 of those found effects. However, those studies did not control for pollutant co-exposures. The weight of the evidence is inconclusive on ozone effects on respiratory function in the general population, but there is an indication that ozone reduces respiratory function in asthmatics, people with smaller airways, and those who spend appreciable time outdoors.

Two studies showing apparently definitive effects with good control for confounding (Tager et al., 2005; Peters et al., 2006) were cross-sectional in design in which the time-ordering events (exposure --> outcome) cannot be confirmed. Tager et al. (2006) also demonstrated qualitative effect modification by gender. The authors explored this finding but incompletely documented their analysis in the paper. Peters et al. (2006) main analysis reported effects for females only, but respiratory function decrements were found in both sexes when the analysis was restricted to children who spent considerable time outdoors. The remainder of the studies essentially reported no effects from ambient ozone exposure.

Intrinsic Quality Evaluation of the Epidemiology Data

Overall, this field of studies is moderately reliable but the findings were inconsistent, sometimes generating more questions than answers. Regarding positive findings (respiratory function decrement), even if those results were statistically significant, clinical judgment is necessary to assess the clinical relevance of the decrement. On weight, these studies of respiratory function do not support a causal inference between ambient levels of ozone and diminished respiratory function.

Table 3. Key studies of the effects of chronic ozone exposure on respiratory function.

Study/Design	Study Population	Measured Parameter(s)	Effects?	Effect estimate	Ozone metric/ conc	Co-exp in model(s)	Confound control, other	Exposure Assessment	Study Reliability	Comments
Forbes et al (2009) Cross-sectional study, 1996, 1996, 1997, & 2001	General English population age 16+ N = 57,000	FEV ₁	Inconclusive	-10 ml (-41 - 61 ml)	Annual average O ₃ concentration ~102 ppb	PM ₁₀ , NO ₂ , SO ₂ (as interaction terms)	Fair overall; no individual data for asthma hx or in/outdoor exp	Monitoring stations, dispersion modeling	Low	Different results between random effects model (shown here) and fixed effects model which indicated a 22 ml improvement in FEV ₁ . Cross-sectional design not strong; time-ordering of events uncertain. Adverse events not measured. Participation high (92%).
Gauderman et al (2000) Cohort study, 1993-1998	3 cohorts of California children, grades 4,7, or 10 in 1993 N = 3,025	FEV ₁ FVC MMEF FEF	No	C-R function: R = -0.13; p = 0.64	Annual avg O ₃ 1000-1800 Range: 20-68 ppb	PM ₁₀ PM _{2.5} PM _{10-2.5} NO ₂ inorganic acid	Good	Air monitors placed in cohort neighborhoods	Moderate	C-R function for each pollutant assumes other pollutants held at site-specific mean levels during study pd. Effect estimate from linear C-R model, which was essentially flat. Outcome measured: annual community FEV ₁ growth rate vs mean concentrations over study pd. No negative effects noted in the other parameters (all had + coefficients). Outdoor exposure accounted for.
Gauderman et al (2002) Cohort study, 1996-2000	Cohort of California children, 4th grade in 1996 N = 2,081	FEV ₁ FVC MMEF MMEF/FVC PEFR	No No No No Yes*	-1.21 (-2.06, -0.36)	Annual avg O ₃ 1000-1800	None	Good	Air monitors placed in cohort neighborhoods	Moderate	* Effects also observed in outdoor-exposed group for FVC as well as for PEFR; estimates per 37 ppb increase in O ₃ and exposure contrast between least and most polluted community. C-R for FEV ₁ & MMEF growth rates had negative slopes but poor model fit.

Study/Design	Study Population	Measured Parameter(s)	Effects?	Effect estimate	Ozone metric/ conc	Co-exp in model(s)	Confound control, other	Exposure Assessment	Study Reliability	Comments
Gauderman et al (2004) Cohort study, 1993-2001	Cohort of children, avg age 10 yrs, from 12 S. Calif communities N = 1,759	FEV ₁ FVC MMEF	No*	C-R function: R = 0.04; p = 0.89	Annual avg O ₃ 1000-1800; range: 25-65 ppb	PM _{2.5} PM ₁₀ NO ₂ Elemental C Organic C	Good overall, but no data on outdoor exposure	Air monitors placed in cohort neighborhood hoods	Moderate	* Statistically non-significant decrements seen for FVC and FEV ₁ ; highly imprecise estimates. FEV ₁ was primary parameter reported. Co-pollutants held at their constant level.
Gauderman et al (2007) Cohort study, 1993-1997	Cohort of children, avg age 10 yrs, from 12 S. Calif communities N = 3,677	FEV ₁ MMEF	No*	-13 mL	Annual avg O ₃ 1000-1800; range not given	PM _{2.5} PM ₁₀ NO ₂ Elemental C	Good overall, but no data on outdoor exposure	Dispersion model derived from single air monitor per community	Moderate	* No regional pollutant effect, but a statistically significant FEV ₁ decrement per 38 ppb O ₃ of -81 mL observed in children living <500 m from a local freeway. Models adjusted at mean concentration of other pollutants.
Ihorst et al (2004) Cohort study, 1994-97 (Aus) 1996-99 (Ger)	Cohort of school-children, grades 4,7, or 10 in 1993 from 15 study sites N = 2,153	FVC FEV ₁	No*	**FVC: -19.2 mL/100d **FEV ₁ : -18.5 mL/100d	30-min mean seasonal O ₃ concentrations; range: 22-54 ppb (summer), 4-36 ppb (winter)	NO ₂ SO ₂	Good overall, but no data on outdoor exposure	Fixed monitors placed in vicinity of study sites	Moderate	* Pertains to long-term effects; none were observed over the 3.5-yr follow-up. ** Effects expressed as lung growth (between measurements) for mean O ₃ summer exposure at 46-54 ppb vs 22-30 ppb, i.e., high exp group experienced less lung growth per 100-d period than the next lowest exp group. Suggestive of medium-term effects.

Study/Design	Study Population	Measured Parameter(s)	Effects?	Effect estimate	Ozone metric/ conc	Co-exp in model(s)	Confound control, other	Exposure Assessment	Study Reliability	Comments
Mortimer et al (2008) Cohort study (retro),	Cohort of asthmatic children in Calif.	FVC FEV ₁ PEF FEV₁/FVC FEF ₂₅₋₇₅ /FVC FEF ₂₅ FEF ₇₅	No*	* -0.025 * -0.034	Avg O ₃ 1000-1800 lifetime Avg ^{24-hr} age 0-3 yrs	CO	Very good; but no outdoor exposure data	Fixed monitors, data mapped to residences or zip code centroids	Low (for O ₃)	* Estimates are from an unreliable model (R ² = 0.13) and is of uncertain clinical significance, but are statistically significant. Ozone terms in models for other respiratory function parameters were statistically non-significant.
Peters et al (1999) Cross-sectional study	Cohort of school-children, grades 4, 7 or 10 in 1993 from 12 communities N = 3,293	PEFR FVC FEV ₁ MMEF	Yes - females only	* PEFR: -251 mL * MMEF: -125 mL	Peak O ₃ ; range not given	None	Good; qualitative data on in/out- door exposure	Not stated	Low	* Based on 1994 air monitoring data; decrement slightly greater than from 1986-1990 data. Secondary female analysis including co-pollutants indicated that O ₃ and PM ₁₀ contributed about equally to MMEF decrement. Statistically significant FVC & FEV ₁ decrement in asthmatic girls & in boys spending more time outdoors.
Tager et al (2005) Cross-sectional study	Freshman at UC-B, life-long residents of SF or LA (convenience sample) N = 255	FVC FEV ₁ FEF₂₅₋₇₅ FEF₇₅	Yes	See Comments	Monthly avg lifetime O ₃ 1000-1800 = 34 ppb; IQR 29-45 ppb	None	Excellent; data on time spent outdoors	Spatial interpolation based on static monitors = lifetime dose	Moderate	O ₃ effects on parameters in bold modified by airway diameter (FEF ₂₅₋₇₅ /FVC); decrements observed in 1st (smallest) vs 4th FEF ₂₅₋₇₅ /FVC quartile. Qualitative interaction (effect mod) in females. Adjusted R ² for models range: 0.57-0.67. Time-ordering of exposure-PFT good for a cross-sectional study.

4. Toxicology and Mechanistic Studies

We identified 6 key studies in rats or mice and 3 key studies in primates that examined long-term exposure to ozone and health effects. In a number of studies, exposure to ozone-only was included as a treatment control for the larger initiation/promotion study design. In general, the studies indicated that ozone is not tumorigenic but may be a contributing factor to lung remodelling. A summary of the results of these studies is found in Tables 4 and 5 along with the study reliability categories (i.e. Klimisch scores).

4.1 Studies in Rodents

In one of the earlier studies on toxicity of chronic ozone exposure (Hasset et al., 1985), exposed 6 week old female A/J mice under two paradigms. In paradigm 1, ozone only control mice were exposed to 0.31 ± 0.1 ppm ozone for 103 hours/week every other week for 6 consecutive months. Five months after final ozone exposure, the animals were sacrificed. For the second paradigm, animals were exposed to 0.50 ± 0.02 ppm ozone for 102 hours during the first week of each month for 6 consecutive months. Animals were sacrificed three months after the final ozone exposure. Both groups had independent control groups. After sacrifice each inflation fixed lung was divided into the 5 individual lobes and surface counted for tumor nodules. A chi squared analysis of results from paradigm 1 indicates that exposure to 0.31 ppm ozone under these exposure conditions results in an increase in lung tumors relative to the controls. For the second paradigm, animals exposed to 0.50 ppm ozone experienced an increase in lung tumor frequency which was statistically significant relative to controls. The A/J mouse strain has been developed as a sensitive animal strain and indicates that chronic ozone exposure has the potential to result in lung neoplasia. However, the exposure paradigms are of questionable relevance to low level chronic exposure since intermittent exposure by week to relatively high ozone was used.

A study conducted by Last et al., 1987 in male mice also utilised A/J mice as well as Swiss Webster mice. In this study, animals were examined for the impact of ozone with urethane exposure. Ozone control animals received a saline injection only and were exposed to 0.4 ppm or 0.8 ppm ozone. Animals were exposed 8 h/night, 7 nights/week for 18 weeks. For Swiss Webster mice, exposure to ozone had no effect on the tumor incidence relative to control. In A/J mice exposure to 0.4 ppm did not alter the tumor incidence; however exposure to 0.8 ppm resulted in a significantly higher incidence of lung tumors compared with control animals. Histopathological investigations of lungs from mice exposed to 0.4 ppm ozone exhibited mild to moderate bronchiolar epithelial hyperplasia. At 0.8 ppm lesions in mice included prominent peribronchiolar lymphoid nodules. Additionally mild to moderate infiltrate of macrophages and neutrophils was present in bronchioles and surrounding tissues. Larger airways were not affected and there was no difference between Swiss Webster and A/J mice. Lung hyperplasia is consistent with long term exposure to an oxidant air pollutant and may help explain the presence of tumors in A/J mice at high dose. One possibility is the oxidant injury changes the cell population, increasing the number of susceptible cells that can undergo spontaneous transformation. This is supported mechanistically since ozone injury to the respiratory tract is not linear or uniform. The ciliated cells found in the upper and lower airways are susceptible to damage resulting in focal lesions (Mustafa, 1990).

Ozone has been the subject of an investigation by the National Toxicology Program (1994). Portions of the work have also been published in peer reviewed journals, such as the one authored by Boorman et al., 1995. Ozone exposure was assessed in F344/N rats and B6C3F1 mice in both 2 year bioassays as well as lifetime studies. The two year bioassay in rats was conducted at concentrations 0, 0.12, 0.5 and 1.0 ppm. The concentration of 0.12 was used because it was the U.S. ozone standard when the study was conducted. Body weights of the 0.12 and 0.5 ppm rats were unchanged from controls while at 1.0 ppm there was a slight reduction relative to controls. Ozone-induced metaplasia in the nose and lungs was observed at 0.5 and 1.0 ppm as has been noted in other studies. However there was no increase in the incidence of neoplasms at any site, including the lung in either male or female rats exposed to ozone. In the lifetime study (125 weeks) only two concentrations were investigated (0.5 and 1.0 ppm) and similar metaplastic lesions were observed as were seen in the 2 year study. An additional finding in the lifetime study was the presence of inflammation (histiocytic infiltration) and interstitial fibrosis which was observed at both exposure concentrations. Inflammation and interstitial fibrosis was observed at both doses in the lifetime study. For the mice, the two year bioassay was also conducted at 0, 0.12, 0.5 and 1.0 ppm ozone. Survival rates were generally similar across all treatment concentrations, with the 1.0 ppm females exhibiting a greater survival rate than controls. This is of interest because 1.0 ppm had been selected as the maximum concentration compatible with long term survival. Concentrations of 4.0 – 10.0 ppm of ozone can cause mortality within hours of exposure (Mustafa and Cross, 1974). The body weights of males at 1.0 ppm were lower than controls while females at all treatment concentrations had lower body weights than control. Consistent with the rat portion of the study, and other studies, metaplasia was observed in the lungs and nose of mice exposed to 0.5 and 1.0 ppm ozone. There was a marginal increase in the adenomas or carcinomas of 0.5 and 1.0 ppm males (0 ppm 14/50, 0.12 ppm 13/50, 0.5 ppm 18/50 and 1.0 ppm 19/50) where increases were also seen at 1.0 ppm with the females (0 ppm 6/50, 0.12 ppm 7/50, 0.5 ppm 9/49, and 1.0 ppm 16/50). In the lifetime study (130 weeks) mice were exposed to 0.5 and 1.0 ppm ozone. The body weights at 1.0 ppm were lower than control animals. The incidence of alveolar/bronchiolar adenoma and carcinoma were marginally increased in exposed males and females. Summarising the findings from the NTP investigation into ozone, the authors concluded there was no evidence of carcinogenic activity in male or female rats. In male mice, equivocal evidence of activity was observed, and some evidence of carcinogenic activity was noted for female mice. There was, however, consistent metaplasia observed in the nose and lungs of animals exposed to ozone at concentrations of 0.5 and 1.0 ppm. There was no significant finding in the 2 year study at 0.12 ppm. This indicates there is likely a threshold dose below which toxicity is not observed.

In a follow up study to the National Toxicology Program (NTP) investigation into chronic ozone toxicity, which demonstrated no or equivocal association for most of the exposure groups, Witschi et al., 1999 investigated the impact of ozone exposure in the reportedly more sensitive A/J mouse strain. Like the NTP study, the concentrations selected were 0.12, 0.5, and 1.0 ppm ozone for 6 h/d, 5 d/w for 5 months, 9 months, or 5 months of ozone + 4 months filtered air recovery. Like the study by Hasset et al., 1985, female mice were used. Following sacrifice, fixed lungs were examined and tumor nodules on the lung surfaces were counted. Ozone exposure did not result in any deaths or any changes in body weight compared with controls. In the 1.0 ppm dose group exposed for 5 months there was an increase in tumor

multiplicity, but it was not statistically significant. The highest incidence of statistically significant lung tumors was observed in the mid dose group, thus there was no demonstrated dose response. In animals exposed to ozone for 9 months there was no increase in tumor multiplicity. For the animals exposed for 5 months and allowed to recover in filtered air for 4 months, the highest prevalence of lung tumors and tumor multiplicity was seen in the 0.12 ppm group and decreased with increasing dose, indicating a reverse dose response. Changes in lung morphology in any of the three groups as indicated by increased tissue volume in the septal tip, did not reach statistical significance due to the large standard deviations. Therefore, due to the lack of a dose response and the lack of disease progression with prolonged exposure brings the toxicological significance of the findings into question. The one measure of change in lung morphology, which did not reach statistical significance due to the large standard deviation, may have some biological relevance if further measures were examined. An earlier study by Witschi et al., 1993 used male Syrian golden hamsters to examine lung tumors. Animals were exposed to 0.8 ppm ozone 23 hours per day, 7 days a week for 6 months followed by 1 month of recovery in filtered air. No lung tumors were observed in ozone treated animals. The authors did note deep lung remodelling with bronchiolar alveolar ducts along with extensive bronchiolar epithelial hyperplasia. These findings indicate that ozone is not tumorigenic but is a contributing factor to lung remodelling.

Another study (Kim and Cho, 2009) investigating the carcinogenic potential of ozone was conducted in B6C3F1 mice. The study examined the effect of ozone alone or in combination with other toxicants. In the ozone alone portion of the study, male and female mice were exposed to 0.5 ppm ozone 6 hours per day, 5 days a week for 1 year in whole body inhalation exposure chambers. No significant changes were observed in body weights for ozone exposed animals, although a significant increase in relative kidney weight and absolute liver weight was observed as was a statistically significant decrease in relative testis weight. The biological significance of these findings with regard to ozone exposure is unclear. The authors also noticed metaplasia in the nose and lungs with extension of the squamous epithelium in the anterior portion of the nasal and bronchial regions. These findings are consistent with other studies utilising ozone at these doses, suggesting that ozone contributes to airway remodelling. There was no evidence of tumorigenesis in response to this exposure paradigm, although this strain of mouse did exhibit neoplastic lesions in the NTP study.

A more recent study by Chuang et al., 2009 looked, in part, at the effects of ozone exposure on a cardiovascular endpoint. One section of the study exposed male ApoE ^{-/-} mice to 8 weeks of cyclic ozone exposure (5 days of ozone and 2 days of filtered air) for 8 hours a day at a concentration of 0.5 ppm. The lack of a functional ApoE gene renders these mice unable to produce a key glycoprotein, apoE (apolipoprotein E), which is essential for the transport and metabolism of lipids. The mice are healthy when born, but have a markedly altered plasma lipid profile compared to normal mice, and rapidly develop atherosclerotic lesions. Following sacrifice, aortic slices were fixed then stained with Oil red-O and imaged under microscopy. Animals exposed to Oil red-O had a statistically significant increase in staining which indicates an increase in areas of aortic lesion. The findings of this study were conducted at an elevated level of ozone, not expected to be present at background levels. Although indicative of a cardiovascular effect, the findings from this mouse study should be viewed with caution because various factors have the potential to impact lesion formation in this mouse model (e.g. fat content of diet).

4.2 *Studies in Primates*

There have been other studies conducted in primates that have investigated the effects of ozone on lung development and remodelling. One study by Evans et al., 2003 examined the effects of ozone in infant rhesus monkeys. The study utilized filtered air, ozone, allergen, and ozone with allergen. For the purposes of this review, only the data concerning filtered air and ozone controls are used. Animals were exposed to 0.5 ppm for 5 days and then allowed to recover for 9 days. The cycle was then repeated 11 times resulting in 5 months of cyclic exposure. Animals began the exposures at 30 days of age and were 6 months old when exposures ended. The authors examined tracheal slices for changes in the Basement Membrane Zone (BMZ) which is the central structure of the epithelial-mesenchymal trophic unit. The development of the BMZ has been shown to develop postnatally in the rhesus monkey (Evans et al., 2002). During development collagen I, perlecan, fibroblast growth factor-2 (FGF-2) are found in the BMZ and in ozone exposed animals alterations in these levels are noted; namely the thickness of collagen 1 appeared thin and irregular, perlecan appeared to not be depleted or not incorporated, and FGF-2 appeared to be absent due to the lack of immunohistochemical staining. Since FGF-2 is stored in the BMZ by binding to perlecan and perlecan was selectively not incorporated into the airway epithelial BMZ, it appears ozone may target the airway cells that produce perlecan. The absence of FGF-2 presence and/or storage in the BMZ may help explain the altered lung development seen following ozone exposure.

Another study building on this work is one by Fanucchi et al., 2006, which examined the effects of cyclic ozone exposure in infant rhesus monkeys. Animals were exposed to 0.5 ppm for 5 days and then allowed to recover for 9 days. The cycle was then repeated 11 times resulting in 5 months of cyclic exposure. Animals began the exposures at 30 days of age and were 6 months old when exposures ended. Airway branches were counted until alveolar outpocketing was observed. In exposed animals the alveolar region began after 10 branches while for control animals outpocketing was evident after 13-14 branches. This did not appear to affect the overall lung development or relative lung weight. Other findings due to ozone exposure were reductions in the size of the distal airways and alteration in smooth muscle bundle orientation. Exposure to ozone during postnatal lung development resulted in a marked increase in baseline airway resistance (two-fold), which may be due in part to the decreased diameter of distal airways and altered smooth muscle alignment. The authors postulate that alterations of the basement membrane during ozone exposure could lead to the changes they observed. The validity of these findings to ambient chronic ozone exposure are questionable since the mechanism of action is likely due to the oxidant nature of ozone and at lower levels, the antioxidant capacity of the lung may not be overwhelmed and similar damage may not be observed.

Infant rhesus monkeys were also used to investigate the chronic effects of ozone (Carey et al., 2007). Infant (90 day and 180 day old) rhesus macaques were used as an animal model for toxic effects in children due to the gross and microscopic similarities in the nasal airways. Acute (5 days at 0.5 ppm) and episodic (5 biweekly cycles of 9 days Filtered Air and 5 days at 0.5 ppm Ozone) exposures were utilized. Three dimensional magnetic resonance imaging (MRI) was used to create epithelial maps to evaluate damage from ozone exposure used in conjunction with traditional histopathological evaluations. The principal nasal lesions noted

by the authors were neutrophilic rhinitis, along with necrosis and exfoliation of the epithelium lining the anterior maxilloturbinate. These findings were present in both the acute and the episodic exposures. The MRI images helped confirm that there is site-specificity nasal injury damage following ozone exposure. These findings are consistent with other findings of epithelial damage following exposure to this concentration of ozone.

Table 4. Key studies of the effects of chronic ozone exposure on respiratory function

Study	Species	Doses/route	Duration	Endpoint	N(L)OAEI	Study Reliability
NTP, 1994 Boorman et al. 1994	Fischer 344 Rats B6C3F1 Mice	Rats/Mice: 0, 0.12, 0.5, 1.0 ppm ozone; whole body inhalation Rats/Mice: 0, 0.5, 1.0 ppm ozone ; whole body inhalation	6hrs/day; 5 days/week;2 year (104 weeks); 6hrs/day; 5 days/week; lifetime (125-130 weeks)	2 year/lifetime Rats: goblet cell hyperplasia and squamous metaplasia in the nose; squamous metaplasia in the larynx; and metaplasia and interstitial fibrosis in the lung 2 year/lifetime Mice: hyperplasia and squamous metaplasia in the nose and inflammation and metaplasia of the lung	Rats: LOEL 0.12 ppm Mice: NOEL 0.12 ppm	High; Klimisch 1
Hassett et al., 1985	A/J Mice (Females Only)	Paradigm 1: 0, 0.31±0.1 ppm ozone; whole body inhalation Paradigm 2: 0, 0.50±0.02 ppm ozone; whole body inhalation	103 hrs/wk every other week for 6 months (5 month recovery) 102 hrs/wk (1 st week of month only) for 6 months (3 month recovery)	Increased number of pulmonary adenomas Increased frequency of pulmonary adenomas	LOEL: 0.31±0.1 ppm LOEL: 0.50±0.02 ppm	Medium; Klimisch 2
Last et al., 1987	A/J Mice Swiss Webster Mice	0, 0.4, 0.8 ppm ozone; whole body inhalation 0, 0.4, 0.8 ppm ozone; whole body inhalation	8hrs/night for 18 weeks 8 hrs/night for 18 weeks	Increased incidence and multiplicity of lung tumors (including both Clara cell tumors and type II alveolar cell tumors); peribronchiolar lymphoid nodules No increase in lung tumors observed	NOEL: 0.4 ppm	Medium; Klimisch 2

Study	Species	Doses/route	Duration	Endpoint	N(L)OAEI	Study Reliability
Witschi et al., 1999	A/J Mice	0, 0.12, 0.5, 1.0 ppm ozone; whole body inhalation	6hrs/day; 5 days/week; 5 or 9 months, 5 months with 4 month recovery	Lung tumor incidence (mainly alveolar/bronchiolar adenomas) increased in mid dose only in 9 month exposure group Lung tumor incidence increased in low dose only, recovery group.	Undetermined	Medium; Klimisch 2
Witschi et al., 1993	Syrian Golden Hamsters	0, 0.8 ppm ozone; whole body inhalation	23 hours/day; 7 days/week 6 months with 1 month recovery	No significant increase in lung neoplasms observed	NOEL: 0.8 ppm	Medium; Klimisch 2
Kim and Choo, 2009	B6C3F1 Mice	0, 0.5 ppm ozone; whole body inhalation	6hrs/day; 5 days/week; 1 year	Significant increase in male absolute liver weight and relative kidney and testis weight; significant increase in female absolute liver weight and relative lung, kidney, adrenal, and ovary weights; no increase incidence of lung neoplasms observed	LOEL: 0.5ppm	Medium; Klimisch 2

Table 5. Key primate studies of the effects of chronic ozone exposure on respiratory function

Study	Species	Doses/route	Duration	Endpoint	N(L)D(A)EL	Study Reliability
Evans et al., 2003	California Regional Primate Research Center colony-born infant (30 days old) rhesus macaques (<i>Macaca mulatta</i>)	0, 0.5 ppm ozone	8 hours/day, 5 days with 9 days of recovery repeated 11 times	Altered incorporation of perlecan into the basement membrane zone (BMZ) resulting in atypical development of the BMZ.	LOEL 0.5 ppm	Medium; Klimisch 2
Fanucchi et al., 2006	California Regional Primate Research Center colony-born infant (30 days old) rhesus macaques (<i>Macaca mulatta</i>)	0, 0.5 ppm ozone	8 hours/day, 5 days with 9 days of recovery repeated 11 times	Fewer nonalveolarized airway generations, hyperplastic bronchiolar epithelium, and altered smooth muscle bundle orientation in terminal and respiratory bronchiole	LOEL: 0.5 ppm	Medium; Klimisch 2
Carey et al., 2007	California Regional Primate Research Center colony-born infant (90 day and 180 days old) rhesus macaques (<i>Macaca mulatta</i>)	0, 0.5 ppm ozone	8 hours/day, 5 days or 5 days with 9 days of recovery repeated 5 times	Neutrophilic rhinitis, necrosis and exfoliation of the epithelium lining the anterior maxilloturbinate observed with both exposure regimens	LOEL: 0.5 ppm	Medium; Klimisch 2

4.3 Data Evaluation Considerations

Evaluating the carcinogenic potential of chronic ozone toxicity from the available studies is challenging. Positive findings in the US National Toxicology Program with female B6C3F1 mice was not supported in the 1 year study by Kim and Cho (2009). This may be due to the decreased length of exposure indicating that perhaps continuous, prolonged exposure is required for neoplastic lesions to occur. Hasset et al. (1985) documented tumorigenesis in the sensitive A/J strain (Hasset, Mustafa et al. 1985) at 0.3 and 0.5 ppm ozone that was not seen in the low dose group (0.4 ppm) by Last et al. (1987). Taken together this data indicates a lack of a dose response. However, the higher dose group (0.8ppm) used by Last et al. did demonstrate evidence of neoplastic lesions. The doses used in the NTP were also repeated by Witschi in the sensitive A/J mouse strain without evidence of tumors following 9 months of exposure or with a prolonged recovery period. In addition, tumors were neither observed in male Syrian golden hamsters after 7 months of ozone exposure nor were they observed in 2 year or lifetime bioassays in F344/N rats. The inability to consistently reproduce tumors within a mouse strain and the lack of evidence in a second species provides evidence that the carcinogenic potential of ozone should be considered to be negative to equivocal.

The identification of toxic endpoints following chronic exposure to ozone is often represented by the expression of metaplastic lesions of the nasal airways and the lung. In addition there have been developmental studies which examined the impact ozone has on the non-human primate infant lung. These findings have indicated that airway remodelling does indeed take place following chronic exposure; however, the biological significance of these findings is in question since the lung function does not appear to be altered by the changes in lung development. The metaplasia in the lungs is used to help explain one of the possible mechanisms of action by which ozone can progress to adenoma or carcinogenic lesions. It is hypothesized that there may be cell populations that are susceptible to ozone. Since there is an increase in cell number or potentially an increase in susceptible cell types, such as ciliated cells (Mustafa, 1990). As these cells slough from the bronchial epithelial they are rapidly replaced by non-ciliated cells which appear to be more resistant to oxidant injury. The combination of effects leading to higher cell turnover could increase the chances of a spontaneous mutation leading to a neoplastic lesion. However, ozone has also been shown to cause chromosome aberrations and DNA damage, which while not directly evidence of cancer, are indicators of carcinogenic potential.

One of the other problems encountered in assessing the ozone toxicity in animal studies is the lack of multiple and relevant concentrations that have been used in the chronic ozone studies. Most of the studies have utilised at 0.5 and 1.0 ppm ozone concentration in their investigation. This has contributed to a lack of data points on which to base an extrapolation to lower dose effects. Only the National Toxicology Program and the follow up study used a lower dose (0.12) which was the ozone standard at the time of the investigation. This concentration did not produce any significant metaplasia in either the nose or the lung. There was also no evidence of neoplastic lesions at this concentration. One plausible mechanistic explanation may involve antioxidant capacity. Ozone is one of the most powerful oxidants known with a redox potential of 2.076 V (Lide 1993). This makes ozone the 271st most reactive substance of the 289 substances having values more positive than that of the standard hydrogen electrode. The high oxidising potential of ozone makes it capable of

interacting with a variety of biological systems. As reviewed by Mustafa (Mustafa 1990), acute investigations of ozone toxicity indicate that the mechanisms can be grouped into six main mechanisms of action.

1. Formation of free radicals and reactive intermediates
2. Initiation of lipid peroxidation chain reactions
3. Oxidative loss of function groups and activities of biomolecule, including enzymes
4. Alteration of membrane permeability and functions
5. Induction of inflammation
6. Initiation of secondary processes

Although these mechanisms have been identified for acute toxicities, it is likely that similar mechanisms occur with chronic ozone exposure. The likely impact from long term exposure will depend on both the duration and the dose and based on the limited chronic ozone toxicity studies, it appears there is a threshold below which toxicity will not occur.

4.4 *Summary of Conclusions*

Toxicity assessments following chronic exposure to ozone have been studied by multiple investigators for the purposes of hazard identification. The most common concentrations used by investigators in the bioassays are 0.5 and 1.0 ppm ozone. In the U.S. National Toxicology Program investigation into chronic effects of ozone, the former ozone standard of 0.12 ppm was also used, which represents the lowest ozone concentration that has been used in chronic ozone toxicity assays. Many chronic ozone studies have investigated ozone as a single toxicant as well as its effect as a co-pollutant to determine if it has the potential to exacerbate the effects seen from other well understood toxicants. The majority of these studies indicate that co-exposure to ozone did not result in any additional toxicity, and in some cases was able to decrease the toxicity of the other compound. For the purposes of this toxicological review, however, the data from the ozone only controls was used to assess the toxicological impact of chronic ozone exposure and results from concomitant exposure was not considered.

Findings from the studies, considered in a weight of evidence approach, indicate that the hazards from chronic high concentration ozone exposure include airway damage and remodelling but are not convincing that ozone is an animal carcinogen. For the nasal airways, cuboidal cell metaplasia has been noted to extend into the anterior nasal passage while the bronchial epithelial cells have been noted to extend into the alveolar region. In addition to the hyperplasia, focal lesions have been noted with cell necrosis and thinning of basement membrane regions. During development, the effects of ozone exposure can be observed in altered lung development, with the authors noting little impact on physiologic lung function. In one publication, a portion of the study evaluated the cardiovascular effect of chronic ozone exposure by examining the atherosclerotic lesions in the aorta. Results indicate an increase in aortic plaque formation. The current EU Air Quality Limit Value of 120 $\mu\text{g}/\text{m}^3$ equivalent to 0.060 ppm (maximum daily 8-hour mean) and the WHO Air Quality Guideline of 100 $\mu\text{g}/\text{m}^3$ equivalent to 0.050 ppm (8-hour mean) are below the levels used in the chronic inhalation studies. This makes extrapolation of the results from studies conducted at higher concentrations difficult because of the potential to overwhelm the antioxidant capacity. Taken

together, the data indicate that chronic exposure to ozone at levels far above the existing air quality standards results in significant airway modifications which are of unknown biological impact at lower ambient levels. Since mortality is not observed at relatively high ozone levels in animal studies, the findings from the animal data do not support the hypothesis that current ambient levels of ozone cause chronic mortality.

5. Weight of Evidence Assessment

5.1 Chronic mortality and morbidity

The Bradford Hill (1965) “viewpoints”¹, or considerations, offer a framework from which to distinguish causal from non-causal associations that are themselves “perfectly clear-cut and beyond what we would care to attribute to the play of chance”. This restriction limits the application of the Hill postulates in this review since the observed statistical associations generally do not meet that basic criterion, i.e., they are too weak to be so definitive. In the few studies in which we have a semblance of statistical clarity, we can then try to answer Hill’s original question:

“In what circumstances can we pass from this observed *association* to a verdict of *causation*?” [original emphasis in (Hill 1965)].

Hill stated that no single set of “rules of evidence” exists regarding causation. Hill (1965) himself did not include consideration of confounding and other forms of bias which have obvious significance when assessing causality. Any contemporary extension of Hill’s viewpoints would certainly include bias, and our individual study critiques include such.

Assessing weight of evidence (WoE) across a *body* of studies solely against the Hill (1965) guidelines is difficult, in part, due to the presence of biases that cannot be reliably quantified. This issue is particularly challenging given the typically weak statistical associations reported in these studies which further increases the potential for any form of bias to engender these associations. Still, the Hill framework offers a starting point when assessing the reliability of individual studies included in this review. While it is not the purpose of this report to review the Hill (1965) guidelines in detail, a brief review is offered below.

- *Strength of the association* [Strong associations are less apt to be explained by some other factor than weaker associations, if all other aspects are considered equal. Strength of the association is usually expressed as a relative risk estimate when assessing etiology.]
- *Consistency of the association* [Causal inference is supported if the association is seen repeatedly in other populations under different investigators, different circumstances, or from a different form of inquiry.]
- *Specificity of the association* [Whether a suspected cause leads to a single effect rather than a myriad of effects; or, that an effect has one cause, not multiple causes]

¹ The term “viewpoints” comes directly from Hill’s paper. Due to researchers’ quest for definitive or prescriptive tenets over the ensuing years, Hill’s viewpoints have misguidedly morphed into “guidelines”, or even “criteria”, for causation.

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- *Temporality* [The cause of the disease must precede its effect. This is the only aspect that is “inarguable” (Rothman et al., 2008)]
 - *Biological gradient* [The presence of an increasing risk with increasing exposure]
 - *Plausibility* [i.e., the biological underpinnings of a potential cause-effect relationship]
 - *Coherence* [When a given cause-effect hypothesis does not conflict with the known natural history or biological underpinnings of the disease. Coherence is often assessed together with biological plausibility.]
 - *Experimental evidence* [This is sometimes interpreted as evidence from human or laboratory animal experiments, but it appears that Hill’s original intent was to indicate that the removal of the causal factor precipitated a subsequent decrease in disease incidence.]
 - *Analogy* [Analogous information from other well-established causal associations that can, at best, generate more elaborate hypotheses that are consistent with the association being evaluated.]

In our view, it is appropriate to consider the Hill framework in help guide a WoE assessment. However, we did not apply each individual criteria or group of criteria as necessary in order to arrive at an overall causal determination. The strength of association guideline remained central to our considerations regarding WoE, as the weak associations provided the opportunity for bias and confounding to generate potentially spurious associations. These biases typify practically all air pollution epidemiology studies and represent a substantial threat to their validity. A key question is this: *How strong, then, must the statistical association be before we are willing to accept that ozone—and not a confounder or measurement error—is etiologically responsible for a health outcome?* Before that question can be answered, several elements from Hill (1965) must be considered. It is noteworthy than in many of the available studies, the potential for various forms of PM to confound the ozone mortality association was either not evaluated at all or only partially measured. Therefore, the potential for PM to confound the ozone mortality association is substantial.

Consistency of association was also brought into our WoE assessment given that the effects of ozone on human health have been examined by different investigators using different study populations, but they predominantly used common study methods. Our premise is that consistency in results across studies provides good evidence for or against a causal relation. Considering that the results of the available studies on ozone mortality and morbidity are mixed with most studies showing a lack of a clear association, the results could be considered to be inconsistent. We also considered the distinct possibility that the modest degree of consistency was spawned by publication bias in which studies showing positive effects are preferentially published by journals. Under this assumption, unpublished studies/analyses showing no effects would have produced additional inconsistency, detracting from this Hill (1965) guideline.

We also considered the temporality aspect, as some studies’ exposure measurement periods were relatively more or less appropriate for the endpoint(s) under study. While no study reversed the exposure-outcome timeline *per se*, not all studies appropriately incorporated a lag period consistent with the natural history of the disease or cause of death under examination.

Concerning coherence and biological plausibility, it is biologically plausible that long term exposure to ozone, a highly reactive gas, could contribute to or cause respiratory morbidity or mortality. Some of the more relevant mechanisms of action by which ozone could produce such effects include initiation of lipid peroxidation chain reactions, oxidative loss of function groups and activities of biomolecules including enzymes, alteration of membrane permeability and functions, and induction of chronic inflammatory processes. However, based on this review of the repeated dose toxicology studies in animals, it does not seem biologically plausible that morbidity and mortality would occur at lower ozone concentrations such as the average levels that exist in ambient air. In long-term studies in rodents and primates, 500 ppb appears to be the threshold for the production of serious effects including hyperplasia in rats and lung remodeling in primates. This contrasts with average levels in ambient air, which generally range from 30-120 ppb. At the high end of this range, in rats exposed to 120 ppb for their lifetime, no significant effects, including no carcinogenicity, were observed.

On the whole, the weakness of the statistical associations in this field of epidemiology studies failed to provide a clear-cut starting point for assessing the WoE using the array of considerations in Hill (1965). As a result, the weight of the evidence rests more on the extent to which we believe those effect estimates are sufficiently reliable for setting air quality standards.

Concentration-Response

Concentration-response (C-R) functions are critical for standard-setting. Even assuming a set of reliable studies reporting health effects from some specified incremental (i.e., added) 'dose' of a pollutant, regulatory agencies need information on where to draw the line for health-based compliance. For the non-cancer endpoints, assuming linearity across the full array of exposure -- theoretically starting at 0 ppb and extending to infinity -- seems biologically implausible. This would suggest that exposure at any concentration is harmful and the relative risk of, say, an additional 10 ppb at the lowest ozone concentrations is the same as that same increase in exposure at the highest levels. Yet this is the functional model of C-R employed by the US Environmental Protection Agency (USEPA 2012). That particular C-R functional model also assumes that the level of risk in the population will be the same for any mix of air pollution that might coexist with a particular value of the single pollutant metric used in that C-R function (McClellan RO, Frampton MW, et al., 2009). Linear models, then, do not yield information that answers the critical question '*At what concentration do population health effects materialize given varying concentrations of co-pollutants?*' Non-linear C-R analyses are essentially missing from the field of human studies on chronic mortality and morbidity. However, the lack of serial C-R examinations may actually be a moot point given the exposure estimation issues that are discussed below. Finally, as pointed out by Brauer et al. (2002), ozone is a highly reactive gas. Levels of ozone indoors are generally much lower than those outdoors. The resulting poor correlation between ambient and personal exposure and resulting exposure misclassification (see below) blurs the ability to assess concentration response relationships and "thresholds" in epidemiology studies relying on ambient monitor measurements.

Exposure Estimation Errors and Misclassification Bias

Contributing heavily to the problem of synthesizing a valid effect measurement for ozone is that the determination of concentrations at the individual level—a mixture of indoor and outdoor exposures—is rarely accomplished. Ambient measurements from area monitors normally provide the exposure data in epidemiologic studies of air pollution, even though most people spend most of their time indoors. Only a small fraction of that ozone permeates indoor spaces due to the highly reactive properties of ozone with household surfaces. It is well known that indoor ozone exposure levels are typically 10%-30% that of outdoor values (Ozkaynak, 1999; Brown et al., 2009). However, few studies in this review collected indoor-outdoor data. The morbidity studies that did so generally found greater asthma risk in those participants who spent more time outdoors.

Epidemiologic studies are dependent on obtaining ambient pollution data from stationary monitors and attempting to use those data as a stand-in for personal (individual-level) exposure levels. However, several studies have shown that ambient monitor readings are not a reliable surrogate for, or index of, individual-level exposure (McClellan et al., 2009; Sarnat et al., 2006). As an example, Sarnat (2006) compared personal air samples to ambient monitor readings in Steubenville, Ohio and concluded that ambient measurements of gaseous pollutants have little ability to represent individual-level exposures. For ozone, the mean personal:ambient ratio was only 0.24 for combined summer and fall readings and approximately 0.17 for summertime readings. Similar findings were reported from studies in Boston and Baltimore (McClellan et al., 2009). Consequently, we have no reliable means of estimating actual exposures at the individual level from ambient monitors. While exposure modeling can often provide relatively reliable exposure estimates at the residential location, epidemiologists seek to assess exposures to people, not houses.

Relatively few epidemiologic studies calculate residential-level pollutant concentrations. While incorporating that particular unit of observation is ostensibly more precise than relying on area-wide readings, that method still cannot provide individual-level exposure estimates. Most individuals frequently move about during the day, from one location to another, and their indoor vs outdoor time (the latter with increased exposure opportunity) also varies. To obtain a time-weighted average of daily individual-level exposure, one cannot simply impose a universal ozone exposure attenuation coefficient based on some factoid (e.g., 'people spend 90% of their time indoors on the average'), since time spent indoors/outdoors can vary significantly between individuals. A 0.9 attenuation coefficient, as an example, would be accurate only for a small subset of the study population for which that truly applies, thus increasing the likelihood of exposure misclassification and its resulting bias on the effect estimate. The direction of the bias would depend on the proportion of the study population that, in reality, falls on either side of an assumed indoor exposure coefficient. This alone could easily be a primary source of both positive and negative statistical relations.

Confounding Bias

Bias is also generated by confounding which can also generate effects that are potentially greater than the effects of the pollutant. Confounding is present to some extent in nearly all observational epidemiologic studies, but air pollution studies are particularly prone. This largely occurs due to the pairing of health outcome data at the individual level with

area/ecologic data on the exposure to a pollutant. Two types of confounding can be present within a single study: (1) unmeasured confounders (i.e., factors which defy measurement or elements for which data were not available or were otherwise absent from an analysis); and (2) 'leftover', or residual, confounding which is often present due to imperfect measurement or estimation of factors that *are* in the analysis. By definition, confounders are statistically related to both the exposure and the health outcome under study. Due to the high prevalence of confounding in air pollution studies, some fraction—likely a significant part—of the reported weak associations is plausibly attributable to confounding variables. Relative risk (RR) estimates on the chronic effects of ambient ozone exposure for mortality are generally below 1.1 per additional increment (commonly 10 ppb) of exposure; RRs for morbidity are generally less than 2. For observational epidemiologic studies, RRs below 2.0 are considered weak effects and are difficult to interpret due to the potential magnitude of statistical noise produced by bias and confounding².

Minimally Reliable Relative Risk

Most scientists would argue against summarily dismissing all results below some rather arbitrary RR, e.g., 2.0. Most would also reason that each study has an unknown minimally reliable RR (MRRR) – a quantitative value independent of statistical certainty, i.e., sample size and probability values. Assuming the presence of confounding and other biases, an MRRR would obviously be equal to or greater than the investigator-reported RR in those studies showing weak positive associations (Nicolich and Gamble, 2011). Stated another way, an MRRR should arguably be the same order of magnitude or greater than the maximum risks associated with confounding and other biases. If the reported RR is smaller than the estimated biases, that RR is unreliable since a low probability exists for a causal association (Nicolich and Gamble, 2011). No single MRRR is applicable across all studies of a particular genre; each study requires separate examination.

Estimating an MRRR for each study in this review is impractical and out of scope for this project. Much of the information needed for such an endeavor is unavailable in most published papers due to page limits and lack of accessible ancillary data on which to attempt to quantify the various types of biases in each study situation. While assigning a single MRRR individually across studies is not defensible, a conservative and robust global MRRR threshold would still be helpful in assessing the reliability of the overall field of studies covered in this review. Lumley and Sheppard (2000) provided such for time-series studies (short-term exposure), stating that a MRRR for a causal association should be greater than about 1.05, as the excess statistical risk (5%) is actually produced by unmeasured factors or measurement inaccuracies, not the pollutant. In other words, those factors themselves generated a relative risk of 1.05 which indicates a relative risk estimate below this would be deemed unreliable. We can find no similar benchmark for chronic exposure, but many of the time-series biases exist in chronic studies as well. As the authors indicated, there is no objective way to quantify many confounders, so the above error factor of 1.05 is only a starting point.

² Some epidemiologists' threshold for reliability is well above 2.0 (see Taubes and Mann, 1995), but 2.0 will be used in this discussion to err on the conservative side.

Based on prior studies on PM_{2.5}, an additional error of 1.10—a plausible summary estimate of risk associated with that pollutant for various health/mortality endpoints—could easily apply to ozone studies that failed to include that important co-exposure (most of them). The total error factor would be $1.05 * 1.10 = 1.16$. Given a reported “uncorrected for error” effect estimate of, say, 1.03 (which is higher than most of the field of mortality studies), the adjusted effect estimate would be $1.03/1.16 = 0.89$. While ozone exposure is most certainly not ‘protective’, this adjusted estimate suggests that the investigator-reported excess risk is generated by factors other than ozone.

Another approach to WoE assessment comes from the legal domain. Consider the following question: *Given a hypothetical RR of 2.0 (which is higher than the vast majority of study-specific RRs), does logic suggest that 50% of a condition-specific mortality or morbidity within a specified study population can reliably be attributed to those ambient readings?*³ The answer is informed by the problems noted, starting with the knowledge that monitor-based ozone readings virtually prohibit valid estimates of individual-level effects. Those ambient readings, however, seem to be a marker for a set of risk factors statistically associated with those health outcomes given the number of studies showing positive associations between those readings and certain health outcomes. However, personal true ozone exposure seems not to be among that set of factors. If one accepts this argument alone, a MRRR below 2.0 would seem unlikely. Furthermore, considering the other limitations discussed above, e.g., confounding, it seems even less likely that a RR of 2.0 would stand up from a legal perspective.

Summary

The noise-to-signal ratio is unacceptably high to accept the recent long-term studies as sufficient evidence that ozone is a causal agent for morbidity and mortality, even under a limited set of conservative assumptions regarding bias and confounding. The findings from these studies are, however, suggestive, indicating that further study is warranted. However, to achieve a desired level of reliability through higher study-specific MRRRs, considerable methodological enhancements are needed in individual exposure estimation and in controlling confounding. Accordingly, the current field of chronic ozone studies is not sufficiently dependable for establishing ambient air quality standards.

5.2 Pulmonary Function

The weight of the evidence is inconclusive on the effects of ozone on respiratory function in the general population. However, the results of short-term single dose human clinical studies, which were not part of this evaluation of repeated, long-term exposure to ozone, provide an indication that near ambient levels of ozone produce transient changes in pulmonary function with a somewhat greater response in asthmatics and people with smaller airways. People who spend more time outdoors are at higher risk. The results from human clinical studies also provide much more accurate assessment of exposure and concentration response. While the responses in human clinical studies are much more definite, the findings in the epidemiology studies require replication before ascribing causal

³ In legal terms, a RR of 2.01 is the threshold for “more likely than not”, i.e., more than 50% of the risk being attributable to a particular agent.

inference to chronic exposure to ozone at 'normal' community ambient levels produces a population shift in pulmonary function. Even then, the key question is how valid are ambient readings from monitors, or modeled exposures at the front door, for quantifying individual-level exposure concentrations?

Annex 1

Critical Review of Individual Epidemiology Studies

Mortality Studies

CRITICAL REVIEW OF ABBEY (1999)

Abbey D, Knutsen SF, Beeson LW, et al

Long-term inhalable particles and other air pollutants related to mortality in nonsmokers

Am J Respir Crit Care Med 159:373-382

Description/Results

The purpose of this study was to assess the effect of long-term ambient inhalable particulate matter (PM₁₀) on various mortality endpoints. The study, from the AHSMOG (Seventh Day Adventist) cohort, also assessed the effects of ozone in a multi-pollutant context.

Findings: Long-term (1973-1992) ambient concentrations of PM₁₀ and other air pollutants--total suspended sulfates, sulfur dioxide, ozone (O₃), and nitrogen dioxide--were related to 1977-1992 mortality in a cohort of 6,338 nonsmokers. In both sexes, PM₁₀ showed a strong association with mortality for any mention of nonmalignant respiratory disease on the death certificate, adjusting for a wide range of potentially confounding factors, including occupational and indoor sources of air pollutants. The adjusted relative risk (RR) for this cause of death as associated with an interquartile range (IQR) difference of 43 d/yr when PM₁₀ exceeded 100 µ/m³ was 1.18 (95% confidence interval [CI]: 1.02, 1.36). In males, PM₁₀ showed a strong association with lung cancer deaths--RR for an IQR was 2.38 (95% CI: 1.42, 3.97). Ozone showed an even stronger association with lung cancer mortality for males with an RR of 4.19 (95% CI: 1.81, 9.69) for the IQR difference of 551 h/yr when O₃ exceeded 100 parts per billion. The statistical association in females was significantly weaker, an RR = 1.39 (not statistically significant). Ozone showed weak effects in males across all categories of mortality. Sulfur dioxide showed strong associations with lung cancer mortality for both sexes. Other pollutants showed weak or no association with mortality.

Analysis

This is a well-conducted study on the AHSMOG Seventh Day Adventist cohort that seems relatively unconfounded by the effects of tobacco (including environmental tobacco smoke), and many other lifestyle factors were accounted for, at least on face. However, given the SDAs proscriptions against smoking, there remains the possibility of underreporting of such behavior, potentially biasing the effect estimates to some extent. The statistical analyses were thoughtfully conducted, confirming that the time-dependent Cox proportional hazards model was in fact an appropriate analytical tool. The analysis for lung cancer used a 3-year lag period for exposure.

Assignment of exposure values acquired from fixed site monitors to individuals within a specified area is always suspect to some degree. Furthermore, the authors chose zip codes as a means of delineating an area covered by a fixed measurement station. Zip codes are irregular in shape, designed for logistics and thus aligned with major thoroughfares. These shapes are often incompatible with spatial analyses, unlike census tracts which are at least polygonal in shape and thus better suited for this type of analysis. Thus, the accuracy of the exposure assignments--already jeopardized to some degree by the use of static monitors--is further eroded by linking these monitoring station measurements to zip code centroids. Neither the magnitude nor the direction of the bias can be reliably estimated however.

This study suggests that ozone alone is relatively strongly associated with lung cancer in males. Given the elevated RR, it is doubtful that perfectly measured confounders would have altered this inference. However, the potential for exposure measurement error/misclassification is high and the results could have been influenced by this. However, neither the direction nor the quantification of this potential bias can be predicted.

CRITICAL REVIEW OF CHEN (2005)

Chen HL, Knutsen SF, Beeson LW, Abbey D, et al

The association between fatal coronary heart disease and ambient particulate air pollution: are females at greater risk?

Environ Health Perspect 13:1723-1729 (2005)

Description/Results

The purpose of this study was to assess the effect of long-term ambient particulate matter (PM) on risk of fatal coronary heart disease (CHD). The study also assessed the effects of ozone, both in a multi-pollutant context (the primary motive) and as a single pollutant. A cohort of 3,239 nonsmoking, non-Hispanic white adults was followed for 22 years. Monthly concentrations of ambient air pollutants were obtained from monitoring stations and interpolated to ZIP code centroids of work and residence locations. All participants had completed a detailed lifestyle questionnaire at baseline (1976), and follow-up information on environmental tobacco smoke and other personal sources of air pollution were available from four subsequent questionnaires from 1977 through 2000. Persons with prevalent CHD, stroke, or diabetes at baseline (1976) were excluded, and analyses were controlled for a number of potential confounders, including lifestyle.

Findings: In females, the relative risk (RR) for fatal CHD with each 10 μm^3 increase in $\text{PM}_{2.5}$ was 1.42 [95% confidence interval (CI), 1.06-1.90] in the single-pollutant model and 2.00 (95% CI, 1.51-2.64) in the two-pollutant model with O_3 ; no positive associations were observed for males. No associations were found in either sex for O_3 in the single-pollutant multivariate-adjusted model with the exception of the post-menopausal female subgroup in which the RR was 1.07 (0.73-1.59). A positive association with fatal CHD was found with all three PM fractions in females but not in males. The risk estimates were strengthened when adjusting for gaseous pollutants, especially O_3 , and were highest for $\text{PM}_{2.5}$.

Analysis

This is a well-conducted study on the AHSMOG Seventh Day Adventist cohort that seems relatively unconfounded by the effects of alcohol or tobacco (including environmental tobacco smoke), and many other lifestyle factors were accounted for, at least on face. However, given the SDAs proscriptions against those behaviors, there remains the possibility of underreporting of such behaviors, potentially biasing the effect estimates. The statistical analyses were thoughtfully conducted, confirming that the time-dependent Cox proportional hazards model was in fact an appropriate analytical tool.

Apparently, this is the only cohort study to have found sex-specific differences regarding cardiopulmonary mortality, and the authors make a strong case for biologic plausibility. They also speculate that the sex-specific differences could be the result of exposure measurement error, discussed below.

Assignment of exposure values acquired from fixed site monitors to individuals within a specified area is always suspect to some degree. Furthermore, the authors chose zip codes as a means of delineating an area covered by a fixed measurement station. Zip codes are irregular in shape, designed for logistics and thus aligned with major thoroughfares. These

shapes are often incompatible with spatial analyses, unlike census tracts which are at least polygonal in shape and thus better suited for this type of analysis. Thus, the accuracy of the exposure assignments--already jeopardized to some degree by the use of static monitors--is further eroded by linking these monitoring station measurements to zip code centroids. Neither the magnitude nor the direction of the bias can be reliably estimated however. Finally, $PM_{2.5}$ was estimated from airport visibility, temperature and humidity, not from direct measurements.

The authors also report that males were more likely to work >5 miles from their residence and thus may have spent more time in heavy traffic during those longer commutes, thus receiving more exposure than females. However, such data were not collected at the individual level, so this potential confounder could not be controlled for in the analysis.

This study suggests that ozone alone has no effect on CHD, but it enhances the detrimental effects of $PM_{2.5}$, at least in females. However, given the relatively weak statistical associations and the potential for exposure measurement error/misclassification, the results may be the result of this bias for which the direction cannot be predicted.

CRITICAL REVIEW OF DOCKERY (1993)

Dockery DW, Pope CA 3rd, Xu X, Spengler JD, Ware JH, Fay ME, Ferris BG Jr, Speizer FE.

An association between air pollution and mortality in six U.S. cities.

N Engl J Med. 1993 Dec 9;329(24):1753-9.

Description/Results

In this prospective cohort study, the authors estimated the effects of air pollution on mortality, while controlling for individual risk factors. Survival analysis, including Cox proportional-hazards regression modeling, was conducted with data from a 14-to-16-year mortality follow-up of 8,111 adults in six U.S. cities.

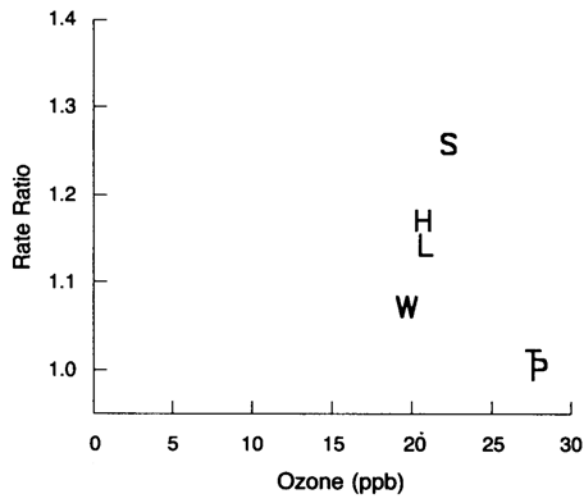
Results: Mortality rates were most strongly associated with cigarette smoking. After adjusting for smoking and other risk factors, statistically significant and robust associations between air pollution and mortality were observed. The adjusted mortality-rate ratio for the most polluted of the cities as compared with the least polluted was 1.26 (95 percent confidence interval, 1.08 to 1.47). Air pollution was positively associated with death from lung cancer and cardiopulmonary disease but not with death from other causes considered together. Mortality was most strongly associated with air pollution with fine particulates, including sulfates. Due to small differences in ozone concentrations among the six cities (range: 19.7-28.0 ppb), the statistical power to analyze ozone was extremely limited. However, the concentration-response function showed no indication of an upward trend in mortality rates with cities having higher levels of ozone. The authors concluded that while the effects of other, unmeasured risk factors could not be excluded with certainty, these results suggest that fine-particulate air pollution, or a more complex pollution mixture associated with fine particulate matter, contributes to excess mortality in certain U.S. cities.

Analysis

Despite the limited range of ambient ozone concentrations among the six cities that further complicated the determination of a concentration-response (C-R) function with just six data points, the C-R graphic (Figure 3, shown below) shows that the cities with the highest ozone concentrations (Topeka and Portage) had the lowest observed mortality rates. Topeka had an average ozone concentration of 27.6 ppb while Portage's average concentration was 28.0 ppb.

Many of the study's limitations (e.g., no consideration of population mobility, validation of the appropriateness of the proportional hazards model, inclusion of ecologic covariates) were addressed in the Health Effects Institute (2000) Reanalysis of the Harvard Six Cities Study and the American Cancer Society Study of Particulate Air Pollution and Mortality. Most of the attention was directed towards fine particles and sulfate rather than ozone, thus the results from the original study relative to ozone remain unchanged. This study does not suggest an effect of ozone on overall mortality.

Figure 3 from Dockery et al: Estimated adjusted mortality-rate ratios and pollution levels in the six cities.



CRITICAL REVIEW OF JERRETT (2009)

Jerrett M, Burnett RT, Pope CA III, Ito K, Thurston G, Krewski D, Shi Y, Calle E, Thun M |

Long-Term Ozone Exposure and Mortality

NEJM 2009;360:1085-95 (with Supplemental Material)

Description/Results

This study uses data from the American Cancer Society Prevention Study II (CPS II) cohort which enrolled volunteers in 1982 and 1983 to test their hypothesis that "ozone might have a primary effect on the risk of death from respiratory causes". Upon enrollment, subjects completed a confidential questionnaire that included questions on demographic characteristics (e.g., education) and health-related behaviors (e.g., smoking history, alcohol use, diet). Deaths within this cohort were ascertained through the year 2000 via conventional methods that were used during those time periods. A total of 448,850 study subjects contributed information and follow-up time to the study. Data on ecological risk factors (e.g., educational level, economic variables, race, meteorological data) at the metropolitan level were obtained from the 1980 U.S. Census.

Subjects' home addresses were linked with at least one air pollution monitor in their metropolitan area. Ozone exposure data were obtained for 1977-2000 from the EPA's AIRS on an hourly basis. The daily maximum value for the site was determined and used for the analysis, and these values were averaged over each quarter year for each monitor. Only the warm months (April-September) were included in the subsequent time-series analysis since that is when ozone concentrations are most elevated, and fewer data were available for the cooler seasons. PM_{2.5} data were also collected for 1999-2000 and included in the analyses for the purpose of differentiation of effects from those of ozone.

Study subjects were matched by age, sex, and race for the purposes of the analyses. Standard and multi-level random effects Cox proportional hazards models were used to assess the risk of death in relation to pollution exposures. A total of 20 variables/44 terms were used to control for individual characteristics; seven ecological covariates were also included in the models. Models were estimated for ozone and PM_{2.5} separately as well as together. Additionally, the models were adjusted for community-level random effects to take into account residual variation in mortality among communities. A formal analysis was done to assess whether a threshold existed in the concentration-response (C-R) function. The analysis also included an investigation of whether specific time windows were associated with health effects. This was done by matching exposures with each multi-year time period, then testing using a 10-year average exposure on the basis of the 5-year follow-up period along with the 5 years before the follow-up period.

RESULTS

Ambient ozone data were available from all 96 areas; PM_{2.5} data were available from 86 areas. Average ozone concentrations from 1997-2000 ranged from 33.3 ppb to 104.0 ppb with the seaboard areas typically generating the highest concentrations. There were 118,777 deaths occurring within the 18-yr follow-up period; 1.5% of the cohort died each year on average.

In single-pollutant models, ozone was significantly correlated with an increase in the risk of cardiopulmonary, cardiovascular, ischemic heart disease, and respiratory-caused mortality. Inclusion of PM_{2.5} attenuated the associations for endpoints other than respiratory causes, but the correlation with ozone created model instability for both pollutants. There was "limited evidence that a threshold model specification improved model fit as compared with a non-threshold linear model (P= 0.06).

The most relevant study findings are presented below, taken from the paper:

<i>Table 1. Extracts from Jerrett et al., Tables 3 & 4: RR (95% CI) of death attributable to a 10 ppb change in ambient ozone</i>				
	Single pollutant models		Two-pollutant models	
	Ozone (86 MSAs)	PM _{2.5} (86 MSAs)	Ozone (86 MSAs)	PM _{2.5} (86 MSAs)
Any cause	1.001 (0.996-1.007)	1.048 (1.024-1.071)	0.989 (0.981-0.996)	1.080 (1.048-1.113)
Cardiopulmonary	1.016 (1.008-1.024)	1.129 (1.094-1.071)	0.992 (0.982-1.003)	1.153 (1.104-1.204)
Respiratory	1.027 (1.007-1.046)	1.031 (0.955-1.113)	1.040 (1.013-1.067)	0.927 (0.836-1.029)
76% of subjects in these 3 strata	<i>Regional strata:</i>			
	NE: 0.99 (0.92-1.07)			
	IMW: 1.00 (0.91-1.09)			
	SE: 1.12 (1.05-1.19)			
	UMW: 1.14 (0.68-1.90)			
	NW: 1.06 (1.00-1.13)			
	SW: 1.21 (1.04-1.40)			

Table 1. Extracts from Jerrett et al., Tables 3 & 4: RR (95% CI) of death attributable to a 10 ppb change in ambient ozone				
	SCA: 1.01 (0.96-1.07) Temp (°C) strata: <23.3: 0.96 (NS) 23.3-25.3: 0.97 (NS) 25.4-28.7: 1.04 (NS) >28.7: 1.05			
Cardiovascular	1.014 (1.005-1.023)	1.150 (1.111-1.191)	0.983 (0.971-0.994)	1.206 (1.150-1.264)
Ischemic heart disease	1.017 (1.006-1.029)	1.211 (1.156-1.268)	0.973 (0.958-0.988)	1.306 (1.226-1.390)

NE. Northeast; IMW. Industrial mid-west; SE. Southeast; UMW. Upper mid-west; NW. Northwest; SW. Southwest; SCA. Southern California

NS. 95% confidence interval includes the null (1.00) value

Figure 2 from Jerrett et al: Exposure-Response Curve for the Relation between Exposure to Ozone and the Risk of Death from Respiratory Causes

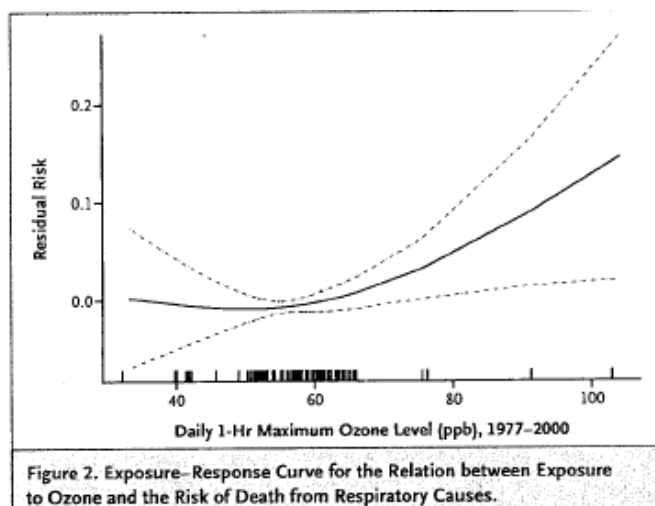


Table 3S from Jerrett et al: -2*log likelihood values based on the threshold concentration response model

Table 3S. -2*log likelihood values based on the threshold concentration response model.[‡]

Threshold Value (ppb)	Without Adjustment for Ecological Covariates	With Adjustment for Ecological Covariates
No Ozone	144,472.51	143,797.72
0	144,420.88	143,758.93
45	144,418.96	143,756.73
50	144,419.60	143,756.72
55	144,418.63 (p=0.134)	143,755.58 (p=0.067)
56*	144,418.39 (p=0.1146) (β=0.00390, se=0.00108)	143,755.39 (p=0.0600) (β=0.00432, se=0.00121)
57	144,418.77 (p=0.1416)	143,755.87(p=0.0802)
60	144,421.82	143,759.48
65	144,425.11	143,763.47

[‡]: -2*log likelihood values based on the threshold concentration response model for ozone concentrations measured from April to September, 1977-2000 in the ACS cohort with follow-up from 1982 to 2000, adjusted for 44 individual risk factors, baseline hazard function stratified by age (single year groupings), gender, and race, with or without adjustment for the ecological covariates. -2*log likelihood values for various threshold model specifications summarize how well the model fits the data. Lower values represent models with a better fit to the data than those models with higher values.

*: Smallest -2*log likelihood values, indicating the best fit to the data.

Analysis

STUDY DESIGN AND CONCEPTUAL ISSUES

This study recycled the same field of ecologic factors collected nearly 30 years ago. There are indications that the ACS study population (ACS volunteers, friends, family) are not particularly representative of the general U.S. population in the same 55-74 general age range. ACS Study participants died at an average rate of 4% each year during the follow-up period (data from CDC WONDER). The ACS cohort average yearly death rate was 1.5% during the follow-up period, meaning that the cohort lived substantially longer than the general population as a whole. Such a difference in mortality may have impacted the statistical findings, as the ACS cohort may be proportionately more 'alive but frail' than the general population. Thus the study design may, for this cohort, be measuring short-term effects of pollutants on a particularly susceptible segment of the population. This would contrast with the authors' intent to model the long-term effects of ozone.

Even if the effects were indeed from chronic ozone exposures, the study limitations discussed below bring into question whether the study measures the effects of air pollutants or effects from some factor correlated with those pollutants. For instance, ozone formation is a function of sunlight (correlated with heat, a risk factor for mortality) and traffic (NO_x), not measured in this study. Together the heat-traffic combination could produce psychological stresses over a lifetime or be a proxy for such. Additionally, the stresses may provoke coping behaviors (e.g., smoking, alcohol consumption) that contribute to mortality. While the magnitude of the effect of this example is unknown, even a small effect could impact the already small RRs reported in this study.

The authors try to explain the decreases in cardiopulmonary, cardiovascular, and ischemic heart disease risks in the combined ozone and PM_{2.5} model. When the effect measure estimates dip below the null (1.00), they are deemed not to be biologically plausible; however, the authors consider small increases in the effect estimate as being plausible. The results overall suffer from a lack of coherence, as it is biologically unlikely that chronic exposure to ozone along with PM_{2.5} decreases the risk of cardiopulmonary-related mortality while short-term joint exposures increases acute cardiopulmonary mortality, as shown in prior studies. It is likewise of questionable plausibility that, from prior studies, acute ozone exposure fails to show positive statistical associations with respiratory mortality while chronic exposures as reported in this study increase the risk of respiratory mortality.

Given the high degree of unexplained regional variability and demonstrated heterogeneity ($p = 0.05$) and the geographic/meteorological influence on air pollution, the combined risk estimates are inappropriate. Likewise, basing a national standard in the face of regional heterogeneity--without a plausible explanation--is likewise improper.

EXPOSURE MEASUREMENT AND MISCLASSIFICATION

Assigning individual ambient air pollutant exposures from ecological measurement inevitably introduces measurement error at the residential level. Added to that inaccuracy is the problem of deriving individual exposure even if the true ambient exposure at a particular residence were known. Ozone levels at the doorstep decrease precipitously after that gas

enters the indoors threshold, rapidly oxidized by contact with household surfaces. Scientific literature indicates that people spend between 70%-90% of their time indoors, thus ecologic ambient measurements cannot accurately depict actual ozone exposure. Many scientists assert that this type of measurement error, namely Berkson error^{*}, does not bias the effect estimate; however, recent simulations and assessments suggest otherwise (see Rhomberg et al., 2011). Like air pollution studies in general, this study has a combination of Berkson and Classical errors that cast doubt on the reliability of the findings.

Inherent exposure measurement notwithstanding, perhaps the most significant weakness of this important study is the high probability of exposure misclassification. While each instance may have biased the effect measure estimates just slightly, the cumulative impact of all misclassification occurrences on such small RRs could have been significant. Sources of exposure misclassification are discussed below.

The authors state that, while the RRs are modest, the risk of dying from a respiratory cause is more than 3 times as great in the metropolitan areas with the highest ozone concentrations vs those with the lowest concentrations. But they had no way of knowing if any study subject actually lived in a specified area for any specific length of time. Individual data, including residential addresses, were collected upon enrollment decades ago and were not subsequently updated.

The authors concede that not accounting for geographic mobility of the population during the follow-up period a study limitation but assert that any resulting bias would have attenuated the effects of ozone on mortality, i.e., the product of non-differential exposure misclassification. Assuming that the misclassification is indeed non-differential, the statistical effects of such cannot be assumed to be biased towards the null, based on recent simulations (Jurek, et al., 2008). In this study, however, such exposure misclassification may *not* have been non-differential, as individuals in this cohort may generally have had the inclination and means to move away from highest ozone areas to environs with less traffic and lower pollution levels. While an individual could have been living in an area with low ozone (or PM) concentrations for many years/decades before death, his/her residence upon enrollment--forever assigned to each individual in the cohort database--may have remained classified as highly exposed. The authors cite data indicating that only 3-4% of the general U.S. population relocates but the ACS cohort has unique socio-demographic characteristics that may increase or decrease residential mobility.

Again, due to the exclusive dependence on the 1980 Census for much of the study data, a person's originally assigned SES-related characteristic was permanent. A relatively high 'migration prevalence' within the ACS cohort, could have biased the risk estimate if individuals relocated to a higher SES zip code or metropolitan area but retained unhealthy behaviors/ lifestyles acquired in their original locales where such lifestyles were more commonly practiced. This scenario is not merely hypothetical, as it is not uncommon for individuals to flee the high traffic, high crime areas for more desirable and less polluted

^{*} Berkson error occurs when only a particular aspect of the true exposure is measured, as opposed to classical error that often occurs as a result of systematic exposure measurement errors, say, from imprecise monitor readings. Berkson-type errors are typified by the measured values having lower variability than the true values while for Classical errors, the variance of the true values is less than that of the measured values.

areas. Given the design of this study, this scenario would increase the likelihood of exposure misclassification and, thus, produced biased estimates.

CONFOUNDING

The effort to account for potential confounding effects of PM_{2.5} was not adequate. First, the authors included only 2 years of PM_{2.5} data, versus 23 years for ozone, and the PM_{2.5} data they did have was in the later time period of the study (1999-2000). There have been dramatic reductions in the ambient levels of PM during the period of the time for which ozone was evaluated, 1977-2000. They make the point that the PM_{2.5} risk estimates were likely underestimated due to use of only recent, and lower level, PM_{2.5} data. However, they fail to mention that for the same reason, the control for potential confounding by PM_{2.5}--which EPA states is known to cause cardiopulmonary mortality--is also under-controlled. Secondly, while the approach to assess ozone exposure focused on summer ozone when levels are higher, the exposure metric for PM_{2.5} was the *annual average*. It is well-known that in the summer, when ozone levels peak, the ambient levels of PM_{2.5} also rise significantly. Using the lower yearly average metric decreased the ability to account for potential confounding by PM_{2.5}. Third, the ozone exposure assessment was based on the *daily maximum values*, versus the yearly average PM_{2.5} values. Overall, this analytical approach maximized the effect measure estimation for ozone. Finally, SO₂, which significantly decreased the risk for ambient PM_{2.5} in the ACS data set to non-statistically significant levels, was not included in the statistical model.

Given that ozone data from 1977-2000 were used in the analysis, exposure values for cohort members who died during the period are based mostly to partly on data that were obtained *after* death had occurred. This was identified by the authors and dealt with by analyzing specific time periods. This analysis revealed no significant difference between the effects of earlier and later time windows which, on face, offers a degree of comfort that more current exposures are reflective of past exposures. However, PM_{2.5} data were far more sparse and could not be assessed by time period, leaving the time window analysis confounded. Additionally, models with PM_{2.5} added as a co-exposure were judged to be unstable due to both the strong correlation between the two pollutants and the sparse PM_{2.5} data issue.

The authors state that "measurement at central monitors probably represents population exposure to PM_{2.5} more accurately than it represents exposure to ozone". Additionally, they state that the ozone effects could have been confounded by the presence of PM_{2.5} because, in the presence of high density local traffic, the measurement error is probably higher for ozone exposure than for PM_{2.5} exposure. This speaks to model misspecification which casts doubt on the validity of the results from those models.

There is significant regional variability in the risk estimates, with 4/7 regions having a risk coefficient below null and the risks are clearly statistically significant in only 2/7 regions--the southwest and southeast. A trend towards higher risks in regions with higher temperatures is evident, with statistically meaningful differences between the lowest and highest temperature categories. While temperature seems to explain some of the regional heterogeneity in effect estimates, most of it remains unexplained, an argument against making a causal determination. Is temperature, an independent risk factor for mortality, not adequately controlled? When this kind of variability is observed for PM_{2.5}, the authors often

hypothesize that it is due to the "differences in PM chemistry." One cannot make the same hypothesis to explain away the variability for ozone in singularity.

THRESHOLD EFFECT ANALYSIS

Perhaps the most egregious 'intentional' bias in this study is the near black-out of the threshold effect analysis. The authors give it short shrift in the main paper: "There was limited evidence that a threshold model specification improved model fit compared with a non-threshold linear model ($P=0.06$)". Nothing more. They relegated the matter to the Supplemental Material (shown above) wherein they established a threshold estimate of 56 ppb based on minimizing the log-likelihood. However, the 95% confidence interval included 0 ppb, a value less than the minimum ozone concentration of 33 ppb in this study. Based on the p-value and 95% CI, the authors concluded here that "the threshold model is not clearly a better fit to the data ($p>0.05$) than a linear representation of the overall ozone-mortality association". The authors, however, provide no proof that the non-threshold model was in fact a better model. It is not clear how the authors chose a threshold estimate of 56 ppb based on minimizing the log-likelihood (should it rather be maximizing the log-likelihood the $-2*\log$ likelihood?). The authors also fail to explain how they derived the 95% confidence interval based on the log-likelihood of 0 to 60 ppb. While the threshold effect was statistically insignificant, it was at least consistent with the authors' exposure-response curve in Figure 2 (shown above). That is, a threshold of residual risk is apparent just below the 60 ppb ozone level, although most of the curve in that area appears not to produce clear results.

Also, the combination of Classical and Berkson errors has been demonstrated in simulations to obscure true concentration-response thresholds (Rhomberg et al., 2011).

SUMMARY

Overall, this study failed to confirm the authors' hypothesis that long-term ozone exposure causes the outcomes under study. Once again, an air pollution epidemiologic study has generated RRs in the 1-4% range, effect measures potentially overwhelmed by even a moderate degree of exposure measurement error and residual confounding. The effect measure estimates are regionally heterogeneous, seriously challenging the notion of presenting a nationwide effect estimate--and subsequently a national ambient air quality standard--from these data. Even in the absence of such heterogeneity, the population-to-individual ozone exposure quantification alone could easily be off by 50%, biasing the estimates in an unknown direction. Geographic mobility was systematically excluded from the analysis, another potential exposure measurement error. Confounding by $PM_{2.5}$ was inadequately controlled in the statistical models, and potential socio-demographic and behavioral confounders--individual or ecologic--were measured once, at the beginning of the study, and were thus likely to have changed over time. Finally, the authors' exploration of a possible threshold concentration-response model seemed inappropriately abbreviated. Accepting these RRs on face--without considering the overpowering methodological and statistical uncertainties accompanying the data--would be imprudent. Given the seemingly unreliable nature of the findings, they should not be used for setting public policy.

Jurek AM, Greenland S, Maldonado G. How far from non-differential does exposure or disease misclassification have to be to bias measures of association away from the null? *Int J Epidemiol* 2008; 37:382-5

Rhomberg LR, Chandalia JK, Long CM, Goodman JE. Measurement Error in Environmental Epidemiology and the Shape of Exposure-response Curves (in press - need citation)

CRITICAL REVIEW OF KREWSKI (2009)

Krewski D, Jerrett M, Burnett RT, et al

Extended follow-up and spatial analysis of the American Cancer Society study linking particulate air pollution and mortality

Res Rep Health Eff Inst. 2009 May;(140):5-114; discussion 115-36.

Description/Results

Krewski et al (2009) conducted an extended follow-up and spatial analysis of the American Cancer Society (ACS) Cancer Prevention Study II (CPS-II) cohort in order to further examine associations between long-term exposure to particulate air pollution and mortality in large U.S. cities. The current study sought to clarify outstanding scientific issues that arose from the earlier HEI-sponsored Reanalysis of the original ACS study data. Specifically, this reanalysis examined (1) how ecologic covariates at the community and neighborhood levels might confound and modify the air pollution-mortality association; (2) how spatial autocorrelation and multiple levels of data (e.g., individual and neighborhood) can be taken into account within the random effects Cox model; (3) how using land-use regression to refine measurements of air pollution exposure to the within-city (or intra-urban) scale might affect the size and significance of health effects in the Los Angeles and New York City regions; and (4) what exposure time windows may be most critical to the air pollution-mortality association. The 18 years of follow-up (extended from 7 years in the original study [Pope et al. 1995]) included vital status data for the CPS-II cohort (N ≈1.2 million) with multiple cause-of-death codes through December 31, 2000 and more recent exposure data from air pollution monitoring sites for the metropolitan areas. In the Nationwide Analysis, the influence of ecologic covariate data (such as education attainment, housing characteristics, and level of income; data obtained from the 1980 U.S. Census) on the air pollution-mortality association were examined at the zip code area (ZCA) scale, the metropolitan statistical area (MSA) scale, and by the difference between each ZCA value and the MSA value (DIFF).

Results: In contrast to previous analyses that did not directly include ecologic covariates at the ZCA scale, risk estimates increased when ecologic covariates were included at all scales. The ecologic covariates exerted their greatest effect on mortality from ischemic heart disease (IHD), which was also the health outcome most strongly related with exposure to PM_{2.5}, and SO₂, and the only outcome significantly associated with exposure to nitrogen dioxide.

The HRs and 95% CIs from this study that pertain to ozone concentrations during the summer of 1980 are shown in the table below. HRs represent the hazard associated with a 10 ppb increase in ambient ozone concentrations. Note that all models were adjusted for the 44 individual-level confounders recorded via questionnaire at the beginning of the study in 1982. In each of the ecologically adjusted models, the ozone HR decreased vs the original model without such adjustment.

Table from Krewski et al:

Hazard Ratios (95% CIs)		HRs (CIs) - Models with ecologic covariates		
Model without ecologic covariates		ZCA	MSA	MSA & DIFF
<i>Mortality category</i>				
All causes	1.016 (1.008-1.024)	1.014 (1.006-1.023)	1.006 (0.998-1.015)	1.008 (0.999-1.017)
Cardiopulmonary	1.028 (1.016-1.041)	1.027 (1.014-1.040)	1.015 (1.002-1.028)	1.016 (1.002-1.029)
IHD	1.01 (0.99-1.02)	Not reported	Not reported	Not reported

Intra-Urban Analyses were conducted for the New York City and Los Angeles regions. The results of the Los Angeles spatial analysis, with high exposure contrasts within that region, showed that air pollution-mortality risks were nearly 3 times greater than those reported from earlier analyses. PM_{2.5} was the pollutant of primary interest; ozone was treated as a co-pollutant. Controlling for ozone exposure (both peak daily and average concentrations) had negligible impact on the PM_{2.5} HRs for all causes, IHD, CPD, and lung cancer. The HR for ozone in the co-pollutant model were below 1.00.

Analysis

The most important contribution to the science that this detailed analysis provides is the enhancement to the Cox models that enabled the model to handle multiple levels of spatial clustering and autocorrelation that the added geospatial/ecological covariates possess. Despite the enhanced statistical methodology afforded by the modified Cox models, the HEI reviewers noted that population migration and ambient temperature were not assessed in this analysis. These covariates had been part of the Reanalysis and both had been shown to be determinants of mortality, with some evidence of confounding. Their exclusion in this Extended Analysis "amplifies the uncertainty due to possible residual confounding by ecologic covariates that were not included in the models" according to the reviewers. The impact of the new analytical methods on ozone could not be examined fully, as the analysis was focused on PM_{2.5}, not ozone. However, the analysis based on 1980 summertime data did not suggest a major geospatial/ecological statistical influence.

It should be noted that the summer of 1980 was particularly hot, increasing both the ozone concentrations and heat-related mortality in many of the cities. (See NOAA chart on the last page). The heat wave began in June when a strong high pressure ridge began to build in the central and southern United States allowing temperatures to soar to 90 °F (32 °C) almost every day from June to September. This phenomenon--uncontrolled for in the analysis--may have been statistically influential on the linear model's upward trajectory given the high positive correlation between ambient temperature, ozone concentrations, and heat-related mortality. (However, debate exists on the correlation between the effect modification of temperature on ozone-related effects). A high proportion of heat-related deaths likely appeared on the death certificates as "cardiopulmonary" as well as other causes, thus creating some of the statistical associations reported above.

Limitations aside, this extended analysis was expertly done; however, some key data elements are recycled from previous analyses of this cohort. The 44 individual-level covariates (some are potentially key confounders) are derived from the original 1982 questionnaire at enrollment. Some of these are time-dependent such as residential location, and this could have significant influence on the HRs given the weak statistical associations observed. Likewise, relying solely on the 1980 ozone data for directly assessing the effects of ozone over the succeeding years in the study undoubtedly introduces exposure misclassification that could have had a material impact on the HRs. The precision of the estimates, a function of the study's large sample size, should not be construed as a guarantor of reliability. The potential for many other measurement errors in this study is high.

As the Health Effects Institute Health Review Committee suggests, his cohort may not be representative of the general population, as the participants were friends and family members of ACS volunteers. Another study limitation articulated by the Committee is the study's reliance on EPA air pollution compliance monitors. Such data cannot reliably depict actual ambient exposures (i.e., at the front door), much less individual-level ozone exposures. However, the exposure estimation concern is attenuated in this Extended Analysis which used land use regression (LUR) modeling for the New York City and Los Angeles areas, an improvement over assigning exposure values based solely on static area monitoring stations. While the final PM_{2.5} LURs predicted 66% and 69% of the variation in monitor-based concentrations for NYC and LA, respectively, validation of the LUR for ozone was not reported. It is unlikely, however, that the LURs for ozone were as reliable as those for PM_{2.5}.

Regarding the causes of death, of particular concern is the cardiopulmonary category -- deaths from both cardiovascular and pulmonary/respiratory (not including lung cancer). This aggregation thwarts attempts to specify a causal model based on known or hypothesized mechanisms of action. This too is a threat to the study's validity.

On balance, the study's limitations and weaknesses seem to outweigh its strengths. Thus, the weak statistical associations could easily be an artifact of those limitations. Regarding ozone specifically, the series of re-/extended analyses focuses on PM_{2.5}, thus limiting its relevance on that pollutant.

CRITICAL REVIEW OF LIPFERT (2002)

Lipfert FW and Morris SC

Temporal and spatial relations between age specific mortality and ambient air quality in the United States: regression results for counties, 1960-97*Occup Environ Med.* 2002 Mar;59(3):156-74.**Description/Results**

This study investigated longitudinal and spatial relations between air pollution and age-specific mortality for United States counties (except Alaska) from 1960 to the end of 1997. The authors used cross-sectional regressions for five specific periods using published data on mortality, air quality, demography, climate, socioeconomic status, lifestyle, and diet. Outcome measures are statistical relations between air quality and county mortalities by age group for all causes of death, other than AIDS and trauma.

Results: A specific regression model was developed for each period and age group, using variables that were significant ($p < 0.05$), not substantially collinear, and had the authors' expected algebraic sign. Models were initially developed without the air pollution variables, which varied in spatial coverage. Residuals were then regressed in turn against current and previous air quality, and dose-response plots were constructed. The validity of this two-stage procedure was shown by comparing a subset of results with those obtained with single-stage models that included air quality (correlation=0.88). On the basis of attributable risks computed for overall mean concentrations, the strongest associations were found in the earlier periods, with attributable risks usually less than 5%. Stronger relations were found when mortality and air quality were measured in the same period and when the locations considered were limited to those of previous cohort studies. Thresholds were suggested at 100-130 $\mu\text{g}/\text{m}^3$ for mean total suspended particulate (TSP), 7-10 $\mu\text{g}/\text{m}^3$ for mean sulfate, 10-15 ppm for peak (95th percentile) CO, 20-40 ppb for mean SO₂. Contrary to expectations, associations were often stronger for the younger age groups (<65 y). Responses to PM, CO, and SO₂ declined over time; responses in elderly people to peak ozone increased over time as did responses to NO₂ for the younger age groups. These results generally agreed with previous prospective cohort and ecological studies for comparable periods, age groups, and pollutants, but they also suggest that the results of those previous studies may no longer be applicable. The authors concluded that spatially derived relations between air quality and mortality vary significantly by age group and period and may be sensitive to the locations included in the analysis.

Analysis

This study was largely a modeling exercise which generated attributable risks (ARs), not the standard relative risks (RR), associated with exposure to many air pollutants including ozone. ARs were used because of the need to compare risks of mortality by pollutant using a metric that is independent of the differing units of measurement. The way to interpret attributable risks in this paper is as such: the incremental mortality risk associated with the presence of a specified pollutant (and period), based on the mean concentration of each pollutant for the largest datasets for each pollutant and period. This is difficult to interpret, and the propriety of the authors' comparison of ARs to RRs generated from other studies is questionable. Also, there were several holes and "caveats" in the data coverage from the AIRS system. For example,

ozone annual averages refer to the annual average of the daily maxima, not based on all readings.

Ozone was the only pollutant for which the expectation of increased risk of mortality with increasing age actually materialized. This is biologically inconsistent with the concept that chronic effects would more likely result from cumulative lifetime exposures and, ozone was the only pollutant for which the AR did not decrease over the time periods included in this study.

Given the issues above, this study is not particularly useful for policy considerations despite the inclusion of many key potential confounders not typically included in air pollution studies.

CRITICAL REVIEW OF LIPFERT (2006a)

Lipfert FW, Wyzga RE, Baty JD, Miller JP

Traffic density as a surrogate measure of environmental exposures in studies of air pollution health effects: Long-term mortality in a cohort of US veterans*Atmospheric Environment* 40 (2006) 154-169**Description/Results**

In this paper, the authors claim that vehicular traffic is an ubiquitous source of air pollution in developed nations, yet relatively few epidemiology studies have considered its long-term health effects. This study addresses that information gap by using an areal measure of traffic density as a surrogate index of exposure to vehicular traffic. This study extends the mortality follow-up of the EPRI Veterans Cohort from 1996 (Lipfert et al., 2003) to 2001.

Results: The authors present associations between county-level traffic density (annual vehicle-km traveled/km²), ambient air quality, and mortality in a cohort of about 70,000 male US veterans (the Washington University-EPRI Veterans Cohort) who were enrolled in 1976 and followed through 2001. Traffic density was seen to be a significant and robust predictor of survival in this cohort (relative risk = 1.156 (95% CI: 1.067-1.253) in a single pollutant model), more so than ambient air quality, with the exception of ozone. In a single-pollutant model, the relative risk of mortality increased by 3.5% per a 38 ppb increase in peak ozone concentrations. In a 2-pollutant model with (log)traffic-density, the relative risk for ozone was slightly lower at 1.033. In a 3-pollutant which added PM_{2.5}, the relative risk was 1.030. None of the 3 effect estimates for ozone were statistically significant, but the lower bounds of those 95% CIs were 0.91 or 0.92. Stronger effects of traffic density was seen in the counties that have ambient air quality monitoring data, which also tend to have higher levels of traffic density. The proportional-hazard modeling results indicate only modest changes in traffic-related mortality risks over time, from 1976–2001, despite the decline in regulated tailpipe emissions per vehicle since the mid-1970s. The authors speculate that other environmental effects may be involved, such as particles from brake, tire, and road wear, traffic noise, psychological stress, and spatial gradients in socioeconomic status.

Analysis

The authors make a compelling argument in favor of their novel method for characterizing exposure to traffic as a function of traffic density over counties. Part of the calculation of traffic density requires dividing the total population within a designated area by the actual area in terms of geographic size, i.e., square miles. They hypothesize that an area-wide measure of traffic density maybe more representative of actual population exposures than a local highway flow rate, but they also recognize that the validity of an area-averaged density measure depends on the homogeneity of the area, including whether or not a particular area is partially vacant which would reduce the 'effective' area. To enhance the validity of this measurement, the authors suggest that it is advantageous to choosing the smallest possible geographic unit for analysis. Yet they chose counties as the unit of analysis which seem to threaten the validity of the study's findings since a county is typically a relatively large and heterogeneous entity. The CO analysis alone, which showed poor correlation with traffic density, suggests that this paper's complex methodology may not be reliable.

Furthermore, many of the data elements required for deriving traffic density were unavailable at the county level and several caveats exist for the data that were available. While these researchers were creative in stringing together disparate data--while making several assumptions--to stand in for actual county-level measurements, there is simply no practical way to validate their exposure data. While this study may have been successful as a proof-of-concept, it is not sufficiently reliable to use for standard-setting.

CRITICAL REVIEW OF LIPFERT (2006b)

Lipfert FW, Baty JD, Miller JP, Wyzga RE

PM_{2.5} constituents and related air quality variables as predictors of survival in a cohort of U.S. military veterans*Inhalation Toxicology* 18:645-657, 2006**Description/Results**

In this study, air quality data on trace metals, other constituents of PM_{2.5}, and criteria air pollutants were used to examine relationships with long-term mortality in a cohort of hypertensive male U.S. military veterans, along with data on vehicular traffic density (annual vehicle-miles traveled per unit of land area). The analysis used county-level environmental data for the period 1997-2002 and cohort mortality for 1997-2001. The proportional hazards model included individual data on age, race, smoking, body mass index, height, blood pressure, and selected interactions; contextual variables also controlled for climate, education, and income.

Results: Overall, excess mortality risks were seen in the northeastern U.S. where traffic densities are high, and in Southern California where aerosol nitrates are elevated. In single-pollutant models, traffic density appears to be the most important predictor of survival, but potential contributions are also seen for NO₂, NO₃⁻, elemental carbon, nickel, and vanadium. The effects of the other main constituents of PM_{2.5}, of crustal particles, and of peak levels of CO, O₃, or SO₂ appear to be less important. Traffic density is also consistently the most important environmental predictor in multiple-pollutant models, with combined relative risks up to about 1.2. The strongest "achievable effect" for any combination of pollutants was seen with peak ozone and traffic density (RR=1.25). The authors assert that, with these findings, it is not possible to discern which aspects of traffic (pollution, noise, stress) may be the most relevant to public health or whether an area-based predictor such as traffic density may have an inherent advantage over localized measures of ambient air quality. They speculate that traffic density could be a marker for unmeasured pollutants or for geographic gradients per se. The authors conclude that pending resolution of these issues--including replication in other cohorts--it will be difficult to formulate additional cost-effective pollution control strategies that are likely to benefit public health.

Analysis

As with another 2006 paper on traffic density and mortality, using counties as the unit of analysis for traffic density is suboptimal. Rather, smaller areas are preferable due to the typical heterogeneity within counties on a number of key factors ranging from the socio-demographic to weather. Given this limitation, the study's findings that portray traffic density as a better predictor of mortality than the other pollutants and PM_{2.5} species may not hold up as the science advances on this issue.

Ozone had a negative coefficient in a single-pollutant model, but showed a weakly positive association with mortality in multi-pollutant models, which generally comports with previous studies. However, ozone had a negative coefficient in the single-pollutant model. The multi-pollutant models, typically preferable in order to elicit the independent effect of a mix of pollutants, may not be the best means of comparison in this particular study. While this study was notable in that it used the EPA's Speciation Trends Network (STN) for PM_{2.5} constituents,

490 of the 627 STN sites do not monitor gaseous pollutants such as ozone. Thus, the counties contributing to the ozone analyses differ significantly from the counties used in the PM_{2.5} analyses, casting some doubt on the validity of the effect estimates.

This paper bases its effect estimates on the achievable level. This novel concept was developed as a means to compare the relative strengths of the air quality and traffic predictors, based on "maximum fractional changes in mortality that might be expected as a result of extreme control measures", given the wide range of typical concentration levels of the various air quality predictors that defied a fair comparison of effect. From the paper, it is difficult to discern whether or not the relative risks representing the achievable effect are truly comparable to the RRs resulting from the more standard type of analyses which generate risks associated with a specified incremental change in exposure (e.g., interquartile range, per 10 units of measurement). Thus, the relatively higher coefficients seen in this study vs those reported from previous studies may be an artifact of the authors' conceptualization of effect measurement.

As the authors state, exposure measurement errors are inevitable in air pollution epidemiologic studies. Such inaccuracy is particularly relevant to both the gaseous pollutants such as ozone and to traffic density. In each, considerable exposure heterogeneity exists within a specified area, enough perhaps to create a bias in the effect estimation in an unknown direction. Given the weak effects reported in this study, exposure measurement errors could have a material impact on the analytical results. Overall, this study offers new insights on PM_{2.5}- associated mortality in particular, but does not make a material contribution to the literature on ozone-related mortality.

CRITICAL REVIEW OF MEDINA-RAMÓN (2007)

Medina-Ramón M & Schwartz J

Who is more vulnerable to die from ozone air pollution?*Epidemiology* 2008;19:672-679**Description/Results**

This study was conducted to explore if certain subpopulations are more susceptible to death related to ozone. The two researchers conducted a case-only study in 48 US cities to identify subpopulations particularly vulnerable to ozone air pollution. Mortality and ozone data were obtained for the period 1989-2000 (May through September of each year) for 2,729,640 decedents. For each potential effect modifier, they fitted city-specific logistic regression models and pooled the results across all cities. Additionally, they examined differences in susceptibility factors according to several city characteristics using a meta-regression.

Results: For each 10 ppb increase in ozone (average of lags 0 to 2 days), people aged ≥ 65 years had a 1.10% (95% confidence interval = 0.44% - 1.77%) additional increase in mortality compared with younger ages. Other groups that were particularly susceptible were black people (additional 0.53% [0.19% - 0.87%]), women (additional 0.58% [0.18% - 0.98%]), and those with atrial fibrillation (additional 1.66% [0.03% - 3.32%]). Susceptibility factors had a larger effect in cities with lower ozone levels. For instance, the additional increase in ozone-related mortality for the elderly was 1.48% (0.81% to 2.15%) in a city with a mean ozone level of 42 ppb versus 0.45% (-0.27% - 1.19%) in a city with a level of 51 ppb. Differences in vulnerability were particularly marked in cities with lower ozone concentrations. The authors assert that they confirmed the susceptibility of the elderly to die of ambient ozone and identified other vulnerable subpopulations including women, blacks, and those with atrial fibrillation.

Analysis

This was a well-executed study that used a novel study design (case-crossover) in which each decedent served as his/her own control on days when 'he/she didn't die'. While there are variations of this type of design, these authors selected a scheme for selecting control days that has been demonstrated to provide the least biased effect estimates (Janes, Sheppard & Lumley, 2005). This study design eliminates much of the potential for confounding by individual-level factors. The authors chose the 0-2 lag period for their analyses because previous studies have shown that the effects of ozone are strongest over this initial cumulative 3-day period. Given that they chose this lag *a priori* (not from exploratory model runs), the study's findings are not attributable to multiple testing.

The susceptibility factors under study, except for being black, showed substantial heterogeneity in city-specific estimates. But generally speaking, "susceptibility factors had a more marked effect in cities with a low average ozone level". However, the difference between low (25th percentile) and high (75th percentile) in this study was only 9 ppb (51 ppb vs 42 ppb), so the reported effect differences seem high in comparison to the exposure contrast. Nevertheless, there are no apparent biases that threaten the reliability of these findings from ozone-only models. The exclusive use of single-pollutant models was a noted study limitation, as particulate matter exposure was uncontrolled in the analyses. The authors suggest, however, that this would not be a serious limitation unless concentrations of PM and ozone peaked

simultaneously and some of the subpopulations studied were particularly vulnerable to the effects of PM; this set of conditions would have generated overestimates. The authors assert that previous studies have shown that PM is not an important confounder of the ozone-mortality relation, so the above scenario does not seem likely.

Another limitation that the authors discuss is the use of administrative data from NCHS for case ascertainment, as the accuracy of such data is objectively speculative. They correctly identify the potential for misclassification of both the underlying cause of death and the contributing cause of death, and also point out that if these factors are indeed operating, they are not likely to vary with daily air pollution levels. Thus, this would not be a likely or significant source of bias.

Overall, this is a reliable study that seems relatively free from bias. While including co-pollutants would have made the results more convincing, this analysis clearly shows a statistical relation between ozone mortality and certain susceptibility factors.

CRITICAL REVIEW OF POPE (2002)

Pope CA III, Burnett RT, Thun MJ, Calle EE, Krewski D, Ito K, Thurston GD

Lung cancer, cardiopulmonary mortality, & long-term exposure to fine particulate air pollution

JAMA 2002;287:1132-1141

Description/Results

This study assessed the relationship between long-term exposure to fine particulate air pollution and all-cause, lung cancer, and cardiopulmonary mortality. Vital status and cause of death data were collected by the American Cancer Society as part of the Cancer Prevention II study, an ongoing prospective mortality study, which enrolled approximately 1.2 million adults in 1982. During enrollment in 1982, participants completed a questionnaire detailing individual risk factor data (age, sex, race, weight, height, smoking history, education, marital status, diet, alcohol consumption, and occupational exposures). The risk factor data for approximately 500,000 adults were linked with air pollution data for metropolitan areas throughout the United States and combined with vital status and cause of death data through December 31, 1998. The metric for ozone was daily 1-hour maximum readings, calculated separately for each full year (from 1982-1998) and for the 3rd quarter of each year. The statistical approach extending the standard Cox proportional hazards survival model by including a spatial random effects component intended to control for spatial autocorrelation (i.e., survival times of people living densely populated areas may be more similar than in people living in more sparsely populated areas). Additions to the original study (Pope et al., 1995): doubles follow-up time to more than 16 years; triples the number of deaths; includes gaseous copollutants; improved control of occupational exposures; includes dietary variables.

Results: Fine particulate and sulfur oxide--related pollution were associated with all-cause, lung cancer, and cardiopulmonary mortality. Each 10 $\mu\text{g}/\text{m}^3$ elevation in fine particulate air pollution was associated with approximately a 4%, 6%, and 8% increased risk of all-cause, cardiopulmonary, and lung cancer mortality, respectively. Measures of coarse particle fraction and total suspended particles were not consistently associated with mortality. Ozone showed a nearly statistically significant relative risk (RR) of approximately 1.1 for cardiopulmonary mortality, based on 3rd quarter maximum 1-hr readings. For full-year readings, the RR was approximately 1.08 with a wider 95% confidence interval. The authors concluded that long-term exposure to combustion-related fine particulate air pollution is an important environmental risk factor for cardiopulmonary and lung cancer mortality. They did not state a conclusion regarding ambient ozone.

Analysis

Perhaps the major weaknesses of this study and all other ACS-based studies is its semi-ecological design, with *ecological* exposure/pollutant data combined with *individual*-level data on health outcomes (e.g., lung cancer death), health behaviors (e.g., smoking) and individual characteristics (e.g., education, BMI). Also, ACS studies rely upon data obtained from questionnaires administered during the study enrollment in 1982. Several key individual-level covariates are time-dependent (e.g., smoking status, body mass index, alcohol consumption, occupational exposures, diet, and residential location), thus they are not correctly specified in the Cox models. The statistical models for ozone did not include co-pollutants such as PM_{2.5} and

sulfate particles which have been positively associated with mortality, not only in other studies, but in this study as well. Under the reasonable assumption that one or more of these factors are etiologically related to the health endpoints measured and also associated with exposure (i.e., confounding), the validity of the study's findings is questionable given the weak statistical associations. Such correlations could easily have been the result of misclassification bias and the uncontrolled confounding.

The ACS Study data are themselves a limitation. The subjects were not selected randomly; rather, they were friends, relatives and neighbors of the ACS volunteers. As such, they were not representative of the population of any particular city. This sampling bias produced a study population that was more white and more educated than the general U.S. population. It is not known if the sampling bias confounded the ability to generalize these findings to the general population. (HEI, 2000)

The analytical approach using the enhanced Cox model to account or control for spatial autocorrelation was a study strength, particularly given the presence of such. Also, and unlike the particulate matter analyses, ozone monitoring data were available for the entire study period. However, the readings from the stationary monitors are specific to neither individuals nor residential locations, introducing an unquantifiable degree of exposure misclassification. Likewise, the resulting bias is unknown in both magnitude and direction.

Despite the prospective nature of this cohort study, the issues discussed above weaken the reliability of its findings, particularly for ozone which is particularly susceptible to the effects of these limitations.

CRITICAL REVIEW OF WANG (2009)

Wang XY, Hu W, Tong S

Long-term exposure to gaseous air pollutants and cardio-respiratory mortality in Brisbane, Australia*Geospatial Health* 3(2):257-263***Description/Results***

This study examines the association of long-term exposure to gaseous air pollution with cardio-respiratory mortality in Brisbane, Australia, in the period 1996-2004. The pollutant concentrations were estimated using geographical information system (GIS) techniques at the statistical local area (SLA) level. The estimates were based on an inverse distance weighted (IDW) methodology. The generalized estimating equations model was used to investigate the impact of nitrogen dioxide (NO₂), ozone (O₃) and sulphur dioxide (SO₂) on mortality due to cardio-respiratory disease after adjusting for a range of potential confounders.

Findings: An increase of 4.7% (95% confidence interval = 0.7-8.9%) in cardio-respiratory mortality for 1 part per billion (ppb) increment in annual average concentration of SO₂ was estimated. However, there was no significant association between long-term exposures to NO₂ or O₃ and death due to cardio-respiratory disease. For ozone, the relative risk (RR) = 1.002 (95% CI 0.989-1.015) per 1 ppb increase in exposure for the single pollutant model and RR = 0.999 (0.986-1.012) for the multiple pollutant model (included all 3 gases listed above). The main finding is that the annual average concentration of SO₂ is associated with cardio-respiratory mortality at the SLA level and this association appears to vary with the geographical area.

Analysis

This is a well-executed ecological study of long-term effects of ozone at low ambient concentrations (26.55 ppb - 34.01 ppb) only, for which the effects of ozone on cardio-respiratory mortality were practically undetectable.

The IDW-based exposure modeling that generated annual average potential concentrations seemed appropriate given the study objectives and the gaseous form of the pollutants under study. The analysis appeared sound, as many key potential confounders were included. Particularly notable was the inclusion of the socioeconomic index for areas (SEIFA), a composite index of socioeconomic status that also "reflects the influence of some unmeasured factors such as smoking and physical inactivity". The statistical model was also appropriate for this type of study.

As with any ecological study, exposure misclassification, to some extent, is inevitable (as the authors state). There are 2 sources of measurement error: (1) the models that produced the exposure concentration estimates, and (2) fixing the exposures (as measured) to the residence and not accounting for daily mobility (e.g., working in an area with higher/lower concentrations). However, the IDW models have seemingly performed well in other studies, and most of the deaths occurred in the elderly who are less mobile than the working-age population. Thus, as the authors believe, the extent of misclassification bias would seem to be minimal. Perhaps the most significant study limitation is the seasonal mortality patterns were not examined since the modeled ambient concentrations were averaged over the full year. This is particularly relevant to ozone given its warm weather correlation.

CRITICAL REVIEW OF ZANOBETTI (2011)

Zanobetti A and Schwartz J

Ozone and survival in four cohorts with potentially predisposing diseases*Am J Respir Crit Care Med* 2011; doi:10.1164/rccm.201102-0227OC, with on-line supplement***Description/Results***

These authors investigated whether ozone was associated with survival in four cohorts of persons with specific diseases in 105 US cities, treating ozone as a time-varying exposure. They used Medicare data (1985-2006), and constructed cohorts of persons hospitalized with chronic conditions that might predispose to ozone effects: chronic obstructive pulmonary disease (COPD), diabetes, congestive heart failure (CHF), and myocardial infarction (MI), based on the primary hospital discharge diagnosis. Subjects alive on the first of May of the year following their index admission were entered into the cohort. Yearly warm-season average ozone was merged to the individual follow-up in each city. The analysis applied Cox's proportional hazard model for each cohort within each city, adjusting for individual risk factors, temperature and city specific long-term trends.

Results: The authors found significant associations with a hazard ratio for mortality of 1.06 (95% confidence interval (CI): 1.03-1.08) per 5 ppb increase in summer (May-Sept) average ozone for persons with CHF, of 1.09 (95% CI: 1.06-1.12) with MI, of 1.07 (95% CI: 1.04-1.09) with COPD, and of 1.07 (95% CI: 1.05-1.10) for diabetics. They also found that the effect varied by region, but that this was mostly explained by mean temperature, which is likely a surrogate of air conditioning use, and hence, exposure. The hazard ratios for the same outcomes decreased by 4%-5% during the transition (spring-autumn) season, and these effects were controlled in the analysis for summertime exposures. The authors concluded that this is the first study that follows persons with specific chronic conditions, and the results show that long-term ozone exposure is associated with increased risk of death in these groups.

Analysis

Analytically, this is a particularly strong study that was enhanced by its large study population and precision. Given that the authors used the entire U.S. population over age 65 as their study base, the potential for sampling bias was removed. The conceptualization of the two-stage statistical modeling was well thought-out and it served to eliminate or reduce the field of confounders that typically plague air pollution studies, whether short- or long-term studies. As such, their approach offers several advantages of both types of studies. For instance, city-specific regressions eliminated potential confounding by factors that vary across a given city. Within-city long-term trends were controlled in the analysis, and potential confounding by cross-sectional factors that vary by city that are problematic in time-series studies was avoided.

A key novel finding facilitated by the control for long-term trends was that year-to-year ozone fluctuations--not just exceptionally long-term exposures--can influence survival. Also, regional heterogeneity of effect was a function of mean temperature, a likely indicator of air conditioner usage which would reduce ozone exposure. Thus, temperature explains regional heterogeneity to a large extent, assuming the results of this study are reliable. Another notable finding is they found an effect for the transitional season (summer/spring), even after controlling for summertime exposure. However, that reliability of this finding is questionable since they did not control for PM_{2.5} (data were not available). That limitation affects all reported findings from

this paper, as other studies, (e.g., Jerrett, 2009) have shown that the effect estimates for ozone decrease when PM is added to the model as a co-exposure.

The effect estimates (hazard ratios) are based on a 5 ppb increase in ambient ozone concentrations, thus they are remarkably higher than effect estimates from previous cohort studies. For instance, Jerrett et al. (2009) used increments of 10 ppb on which to base their effect estimates. Reasons for the elevated HRs in the Zanobetti & Schwartz paper will be discussed below.

While a strong study, it is not without its limitations. First of all, this is a cohort of obviously susceptible people by design. This alone resulted in hazard ratios that were higher than those from the ACS cohort study (Jerrett, 2009) for a similar constellation of conditions (causes of death), as the ACS cohort was comprised of individuals, healthy or otherwise, 30+ years of age. The Jerrett et al. (2009) study examined all-cause, cardiopulmonary, respiratory, cardiovascular, and ischemic heart disease mortality; Zanobetti and Schwartz (2011) looked at COPD, diabetes, CHF, and MI. As the authors point out, this study was of semi-chronic effects which include a bit of both chronic and acute responses to the exposure. Given both the difference in the composition of the 2 study populations and the inclusion of acute effects in the Zanobetti and Schwartz (2011) study, this most recent study was destined to show greater effects than those from Jerrett et al. (2009) even had the Zanobetti study's effects had been based on the same incremental increase in ozone exposure. Note that the comparisons with Jerrett et al. are based on their single-pollutant model, the only type of model used in Zanobetti & Schwartz.

Besides the limitation imposed by the single-pollutant model, other potentially confounding variables such as smoking history and socioeconomic status were not available in the Medicare administrative information system. Such factors were controlled to some extent by the ecologic adjustment in the statistical models, but one may assume that considerable uncontrolled confounding still remained, biasing the results to an unknown degree and direction. This alone casts doubt on the reliability of those findings.

Another limitation of this study and most others in air pollution epidemiology is the reliance on area monitoring stations for assigning individual-level exposure values. This is particularly problematic for ozone and we should assume some degree of exposure measurement error and misclassification. These inaccuracies generally bias the effect estimates towards the null, but such directionality is not a given.

An important point that these authors discussed, often absent from such studies, is that "The biological mechanism by which ozone can affect mortality is still under examination". They offer some seemingly plausible mechanisms.

Had this study included co-exposure by PM_{2.5}, the reported effect estimates for ozone exposure would be sufficiently reliable. However, without controlling for that co-pollutant in the analysis, we cannot assess the independent effects of ozone, particularly in this elderly population predisposed to the medical conditions under study.

Morbidity Studies

CRITICAL REVIEW OF BEESON (1998)

Beeson WL, Abbey DE, Knutsen SF

Long-term concentrations of ambient air pollutants and incident lung cancer in California adults: Results from the ASHMOG study*Environ Health Perspect.* 2004; 351:1057-67**Description/Results**

The purpose of this study was to evaluate the relationship of long-term concentrations of ambient air pollutants and risk of incident lung cancer in nonsmoking California adults. A cohort study of 6,338 nonsmoking, non-Hispanic, white Californian adults, ages 27-95, was followed from 1977 to 1992 for newly diagnosed cancers as ascertained from cancer registries and medical records from self-reported hospitalizations. Data on respiratory symptoms and lifestyle factors (e.g., smoking, occupation and associated exposures) were obtained by questionnaires. Monthly ambient air pollution data were interpolated to zip code centroids according to home and work location histories, cumulated, and then averaged over time. Time-dependent, gender-specific Cox proportional hazards models were used to assess the association between lung cancer incidence and the selected air pollutants, adjusting for potential confounding effects of the other covariates. Attained age was used as the time variable which allowed each lung cancer case to be compared only to non-lung cancer cases of the same age. The authors chose annual average number of *hours* in excess of 100 ppb of ozone as the primary metric for developing statistical models, partly (as they state) because this metric filtered out lower background levels and showed the strongest association with respiratory cancer in previous analyses. They imposed a 3-yr lag period between the cumulated exposures and the diagnosis of lung cancer.

Results: The increased relative risk (RR) of incident lung cancer in males associated with an interquartile range (IQR = 556 hr/yr) increase in time exposed to 100+ ppb ozone (O₃) was 3.56 [95% confidence interval (CI), 1.35-9.4, controlling for pack-years of past cigarette smoking, educational level, and current alcohol use. However, in females the RR was 0.94. Increased risks of incident lung cancer were associated with elevated long-term ambient concentrations of PM₁₀ and SO₂ in both genders and with O₃ in males. In models restricted to subjects from high density (urban) areas, the male RR increased to 10.18 (2.44-42.45), while the female results remained unremarkable. Models were also run for other ozone exceedance levels, that is, 60 ppb, 80 ppb . . . 120 ppb, 150 ppb, and all RRs approached or exceeded 3.0 except for the 60 ppb level which had a RR of 2.14. The latter was also the only level at which statistical significance was not achieved.

Analysis

The researchers seemingly conducted the proper preliminary analyses to validate their use of the Cox PH models. The results are 3+ times higher than effect measures reported by other cohort mortality studies; however, this is not surprising given that the metric was based on the number of hours exposed to concentrations above 100 ppb. Also, the reported RRs are also likely to have been biased upwards (discussed below).

Despite the moderately high strength of the statistical associations, these effect estimates are imprecise. Consider the main finding in men that was described above (RR = 3.56). The lower

limit of the 95% CI, RR = 1.35 (3.86 on the original logarithmic scale) is a weak association that could easily have been the result of confounding or misclassification. The upper limit RR of 9.42 (12,333 on the log scale, which is about 3,200 times higher than the lower limit) is however a strong association that would be impervious to practically any form of bias. The wide range of possible true associations within the confidence interval suggests that any of those RRs within the interval would not be reliable risk estimates.

The primary source of bias is the potential underreporting of alcohol and tobacco use, both of which are highly discouraged by the Seventh Day Adventists from which this cohort originates. The authors recognized this issue and offered arguments that if smoking and alcohol use were underreported non-differentially (the most likely scenario) across all exposure levels by 50%, the RR would have been higher than what was observed. Thus, they imply that underreporting did not generate a spurious statistical association between ozone exposure and mortality. Their stance on this issue is speculative given that spurious associations can result from non-differential underreporting of confounders (Shapiro et al., 1996 – cited in this paper) which produces an upward bias. The authors do not discuss the potential limitations on the use of periodic questionnaires to acquire data on likely confounders. This method of data acquisition poses inherent concerns regarding accuracy of those responses which can significantly impact the statistical adjustment for confounding.

Another likely source of inflationary bias in the effect estimates is the investigators' use of single-pollutant models, which they recognize as a potential limitation. As they correctly state, the high RRs seen for ozone may be due, in part, to the lack of control/adjustment for confounding by PM₁₀ and SO₂ which are highly correlated with ambient ozone. Additionally, this study was based in an area of California which has some of the highest ambient ozone concentrations in the U.S., and the authors chose the *annual average number of hours in excess of 100 ppb* of ozone as their exposure metric. This combination generated RRs that are not representative of effect measures throughout the entire country.

In addition to the unique and limited geographic setting for this study, the study population is likewise unique due to its socio-demographic homogeneity. This characteristic limits the application of the study's findings to the vastly diverse general population.

Gender differences for the O₃ results were hypothesized by the authors to be partially due to their differences in exposure (i.e., males spend more time outdoors), and this theory is supported by questionnaire data on outdoor exercise in the summer, when ozone levels are highest. Hormonal differences were also discussed in relation to the gender differences, with prior research supporting this conjecture.

Another limitation is that measured ambient concentrations at monitoring sites may not reflect true individual exposures, resulting in exposure misclassification. The authors used an indoor adjustment factor of 0.5, based on previous exposure studies, and the RR fell to that of the mean ozone concentration, an unadjusted RR = 2.23 (0.79-6.34).

In the incremental exceedance level analysis described above, the relative consistency of the RRs across all levels suggests that the statistical models may not have been properly specified, e.g., key variables/confounders may have not have been included. As the authors mention, their multi-pollutant analyses were constrained due to unavailability of monitoring data. For instance, suspended sulfates were not evaluated because these data were only collected and

available since 1977 which does not allow sufficient time for latency. The investigators also discuss the limitation of using interpolations of personal exposure based on data from the fixed site monitors. However, when the analysis was restricted to those men living within 20 miles of a monitoring station (logically improving the accuracy of the interpolations) the RR declined only slightly.

The inflationary influences of the study setting (i.e., high exposures, small geographic area) along with bias from underreporting of alcohol and tobacco use threaten the validity of the results. In summary, this study is not suitable for promulgating air quality standards across broad populations with lower and more varied ambient ozone exposure ranges over a larger geographic area.

CRITICAL REVIEW OF ISLAM (2007)

Islam T, Gauderman WJ, Gilliland, et al.

Relationship between air pollution, lung function and asthma in adolescents

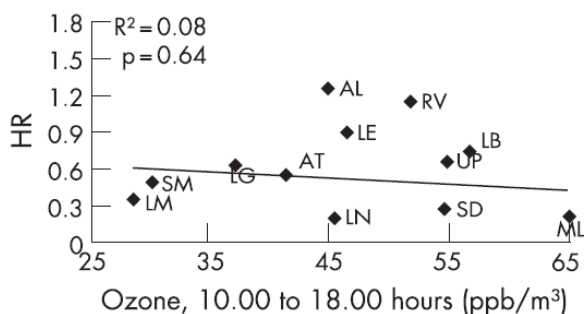
Thorax 2007;62:957-963

Description/Results

This study was undertaken to determine whether lung function is associated with new onset asthma and whether this relationship varies by exposure to ambient air pollutants. A cohort of children aged 9-10 years without asthma or wheeze at study entry were identified from the Children's Health Study and followed for 8 years. The participants resided in 12 communities with a wide range of ambient air pollutants that were measured continuously. Spirometric testing was performed and a medical diagnosis of asthma was ascertained annually. Proportional hazard regression models were fitted to investigate the relationship between lung function at study entry and the subsequent development of asthma and to determine whether air pollutants modify these associations.

Results: From this cohort, 212 incident cases of asthma were observed, based on physicians' diagnoses; the incidence rate was 16.1 per 1,000 person-yrs. The level of airway flow was associated with new onset asthma. Over the 10th-90th percentile range of forced expiratory flow over the mid-range of expiration (FEF₂₅₋₇₅, 57.1%), the hazard ratio (HR) of new onset asthma was 0.50 (95% CI: 0.35 - 0.71). This protective effect of better lung function was reduced in children exposed to higher levels of PM_{2.5}. Over the 10th-90th percentile range of FEF₂₅₋₇₅, the HR of new onset asthma was 0.34 (95% CI: 0.21 - 0.56) in communities with low PM_{2.5} (<13.7 µg/m³) and 0.76 (95% CI: 0.45 - 1.26) in communities with high PM_{2.5} (≥13.7 µg/m³). A similar pattern was observed for forced expiratory volume in 1 second. Little variation in HR was observed for ozone (see graph, each diamond represents a community's results from a single-pollutant model). The authors suggest that exposure to high levels of PM_{2.5} attenuates the protective effect of better lung function against new onset asthma.

Figure 1. Extract from Islam et al, Figure 1: Community-specific hazard ratio (HR) of newly diagnosed asthma over 10th-90th percentile range (57.1%) of forced expiratory flow over the mid-range of expiration (FEF₂₅₋₇₅) by average levels of different ambient pollutants.



Analysis

This well-conducted study generated a new finding/observation: an attenuation of the protective effect of enhanced lung function on the development of asthma with increasing levels of particulate and related non-ozone pollutants. The major limitations of this study were the relatively low statistical power, limited control for known confounders such as ambient temperature, and potential exposure misclassification. Also, while the statistical analysis was thorough for the most part, the authors did not provide any evidence that the assumptions of the Cox proportional hazards model were met. Thus, we are left with assuming that the model was appropriate for these data.

In asthma studies, the issue of diagnostic accuracy is a potential methodological concern. To address this concern, the investigators conducted a sensitivity analysis to assess the potential misclassification of new onset asthma and those results did not differ appreciably from the original findings.

The authors did not precisely quantify the loss to follow-up; however, they reported that the children were followed for 79% of the possible time of the observation over the 8-yr period. They also reported that the completeness of follow-up did not vary substantially across any of the various subgroups (e.g., age at entry, race, gender, allergy history, household smoking, family income). Furthermore, the authors also determined that most of the attrition was the result of employment-related moves of families out of the school catchment area, thus the losses were random. Given this information, the loss to follow-up is a minor limitation at best.

As with most air pollution epidemiologic studies, static air monitors provided the exposure data. Assigning exposures from stationary monitors is particularly prone to measurement error and exposure misclassification, particularly for gases. Additionally, there was no method of adjusting for time spent outdoors--singularly and particularly relevant for ozone--which could possibly vary by monitored area (i.e., children in some communities may spend more time outdoors than those in other communities due to outdoor recreational availability or lifestyle/cultural differences).

Overall, this is a credible study despite some of the usual limitations associated with air pollution observational epidemiologic studies. The results, as limited as they are for ozone, do not suggest an ozone-related effect as was observed for PM_{2.5}.

CRITICAL REVIEW OF LIN (2008)

Lin S, Liu X, Le LH, Hwang S-A

Chronic exposure to ambient ozone and asthma hospital admissions among children*Environ Health Perspect*;116:1725-1730 (2008)***Description/Results***

This study investigated the impact of chronic exposure to high ozone levels on childhood asthma admissions in New York State. The researchers followed a birth cohort born in New York State during 1995-1999 to first asthma admission or until 31 December 2000. They identified births and asthma admissions through the New York State Integrated Child Health Information System and linked these data with ambient ozone data (8-hr maximum) from the New York State Department of Environmental Conservation. Chronic ozone exposure was defined using three indicators: mean concentration during the follow-up period, mean concentration during the ozone season, and proportion of follow-up days with ozone levels >70 ppb. Mean ambient ozone concentrations between 10AM and 6PM during the 6-year follow-up period ranged from 37.5 to 47.8. Logistic regression analysis was performed to adjust for child's age, sex, birth weight, and gestational age; maternal race/ethnicity, age, education, insurance status, smoking during pregnancy, and poverty level; and geographic region, temperature, and co-pollutants.

Results: Asthma admissions were significantly associated with increased ozone levels for all chronic exposure indicators (odds ratios of 1.16-1.68; see Table 2), with a positive dose-response relationship (see Figure 2).

Table 2 from Lin et al

Table 2. Association between ozone exposure indicators, birth and maternal risk factors, and asthma hospitalizations.

Characteristic	Adjusted OR (95% CI)
Ozone exposure (1-ppb increase/day) ^a	
Mean concentration during the follow-up period	1.16 (1.15–1.17)
Mean concentration during the ozone season	1.22 (1.21–1.23)
Exceedance proportion (%) > 70 ppb with IQR increase ^b	1.68 (1.64–1.73)

^aAdjusted for geographic region, child’s sex, child’s age, birth weight, gestational age, maternal race, ethnicity, maternal age, education, maternal insurance, smoking status during pregnancy, poverty level, and temperature. ^bIQR is a 2.51% increase. ^cORs are from the model including the mean concentration during the follow-up period.

Figure 2 from Lin et al

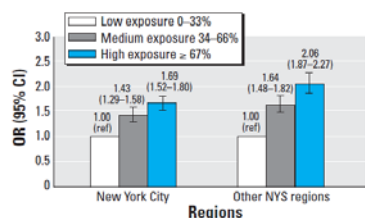


Figure 2. Ozone–asthma dose–response relationship using the mean concentration during the entire follow-up period, adjusted for child’s sex, age, birth weight, and gestational age; maternal race, ethnicity, age, education, insurance, and smoking status during pregnancy; and regional poverty level and temperature. NYS, New York State. ref, referent.

Additionally, the authors examined the ozone-asthma association during the entire follow-up period after controlling for the air quality index and other confounders. The adjusted OR after AQI control increased slightly to 1.24 (95% CI, 1.23-1.25). Stronger associations were observed among younger children, low socio-demographic groups, and New York City residents as effect modifiers. The authors concluded that chronic exposure to ambient ozone may increase the risk of asthma admissions among children, with younger children and those in low socioeconomic groups having a greater risk than do other children at the same ozone level.

Analysis

This was a well-conducted case-control study that examined the probability of an event (asthma hospitalization) rather than time to that event, and this study reported a 6-year risk of such. Despite its incorporation of high quality administrative data sources for case ascertainment and acquiring individual-level information--and Census data for regional/ecological characteristics--some limitations are apparent. The set of personal-level factors did not include many well-known risk factors for asthma (e.g., pets, allergies, water intrusion into homes). Maternal smoking was assessed by self-report and is likely to be underreported; post-partum smoking was not assessed. The authors used an impressive array of ecological variables as stand-ins for many such factors, but this could not have captured the full extent of their influence on effect measurement, e.g., individual level of activity. Thus, some degree of uncontrolled or residual confounding was inevitable given the individual-ecological data mixture.

Exposure measurement and assignment emanated from static air pollution monitors, with one monitor's readings used to assign ambient ozone concentrations across a large geographic expanse in many cases. For a gaseous pollutant such as ozone, poor correlation would be

expected between levels measured at the monitor and actual levels at individuals' residential locations--not to mention the indoor-outdoor disparity in ozone exposures. The assigned exposure value came from monitors in the area where the mother lived at registration, not necessarily the child's actual residence. Given the above considerations, this study seems particularly prone to exposure measurement error and misclassification. The direction of such a bias tends to be towards the null, but simulations have demonstrated that this is not a certainty.

The reported dose-response finding is not true for New York City, as the confidence intervals for the medium and high ozone exposure categories overlapped. Also, other criteria for a dose-response relationship were not met (ref. Breslow and Day, 1987 *in* Maclure and Greenland, 1992). The only statement one can make in this instance is that the medium and high categories differed from the low exposure group. This does not constitute a "significantly positive dose-response relationship". Such a claim is also baseless for other New York State regions, given the authors' analytical methods.

In summary, this is a high quality case-control study but with many of the limitations associated with observational studies of this type. While one can argue about the reliability of the reported ORs, this paper does indicate that the risk of asthma hospitalization in New York State increased to some extent with increasing levels of ozone.

CRITICAL REVIEW OF McCONNELL (1999)

McConnell WF, Abbey DE, Nishino N, Lebowitz MD

Long-term ambient ozone concentration and the incidence of asthma in nonsmoking adults: the AHSMOG study*Environmental Research Section A* 80,110-121 (1999)***Description/Results***

This is a prospective study of a cohort of 3,091 nonsmokers, ages 27 to 87 years, that evaluated the association between long-term ambient ozone exposure and development of adult-onset asthma. This cohort was randomly drawn from California 7th Day Adventists, enrolled in 1977 and followed through 1992. One of the study entry requirements was to have lived within 5 miles of the San Francisco, San Diego, or South Coast air basins for 10+ years, an area described as having increased ambient ozone concentrations. Individuals smoking at the time of enrollment or who reported actively smoking at any time during the study were excluded from all analyses. Participants completed questionnaires in 1977, 1987, and 1992 which ascertained past residence and work locations histories, lifestyle factors potentially affecting exposure to air pollutants, past smoking history, years worked/lived with a smoker, occupational exposures and the presence of symptoms and doctor diagnosis of asthma and other respiratory diseases. Of the 1,914 participants physically able to be tested for lung function, 79% did so which permitted a validation of the questionnaire-based responses regarding asthma symptoms. The standardized questions were taken from the American Thoracic Society questionnaire. Participants were considered incident cases if they did not indicate doctor-diagnosed asthma in 1977 but did so in either the 1987 or 1992 follow-up survey. Air pollution data came from monitoring stations in the aforementioned air basins. These readings were interpolated to surrounding zip code centroids according to residential and work location histories and averaged over time. Average 8-h ozone concentration for the study participants was 46.5 ppb while the mean concentration overall was 25.7 ppb. Logistic regression was used for gender-specific model-building and the final statistical analyses. The odds ratios from these models were adjusted downward to more closely approximate relative risk because incident cases of asthma were not rare.

Results: Over a 15-year period, 3.2% of males and 4.3% of females reported new doctor diagnoses of asthma. For males, the researchers found a significant relationship between report of doctor diagnosis of asthma and 20-year mean 8-h average ambient ozone concentration (relative risk (RR) = 2.09 for a 27 ppb (the inter-quartile range) increase in ozone concentration, 95% CI: 1.03-4.16). No such relationship was observed for females. When 8-h average ozone concentrations were analyzed as tertiles, (0.0-34.9 ppb, 35.0-54.9 ppb, 55.0-74.9 ppb), adjusted RRs in the 2 highest tertiles were 4.44 and 4.01 (statistically significant), respectively. Other variables significantly related to development of asthma were a history of ever-smoking for males (RR = 2.37, 95% CI: 1.13-4.81), and for females, number of years worked with a smoker (RR = 1.21 for a 7-year increment, 95% CI: 1.04-1.39), age (RR = 0.61 for a 16-year increment, 95% CI: 0.44-0.84), and a history of childhood pneumonia or bronchitis (RR = 2.96, 95% CI: 1.68-5.03). Addition of other pollutants (PM₁₀, SO₄, NO₂, and SO₂) to the models did not diminish the relationship between ozone and asthma for males. The authors concluded that long-term exposure to ambient ozone is associated with development of asthma in adult males.

Analysis

This was a well-executed rigorous study that included a thoughtful and methodologically sound analytical scheme. Several secondary analyses tested the reliability of the findings and, based on these analyses, the results seem valid. The analysis of ozone concentrations by tertile clearly indicated increased risk of asthma incidence at low concentrations, as the middle tertile's midpoint was 45.6 ppb. Despite the study's overall strength, some issues exist that could move the RRs in either direction. However, with one exception that follows, any such movement would be very unlikely to alter the study's causal inference.

The statistical models included many potentially confounding covariates; however, some known asthma risk factors were not accounted for in the questionnaires/data. Information on atopy, dust mite allergies, pet dander, and genetics was unavailable. This deficiency--potentially creating an upward bias in the effect estimation--is perhaps the study's greatest limitation, perhaps the only one that could materially affect the relative risks.

The male-only association with ozone, on face, seems to discredit the study's findings overall. However, the such concern is allayed to some extent by the males' increased opportunity exposure for ozone exposure due to more time spent outdoors compared to females as well as a greater prevalence of various types of occupational exposures and ever having smoked. However, one has to suspect underreporting of smoking due to religious prohibitions against this practice. Given that the study population is exclusively 7th Day Adventists, participants may have been reticent to admit to such proscribed behaviors. Also, the losses to follow-up were greater in males which--assuming their outdoor exposure was the same as those who were followed up--further accentuated the gender difference in risk due to ozone. The authors also cite literature that supports a male-dominant risk of asthma due to a number of factors, both endogenous and exogenous.

The authors cite studies that validate their pollutant exposure interpolation method using zip code centroids. Zip codes are not based on natural geography. Rather, they are designed for logistical purposes (delivering mail and packages), thus their shape (which follows no specification) is often highly irregular, in contrast to census tracts which are always polygonal in shape and conform to the local geography/topography. While prior exposure model validation studies showed high correlations between exposure estimates and actual exposures, one has to wonder if these correlations are always applicable, particularly to the unique California setting.

Overall, this is a credible study; however, like-type studies should be conducted in other populations/areas to confirm these statistical associations on a broader scale.

CRITICAL REVIEW OF McCONNELL (2002)

McConnell R, Berhane K, Gilliland F, London SJ, Islam T, Gauderman JW, Margolis HG, et al.

Asthma in exercising children exposed to ozone: a cohort study

Lancet 359:386-91

Description/Results

This prospective study--part of the Southern California Children's Health Study (SCCHS)--investigated the relation between newly-diagnosed asthma and team sports in a cohort of children exposed to different concentrations and mixtures of air pollutants, including ozone. More specifically, the authors examined the effect of exposure to air pollution during exercise or time spent outdoors on the development of asthma. A total of 3,535 children with no history of asthma were recruited from schools in 12 communities in southern California and were followed up for up to 5 years; 265 children reported a new diagnosis of asthma during follow-up. The authors assessed the risk of asthma in children playing team sports at study entry in six communities with high daytime ozone concentrations, six with lower concentrations, and in communities with high or low concentrations of NO₂, particulate matter, and ozone. Ozone metrics included both 4-yr average concentrations based on 24-hr mean concentrations and average concentrations between 10AM and 6PM (ozone₁₀₋₁₈), a period when team sports are played in California. The exposure categorization scheme is shown below with the actual concentration ranges:

	Low pollution communities (n=46)		High pollution communities (n=46)	
	Concentration (mean [SD])	Median (range)	Concentration (mean [SD])	Median (range)
Maximum 1-h ozone (ppb)	50.1 (11.0)	47.6 (37.7-67.9)	75.4 (6.8)	73.5 (69.3-87.2)
Ozone ₁₀₋₁₈ (ppb)	40.0 (7.9)	40.7 (30.6-50.9)	59.6 (5.3)	56.9 (55.8-69.0)
24-h ozone (ppb)	25.1 (3.1)	25.1 (20.6-28.7)	38.5 (11.0)	33.1 (30.7-59.8)
PM ₁₀ (mg/m ³)	21.6 (3.8)	20.8 (16.2-27.3)	43.3 (12.0)	39.7 (33.5-66.9)
PM _{2.5} (mg/m ³)	7.6 (1.0)	7.7 (6.1-8.6)	21.4 (6.0)	21.8 (13.5-30.7)
NO ₂ (ppb)	10.8 (4.6)	12.1 (4.4-17.0)	29.2 (8.5)	29.5 (17.9-39.4)
Acid (ppb)	1.8 (0.7)	1.7 (0.9-2.6)	3.9 (0.7)	3.7 (3.3-4.9)

*These are the same six high and six low communities for PM₁₀, PM_{2.5}, NO₂, and acid, but not for other pollutants. Ppb=parts per billion; Acid=inorganic acid vapour.

Table 3: 4-year pollution concentrations in high and low pollution communities*

Children with preexisting medically diagnosed asthma were excluded from the study, as ascertained from a preliminary questionnaire administered to parents. Asthma incidence was determined/defined by a positive response to the "Has a doctor ever diagnosed this child as having asthma?" question on any of the yearly questionnaires that were administered by trained personnel. Exposure measurements were based on a SCCHS-unique monitoring network, with monitors located within the "stable middle-income communities" where the participants lived. Other exposure information (e.g., sports/team participation, demographic information, allergy history, time spent outdoors, maternal smoking health insurance, family income) were ascertained via the baseline questionnaire.

Findings: In communities with high ozone concentrations, the relative risk of developing asthma in children playing three or more sports was 3.3 (95% CI 1.9-5.8), compared with children playing no sports. Sports had no effect in areas of low ozone concentration (RR = 0.8, 0.4-1.6). Time spent outside was associated with a higher incidence of asthma in areas of high ozone (RR = 1.4, 1.0-2.1), but not in areas of low ozone. Exposure to pollutants other than ozone did not alter the effect of team sports. The risk of asthma development was not greater overall in children living in the 6 high pollution (not necessarily ozone) communities compared to those living in the 6 low pollution communities after adjustment/stratification on age, sex, and ethnic origin. Those relative risks were below the null for 2/3 of the ozone concentration metrics (ozone₁₀₋₁₈ and daily maximum) and 1.1 for the 24-hr average ozone. The authors concluded that the incidence of new diagnoses of asthma is associated with heavy exercise in communities with high concentrations of ozone; thus, air pollution and outdoor exercise could contribute to the development of asthma in children.

Table 2 from McConnell et al

	N (incidence)*	RR (95% CI)
Number of sports played		
0	104 (0-022)	1-0
1	90 (0-026)	1-3 (1-0-1-7)
2	36 (0-021)	1-1 (0-7-1-6)
≥3	29 (0-033)	1-8 (1-2-2-8)

N=number of cases of asthma; RR=relative risk (hazard ratio), adjusted for ethnic origin, and for stratified baseline hazards by sex and age group.

*Denominator=person-years of follow-up.

Table 2: **Effect of sports on incidence of asthma diagnoses**

Table 5 from McConnell et al

	Low ozone communities		High ozone communities	
	N (incidence)*	RR (95% CI)	N (incidence)*	RR (95% CI)
Number of sports played				
0	58 (0-027)	1-0	46 (0-018)	1-0
1	50 (0-033)	1-3 (0-9-1-9)	40 (0-021)	1-3 (0-8-2-0)
2	20 (0-023)	0-8 (0-5-1-4)	16 (0-020)	1-3 (0-7-2-3)
≥3	9 (0-019)	0-8 (0-4-1-6)	20 (0-050)	3-3 (1-9-5-8)

N=number of cases of asthma; RR=relative risk, adjusted for ethnic origin, and for stratified baseline hazards by sex and age group. *Denominator=person-years of follow-up.

Table 5: **Effect of number of team sports played on the risk of new asthma diagnosis in high and low ozone communities**

Analysis

Multivariate proportional hazard models were run, and these models included a number of relevant covariates that could potentially act as confounders. The paper, however, provided no confirmatory evidence that such a model was appropriate given the data. The models were single-pollutant which, in essence, repeatedly attributed each incident case of asthma to each pollutant vs distributing asthma incidence proportionately--or, in the presence of joint effects, combined incidence. The authors state that statistical power was too low to rule out an independent effect of pollutants other than ozone on asthma incidence; however, no effect of sports was seen in communities with high concentrations of any other pollutant.

Aside from the ozone measurements and asthma incidence, all other data--some potentially time-dependent--were obtained during the initial interview and were never updated over the 5-yr follow-up. However, given the authors' description of the communities, the impact of using only the original questionnaire data may be low.

The authors discuss the advantages of using a network of monitoring stations created specifically for this study to more closely depict actual ozone concentrations in the communities under study. This is perhaps the study's most notable strength given the longstanding issue of exposure measurement errors in most other studies on ozone. While this may be a relative advantage compared with most other studies, exposure misclassification potential remains high without individual level exposure assessment.

As the authors discuss, the inference of ozone modifying the effect of sports in asthma development is strengthened by the larger effect seen in the higher activity sports (which increases the ventilation rate) and the independent effects of time spent outdoors (which increases the ambient ozone exposure opportunity). But, they too are puzzled regarding the 'protective' effect seen in high pollution communities, as this is not biologically plausible. They investigated the possibility of selection bias but their quest failed to confirm such. While this may have been a worthy pursuit, one needs to look no further than the 95% confidence intervals to see that a true positive association was nearly as likely as the reported negative association. The authors also commented on their exclusion of some 'non-team' sports (e.g., running, bicycling) in the high-activity category which have been associated with asthma. These exemptions may have generated some misclassification leading to a downward bias of the effect estimates for sports participation.

As the authors voice, the increase in asthma with sports may be the result of chronic exacerbation of exercise-induced bronchospasm via sports participation, to the point that medical attention was sought and an asthma diagnosis was made based on symptoms that more sedentary children would not have experienced. Thus a diagnostic/information bias may exist. While that is a plausible bias, there is no logical explanation for the presence of such only in the communities with higher ozone exposure. In short, the observations from this study indicate that the causative agent was more likely to be ozone, not playing sports.

The quality of this study is good and provides useful information that warrants further investigation. Despite the lingering questions and limitations discussed above, this study is highly suggestive that children participating in sports in communities with higher ambient ozone exposure have a greater risk of developing asthma.

CRITICAL REVIEW OF MILLSTEIN (2004)

Millstein J, Gilliland F, Berhane K, Gauderman WJ, McConnell R, Avol E, Rappaport EB, Peters JM
Effects of ambient air pollutants on asthma medication use and wheezing among fourth-grade school children from 12 Southern California communities enrolled in the Children's Health Study

Arch Environ Health; 50(10):505-514

Description/Results

To investigate the effects of 12 monthly average air pollution levels on monthly prevalence of respiratory morbidity, the authors examined retrospective questionnaire data on 2,034 4th-grade children from 12 Southern California communities that were enrolled in The Children's Health Study.

Results: Wheezing during the spring and summer months was associated with community levels of airborne PM₁₀ (odds ratio (OR) = 2.91; 95% confidence interval (CI) = 1.46-5.80), but was not associated with community levels of ozone, nitrogen dioxide, PM_{2.5}, nitric acid, or formic acid. Logistic regression was performed on data stratified into two seasonal groups, spring/summer and fall/winter. Among asthmatics, the monthly prevalence of asthma medication use was associated with monthly levels of ozone, nitric acid, and acetic acid (OR = 1.80 [95%CI = 1.19-2.70]; OR = 1.80 [95%CI = 1.23-2.65]; OR = 1.57 [95% CI = 1.11-2.21]; respectively). These estimates were generated with no lag period. Asthma medication use was more prevalent among children who spent more time outdoors--with consequential exposure to ozone--than among children who spent more time indoors (OR = 3.07 [95%CI = 1.61-5.86]; OR = 1.31 [95%CI = 0.47-2.71]; respectively). The authors concluded that monthly variations in some ambient air pollutants were associated with monthly respiratory morbidity among school children.

Analysis

The questionnaire response rate was 83%; no comparison was made to non-responders. Parents were asked to indicate which months during the previous 12 months that their children had wheezed or had used asthma medications. A "wheeze analysis" was done in children whose parents reported any history of wheezing, but the nature of such testing was not discussed. The authors state that they did a sensitivity analysis to assess possible recall bias--always a concern--but offered no results from that effort. Information on age, gender, ethnicity, tobacco smoke exposure, and other personal characteristics was obtained from the entrance questionnaire which was supplemented by an outdoor activity questionnaire. Data were also obtained on important potential confounders (e.g., pets, allergies, carpet in home water damage in home) but the source of these data was not disclosed.

The analysis was based on a logistic multi-level, mixed-effects model. This facilitated adjustment for possible confounding by community-related factors and months-related factors such as pollen level and respiratory illness outbreaks, while adjusting still for individual-level factors. This approach seems well-suited for the study objective. The reported ORs are for an increase in ozone exposure equal to the interquartile range. In this study, the IQR was 27.83 ppb, a relatively large increment that inflates the measured effect.

Exposure assessment was done in the typical fashion for observational air pollution epidemiologic studies--by a single monitor at a central location within each community. Peak

summertime ozone concentrations ranged from 300 ppb-100 ppb. This method of assigning exposure to individuals generates an unknown amount of measurement error and exposure misclassification that can bias the effect estimate to some extent in an unknown direction. The authors also point out that another study limitation is the potential overestimate of exposure in subjects who spend considerable time indoors. On a positive note, the exposure measurement period matched up with the risk period for the outcomes under study.

Overall, this is a credible study. The inaccuracies articulated above would not be expected to lower the ORs to such extent that they approach the null, particularly in light of the large IQR increment the authors used in their models of effect.

CRITICAL REVIEW OF PETERS (1999)

Peters JM, Avol E, Navadi W, London SJ, Gauderman WJ, Linn WS, Thomas DC, et al.

A study of twelve Southern California communities with differing levels and types of air pollution: I. Prevalence of respiratory morbidity

Am J Respir Crit Care Med 1999;159:760-767

Description/Results

To study possible chronic respiratory effects of air pollutants, Peters et al. initiated a 10-yr prospective cohort study of Southern California children, with a study design focused on four pollutants: ozone, particulate matter, acids, and nitrogen dioxide (NO₂). Twelve demographically similar communities were selected on the basis of historic (1986-1990) monitoring information to represent extremes of exposure to one or more pollutants. Air pollution concentrations were again measured in 1994. The 24-h average ambient ozone concentration for the 12 communities was 32 ppb (range: 18.9-65.8). In each community, about 150 public school students in grade 4, 75 in grade 7, and 75 in grade 10 were enrolled through their classrooms. Informed consent and written responses to surveys about students' lifetime residential histories, historic and current health status, residential characteristics, and physical activity were obtained with the help of the parents; 3,676 students returned questionnaires for a response rate of 76%. The researchers confirmed associations previously reported between respiratory morbidity prevalence and the presence of personal, demographic, and residential risk factors. Rates of respiratory illness were higher for males, those living in houses with pets, pests, mildew, and water damage, those whose parents had asthma, and those living in houses with smokers. Wheeze prevalence was positively associated with levels of both acid (odds ratio [OR] = 1.45; 95% confidence interval [CI], 1.14-1.83) and NO₂ (OR = 1.54; 95% CI, 1.08-2.19) in boys. **For ozone, whether using the historic or more current monitoring data, ORs for ever asthma or current asthma were mildly (ORs of 1.15 and 1.18, respectively) elevated in male children; females showed moderately negative associations. Peak ozone was also weakly associated with bronchitis and wheeze in males, but females had such an association only with bronchitis.** These effect estimates were relatively imprecise, and none reached statistical significance. The authors conclude, based on this cross-sectional assessment of questionnaire responses, that current levels of ambient air pollution in Southern California may be associated with effects on schoolchildren's respiratory morbidity as assessed by questionnaire.

Analysis

The authors describe their study design as quasi-experimental, but this is not apparent in the paper. The description of the study methods was vague on some elements, e.g., the exact study period is unclear. A health effects/risk factor questionnaire was administered in 1993 but apparently not updated during the 10-year span. Some of the data elements (e.g., residential location) were subject to change over the time, opening up the possibility for information bias to threaten the validity of the findings. Regardless of which exposure time period was used in the analysis (1986-1990 or 1994), evidence suggested that ozone moderately elevated the risk of ever and current asthma in males only, with indications of a statistically protective association in females. Likewise, peak ozone was statistically associated with wheeze only in males. The most likely explanation--assuming a causal relation between ozone and these two health endpoints--is that boys are more highly exposed to ozone due to more time spent outdoors. The finding regarding bronchitis is less easily explained, as gender-based exposure

differences are seemingly irrelevant, which does not comport with the findings discussed above. There were no consistent or large excesses of morbidity in participants who lived in the most polluted communities and/or had the highest estimated exposures.

The effects reported in this study are exaggerated by the chosen ozone increment, i.e., ORs per 40 ppb increase in peak ozone. This increment is 3-4 times that of the studies based on average daily ozone, thus the excess risk is proportionately larger, i.e., the effects may seem exaggerated compared to most other study results.

This study's greatest strength was the detail of the questionnaire which included a number of factors that have been associated with respiratory conditions including asthma (e.g., paternal/maternal history of asthma, pets, water damage, active/passive smoke). The major weakness is the potential for exposure misclassification which is a common weakness of air pollution epidemiology studies. However, the manual assignment of residential locations to the most relevant monitoring station reduced this to some extent.

Lung Function Studies

CRITICAL REVIEW OF FORBES (2009)

Forbes LJJ, Kapetanakis V, Rudnicka AR, et al.

Chronic exposure to outdoor air pollution and lung function in adults

Thorax 2009;64:657-663

Description/Results

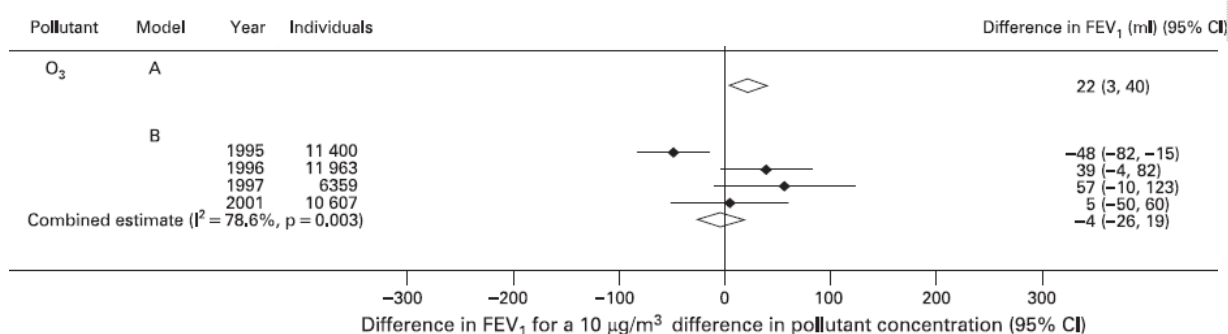
The aim of this cross-sectional study was to measure the association between chronic exposure to outdoor air pollutants and adult lung function. The relationship between measures of lung function, FEV₁ and FVC, and average exposure to PM₁₀, NO₂, SO₂ and O₃ was examined in four representative surveys of the English population aged ≥16 in 1995, 1996, 1997 and 2001. Year-specific estimates were pooled using fixed effects meta-analysis. Exposure was assigned by postal code sector, measured directly at each sector's centroid, under the assumption that the annual average pollutant exposure for people living in each sector was that of its centroid. Annual average background exposure for each 1 km² of England was estimated from an emission inventory by using air dispersion models including the effect of weather conditions

Results: Greater exposure to PM₁₀, NO₂, and SO₂ was associated with lower adult FEV₁. The size of the effect on population mean FEV₁ was about 3% for PM₁₀, and 0.7% for NO₂ and SO₂, for a 10 µg/m³ increase in pollutant concentration. The effects were most marked in men, older adults and ex-smokers. FEV₁ was not associated with ozone concentration. No associations were found between the pollutants and FEV₁ as a percentage of FVC. The authors concluded that chronic exposure to outdoor air pollution is associated with modestly reduced FEV₁ in adults.

Analysis

Cross-sectional studies are not particularly strong from a design perspective, but this study consisted of periodic population-based surveys that were seemingly conducted well. Each survey included in this study consisted of a different sample of the population (with different people almost exclusively), mostly facilitating a trend analysis of prevalent conditions, not an etiological analysis. Yet, when paired with exposure data as in this study, the results become more etiologically meaningful. The multilevel modeling enriched the study's design since such an approach allowed for the possibility that lung function in people living in one postcode sector or one household may be more similar to each other than people living elsewhere because they share various risk factors in addition to pollutant exposures.

For ozone, the authors claim no association with lung function in their study. However, for this conclusion they relied on modeled effect estimates (Model B in Figure 1, below) from all years combined (i.e., a fixed effects meta-analysis), adjusting for pack-yrs of active smoking, passive



smoking, social class, region and season. The results from each survey year were weighted in order to derive the combined effect (diamond).

Model A (above)--all years combined; adjusted for age, sex, height and all their 2-way interactions for all years--showed a modest increase in FEV₁. Model B analyzed and reported on each survey separately. The propriety of reporting a single combined effect is highly questionable given the heterogeneity of effects noted between surveys. For instance, the 1995 sample in Model B indicated a significant decrement in lung function related to ozone exposure. Another stratified analysis by age category reported a -21 ml difference in the 16-44 yr age group for ozone exposure. The next 2 surveys showed increases in lung function, while the final survey was essentially a null finding. The test for heterogeneity (I²) was 79% (p = 0.003), indicating that a single effect estimate is not representative of the full series of surveys. Even in the presence of heterogeneity of effects by survey year, any decrement may not have represented an adverse effect, as no data on accompanying symptoms were collected. Also, the observed decrements may not have been clinically significant with or without symptoms.

While the reporting of effects may not have been done in the most proper manner, the preponderance of the authors' findings support their conclusions that lung function decrements due ozone exposure were not observed in this study.

CRITICAL REVIEW OF GAUDERMAN (2000)

Gauderman WJ, McConnell R, Gilliland F, London S, Thomas D, Avol E, Vora H,
Association between air pollution and lung function growth in Southern California children
Am J Respir Crit Care Med 2000 Oct;162(4 Pt 1):1383-1390

Description/Results

Average growth of lung function over a 4-yr period, in three cohorts of southern California children (N = 3,035) who were in the fourth, seventh, or tenth grade in 1993, was modeled as a function of average exposure to ambient air pollutants, including ozone. This was done within a cohort study for which the follow-up period was 4 years. Because of the non-linearity of lung function growth during adolescence, each grade cohort was analyzed separately using linear regression. The model building itself was methodically conducted. The first model was a linear regression of PFT on age done to obtain a separate intercept and growth rate for each child, with adjustments for subject- and time-specific covariates including height. The second model was a linear regression of the subject-specific adjusted growth slopes estimates from Model 1 on indicator variables for each community, adjusting again for subject-specific covariates. Model 3 used the 12 adjusted community-average lung growth rates from Model 2 to compare graphically on community mean concentrations of the pollutants. This generated the change in annual growth per unit increase in pollutant level. Both single- and two-pollutant models were run.

Results: In the fourth-grade cohort, significant deficits in growth of lung function (FEV(1), FVC, maximal midexpiratory flow [MMEF], and FEF(75)) were associated with exposure to PM₁₀, PM_{2.5}, PM_{10-2.5}, NO₂, and inorganic acid vapor (p < 0.05). No significant associations were observed with ozone as measured by either as a 24-h average or the annual average concentrations between 10AM - 6PM when both ozone concentrations and outdoor individual exposures are highest. The estimated growth rate for children in the most polluted of the communities as compared with the least polluted was predicted to result in a cumulative reduction of 3.4% in FEV(1) and 5.0% in MMEF over the 4-yr study period. The estimated deficits were generally larger for children spending more time outdoors. In the seventh- and tenth-grade cohorts, the estimated pollutant effects were also negative for most lung function measures, but sample sizes were lower in these groups and none achieved statistical significance. The results suggest that significant negative effects on lung function growth in children occur at current ambient concentrations of particles, NO₂, and inorganic acid vapor, but not ozone.

Analysis

Air pollution monitoring stations were placed in each of the 12 communities under study; measurements were available during the entire study period. This is an improvement over many similar studies where monitor placement is less representative of the local area's ambient concentrations. The model-building was done appropriately. Ozone was not shown to restrict growth rates in any of the 3 class cohorts, although the precision of these the effect estimates was not high for any of the measured pollutants. While none of the effect estimates were statistically significant, they were all consistently qualitatively negative across grade sub-cohorts except for ozone which showed "positive" (i.e., lung function growth) results across the 3 sub-cohorts. Moreover, ozone showed no effect modification tendencies in 2-pollutant models.

While assessing the independent effects of individual pollutants was generally difficult due to the correlation between them, long-term average ozone concentrations were not significantly correlated with the other pollutants. This allowed a more reliable estimate of ozone's effect on lung function growth and the results fail to support a substantial long-term effect.

Despite the overall high study quality, some degree of exposure misclassification is inevitable. However, neither the extent nor direction of any bias on the effect estimates resulting from such misclassification can be postulated without additional information.

CRITICAL REVIEW OF GAUDERMAN (2004)

Gauderman WJ, Avol E, Gilliland F, et al.

The effect of air pollution on lung development from 10 to 18 years of age

NEnglJMed 2004; 351:1057-67

Description/Results

In this prospective study 1,759 children (average age, 10 years) were recruited from schools in 12 southern California communities. Lung function was measured annually for eight years. The rate of attrition was approximately 10% per year. The communities represented a wide range of ambient exposures to ozone, acid vapor, nitrogen dioxide, and particulate matter. Linear regression was used to examine the relationship of air pollution to FEV₁ and other spirometric measures.

Results: Over the eight-year period, deficits in the growth of FEV₁ were associated with exposure to nitrogen dioxide (P=0.005), acid vapor (P=0.004), PM_{2.5} (P=0.04), and elemental carbon (P=0.007), even after adjustment for several potential confounders and effect modifiers. Associations were also observed for other spirometric measures. Exposure to some pollutants was associated with clinically and statistically significant deficits in the FEV₁ attained at the age of 18 years. For example, the estimated proportion of 18-year-old subjects with a low FEV₁ (defined as a ratio of observed to expected FEV₁ of less than 80%) was 4.9 times as great at the highest level of exposure to PM_{2.5} as at the lowest level of exposure (7.9% vs 1.6%, P=0.002). For ozone, the linear fit was essentially flat (R = 0.04) and not statistically significant, P = 0.89. Similar results were seen for both the 1-hr maximal level between 10AM and 6PM. The authors concluded that these results indicate that current levels of air pollution have chronic, adverse effects on lung development in children from the age of 10 to 18 years, leading to clinically significant deficits in attained FEV₁ as children reach adulthood.

Analysis

The authors conclusion cannot be generalized for each of the pollutants examined, as FEV₁ decrement was not associated with higher concentrations of ozone. The effect estimates (differences in average growth in lung function over the 8 yrs from the least to the most polluted community) for ozone were negative for FVC and FEV₁ despite the nearly flat linear concentration-response function. The low statistical power to find significant differences was a product of the high loss to follow-up. Since most of the 95% confidence interval for ozone's effect estimate was on the negative side of the null line, perhaps a higher follow-up percentage would have been more convincing of a detrimental effect. However, even if statistically significant, would a decrement of 51 ml and 23 ml in FVC and FEV₁, respectively, have been clinically significant?

The air pollution data came from static monitoring stations in each of the 12 communities, introducing some degree of measurement error and exposure misclassification. Such errors are more likely for the gaseous pollutants, so the ozone results could have been biased towards the null in a non-differential misclassification scenario. However, the direction of such bias, while most often towards the null, is not always in that direction. Additionally, there was no method of adjusting for time spent outdoors--singularly and particularly relevant for ozone--which could possibly vary by monitored area.

The study design was sound and the analyses were conducted appropriately. However, the study suffered from a 58% loss to follow-up. The authors ran additional analyses to assess the impact of the attrition. They reported that the length of follow-up was significantly associated with factors related to the population sample's mobility (e.g., race, ethnicity, parents' education) but was not significantly associated with baseline lung function or the level of exposure to air pollution. From these secondary analyses the authors suggest that those who dropped out were not different with respect to the primary variables of interest. However, the mobility-related variables were not the only variables that would seem to be of primary interest, e.g. histories of asthma and allergy. These data were not even collected for this study, thus adding to the inevitable presence of confounding. Also, the authors did not state whether their assertion held for each of the pollutants, rather using the term "exposure to air pollution". Data for the two-pollutant models were not shown, but the authors state that adjustment for ozone did not substantially alter the effect estimates or the levels of statistical significance.

In summary, while this study provides little evidence of a detrimental effect of ozone exposure on lung development during adolescence, the limitations/problems described above render these results unreliable for standard-setting regarding ambient ozone.

CRITICAL REVIEW OF GAUDERMAN (2007)

Gauderman WJ, Vora H, McConnel R, Gilland F, Thomas D, Avol E, Kunzli N, Jerrett M, Peters J
Effect of exposure to traffic on lung development from 10 to 18 years of age: a cohort study
Lancet 2007;369:571-77

Description/Results

This study investigated the association between residential exposure to traffic and 8-year lung-function growth. In this prospective study, 3,677 children (mean age 10 years [SD 0.44]) participated from 12 southern California communities that represent a wide range in regional air quality. This paper is one of many from the Children's Health Study in that state. Children were followed up for 8 years, with yearly lung function measurements recorded. For each child, the investigators identified several indicators of residential exposure to traffic from large roads. Regression analysis was used to establish whether 8-year growth in lung function was associated with local traffic exposure, and whether local traffic effects were independent of regional air quality.

Results: An average of 6.2 pulmonary function tests were done per child, with the maximum possible number of tests being 8. Children who lived within 500 m of a freeway (motorway) had substantial deficits in 8-year growth of FEV₁, -81 mL, p=0.01 [95% CI -143 to -18]) and maximum midexpiratory flow rate (MMEF), -127 mL/s, p=0.03 [-243 to -11], compared with children who lived at least 1500 m from a freeway. Joint models showed that both local exposure to freeways and regional air pollution had detrimental and independent effects on lung-function growth. Pronounced deficits in attained lung function at age 18 years were recorded for those living within 500 m of a freeway, with mean percent-predicted 97.0% for FEV₁ (p=0.013, relative to >1500 m [95% CI 94.6-99.4]) and 93.4% for MMEF (p=0.006 [95% CI 89.1-97.7]). The authors concluded that local exposure to traffic on a freeway has adverse effects on children's lung development, which are independent of regional air quality, and which could result in important deficits in attained lung function in later life. Ozone_{10AM-6PM} was not associated with reduced pulmonary function, as the regional effect was -13 mL. However, living less than 500 m from a local freeway was associated with a statistically significant FEV₁ decrement of -81 mL with no indication of statistical interaction with local freeway distance overall.

Analysis

For this genre of air pollution epidemiologic studies--focusing on traffic volume/density or distance from roadways--this is a high quality study. For ozone specifically, the quality suffers somewhat due to inherent ambient measurement errors. However, subjects' exposure assignment accuracy is likely benefited by the use of dispersion modeling vs ecologically assigning exposures from a single monitoring site. Still, the exposure assessment/assignment does not account for differences in time spent outdoors, which is a key determinant of ambient ozone exposure potential. Otherwise, control for confounding was relatively complete. One should also be cognizant of the nature of the statistical analysis: the authors developed prediction models, not etiological models. Factors that predict respiratory dysfunction are not necessarily factors that cause the growth deficit. Many predictive variables are merely surrogates of an etiologically relevant exposure. The study's major strength is the prospective cohort design which enhances the reliability of observational findings.

The key feature of this study was to examine modification of each pollutant's effect (called "regional pollutant effect") on lung function by residential distance from either freeways or non-freeway roads. The main effects of the pollutants were reported alongside joint effects of each pollutant and distance from a freeway. The only finding regarding ozone_{10AM-6PM} via formal testing was essentially a null regional effect (of a 37.5 ppb increase in O₃) on FEV₁ with no indication of statistical interaction with freeway distance. However, there was an independent effect of distance from a freeway when comparing the community with the highest ozone concentrations vs the lowest concentrations. Therefore, proximity to freeway traffic was the best predictor of pulmonary function deficits associated with ozone.

Given the overall study quality and the issues described above, this study generated relatively reliable findings for ambient ozone.

CRITICAL REVIEW OF IHORST (2004)

Ihorst G, Frischer T, Horak F, et al.

Long- and medium-term ozone effects on lung growth including a broad spectrum of exposure

Eur Respir J 2004;23:292-299

Description/Results

The effects of semi-annual and 3.5-yr mean ozone (O₃) concentrations on children's FVC and FEV₁ were assessed over a study period of 3.5 yrs in 2,153 schoolchildren from 15 study sites in South Western Germany and Lower Austria. Spirometric parameters were assessed twice a year, and differences between consecutive measurements divided by days were considered as a measure of lung growth. Exposure was analyzed in four classes, separately for winter and summer (semi-annual mean O₃ concentrations: 22-30, 30-38, 38-46, 46-54 parts per billion (ppb) in summer and 4-12, 12-20, 20-28, 28-36 ppb in winter).

Results: Regression methods for repeated measurements were used, and these revealed a significantly lower FVC (FEV₁) increase estimated at -19.2 (-18.5) mL/100 days for semi-annual mean O₃ exposure in summer between 46 and 54 ppb compared to exposure between 22 and 30 ppb. However, in winter, the estimated difference in FVC (FEV₁) was 16.4 (10.9) mL/100 days between the semi-annual O₃ class 28-36 ppb and the 4-12 ppb class. By means of linear regression the study found that there was no association between growth rates and mean summer O₃ for FVC and FEV₁ over a 3.5-yr period. The authors conclude that medium-term effects on schoolchildren's lung growth are possibly present, but are in the long-term not detectable for FVC and FEV₁ over a 3.5-yr period due to partial reversibility. An effect of summer ozone exposure on the growth-related increase in FEV and FEV₁ was reported in for the highest ozone concentrations during the first 2 yrs of the study. In the following winter seasons, the pattern was reversed, leading the authors to also conclude that medium-term effects on lung growth are possibly present.

Analysis

Of the 2,153 children who were enrolled in the study, 40% were lost to complete follow-up (7 spirometrics); 1,811 (85%) contributed at least 5 such test results. The authors did not provide any information on which to compare those children who remained in the study vs those lost due to attrition. This is a key piece of information on which to assess a study's validity, as these two groups could have differed on some key attributes. This concern is, however, attenuated by the high percentage of enrollees that contributed 5 or more data points. The power of this study was enhanced via the pooling of two large datasets from Germany and Austria. Various analyses did not indicate any systematic methodological differences between the two countries.

This longitudinal/panel study--a rigorous design--appeared to have been meticulously conducted, and data were collected on many potential confounders that were controlled via regression analysis (e.g., NO₂, SO₂, asthma, allergies, pollen sensitization, sex, age & height at start, passive smoke exposure, short-term O₃ exposure at start and end of the time period, and time period). Asthma prevalence ranged from 1.2% to 12.8% among the 15 sites, thus this was an important covariate. An age-stratified analysis was done to assess whether younger children

were more vulnerable to the effects of ozone exposure; no differences were found. Several potential statistical interactions were assessed, but none were statistically relevant.

As with most air pollution epidemiologic studies, static air monitors provided the exposure data. Assigning exposures from static monitors is particularly prone to measurement error and, with gases, exposure misclassification. Additionally, there was no method of adjusting for time spent outdoors--singularly and particularly relevant for ozone--which could possibly vary by monitored area (i.e., children in some communities may spend more time outdoors than those in other communities due to outdoor recreational availability or lifestyle/cultural differences).

Overall, this is a credible study despite some of the usual limitations associated with air pollution observational epidemiologic studies. The evidence for medium-term effects is suggestive at best, and support for long-term effects is not offered by these results.

CRITICAL REVIEW OF MORTIMER (2008)

Mortimer K, Neugebauer R, Lurmann F, et al.

Air pollution and pulmonary function in asthmatic children: effects of prenatal and lifetime exposures

Epidemiology 2008;19:550-557

Description/Results

The objective of this study was to examine the association of prenatal and lifetime exposures to air pollutants with pulmonary function in a cohort of children with asthma, based on the premise that prenatal and early life periods represent critical windows for oxidant pollutant-induced lung remodeling. Prenatal and lifetime exposure to several air pollutants was reconstructed for 232 children with asthma from the San Joaquin Valley of California, USA. Prenatal and lifetime residences were geocoded. Data were obtained on monthly average ozone, CO, NO₂, and PM₁₀ concentrations. Metrics were created for key developmental periods. Predictive models were developed for 8 pulmonary function measures. A newly-developed stepwise model selection procedure--the Deletion/Substitution/Addition (DSA) algorithm--was implemented and results were compared with those obtained using traditional stepwise methods.

Results: Second-trimester exposure to NO₂ negatively affected forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV₁), and first trimester exposure to PM₁₀ negatively affected peak expiratory flow (PEF) rate. Exposure to CO in early years of life also had a negative effect on FEV₁/FVC and forced expiratory flow between 25% and 75% of FVC (FEF₂₅₋₇₅)/FVC. Second trimester exposure to PM₁₀ and exposure to CO in the first 6 years of life had negative effects on forced expiratory flow at 25% of FVC. Prenatal, but not trimester-specific, exposure to CO was negatively associated with FEF₂₅₋₇₅. Effects were limited to subgroups, such as children who were African American, those diagnosed with asthma before the age of 2 years, and those exposed to maternal smoking during pregnancy. The authors concluded that prenatal and early-life exposures to CO, PM₁₀, and NO₂ have a negative effect on pulmonary function in subgroups of asthmatic children. Regarding ozone, this study did not find a negative effect in this particular cohort.

Analysis

The only parameter for which ozone exposure appeared to have exerted a negative (deleterious) effect was for the FEV₁/FVC parameter, with a coefficient of -0.02 (10AM-6PM average, lifetime) and a standard error of 0.0069. The 24-hr average ozone at age 0-3 yrs also generated a statistical effect, with a coefficient of 0.034 (0.0086). Unlike 'original' objectively measured parameters, e.g., FEV₁, this derived metric has no direct biologically based unit of measurement such as liters per second, thus the coefficient lacks a clear interpretation. Of note is the model's adjusted R-square of only 0.13, a poor fit to the actual data. Given this set of circumstances, Mortimer (2008) has little relevance for assessing the effects of ozone on respiratory function. As a result, the remaining comments relate primarily to the other pollutants examined.

While the DSA analytical method was a better choice on principle, the results were said to have varied little from traditional stepwise regression techniques. The latter modeling methodology

is fraught with problems, e.g., model over-fitting and multiple testing. This particular study seems particularly vulnerable to these problems given the number of separate analyses run and the number of covariates. The DSA method, while preferable over step-wise, cannot safeguard against these potential problems. However, the authors claim to have used subject matter knowledge to select the most appropriate set of covariates for each respiratory function tested. The reported results from the traditional step-wise analysis are likely reliable, assuming that such knowledge was applied. Given that assumption, the large number of covariates in the models offered excellent control for nearly all of the factors that could have confounded the results.

There are other limitations to this study. First, the study subjects were asthmatic children, so the results may not apply to non-asthmatic children. Second, it is inevitable that some degree of pollutant exposure misclassification occurs when basing those estimates on static monitors. Thirdly, this is an observational study that comes with inherent biases due to observed and unobserved confounding that cannot be adequately quantified. However, despite the above limitations, the authors' careful study design and analytical scheme seem to override most of those concerns.

CRITICAL REVIEW OF PETERS (1999b)

Peters JM, Avol E, Navadi W, London SJ, Gauderman WJ, Linn WS, Thomas DC, et al.

A study of twelve Southern California communities with differing levels and types of air pollution: II. Effects on pulmonary function

Am J Respir Crit Care Med 1999;159:760-767

Description/Results

To study the possible chronic respiratory effects of air pollutants, Peters, et al. designed and initiated a 10-yr prospective study of Southern California public school children, grades 4, 7 & 10, living in 12 communities with different levels and profiles of air pollution. Pulmonary function tests were completed on 3,293 subjects. The effects of air pollution exposures were evaluated cross-sectionally based on data collected in 1986-1990 by existing monitoring stations and data collected by the study team in 1994 via questionnaire. This instrument collected data (parents completed them for the younger children) on medical history, residential history, and housing characteristics. The older groups were also asked privately about smoking habits, recent illness, and recent exercise. Lung function tests were done in the morning hours of spring in order to avoid daily and annual peak pollution levels.

Two-stage multiple regression models were used to investigate the relationship between PFT and air pollutants. The first stage assessed mean community PFT adjusted for personal variables. These adjusted community means were then utilized in a second ("ecologic") model in which community-level PFTs were the dependent variable and community pollutant measurements were the independent variable. The parameter of interest (β) represented the slope coefficient for the relationship between community mean PFT and the pollutant level.

Results: Expected relationships were seen between demographic, physical, and other environmental factors and pulmonary function values. When the data were stratified by sex, an association was seen between pollution levels and lower pulmonary function in female subjects, with the associations being stronger for the 1994 exposure data than the 1986-1990 data. After adjustment, O₃ was associated with lower PEF_R and MMEF. Effects were generally larger in those girls spending more time outdoors. NO₂ was most strongly associated with lower FVC ($r = -0.74$, $p < 0.01$), PM_{2.5} with FEV₁ ($r = -0.72$, $p < 0.01$), O₃ with PEF_R ($r = -0.75$, $p < 0.005$), and PM_{2.5} with MMEF ($r = -0.80$, $p < 0.005$). There was a statistically significant association between ozone exposure and decreased FVC and FEV₁ in girls with asthma. For boys, significant associations were seen between peak O₃ exposures and lower FVC and FEV₁, but only in those spending more time outdoors. Additional multivariate analyses of the female data using 2-pollutant models for MMEF showed that peak ozone in combination either PM₁₀ or NO₂ was a better fit than the ozone-only model; the model which included PM₁₀ was the best fit. That particular model indicated that both pollutants (O₃ and PM₁₀) contributed about equally to the MMEF decrement. The only conclusion given by the authors was that, given the limitations associated with cross-sectional data, this cohort should be studied using prospective data.

Analysis

The statistical methods were described clearly, and they were appropriate for assessing the effects of continuous data such as PFT. The model-building included/excluded potentially confounding covariates from the two-stage process described above, except for the existence of

other pollutants; the primary models were single-pollutant. Surprisingly, active smoking actually improved 3 of the 4 PFTs, an inexplicable finding that sheds some doubt on the study's reliability.

The list of potential confounders accounted for 86-87% of the variation in FVC and FEV₁ across the study population, substantially less for PEF and MMEF, 73% and 59%, respectively. Gender, height, and weight were the primary predictors of FVC and FEV₁, but were far less predictive for PEF and MMEF. Instead, peak ozone exposure was the primary predictor, but only in females. This finding contrasts the findings from the first study (see critique for Peters, 1999a) in which ozone was reported to increase the risk of respiratory morbidity for males only. In this second study, increased outdoor exposure negatively impacted PEF and MMEF for males only, a finding that corresponds to the first study.

The authors note that their analysis does not permit a specific pollutant to be identified, with any degree of certainty, as having broad PFT effects; a different pollutant was found to be the most strongly associated for each of the four PFT outcomes. They also concede the limitations of the exposure data, not only for pollutant levels which likely vary spatially in microenvironments, but also behaviors such as time spent outdoors. Another limitation noted by the authors is that their study design could not distinguish acute reversible effects of recent air pollution exposure from chronic effects of interest.

The limitations imposed by the study design and data availability weaken the study's reliability. No health outcomes were ascertained and even the PFT findings were inconclusive. In short, this study is not suited for setting air quality standards.

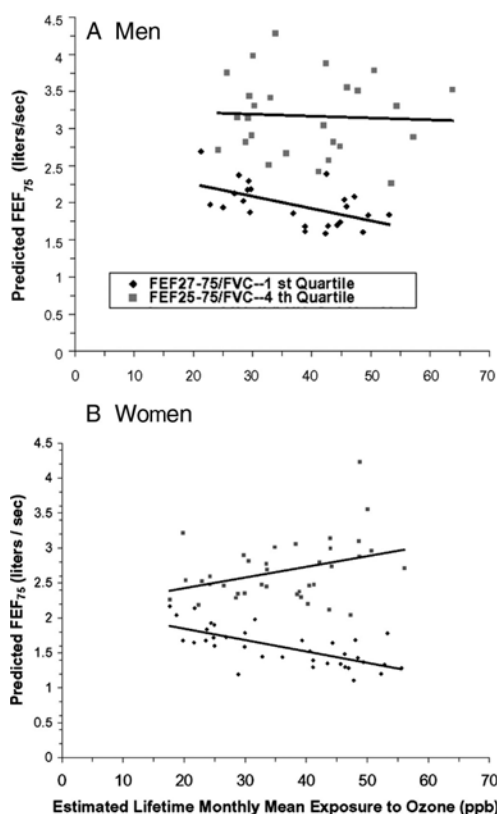
CRITICAL REVIEW OF TAGER (2005)

Tager I, Balmes J, Lurmann, et al.

Chronic exposure to ambient ozone and lung function in adults*Epidemiology* 2005;16:751-759***Description/Results***

This study investigated the effects of long-term exposure to ozone (O₃) on lung function in college freshmen, based on prior studies suggesting that chronic exposure is associated with decreased lung function in children and adolescents. University of California-Berkeley students (n=255) who were lifelong residents of the Los Angeles and San Francisco Bay areas and who never smoked were recruited into the study. Information on residential history, past history of pneumonia and other lower respiratory track illnesses, allergy history, history of asthma-related symptoms, physician diagnosis of asthma, personal smoking history, second-hand smoke and family history of chronic respiratory diseases were acquired via questionnaire. Lifetime exposures to O₃, PM₁₀, and nitrogen dioxide (NO₂) were based on spatial interpolation of compliance monitor measurements to all residences at which students lived. Spirometry was performed between February and May, times when students would not have had recent exposure to increased levels of O₃.

Results: Lifetime exposure to O₃ was associated with decreased levels of measures of small airways (<2 mm) function (FEF₇₅ and FEF₂₅₋₇₅). There was an interaction with the FEF₂₅₋₇₅/FVC ratio, a measure of intrinsic airway size. Subjects with a large ratio were less likely to have decreases in FEF₇₅ and FEF₂₅₋₇₅ for a given estimated lifetime exposure to O₃. This association was not altered by history of chronic respiratory disease, allergy, second-hand exposure to environmental tobacco smoke, exposure to PM₁₀ and NO₂, or measurement errors in exposure assessment. The authors concluded that a history of increased level of lifetime exposure to ambient O₃ is associated with decreased function of airways in which ozone deposition in the lungs is the greatest. Adolescents with intrinsically smaller airways appear to be at greatest risk. Environmental or genetic factors leading to reduced airway size may lead to increased susceptibility to the adverse effects of ozone.



Analysis

This study was executed very well, and the analytical results support the authors' conclusions in general. However, the effect modification --that is, the influence of airway size on ozone's effect on pulmonary function--was most obvious in females for which the statistical interaction was qualitative, i.e., concentration-responses going in opposite directions for the 1st vs 4th quartiles of the FEF₂₅₋₇₅/FVC ratio (see figures at left). These gender differences were not strong according to the authors (formal testing was not reported). The authors also state that the level of the FEF₂₅₋₇₅/FVC ratio at which "no effect" would be expected was lower for women than for men (1.04 and 1.17, respectively), but this analysis was neither explained nor reported in the paper. Accounting for exposure measurement errors (via an earlier validation) had little impact on the effect measures.

A notable aspect of this study is their incorporation of 2 basic models to estimate lifetime pollutant exposure: a time-outdoors model and an ecological model. The first model included age-specific estimates of time spent outdoors at each residence obtained from California Air Resources Board study; they used an indoor-outdoor ozone ratio of 0.2. The latter model omitted the time spent outdoors estimates and used only the residence-specific monthly average, interpolated pollutant concentrations. Both models supported the other's findings which, in turn, support the authors' conclusions. However (as the authors assert), it cannot be said with certainty that O₃ alone was responsible for the observed effects due to modeling limitations. Also, these findings are not consistent with those from other longitudinal studies (Gauderman, et al., 2004; Ihort et al., 2004).

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