March 13, 2014

Dr. H. Christopher Frey
Chair, Clean Air Scientific Advisory Committee
Science Advisory Board
US Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, DC 20460

Dear Dr. Frey:

We urge CASAC to consider the following comments, presenting our recommendations for improvements to the 2014 *Health Risk and Exposure Assessment for Ozone Second External Review Draft* (REA; US EPA, 2014). We received funding from the American Petroleum Institute (API) to prepare these comments, but the recommendations are based on our own independent evaluation of the REA and don't necessarily reflect the views of API.

We found the REA to be a much more thorough and complete risk assessment than the first draft. In the second draft, the United States Environmental Protection Agency (EPA) presented analyses of 12 urban areas, a more detailed discussion of sensitivity analyses, and several sources of uncertainty, and it included confidence bounds for mortality risk estimates. However, we found that EPA took an overly conservative approach and highlighted modeled results without sufficient discussion of the uncertainty. EPA could improve the REA by presenting analyses more clearly and providing a more complete discussion of uncertainty and its impact on the two key questions posed in the REA:

> (a) To what extent is the existing ozone standard protective of public health? and,
> (b) What is the nature and magnitude of additional public health protection provided by the suite of alternative standards under consideration?

EPA made a number of changes we recommended in prior comments (Goodman and Sax, 2012), which we believe improved the analysis. One of these changes is the use of a model-based adjustment procedure instead of the mathematically based quadratic rollback procedure to estimate ozone concentrations that meet current and alternative ozone standards. EPA relied on these estimates for two separate risk evaluations: lung function decrements based on controlled exposure studies, and respiratory morbidity and all-cause mortality endpoints based on epidemiology data.

Exposure and lung function risks assessment based on controlled human exposure studies

In the REA, EPA used an updated model to evaluate lung function decrements – this was one of our recommendations from previous comments (Goodman and Sax, 2012). In addition, EPA used an updated epidemiology study in its evaluation of short-term ozone exposure and risks of all-cause mortality. EPA also evaluated respiratory morbidity outcomes (*i.e.*, emergency department visits for asthma and all respiratory outcomes and hospital admissions for chronic obstructive pulmonary disease, asthma and all respiratory effects, and respiratory symptoms), but it used the same studies as in the first draft REA. Against our recommendation (because of a lack of evidence for causation), EPA added an evaluation of respiratory mortality risks associated with long-term ozone exposure in the second draft REA.

EPA estimated population ozone exposures using version 4.5 of the Air Pollutants Exposure (APEX) Model for four groups of what it considered to be "higher-risk" populations, including school-aged

children (ages 5-18 years), asthmatic school-age children, asthmatic adults (ages 19-64 years), and older adults (ages 65-95 years), but it reported exposure-related lung function decrements only for school-aged children (ages 5-18 years) and adults (19-35, 35-55, and >55 years old). EPA developed concentration-response functions[1] for lung function decrements from several controlled human exposure studies. The exposure-response functions were based on two models: one used in the 2007 ozone National Ambient Air Quality Standards (NAAQS) review updated with data from more recent studies, and an updated McDonnell-Stewart-Smith model (McDonnell *et al*., 2012).

EPA estimated exposures over three "benchmark" levels in its exposure evaluation, noting that an 8-hour exposure level of 60 parts per billion (ppb) was the lowest level at which ozone-related effects have been observed in human exposure studies. In reporting lung function impacts from ozone exposure, EPA evaluated the percentage of people with a single occurrence of a lung function decrement over a cutoff value (*i.e.*, ≥ 10, 15, or 20%) in one year.

Mortality and morbidity risk assessment based on epidemiology studies

EPA based its analyses of respiratory morbidity and mortality on data from epidemiology studies. EPA calculated risks using the environmental Benefits Mapping and Analysis Program for 12 US cities and also calculated a national assessment of mortality risks to compare risks in these cities to the US as a whole. EPA calculated "total" risks modeled down to a zero ozone level for the air quality years 2007 and 2009 and scenarios in which ozone concentrations meet the current standard (75 ppb). It also calculated the incremental risk reductions that would occur by achieving lower proposed standards (60, 65, and 70 ppb) *vs*. the current level of the standard.

EPA conducted a limited uncertainty evaluation for each analysis. EPA reported confidence bounds for mortality risk estimates from short- and long-term ozone exposure only, and only a small portion of the uncertainty was represented by these confidence bounds. EPA discussed other sources of uncertainty associated with the exposure, lung function, and respiratory morbidity risk assessments only qualitatively. In addition, EPA conducted only a limited number of sensitivity analyses.

Based on the results from the various risk evaluations, EPA (2014) concluded:

> [E]xposures and risks remain after just meeting the existing standards, and…in many cases, just meeting alternative standard levels results in reduction in those exposures and risks.

EPA appears to base this conclusion largely on evaluations of standards that would result in children experiencing one time in a year (1) exposures above 8-hour average concentrations of 60 ppb ozone or (2) ≥ 10% lung function decrements. Notably, EPA concluded that "[m]ortality from short- and long-term O_3 exposures and respiratory hospitalization risk is not greatly affected by meeting lower standards" (US EPA, 2014, p. 9-47).

Recommendations

Our evaluation shows that the REA likely overestimated exposure and lung function risks, and small reductions in exposure and risks from lower ozone standards are likely to be well within model uncertainty – particularly when considering that the current ozone standard is health protective. We discuss specific recommendations in response to CASAC charge questions.

[1] EPA refers to the concentration-response function as an "exposure-response function" for controlled exposure studies.

Exposure and lung function risk assessment based on controlled human exposure studies

With regard to the exposure and lung function risk assessment, EPA posed the following question to CASAC:

> ***To what extent does the Panel find the assessment, interpretation, and presentation of the methods and results of the updated and expanded population-based exposure analysis to be technically sound, appropriately balanced, and clearly communicated?***

- EPA's exposure assessment approach was conservative on many levels, and it should not be.
  - EPA used benchmark ozone concentrations down to an 8-hour average of 60 ppb ozone when data do not support health effects at these low levels of exposure (*e.g.*, Goodman *et al*., 2013). EPA should analyze exposures and risks above a higher benchmark, such as 70 ppb, as the core analysis.
  - EPA highlighted analyses of a single day in a year with exposures above benchmark levels, but it would be more appropriate to consider multiple days as the core analysis.
- With regard to the exposure analyses, results indicate that lower ozone standards would lead to small reductions in exposure above EPA benchmarks, particularly at the mid-level benchmark (Figure 1), but reductions in the number of exposed individuals are likely well within model uncertainty. EPA should highlight these findings instead of findings at lower more uncertain benchmarks which are of questionable adversity (*i.e.*, single exceedances at 60 ppb, Figure 2).
- With regard to the lung function risk analysis, EPA highlighted risks of experiencing small lung function decrements ($\geq$ 10% and < 20%), measured as forced expiratory volume in 1 second (FEV_1), but these are not likely to be adverse according to ATS and others if not accompanied by other respiratory symptoms (ATS, 2000; Pellegrino *et al*., 2005). Also, compared to multiple occurrences, single FEV_1 decrements in a year are more likely to be due to factors other than ozone or within normal variability. Therefore, EPA should only report results for multiple occurrences of FEV_1 decrements.

With regard to the uncertainty analysis, EPA posed the following question to CASAC:

> ***To what extent does the Panel find that the discussion of uncertainty and variability have covered important sources of uncertainty and variability and appropriately characterized their relationship to the exposure estimates?***

- EPA conducted only quantitative analysis for most sources of uncertainty in the exposure and risk assessments.
  - Even though EPA appears to have identified the major sources of uncertainty, it needs to better identify which are expected to have the most influence on exposure and risk estimates.
  - Also, EPA should more clearly state the expected direction and magnitude of bias with examples.
  - EPA should also provide confidence bounds for exposure estimates that reflect all the uncertainty in the modeled estimates that can be quantified. This should, at a minimum, be part of the core analyses.

- Sensitivity analyses EPA performed in the REA suggest that key model assumptions, including age-adjustments and ventilation rate assumptions, led to overestimates of risks – particularly for children. Key results are imbedded in appendices and need to be highlighted more in the main REA document.
- As with the exposure estimates, EPA should incorporate confidence bounds for risk estimates in the core and sensitivity analyses. This would allow proper comparison between risk estimates for meeting the current and alternative standards.
- Despite having no confidence bounds for risk estimates, results suggest very small reductions in risk with lower ozone standards, particularly when considering risk levels that are more likely to be adverse (*e.g.*, multiple days with FEV_1 decrements). EPA should highlight these findings, which indicate that the current standard is health protective and no significant additional benefits will be achieved from lower ozone standards.
    - For example, Table 6-4 from the REA (reproduced as Table 1 below) shows that not more than approximately 6% of simulated individuals (representing worst-case conditions) would have multiple (> 6 days) of lung function decrements ≥ 10% at current levels of the ozone standard (75 ppb) compared to 9% at baseline ozone levels.
    - Also, Table 6-5 from the REA (reproduced as Table 2 below) shows that not more than about 1% of simulated individuals (representing worst-case conditions) have multiple days of lung function decrements ≥ 15% at the current level of the ozone standard compared to about 3% for baseline conditions.
    - Lower ozone standards do not significantly change these numbers, and these changes are more than likely within model uncertainty.

Mortality and morbidity risk assessment based on epidemiology studies

With regard to the mortality risk assessment, EPA posed the following question to CASAC:

> ***To what extent does the Panel find the assessment, interpretation, and presentation of the methods and results of the updated epidemiology-based risk assessment to be technically sound, appropriately balanced, and clearly communicated?***

- Instead of evaluating mortality and respiratory morbidity risks down to a zero ozone concentration, EPA should only evaluate risks above ozone threshold levels (either background ozone or thresholds determined by evidence from controlled human exposure studies).
- EPA's presentation of mortality rates in graphics is sometimes misleading and should be updated.
    - For example, although the data are shown in tables, EPA should present mortality rate results in figures with confidence bounds for current and alternative levels of the ozone standard. As we show below (Figure 3), in contrast to results as presented by EPA in the REA (Figure 4 below), this approach more clearly demonstrates that there is no apparent statistical difference in mortality at alternative ozone levels compared to current levels when considering uncertainty.
    - EPA should consistently show confidence bounds in summary figures in the main REA document.

Regarding a specific approach in the risk analysis, EPA posed the following question to CASAC:

> ***What are the views of the Panel on the characterization of the degree to which these changes in spatial patterns of O_3 introduce uncertainty in risk estimates when effect estimates based on one spatial/temporal pattern of O_3 (the pattern in the epidemiology study) are applied to a substantially different spatial/temporal pattern of O_3 concentrations?***

- EPA should not have increased the air quality monitoring area evaluated in the risk assessment beyond the area included in the underlying epidemiology studies in the core analysis. This introduced considerable uncertainty.

With regard to uncertainty in the mortality risk assessment, EPA posed the following question to CASAC:

> ***To what extent does the Panel find that the discussion of uncertainty and variability have covered important sources and appropriately characterized the relationship of those sources of uncertainty and variability to the risk estimates?***

- EPA did not, but should, explicitly discuss the magnitude and direction of bias from uncertainty not included in the confidence bounds for mortality risk estimates (*e.g.*, shape of the CRF, confounding, model specification, exposure and outcome measurement error).
  - For example, Figure 5 shows that mortality estimates that were positive in core analyses are not positive when PM_{10} is included in the model.
- EPA should discuss the rational for the respiratory morbidity risk assessment more fully, as it is based on much more limited data (*e.g.*, primarily single-city studies) compared to the mortality risk evaluation. Also, EPA should include confidence bounds for morbidity risks.
- EPA should not include an evaluation of respiratory mortality associated with long-term ozone exposure. EPA cites a single study, Jerrett *et al*. (2009), as support of evidence for respiratory mortality, when other studies and a larger body of evidence do not support a causal association with this endpoint. Thus, this analysis should not be included in the REA.
- EPA should be consistent in its approach for evaluating higher-risk individuals. For example, it justified using data based on adults for children by acknowledging a similar lung function response, but then used inappropriate age adjustments. This led to overestimated risks.

If the REA is updated to address these recommendations, it will be evident that ozone exposure and risk assessments do not indicate additional public health protection from lowering the ozone standard compared to the current, health-protective standard.

Thank you for your consideration.

Sincerely,

GRADIENT

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Table 1 Percents of Simulated Individuals with One or More Days in the Ozone Season with Lung Function Decrement (ΔFEV_1) Greater than 10%. Table shows the minimum and maximum percents across all cities and years (US EPA, 2014, Table 6-4).

		percent experiencing ≥ 1 day with $\Delta FEV_1 \geq 10\%$		percent experiencing ≥ 6 days with $\Delta FEV_1 \geq 10\%$	
Age group	**Scenario**	**minimum**	**maximum**	**minimum**	**maximum**
5 to 18	base	11%	31%	1%	9%
5 to 18	75	11%	22%	1%	6%
5 to 18	70	8%	20%	1%	5%
5 to 18	65	2%	18%	0%	4%
5 to 18	60	4%	13%	0%	3%
19 to 35	base	3%	13%	0%	1%
19 to 35	75	3%	9%	0%	1%
19 to 35	70	2%	8%	0%	1%
19 to 35	65	1%	6%	0%	1%
19 to 35	60	1%	5%	0%	0%
36 to 55	base	1%	4%	0%	0%
36 to 55	75	1%	2%	0%	0%
36 to 55	70	0%	2%	0%	0%
36 to 55	65	0%	2%	0%	0%
36 to 55	60	0%	1%	0%	0%
> 55	All	0%	0%	0%	0%

Table 2 Percents of Simulated Individuals with One or More Days in the Ozone Season with Lung Function Decrement (ΔFEV_1) Greater than 15%. Table shows the minimum and maximum percents across all cities and years (US EPA, 2014, Table 6-5).

		percent experiencing ≥ 1 day with $\Delta FEV_1 \geq 15\%$		percent experiencing ≥ 6 days with $\Delta FEV_1 \geq 15\%$	
Age group	**Scenario**	**minimum**	**maximum**	**minimum**	**maximum**
5 to 18	base	2%	12%	0%	3%
5 to 18	75	2%	6%	0%	1%
5 to 18	70	2%	5%	0%	1%
5 to 18	65	0%	4%	0%	1%
5 to 18	60	0%	3%	0%	0%
19 to 35	base	0%	3%	0%	0%
19 to 35	75	0%	2%	0%	0%
19 to 35	70	0%	1%	0%	0%
19 to 35	65	0%	1%	0%	0%
19 to 35	60	0%	1%	0%	0%
36 to 55	base	0%	1%	0%	0%
36 to 55	75	0%	0%	0%	0%
36 to 55	70	0%	0%	0%	0%
36 to 55	65	0%	0%	0%	0%
36 to 55	60	0%	0%	0%	0%
> 55	All	0%	0%	0%	0%

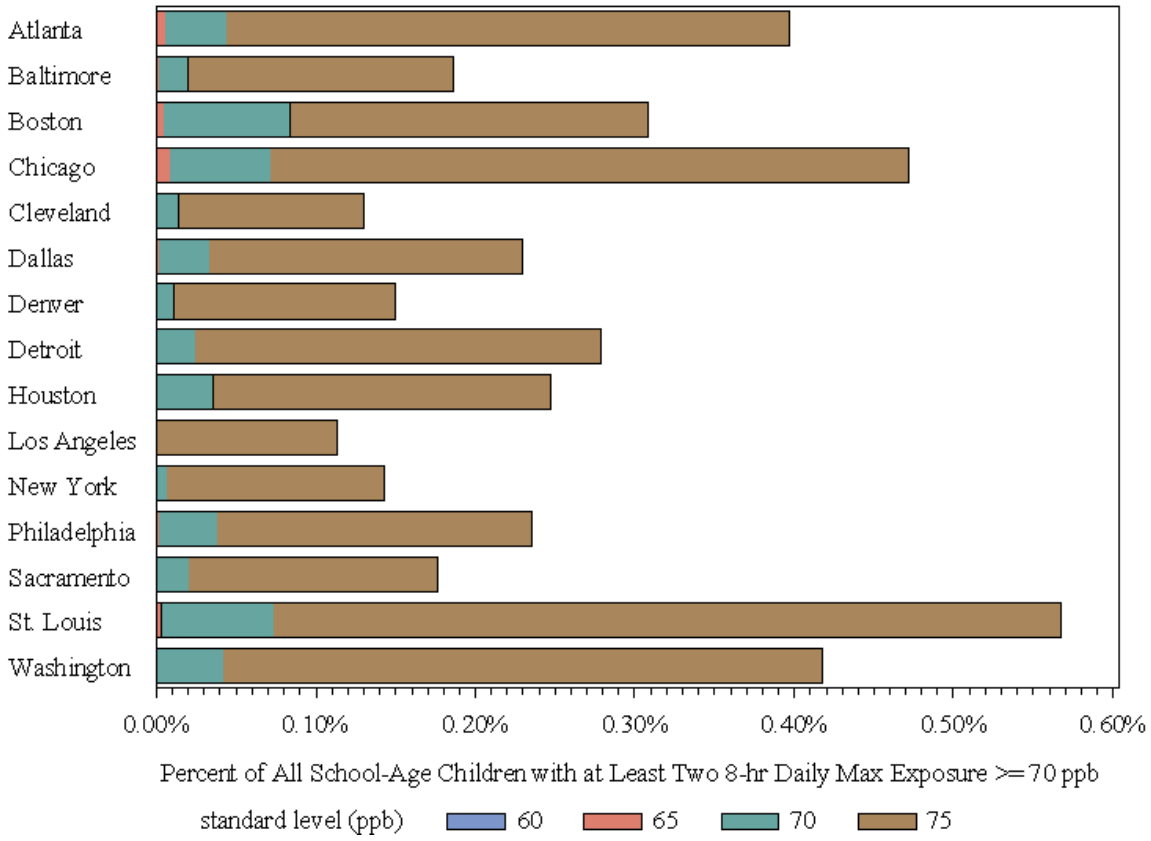


Figure 1 Average Percent of All School-age Children with at Least Two Daily 8-hr Maximum Exposures Greater than or Equal to 70 ppb for Air Quality Scenarios of Just Meeting the Current and Alternative Ozone Standards. Figure reproduced from EPA (2014) Figure 5F-13, Appendix 5F.

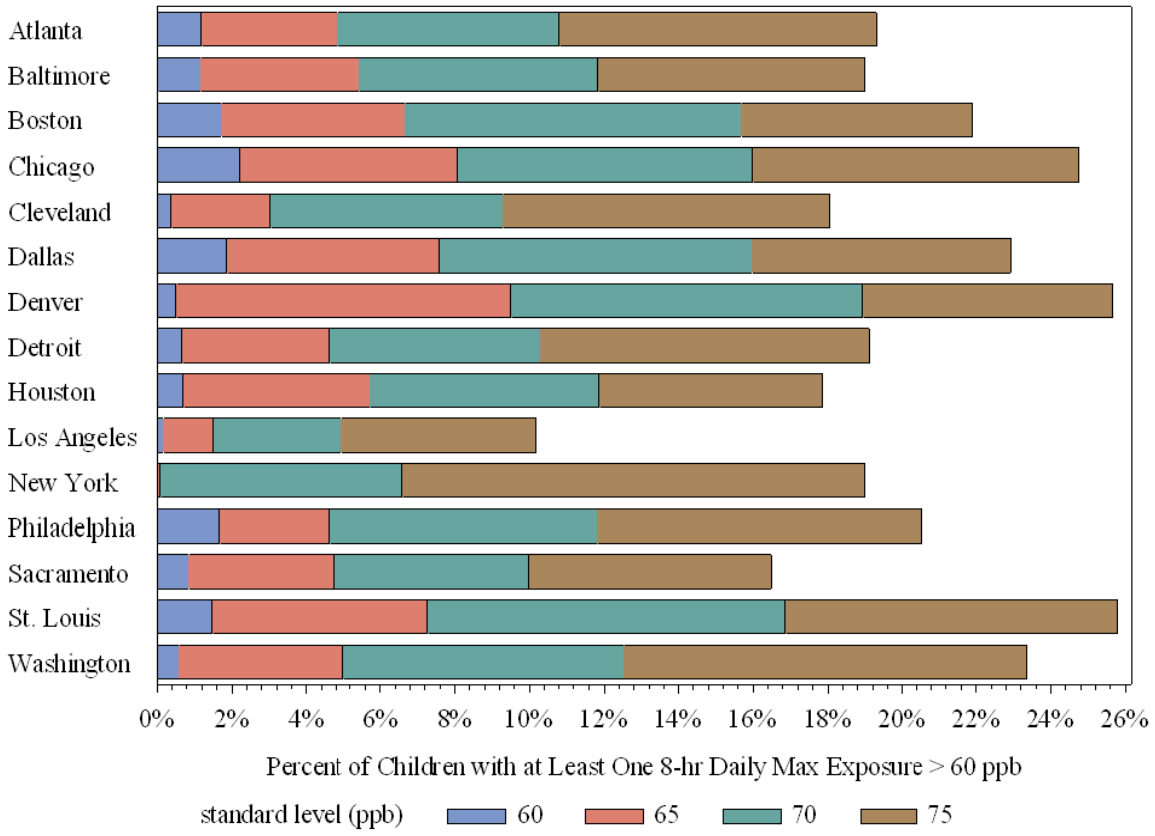


Figure 2 Average Percent of All School-age Children with at Least One Daily 8-hr Maximum Exposures Greater than or Equal to 60 ppb for Air Quality Scenarios of Just Meeting the Current and Alternative Ozone Standards. Figure reproduced from EPA (2014) Figure 9-3.

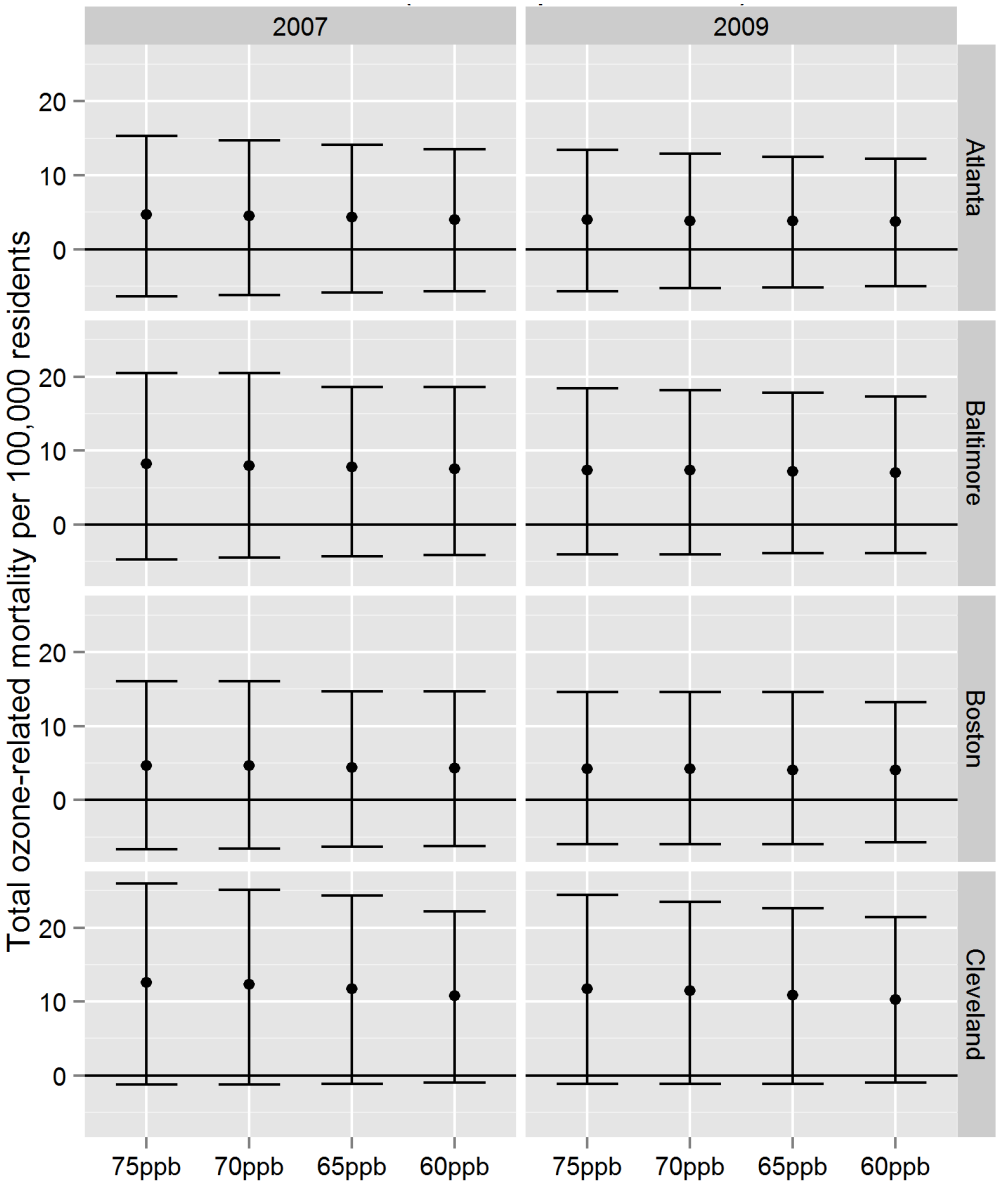
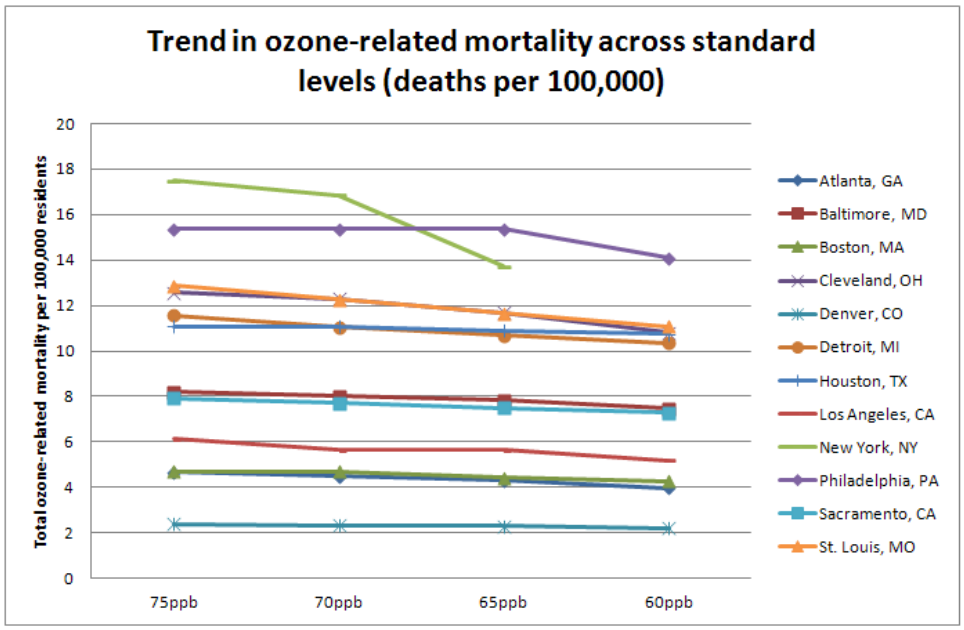


Figure 3 All-cause Mortality Rates (per 100,000 people) with 95% Confidence Intervals for 2007 and 2009. Mortality rates estimated for air quality meeting current and or alternative ozone standard standards in Atlanta, Baltimore, Boston, and Cleveland in 2007 and 2009. Based on data in US EPA (2014) Table 7-7.

2007 Simulation year



2009 Simulation year

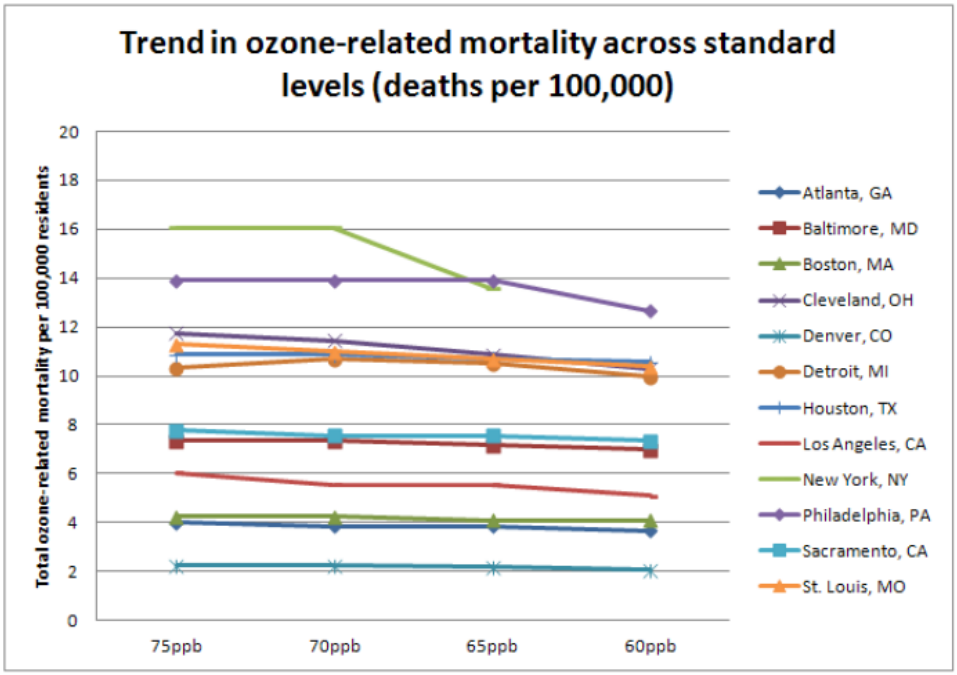


Figure 4 All-cause Mortality Rates for Just Meeting Existing (75 ppb) and Alternative Standards for 2007 and 2009. Reproduced from US EPA (2014) Figure 9-6.

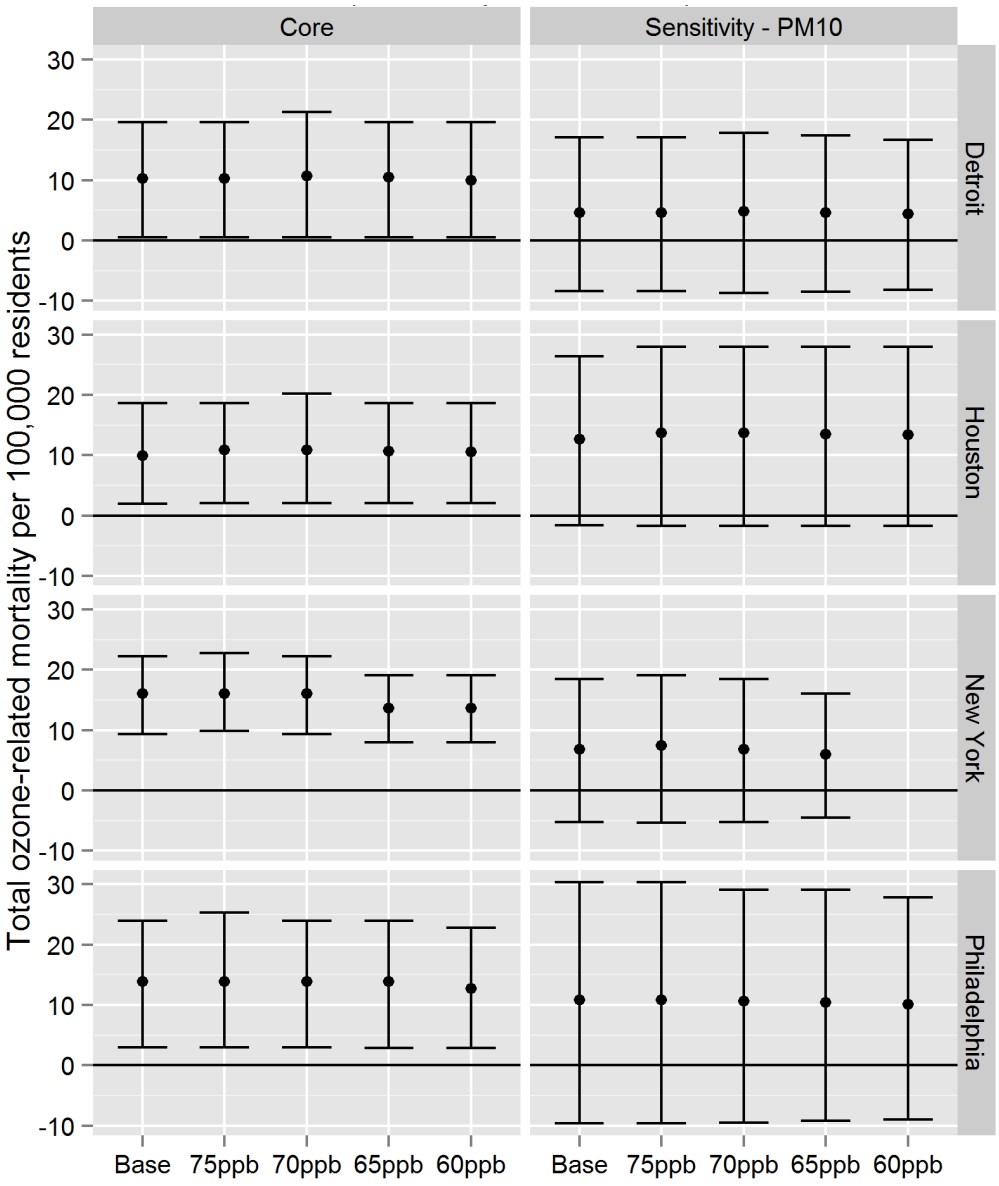


Figure 5 All-cause Mortality Rates (per 100,000 people) with 95% Confidence Intervals for Core and PM_{10}-adjusted Analyses. Mortality rates estimated for air quality meeting current and or alternative ozone standard standards in Houston, New York, Philadelphia, and Detroit in 2009. Core analysis results (based on data in US EPA, 2014, Table 7B-2) compared to sensitivity analyses including PM_{10} (based on data in US EPA, 2014, Table 7C-4).

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Evaluation of adverse human lung function effects in controlled ozone exposure studies

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ABSTRACT: The US EPA is evaluating controlled human ozone exposure studies to determine the adequacy of the current ozone National Ambient Air Quality Standard of 75 ppb. These studies have shown that ozone exposures of 80 ppb and greater are associated with lung function decrements. Here, we critically review studies with exposures below 80 ppb to determine the lowest ozone concentration at which decrements are causally associated with ozone exposure and could be considered adverse using the Adverse Effects/Causation Framework. Regarding causation, the framework includes consideration of whether exposure-related effects are primary or secondary, statistically significant, isolated or independent, or due to study limitations. Regarding adversity, the framework indicates one should consider whether effects are adaptive, compensatory, precursors to an apical effect, severe, transient and/or reversible. We found that, at exposures below 72 ppb ozone, lung function effects are primary effects, but are isolated, independent and not statistically different compared to effects observed during filtered air exposure, indicating a lack of causation. Up to 72 ppb, lung function effects may be precursors to an apical effect, but are not likely adverse because they are transient, reversible, of low severity, do not interfere with normal activity and do not result in permanent respiratory injury or progressive respiratory dysfunction. Overall, these studies do not demonstrate a causal association between ozone concentrations in the range of the current National Ambient Air Quality Standard and adverse effects on lung function. Copyright © 2013 John Wiley & Sons, Ltd.

Keywords: criteria pollutants; inhalation exposure; pulmonary disease; ozone; human exposure; adverse effects; pulmonary function; causality; NAAQS

Introduction

Acute ozone exposure can produce reversible lung function decrements and transient respiratory symptoms, such as coughing, wheezing and chest pain (US EPA, 2006). Studies of humans subjected to ozone under controlled conditions have consistently shown these effects with ozone exposures of 80 ppb and greater (US EPA, 2006), but the lowest ozone concentration at which these effects occur and, importantly, the lowest concentration at which they should be considered to be adverse, is under debate.

The current primary ozone National Ambient Air Quality Standard (NAAQS) set by the United States Environmental Protection Agency (US EPA) in 2008 is 75 ppb (US EPA, 2008), although this is currently under review. In August 2012, US EPA released the first draft Policy Assessment (US EPA, 2012a), in which it stated that evidence presented in the third draft Integrated Science Assessment (US EPA, 2012b) and the first draft Risk and Exposure Assessment (US EPA, 2012c) 'calls into question the adequacy of the current standard and provides strong support for considering potential alternative standards to increase public health protection' (US EPA, 2012a). Part of the evidence cited by US EPA came from human studies with controlled ozone exposures below 80 ppb.

Biological changes in response to an environmental exposure occur along a continuum that may eventually lead to adverse effects, depending on exposure dose and duration [National Research Council (NRC), 2007]. Many types of changes may fall along the continuum, but some changes are mild and transient and do not result in long-term adverse outcomes. For example, if the perturbation of a normal biological pathway is small enough, it will cause an adaptive response to maintain homeostasis. Because the body is able to adapt to various environmental stresses to maintain homeostasis, most substances do not cause adverse effects unless they are at a dose sufficient to overwhelm these processes over a certain period (Goodman *et al*., 2010). Distinguishing non-adverse from adverse effects is critical for determining the dose at which an exposure can result in a detrimental health outcome.

We critically reviewed the recent controlled human studies with exposures below 80 ppb. We applied the recently developed Adverse Effects/Causation Framework (Goodman *et al*., 2010) to assess causality and characterize the degree of adversity of the respiratory effects reported in these controlled human ozone exposure studies.

Methods

Literature Search

We conducted a literature search in PubMed for studies of lung function effects in humans after controlled exposure to ozone. Search terms included expos*, ozone, pulmonary, lung, respiratory, response, function, human and forced expiratory volume in 1 s (FEV_1). We limited our analysis to studies that examined ozone

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concentrations relevant to the current primary ozone NAAQS of 75 ppb and informative for the determination of the Lowest Observable Adverse Effect Concentration (LOAEC) for lung function effects of ozone. Studies that evaluated only high exposures (i.e., > 80 ppb) were excluded from the analysis.

The Adverse Effects/Causation Framework

We evaluated the relevant controlled exposure studies using the Adverse Effects/Causation Framework described by Goodman *et al*. (2010). The framework describes several factors that should be considered for determining both causation and adversity. Regarding causation, primary exposure-related effects are much more reliable indicators of causation than effects that are secondary (e.g., DNA damage because of cytotoxicity). Statistical significance is also a key factor for determining causality. Although there are many reasons that a true association might not be statistically significant in a particular study, if an association is statistically significant, it is much more likely to be indicative of a true association. Similarly, in most cases, if an effect is isolated (occurring in very few study subjects) or independent (occurring in the absence of other effects that are expected via the same mode of action), it is not as likely to be causally related to an exposure. By definition, both isolated and independent effects occur inconsistently and likely reflect biological effects from another cause or measurement error rather than effects from a given exposure (Lewis *et al*., 2002). If observed effects are a result of study limitations (e.g., exposure measurement error, confounding), they are not likely to be indicative of causation.

Regarding adversity, adaptive effects are changes that occur in response to environmental stressors to maintain normal function in an organism and enhance its ability to respond to additional stressors. Adaptive effects are not considered adverse. Compensatory effects also maintain normal function of an organism without enhancement of response to further environmental insults. If compensatory effects occur over a short period, they are not considered adverse. Transient effects are initial responses to an environmental exposure that are not sustained throughout the exposure period. Effects are reversible if the subject recovers completely at some point after exposure has ended. Both transient and reversible effects are less likely to be considered adverse. Early precursors of apical effects, effects of low severity and effects that are not severe (i.e., do not result in functional impairment) are less likely to be adverse. It should also be noted that there could be effects that occur after an exposure that are not homeostatic, but they are unrelated to any toxic effect of that particular exposure. These effects should not be considered adverse.

Sir Austin Bradford Hill (1965) put forth nine guidelines that should be considered when evaluating causation that also apply to evaluations of adversity. He cautioned:

> None of my nine viewpoints can bring indisputable evidence for or against the cause-and-effect hypothesis and none can be required as sine qua non. What they can do, with greater or less strength, is to help us make up our minds on the fundamental question – is there any other way of explaining the facts before us, is there any other answer equally, or more, likely than cause and effect?

The Adverse Effects/Causation Framework should be used and interpreted in the same manner. It should not be used as a checklist, but rather all of the factors should be considered and they should each inform the interpretation of each other. For example, a non-statistically significant association is more likely to be indicative of causation if it occurs with other effects that are expected based on the mode of action.

We evaluated controlled human exposure studies using the framework to determine the No Observable Adverse Effect Concentration (NOAEC) or the level at which no adverse effects are observed, and the LOAEC or the level at which adverse effects are observed because of ozone exposure.

Controlled Exposure Studies

We reviewed studies that evaluated concentrations of ozone below 80 ppb because such concentrations are relevant to the current primary ozone NAAQS of 75 ppb and are informative for determining the LOAEC for lung function effects. These studies involved healthy young adults who were exposed to controlled concentrations of ozone through a facemask inhalation system or via whole-body exposure in an environmental chamber for periods of up to 6.6 h. Because physical activity increases ventilation rate, which in turn increases the dose of inhaled ozone at a given concentration relative to an individual at rest, the subjects performed quasi-continuous exercise (QCE) during the exposure period (i.e., 50 min of continuous exercise followed by 10 min of rest each hour). All of the studies included a control scenario in which subjects were exposed to filtered air (FA) with no ozone.

Lung function and respiratory symptoms were generally assessed once during each hour of exposure. Lung function effects were evaluated via spirometry to determine temporal changes in FEV_1, forced vital capacity (FVC) and the ratio of FEV_1/FVC. FEV_1 is the amount of air that can be exhaled from lungs in the first second of a forced exhalation. FVC is a measure of the maximum amount of air that can be exhaled from lungs after a maximum inspiration. The FEV_1/FVC ratio, also called the Tiffeneau index, is used in the diagnosis of obstructive and restrictive lung disease. In obstructive lung disease, this ratio is reduced because of a reduction in FEV_1. In contrast, for restrictive lung disease, the FVC is reduced because of fibrosis or other lung pathology, and the ratio is larger due to an increased FEV_1. Ventilatory disease, which encompasses aspects of both obstructive and restrictive patterns, can result in decreases of both FEV_1 and FVC, resulting in a ratio similar to 1.

In general, the controlled human exposure studies we reviewed were not designed to assess ozone effects in individuals; rather, they were designed to determine whether, at a given exposure level, the data support ozone as a cause of lung function effects or respiratory symptoms at the group level. The studies deviated primarily in the statistical analyses of the results. The relevant studies that were identified are summarized in Table 1 and described below.

Adams (2002)

Adams (2002) investigated lung function effects and respiratory symptoms in 30 healthy, non-smoking young adults (15 of each sex; mean age of 22 years) exposed to ozone for 6.6 h. He used five experimental protocols with square-wave exposures, in which ozone concentrations were maintained at a constant value throughout the exposure period. Two protocols included exposure to 120 ppb ozone (facemask or chamber exposure); the other protocols included facemask exposures at 80 ppb and 40 ppb ozone and a chamber exposure to FA. The subjects

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Table 1. Controlled human exposure studies of ozone in healthy, exercising adults

Study	n	Mean age (years ± SD)	Ventilation rate (L min^{-1} m^{-2} BSA)	Exp	Ozone exp (ppb)	Exp profile	ΔFVC		ΔFEV_1						RS[d]	NOAEC (ppb)	LOAEC (ppb)
							ΔFVC (%)	SD[a]	ΔFEV_1 (%)	SD[a]	Group mean change (%)[b]	Group SD[c]	SS[d]	CS[e]			
Adams (2002)	30	22.5 ± 1.3	20		FA		0.27	2.95	2.39	4.01							
				FM	40	SQR	−1.24	4.23	−1.15	4.20	−1.24	4.89				80	120
				FM	80	SQR	−4.34	5.25	−3.96	7.50	−6.35	7.40	X		X		
				FM	120	SQR	−11.0	7.88	−13.0	9.21	−15.4	8.91	X	X	X		
				CHBR	120	SQR	−10.7	8.24	−13.3	11.2	−15.6	10.7	X	X	X		
Adams (2006)	30	23.1 ± 1.4	20	CHBR	FA		−0.44	2.15	1.35	2.98							
					40	TRI	−0.74	2.05	1.17	2.97	−0.18	3.54				80	None
					60	SQR	−0.89	3.12	−1.51	4.24	−2.86	4.42					
					60	TRI	−1.72	5.15	−1.43	5.95	−2.78	5.83					
					80	SQR	−4.46	7.26	−4.72	8.65	−6.07	8.29	X		X		
					80	TRI	−4.78	6.23	−5.65	8.08	−7.00	7.76	X		X		
Schelegle *et al.* (2009)	31	21.4 ± 1.5	20	CHBR	FA				0.80	5.01							
					63	TRI			−2.72	8.24	−3.52	8.31				81	88
					72	TRI	NR		−5.34	7.91	−6.14	8.03	X		X		
					81	TRI			−7.02	8.91	−7.82	8.86	X		X		
					88	TRI			−11.4	12.2	−12.2	11.8	X	X	X		
Kim *et al.* (2011)	59	25.0 ± 3.8	20	CHBR	FA		−1.13	1.52	−0.002	3.53						60	None
					60	SQR	−2.32	1.83	−1.71	3.84	−1.71	0.57	X				

BSA, body surface area; CHBR, chamber; CS, clinically significant; Exp, exposure; FA, filtered air; FM, facemask; LOAEC, lowest observed adverse effect concentration; NOAEC, no observed adverse effect concentration; NR, not reported; ppb, parts per billion; RS, respiratory symptoms; SD, standard deviation; SQR, square-wave; SS, statistically significant; TRI, triangular; ΔFEV_1, change in forced expiratory volume in one second; ΔFVC, change in forced vital capacity.

[a]SD derived from SE (= SE × √N) reported by Schelegle *et al.* (2009) and Kim *et al.* (2011).

[b]Group mean change (decrease) in FEV_1 adjusted for effects of exercise in filtered air; $\Delta FEV_{1,Air} - \Delta FEV_{1,O3}$.

[c]SD = √(SD($\Delta FEV_{1,Air}$)2 + SD($\Delta FEV_{1,O3}$)2−2 [A-Za-z0-9,2−2 × ρ × SD; ($\Delta FEV_{1,Air}$) × SD($\Delta FEV_{1,O3}$)]; where r (=0.291) is the average correlation coefficient for $\Delta FEV_{1,O3}$ versus $\Delta FEV_{1,Air}$ for individuals [calculated from individual subject data in Kim *et al.* (2011) and Adams (2006) studies.

[d]Statistically significant if $P \leq 0.05$.

[e]Defined as a decrease in $FEV_1 \geq 10\%$ [based on US EPA (2007)].

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performed QCE during the exposure period, alternating between a cycle ergometer and a treadmill each hour. A minute ventilation rate (V_E) of ~20 L min^{-1} m^{-2} of body surface area was maintained during exercise. After the first 3 h of exposure, the subjects were given a brief lunch break, during which they were exposed to ozone in chamber (but not facemask) protocols. The endpoints examined in this study were percentage changes in FEV_1 and FVC, absolute changes in self-reported total symptom severity (TSS) and pain on deep inspiration (PDI). All endpoints were evaluated before, after and hourly during the 6.6 h experimental protocol.

To analyze the data, Adams (2002) used a two-way analysis of variance (ANOVA) with repeated measures, which tested for ozone concentration and exposure protocol (facemask versus chamber) and time effects on lung function. In this type of analysis, individual subjects are measured multiple times, each serving as his or her control. Upon obtaining a statistically significant F statistic, the Scheffe post hoc test was used to assess differences between the mean values for each time point and for each protocol (a total of five protocols × six time points = 30 mean values), which resulted in (30 × 29)/2 = 435 comparisons.

Adams (2002) reported statistically significant effects on lung function at ozone concentrations of 80 ppb and 120 ppb, but not 40 ppb. Based on the Scheffe test, the mean percentage changes in FEV_1 and FVC in the post-exposure group for the two 120 ppb protocols were greater than for the other three protocols ($P < 0.05$). In addition, the changes were greater for the 80 ppb protocol than the 40 ppb and FA protocols ($P < 0.05$), which did not differ from one another. As shown in Table 1, the group mean decrement in FEV_1 after exposure to 80 ppb ozone relative to FA was 6.35%, and for the 120 ppb protocols, the group mean FEV_1 decrement ranged from 15.4 to 15.6% relative to FA. Post-exposure decrements in FVC ranged from 11.0 to 11.2% for the 120 ppb protocols and 4.6% following 80 ppb ozone relative to FA. Observed changes in FEV_1 and FVC both decreased such that there was little difference in the FEV_1/FVC ratio after exposure. Both post-exposure TSS and PDI were increased with the 80 and 120 ppb protocols compared to the FA protocol ($P < 0.05$). Adams (2002) concluded that the results for the chamber and facemask exposures at 120 ppb were equivalent, but noted high individual subject variability in responses with the 80 ppb protocol.

Adams (2006)

Adams (2006) examined the lung function effects of time-dependent chamber exposures to ozone for 6.6 h in 30 healthy, non-smoking young adults (15 of each sex; mean age of 23 years). Much of the experimental study design followed the structure of his previous study (i.e., Adams, 2002); one difference is the concentration–time profile of exposure because one of the goals of the newer study was to investigate the differences between triangular and square-wave exposure scenarios. In the triangular exposure profile, ozone concentrations were increased step-wise each hour for the first 4 h then decreased in the last 2 h of exposure. This was done to achieve an overall cumulative average equal to a specified level that was also used as a uniform or square-wave exposure in a separate experiment. The triangular exposure profile better approximates the typical pattern of daytime ambient ozone concentrations than the square-wave profile (Adams, 2006; Lefohn *et al.*, 2010). Adams (2006) used six different experimental protocols: standard square-wave concentration profiles at 0 (FA), 60 and 80 ppb ozone and triangular concentration profiles at 40, 60 and 80 ppb ozone. The same endpoints were examined as in the study by Adams (2002) before, after and at 1 h intervals during exposure. The subjects performed QCE to maintain a V_E of 20 L min^{-1} m^{-2} body surface area, a precedent set by earlier studies. Characteristics such as age, height, weight, body fat, VO_{2max} and baseline pulmonary measures (FEV_1, FVC, FEV_1/FVC) were recorded and summarized as a group, but were not directly assessed in the data analyses as possible confounders.

Similar to the Adams (2002) study, the data in this study were analyzed using a two-way ANOVA with repeated measures. The analysis tested for ozone concentration and exposure protocol (square-wave versus triangular) and time effects on lung function and was followed by the Scheffe post hoc test to assess differences between the mean values.

Adams (2006) reported statistically significant decrements in lung function only with the 80 ppb exposure protocols. As shown in Table 1, the post-exposure group mean percentage changes in FEV_1 and FVC for the two 80 ppb protocols were greater than those for the FA, 40 ppb triangular, 60 ppb square-wave and 60 ppb triangular exposure protocols ($P < 0.05$ for all), which did not differ from one another. Specifically, Adams (2006) reported group mean decrements in FEV_1 of 6.1% and 7.0% relative to FA for the square-wave and triangular 80 ppb ozone exposures, respectively. A dose-dependent group mean decrement in FVC was also observed, with maximal FVC change of 4.9% for square-wave and 5.2% for triangular protocols at 80 ppb ozone relative to FA. Changes in FEV_1 and FVC were observed concurrently, and therefore the ratio of FEV_1/FVC remained relatively unchanged across exposure levels. Post-exposure TSS and PDI were increased, relative to the FA protocol, for the two 80 ppb exposure protocols ($P < 0.05$), whereas for all other ozone exposure protocols, post-exposure scores were indistinguishable from those of the FA protocol. Adams (2006) also noted that for a nominal exposure concentration, triangular exposures caused effects on lung function earlier than equivalent square-wave protocols.

Schelegle *et al.* (2009)

Schelegle *et al.* (2009) conducted chamber exposure studies on 31 healthy, non-smoking young adults (16 females, 15 males; mean age of 21 years) using 6.6 h triangular exposure profiles with mean ambient ozone concentrations of 63, 72, 81 and 88 ppb, as well as FA. The subjects performed QCE as in the studies by Adams (2002, 2006), and a 35 min lunch break was given after 3 h in the chamber at the ozone concentration used during the third hour. FEV_1, FVC and TSS were measured at multiple time points up to 6.6 h of exposure, as well as 1 h post-exposure, and, in a subset of 13–17 subjects (depending on concentration), 4 h post-exposure.

Schelegle *et al.* (2009) used three different statistical methods to address different analytical issues, such as repeated measurements, multiple comparisons and non-parametric data. These techniques were mixed-effects two-way ANOVA (with ozone concentration and time as factors) with Tukey's adjustment for multiple comparisons (also known as the Tukey–Kramer adjustment), mixed-effects one-way ANOVA (with ozone concentration as the factor) with Dunnett's adjustment for multiple comparisons against a single control (FA), and Friedman's non-parametric test with Dunnett's adjustment. For the latter two methods, the authors restricted the statistical analyses to the FEV_1 decrements at 6.6 h only. The use of the mixed-effects ANOVA technique addressed subject variability. None of these statistical methods yielded a statistically significant group mean FEV_1 decrement with exposure to 63

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ppb ozone for 6.6 h ($P > 0.05$), yet all were statistically significant for the 72 ($P < 0.01$), 81 ($P < 0.001$) and 88 ppb ($P < 0.0001$) ozone exposure protocols. Similarly, increases in TSS were associated with exposure to 72, 81 and 88 ppb ozone ($P < 0.05$), but not with exposure to 63 ppb ozone. After 6.6 h of exposure to 63 ppb ozone, the authors observed a group mean FEV_1 decrement of 3.5% (relative to FA) versus 6.1% at 72 ppb ozone. Statistically significant group mean decrements in FEV_1 were observed only at 6.6 h at the 72 ppb exposure level, while no statistically significant differences were reported at any time point for FVC at this level. Statistically significant changes in the FEV_1/FVC ratio were only observed at the 5.6 h and 6.6 h time points for the 81 ppb ozone exposure level. In sum, Schelegle *et al.* (2009) found that the lowest mean ozone concentration associated with a statistically significant decrease in FEV_1 after 6.6 h of exposure relative to baseline, without any concomitant decrement in FVC, was 72 ppb.

Kim *et al*. (2011)

Kim *et al*. (2011) exposed 59 healthy, non-smoking young adults (32 females, 27 males; mean age of 25 years) to FA and 60 ppb ozone (square-wave) for 6.6 h under controlled chamber conditions to evaluate the changes in FEV_1, FVC and TSS relative to baseline. As in the studies described above, the subjects performed QCE during the exposure period, with a 35 min lunch break in the chamber. Lung function was measured before beginning the initial QCE period; during the 10 min rest periods after 3, 4.6 and 5.6 h; and immediately after the 6.6 h exposure period. The authors stated that they analyzed only the measurements taken before the first hour and after the sixth hour of exposure because they believed this would limit the need for multiple comparisons. They used linear mixed-effects models with subject-specific random intercepts to account for subject-level variability and repeated measures. Kim *et al*. (2011) reported a statistically significant mean percentage change in FEV_1 (FA adjusted) of -1.7 (95% CI: −3.0, −0.5, $P = 0.008$) and a statistically significant decrement in FVC (FA adjusted) of $1.2 \pm 0.51\%$ ($P = 0.02$). In addition, the authors noted an increase in neutrophilic inflammation of the airways in male subjects. Lastly, exposure to 60 ppb ozone in this study was not associated with any statistically significant increase in TSS.

Results and Discussion

Adverse lung function decrements have been consistently observed with controlled human exposures to ozone of at least 80 ppb, but the evidence for lower exposures is less clear. We previously described a framework that evaluates effects found along a continuum of biological changes to determine whether exposure is likely to be causal and an effect likely to be adverse (Goodman *et al*., 2010). We applied this framework to estimate the lowest ozone concentration that can cause adverse lung function effects in controlled exposure studies.

Causality

We applied the Adverse Effects/Causation Framework to determine the exposure level at which ozone may be causally associated with adverse lung function effects. The framework, described above and in more detail in Goodman *et al*. (2010), specifies several factors that should be considered to determine whether an association between an exposure and a health effect is likely to be causal. These include a consideration of whether an observed effect is a primary effect, isolated or independent, statistically significant and/or a result of study limitations.

A change in FEV_1 or FVC can be considered a primary effect, as they each can be a direct result of ozone exposure. The observed FEV_1 and FVC decrements in the controlled human exposure studies are isolated at exposure levels below about 72 ppb in that they occur in a very small number of study subjects. In addition, these decrements occurred independent of other effects (e.g., respiratory symptoms) at these exposure levels. Independent and isolated effects are not likely indicative of causation.

For an exposure to be considered causal of an effect, the association between the two should be statistically significant based on an appropriate statistical test. To best determine effects at exposures below 80 ppb, one could conduct a meta-regression of all relevant data (at all exposure levels and all time points) in each relevant study (i.e., Adams, 2002, 2006; Schelegle *et al*., 2009; Kim *et al*., 2011). The results presented by study authors, shown in Fig. 1, represent the overall post-exposure FEV_1 changes. Because each study did not provide intermediate data points, we were unable to conduct such an analysis. Therefore, below, we discuss individual studies, as well as a few quantitative evaluations of relevant studies.

Adams (2002, 2006) used the Scheffe post hoc test to assess several ozone protocols and time points. The Scheffe test is a commonly used statistical test to compare mean values because it minimizes false positive results; however, it can also produce false negative results. Nicolich (2007) analyzed the entire FEV_1 data set using a mixed-model ANOVA and Dunnett's post hoc test, which is less likely to produce false negative results than the Scheffe test. We found that the results from this analysis were consistent with the original findings of Adams (2006); there was no statistically significant group mean decrement in FEV_1 after 6.6 h of exposure to 60 ppb ozone versus FA.

In a separate evaluation of controlled human exposure studies, Brown *et al*. (2008) reanalyzed the FEV_1 study results from Adams (2006) using the nonparametric sign test as the primary statistical method to test the hypothesis that FEV_1 responses for the FA and 60 ppb exposure protocols differed. They also used the Wilcoxon signed rank test and paired t-test in their reanalysis. Both the sign test and Wilcoxon signed rank test are nonparametric tests that do not rely on the assumptions of normal distributions.

We note that a major limitation of the Brown *et al*. (2008) analysis is that they used only a subset of the Adams (2006) data. They analyzed the data as if they were from a study that measured only pre- and post-exposure results for FA and the 60 ppb square-wave protocol; data from other protocols and measurements taken at intermediate time points were ignored. Omitting the majority of the data from their analysis likely biased the results away from the null. Based on their statistical approach, Brown *et al*. (2008) reported the same 2.85% decrease in FEV_1 with the 60 ppb square-wave ozone protocol (relative to FA) as reported by Adams (2006), but reported results as statistically significant.

Regarding the study by Schelegle *et al*. (2009), the authors implemented a mixed-effects two-way ANOVA, as well as the Tukey–Kramer adjustment for multiple comparisons, that used all available data. The authors did not find statistically significant effects of ozone on FEV_1 or FVC at 63 ppb using these statistical methods. Schelegle *et al*. (2009) also used two additional

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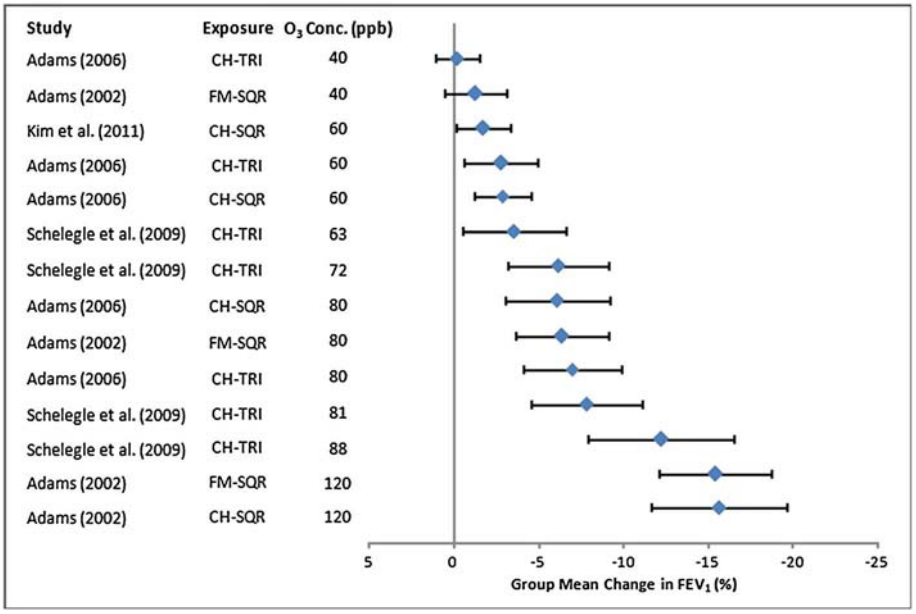

Figure 1. Forced expiratory volume in 1 s (FEV_1) decrements in controlled ozone exposure studies of healthy adults with exercise. Diamonds indicate differences between mean FEV_1 decrements after ozone exposure and FEV_1 decrements after filtered air exposure. Horizontal lines indicate 95% confidence intervals calculated from SDs. CH, chamber exposure; FM, facemask exposure; SQR, square-wave exposure profile; TRI, triangular exposure profile.

statistical approaches (ANOVA and Friedman's test, both with Dunnett's test) to examine only the FEV_1 data for pre-exposure and post-exposure after 6.6 h. The results of these additional analyses using only a subset of the data also indicated that there was no statistically significant FEV_1 decrement after exposure to 63 ppb ozone.

The most recent controlled human exposure study by Kim *et al*. (2011) measured FEV_1 and FVC at several time points during the exposure period, but only analyzed the data from the pre-exposure and post-exposure (i.e., at 6.6 h) time points. Kim *et al*. (2011) reported a statistically significant group mean percentage change in FEV_1 and FVC after 6.6 h of exposure to 60 ppb ozone. Like the analysis conducted by Brown *et al*. (2008), this analysis did not use the full data set, which likely biased the results away from the null.

Lefohn *et al*. (2010) re-analyzed five controlled ozone exposure studies that used triangular exposures, including those by Adams (2006) and Schelegle *et al*. (2009). Lefohn *et al*. (2010) used two-way ANOVA and evaluated statistical significance by using the Tukey's studentized range approach to account for multiple comparisons for least square means. Although they did not subtract the FEV_1 responses with FA from the ozone treatment responses, their methodology can still be considered conservative in that it minimizes type II errors (false negatives). Lefohn *et al*. (2010) did not find any statistically significant changes in FEV_1 at any measurement time associated with the 40 ppb and 60 ppb profiles. In addition, they found that in four of five studies, exposures to FA substantially improved FEV_1 response over the exposure period, indicating that analyses using FA controls, such as those by Adams (2006) and Schelegle *et al*. (2009), may have overestimated FEV_1 changes.

In addition, McDonnell *et al*. (2012) analyzed 23 controlled exposure studies to assess respiratory risks from ozone exposure, including those with no exposures below 80 ppb. In one of their models, McDonnell *et al*. (2012) defined the threshold as 59 ppb in liters of inhaled air per min (accumulated ozone dose), accounting for both the level of exercise and ozone concentration. This is equivalent to near-continuous exercise for 1 h at 60–80 ppb ozone or 2 h at 40 ppb ozone, or rest for 1 h at 180–240 ppb ozone or for 2 h at 120 ppb ozone. The authors found that the threshold model fit the observed data better than a no-threshold model, particularly at earlier time points and at the lowest exposure levels. These findings indicate that there are ozone exposures at which no adverse effects are likely to occur.

Regardless of the statistical test used, several study limitations should be considered when evaluating these results. For example, it is well established that inter-individual variation in FEV_1 measurements can occur in response to other factors, but these potential confounding factors were not accounted for in the controlled exposure studies. Observed lung function decrements could be due to factors unrelated to ozone. Intra-individual variation in FEV_1 measurements can also be substantial and has been estimated to be about 5% for measurements taken from healthy individuals within the same day (Pellegrino *et al*., 2005). This variability is also not fully accounted for in the controlled exposure studies and could explain some of the observed statistically significant lung function changes at lower ozone exposure levels.

Another important point is the level of exertion in these studies. As noted in Folinsbee *et al*. (1988) and McDonnell *et al*. (1991), the exercise regimen (i.e., 40 L min^{-1} for 6–8 h) used in almost all recent human controlled exposure studies conducted below 80 ppb ozone is meant to simulate work performed during a day of heavy manual labor common of outdoor workers. As defined by US EPA (1986), exercise continued at this level and duration should be considered as 'heavy' or 'strenuous work or play' (e.g., US EPA, 1986, table 10–3). The level of exertion and differential impact of exercise on study participants, in terms of its impact on FEV_1 decrements, needs

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to be considered when interpreting results. This is another reason to analyze data from all time points, rather than restricting the analysis to a comparison of pre- and post-exposure results.

Overall, the controlled human exposure data indicate that there are consistent, statistically significant decrements in FEV_1 at ozone concentrations of 72 ppb and above, regardless of the statistical test used (see Fig. 1). These decrements are primary effects of ozone exposure. The FEV_1 decrements observed at 60 (or 63) ppb are isolated and may be attributable to other factors unrelated to ozone. The effects at this concentration are only observed as statistically significant when less appropriate statistical tests are used or when only a subset of the available data are used in the analysis. No statistically significant decrements in FEV_1 have been reported for exposure to 40 ppb ozone, regardless of the statistical test. Although our causality analysis focused only on the more recent studies that included exposures below 80 ppb, the results are consistent with other controlled exposure studies demonstrating statistically significant decrements in FEV_1 at ozone concentrations of 80 ppb (Horstman *et al*., 1990; McDonnell *et al*., 1991; Adams, 2003a, 2003b), 100 ppb (Horstman *et al*., 1990; McDonnell *et al*., 1991) and above (as reviewed by US EPA, 2006).

Adversity

Inhaled ozone can produce vagal stimulation, resulting in physiological changes in pulmonary function, including bronchoconstriction (Schelegle *et al*., 1993). Antioxidants within airway lining fluid have been shown to prevent ozone-mediated cellular and tissue oxidation (Cross *et al*., 1994; Mudway *et al*., 1996; Avissar *et al*., 2000; Samet *et al*., 2001; Ballinger *et al*., 2005). Therefore, only ozone exposure of sufficient duration and concentration can overwhelm antioxidant defenses, allowing oxidative damage to occur in airway epithelial cells (Schelegle *et al*., 2007). Accordingly, a threshold exists below which antioxidant defenses are sufficient to protect against adverse effects from ozone. The question to ask is what is this threshold? To answer this, we used the Adverse Effects/Causation Framework. We determined the degree of adversity of the observed lung function effects in the controlled exposure studies by assessing whether effects were adaptive or compensatory, early precursors of an apical effect (e.g., early precursors of lung disease), transient, reversible, of low severity or did not result in functional impairment.

Ozone-associated pulmonary function decrements appear associated with both inflammatory processes and with the direct activation of nerve cells involved in response to noxious stimuli (Kim *et al*., 2011). Based on their rapid temporal response, small decrements are unlikely to be associated with tissue damage or pathologic changes associated with chronic pulmonary disease and they should be considered adaptive or compensatory responses. In contrast, large decrements in FEV_1 and/or FVC could result in adverse effects, including impaired ventilation, such as that found in restrictive or obstructive lung disease (Witschi *et al*., 2008) or a mixed (restrictive/obstructive) pulmonary abnormality (Pellegrino *et al*., 2005).

Adversity criteria have been established by various groups describing levels of FEV_1 or FVC that should be considered adverse. For example, the American Thoracic Society (ATS) judges a reversible loss of lung function in combination with respiratory symptoms to be adverse but does not indicate that a small and asymptomatic transient change constitutes an adverse effect (ATS, 2000). The European Respiratory Society suggests that short-term changes in FEV_1 exceeding 12% 'may be clinically important' and that changes in FEV_1 measurements should exceed 5% to overcome the intra-day variability of FEV_1 in normal subjects (Pellegrino *et al*., 2005). Clinical studies have often used a cut-off value of at least 20% for a decline in FEV_1 as the definition of a 'mild' asthma exacerbation (Reddel *et al*., 2009). US EPA (2007) considers a moderate decrement in lung function (defined as a decrease in FEV_1 between 10% and 20%) and/or respiratory symptoms to be adverse, although this classification has not been validated for acceptability or against other measures (ATS, 2000). In contrast, a FEV_1 decrement up to 30% of predicted is considered to be 'mild' in the diagnostic classification of ventilatory disorders (Pellegrino *et al*., 2005). The minimal clinically important change for FVC in patients with pulmonary disease is approximately 6% (du Bois *et al*., 2011). Therefore, ozone effects may be indicative of an apical effect only if FEV_1 or FVC decrements are sufficiently large.

As shown in Table 1, Adams (2002) reported mean decrements in FEV_1 of at least 10% at an ozone exposure of 120 ppb, either via facemask (15.4% decrement) or chamber (15.6% decrement), but not at exposures of 40 or 80 ppb ozone. Increased respiratory symptoms were reported with exposure to both 80 ppb and 120 ppb ozone. A mean reduction in FVC greater than 6% was observed only at 120 ppb, where FVC was reduced by 11%. Similarly, Adams (2006) reported no mean decrements in FEV_1 that exceeded 10% or FVC that exceeded 6% with exposure to 40, 60 or 80 ppb ozone, but increased respiratory symptoms with both triangular and square-wave exposures to 80 ppb ozone were found.

Schelegle *et al*. (2009) also reported a mean FEV_1 decrement greater than 10% only after exposure to ozone at a concentration above 80 ppb. The authors reported a mean FEV_1 decrement of 12.2% after exposure to 88 ppb ozone but reported decrements of 7% or less with lower exposures (63, 72 and 81 ppb ozone). No significant change in FVC was reported. Respiratory symptoms were increased after exposure to 72, 81 and 88 ppb but not 63 ppb ozone. Finally, Kim *et al*. (2011) reported a mean FEV_1 decrement of 1.7% and no increase in respiratory symptoms after exposure to 60 ppb ozone, but did not investigate other exposure concentrations.

These findings are consistent with other controlled exposure studies that examined 6.6 h exposures to ozone concentrations between 80 and 100 ppb. At 80 ppb, other studies reported statistically significant group mean decrements in FEV_1 ranging from 6–8% in conjunction with increased respiratory symptoms (Horstman *et al*., 1990; McDonnell *et al*., 1991; Adams, 2003a, 2003b). Similarly, exposures to 100 ppb ozone were associated with statistically significant group mean FEV_1 decrements of 8–14% and increased symptoms (Horstman *et al*., 1990; McDonnell *et al*., 1991).

Based on the ATS (2000), European Respiratory Society (Pellegrino *et al*., 2005) and US EPA (2007) guidelines, we considered the lung function effects of ozone in controlled human exposure studies to be adverse if mean decrements in FEV_1 were at least 10% and accompanied by respiratory symptoms. As such, we defined the NOAEC as the highest level of ozone exposure at which FEV_1 decrements were less than 10% and the LOAEC as the level of ozone exposure at which FEV_1 decrements were greater than 10% and significant respiratory symptoms were observed (Table 1). The 10% criterion may be overly conservative given that the concomitant decrease in FVC was only associated with larger FEV_1 responses (Schelegle *et al*., 2009). As shown in Table 1, the group mean FEV_1 decrements are less than 10% at ozone exposures up to 81 ppb in all studies

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reviewed here, and are greater than 10% only at exposures of at least 88 ppb. In addition, respiratory symptoms were increased only at exposures of 80 ppb and above. A decrease in FVC of greater than 6% was noted only following exposure to 120 ppb. Thus, only ozone exposures of at least 88 ppb should be considered to induce adverse health effects, albeit of low severity.

Studies have also shown that the FEV_1 and FVC decrements observed after controlled human ozone exposures returned to baseline levels following the cessation of both exercise and exposure (e.g., Schelegle *et al*., 2009). In addition, several studies have shown recovery of FEV_1 decrements as the ozone concentration decreases from the peak value in triangular exposure profiles (Lefohn *et al*., 2010). This indicates that the FEV_1 and FVC decrements in response to ozone can be both transient and reversible. This appears related to the removal of nervous stimulus and return to baseline activity of vagal signaling without functional changes to tissue.

It should be noted that all of the studies evaluated were conducted in healthy adults. As discussed above, the vigor and duration of exercise in test subjects exceeded the length and extent likely to occur in the general population. That is, test subject respiratory rate and ventilatory capacity were increased, producing larger ozone exposures at each tested concentration than would be expected in the general population. Therefore, exposure to similar levels of ozone in the general population are unlikely to produce the same magnitude of respiratory changes as those observed in these studies. In addition, there is little evidence of differential effects of ozone exposure on susceptible populations, including asthmatics and children. For example, several controlled human exposure studies have found no differences in lung function effects between asthmatics and non-asthmatics at elevated ozone exposures of 200–400 ppb (Basha *et al*., 1994; Scannell *et al*., 1996; Alexis *et al*., 2000; Mudway *et al*., 2001). With regard to children, several controlled exposure studies have reported that ozone responsiveness is similar or lower in children than in adults. In a controlled exposure study of children, aged 8–11 years, exposed to 120 ppb ozone for 2.5 h, there were similar decrements in FEV_1 and fewer symptoms than those observed in adults in other studies (McDonnell *et al*., 1985). In an analysis of a larger data set of 741 healthy young adults (mean age 23.8 years) from controlled human exposure studies, McDonnell *et al*. (2012) did not find a statistically significant effect of age on the FEV_1 responses to ozone exposures.

In summary, the small decrements in pulmonary function, as represented by slightly decreased mean FEV_1 values with no or slight concomitant changes in FVC, observed at relatively low ozone concentrations, are of low severity because they do not interfere with normal activity and do not result in permanent respiratory injury or progressive respiratory dysfunction. In addition, because the decrements in FEV_1 and FVC are reversible, transient and represent a reflexive nervous response, these small changes represent a lesser degree of adversity than irreversible and sustained changes in cellular composition or in lung function.

Conclusions

Inhaled ozone of sufficient concentration produces vagal nerve stimulation and resulting physiological changes in pulmonary function. These changes, including bronchoconstriction, are measureable apical effects. In addition, antioxidants within airway lining fluid prevent ozone-mediated cellular and tissue oxidation in a time- and concentration-dependent manner; ozone can cause adverse effects only when these defenses are saturated. Therefore, a threshold exists below which nerve activation is minimal and where antioxidant defenses are sufficient to protect against adverse effects from ozone.

Studies of humans exposed to ozone under controlled conditions indicate that ozone can cause decreased lung function and respiratory symptoms at sufficiently high concentrations. We evaluated the data from studies of controlled human exposures below 80 ppb using the Adverse Effects/Causation Framework and found that, below 72 ppb, lung function effects are primary effects, but are isolated, independent and not statistically different in subjects exposed to ozone compared to those exposed to FA, indicating a lack of causation. Up to about 72 ppb, lung function effects are not adverse because they are transient, reversible and of low severity, as they do not interfere with normal activity and do not result in permanent respiratory injury or progressive respiratory dysfunction.

Overall, controlled human exposure studies do not demonstrate a causal association between ozone concentrations in the range of current NAAQS and adverse effects on lung function.

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