

**SUMMARY OF COMMENTS ON THE INTEGRATED SCIENCE ASSESSMENT FOR
OXIDES OF NITROGEN–HEALTH CRITERIA, SECOND EXTERNAL REVIEW
DRAFT**

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EPA seeks to upgrade the causal determination for both short-term and long-term NO₂-attributable respiratory effects despite the fact that recent studies fail to address key uncertainties identified in the last review or to provide an improved basis for quantifying NO₂-attributable risks that are independent of other traffic-related pollutants. Neither the newly available evidence, nor EPA's new approach to evaluating the coherence and biological plausibility of specific respiratory outcomes support these upgrades and any updated risk estimates in the current review would be subject to the same uncertainties identified in the 2008 review.

SHORT-TERM RESPIRATORY EFFECTS

EPA clearly acknowledges that the evidence to inform short-term NO₂-induced respiratory effects levels has not changed substantially since the last NO₂ NAAQS review,¹ yet EPA still seeks to upgrade the causal determination from a “likely to be causal relationship” to a “causal relationship”. EPA bases this upgrade on what it describes as a more specific evaluation of the coherence and biological plausibility for specific respiratory outcomes.² In support of this action, EPA states that together, epidemiologic, clinical, and animal toxicological studies describe a “coherent and biologically plausible pathway by which NO₂ exposure can trigger asthma exacerbation”.³ However, EPA's statement does not accurately describe the body of evidence regarding short-term respiratory effects associated with NO₂, as these studies fail to clearly establish a link between immunological and/or physiological changes and asthma exacerbation at environmentally-relevant NO₂ concentrations.

Short-Term Studies Fail to Link NO₂-Attributable Immunological or Physiological Changes to Asthma Exacerbation

Evidence that NO₂ affects key events in the mode-of-action for asthma exacerbation is mixed at best. NO₂ exposures in experimental studies increase signaling molecules involved in white blood cell recruitment, but are not consistently associated with lung injury or pulmonary oxidative stress.⁴ In the few toxicology studies available, oxidative stress was not consistently induced by environmentally-relevant NO₂ concentrations, nor were antioxidant levels or enzyme activity affected.⁵ These inconsistent findings for key events in the proposed mode-of-action for NO₂-attributable asthma exacerbation weaken the evidence for a causal role for NO₂ exposure in enhancing airway inflammation, a characteristic feature of asthma exacerbation.

¹ EPA, 2015a. Review of the Primary National Ambient Air Quality Standards for Nitrogen Dioxide: Risk and Exposure Assessment Planning Document. EPA-452/D-15-001. p. 2-14.

² EPA, 2015b. Integrated Science Assessment for Oxides of Nitrogen–Health Criteria. Second External Review Draft. EPA/600/R-14/006. p. 1-19.

³ *Ibid.* p. 1-17.

⁴ *Ibid.* p. 5-238.

⁵ *Ibid.* pp. 5-229 and 5-230.

In addition, controlled human exposure studies using environmentally-relevant concentrations of NO₂ suggest that NO₂ exposure does not provide consistent evidence of enhanced airway inflammation in asthmatics.⁶ While brief exposure to NO₂ can enhance allergen responsiveness in individuals with asthma, this has only been demonstrated at concentrations considerably higher (> 260 ppb) than those encountered in the environment. Moreover, even the epidemiology studies cited by EPA as key evidence fail to clearly support that NO₂ induces inflammatory responses in humans at environmentally-relevant concentrations.⁷

EPA Overestimates the Fraction of Asthmatics with NO₂-Induced Airway Responsiveness by Using Inappropriate Calculations and Statistical Methods

Controlled human exposure studies of NO₂-induced airway hyperresponsiveness have reported mixed results, with some asthmatics responding to short-term NO₂ exposure with an increase in airway responsiveness, some responding with a decrease in airway responsiveness and some not responding at all. While the meta-analysis of these controlled human exposure studies conducted for the current NO_x ISA represents an improvement over the one in the 2008 ISA, it is still fraught with problems including a lack of transparency and use of statistical methods and calculation procedures that fail to produce data suitable for determining whether short-term NO₂ exposure exacerbates asthma. EPA states that “In general, meta-analyses show statistically significant effects of NO₂ on the airway responsiveness of individuals with asthma”.⁸ However, this is a misrepresentation of the overall results for a variety of reasons: (1) EPA overestimates (by 4% – 9%) the fraction of asthmatics affected by excluding asthmatics for which NO₂ exposure does not alter airway responsiveness from the calculation; (2) since *increased* airway responsiveness is a characteristic feature of asthma, the relevant research question is whether NO₂ exposure *increases* airway responsiveness (relative to fresh air exposure), but EPA’s statistical analysis instead evaluates whether the number of asthmatics that experienced an increase in airway responsiveness was greater than the number that experienced a decrease after NO₂ exposure, which likely resulted in spurious statistically significant results; and (3) claiming that “...a quarter of the exposed individuals experience a clinically relevant reduction in provocative dose due to NO₂ exposure”⁹ is a misrepresentation because the fraction of asthmatics experiencing a reduction in provocative dose was only evaluated in five of the 24 studies and thus, may not accurately represent the overall percentage of asthmatics experiencing a clinically relevant reduction.

Short-Term Studies Fail to Provide Evidence for an Independent Role for NO₂ in Causing Respiratory Effects

EPA also concludes that the coherence of epidemiologic findings for ambient and indoor NO₂ exposures, combined with effects demonstrated on key events in the mode-of-action for asthma exacerbation, provide strong evidence for an independent effect of NO₂ exposure, but this evidence is not coherent with effects demonstrated on key events. For example: (1) environmentally-relevant NO₂ concentrations fail to consistently increase airway inflammation in asthmatics;¹⁰ (2) while short-term NO₂ exposure may cause small clinically insignificant increases

⁶ *Ibid.* Table 5-17, pp. 5-104 and 105.

⁷ *Ibid.* Table 5-45, pp. 5-250 – 5-254; Holguin et al., 2007. Traffic-related Exposures, Airway Function, Inflammation and Respiratory Symptoms in Children. *Am J Respir Crit Care Med*, 176, 1236–1242; Greenwald et al., 2013. Associations between source-indicative pollution metrics and increases in pulmonary inflammation and reduced lung function in a panel of asthmatic children. *Air Quality, Atmos Health*, 6(2), 487-499; McCreanor et al., 2007. Respiratory effects of exposure to diesel traffic in persons with asthma. *N Engl J Med*, 357(23), 2348-2358.

⁸ EPA, 2015b. p. 5-15.

⁹ *Ibid.* p. 5-16.

¹⁰ Greenwald et al., 2013; McCreanor et al., 2007.

in airway responsiveness in some asthmatics, the response is highly variable and does not demonstrate a dose response;¹¹ (3) recent epidemiologic evidence for NO₂-induced lung function decline does not provide better support for a causal association¹² than was available during the last review, primarily because it cannot be concluded that NO₂ alone is responsible for the lung function decrements;¹³ (4) there is no clear pattern of elevated risk for respiratory symptoms in association with ambient NO₂;¹⁴ (5) evidence for an independent role for NO₂ in causing hospital admissions and Emergency Department visits is lacking because the studies all use ambient monitoring data as surrogates for exposure; and (6) even the studies that measured microenvironment NO₂ concentrations do not provide strong support for an independent role of NO₂ in causing respiratory effects because the studies either do not cause statistically significant effects or statistical significance is lost in multi-pollutant models, with authors often concluding that the effects of the various pollutants cannot be disentangled from one another.¹⁵

A “Causal Relationship” Determination is Not Supported by the Available Evidence for NO₂-Attributable Short-Term Respiratory Effects

The clinical and epidemiology studies that form the bases for the surmised association between short-term NO₂ exposure and asthma exacerbation do not support the “causal relationship” classification for short-term respiratory effects for a variety of reasons. The controlled human exposure studies on airway hyperresponsiveness provide results that are inconsistent, employ a wide variety of methodologies, and are of varying quality, so much so that it is still unclear whether short-term exposures to NO₂ under highly controlled conditions truly cause clinically relevant increases in airway responsiveness or whether the results simply reflect inter-individual variability in the response to the challenge agents or differences in study design/implementation. In addition, many of the epidemiology studies on short-term effects of NO₂ suffer from quality and/or reliability issues, such as: (1) self-performed spirometry, which is known to have high error rates; (2) failure to report participation rates, making it unclear if the studies are biased due to selective participation; (3) use of symptom diaries, which are subject to recall and response bias; (4) failure to include relevant potential effect modifiers (socioeconomic status, ethnicity, indoor sources of pollutants); (5) issues related to the questionable reliability of asthma diagnosis in children below the age of 5 years; and/or (6) evaluation of multiple lag times with no biologic justification, suggesting that the lag-time that maximized either the effect estimate or model goodness-of-fit may have been selected, biasing the results away from the null.

A key component of EPA’s weight-of-evidence determination for a “causal relationship” is the ability to demonstrate health effects in studies in which chance, *confounding*, and other biases can be ruled out with reasonable confidence. However, recent studies fail to provide an improved basis for quantifying short-term NO₂-attributable risks that are independent of other traffic-related

¹¹ Brown, J. 2015. Nitrogen dioxide exposure and airway responsiveness in individuals with asthma. *Inhal Toxicol*, 27(1), 1-14; Goodman et al., 2009. Meta-analysis of nitrogen dioxide exposure and airway hyper-responsiveness in asthmatics. *Crit Rev Toxicol* 39, 719-742.

¹² Smargiassi et al., 2014. Associations between personal exposure to air pollutants and lung function tests and cardiovascular indices among children with asthma living near an industrial complex and petroleum refineries. *Environ Res*, 132, 38-45; Greenwald et al., 2013; Spira-Cohen et al., 2011. Personal Exposures to Traffic-Related Air Pollution and Acute Respiratory Health among Bronx Schoolchildren with Asthma. *Environ Health Perspect*, 119, 559–565; Holguin et al., 2007.

¹³ Delfino et al., 2008. Personal and ambient air pollution exposures and lung function decrements in children with asthma. *Environ Health Persp*, 116(4), 550-558; Martins et al., 2012. Airways changes related to air pollution exposure in wheezing children. *Eur Respir J*, 39, 246–253.

¹⁴ Zora et al., 2013. Associations between urban air pollution and pediatric asthma control in El Paso, Texas. *Sci Total Environ* 448, 56-65; Mortimer et al., 2002. The effect of air pollution on inner-city children with asthma. *Eur Respir J*, 19, 699-705; O’Conner et al., 2008. Acute respiratory health effects of air pollution on children with asthma in US inner cities. *J Allergy Clin Immunol*, 121, 1133-1139; Spira-Cohen et al., 2011; Holguin et al., 2007; McCreanor et al., 2007.

¹⁵ Martins et al., 2012; Greenwald et al., 2013; Spira-Cohen et al., 2011; Holguin et al., 2007; McCreanor et al., 2007.

pollutants by comparison to the last review. Therefore, any updated risk estimates based on information from short-term human exposure and/or epidemiology studies in the current review would be subject to the same uncertainties identified in the 2008 Risk and Exposure Assessment (REA).¹⁶

LONG-TERM RESPIRATORY EFFECTS

According to EPA, the strongest support for an upgrade from “suggestive, but not sufficient to infer a causal relationship” to a “likely to be a causal relationship” between long-term NO₂ exposure and respiratory effects is provided by the consistent associations between long-term NO₂ exposure and increases in asthma incidence, with limited support for biological plausibility provided by a small body of experimental studies.¹⁷ However, neither the association between long-term NO₂ exposure and asthma incidence, nor the biological plausibility of the association are consistently demonstrated in the available studies.

New Epidemiologic Studies on Long-Term Respiratory Effects Fail to Provide Evidence of an Independent Role for NO₂ in Causing Asthma

Despite measuring/estimating exposure near children’s homes, studies reported mostly statistically insignificant associations for asthma incidence¹⁸ and the statistically significant associations that were observed, were either not independent,¹⁹ occurred only in models where appropriate adjustments were not made,²⁰ or occurred in studies where the authors themselves concluded that NO₂ was likely a proxy for other toxic components of the local air pollution mixture.²¹ In addition, of the key studies evaluating associations between long-term NO₂ concentrations and the development of asthma,²² none evaluated associations in co-pollutant models. Therefore, none of the studies provide evidence for an independent role for NO₂ in the development of asthma. Moreover, epidemiological studies provide only weak evidence of an association between NO₂ and airway inflammation, evidence for increases in allergic sensitization at environmentally-relevant NO₂ concentrations is inconsistent,²³ epidemiology studies fail to report significant associations with biomarkers of airway responsiveness,²⁴ and although studies cited by EPA report statistically significant associations for long-term NO₂ concentrations and deficits in lung function growth, a broad range of effect sizes is estimated across the studies and the deficits were not large enough to be considered clinically relevant.

¹⁶ EPA, 2008. Risk and Exposure Assessment to Support the Review of the NO₂ Primary National Ambient Air Quality Standard. EPA-452/R-08-008a. pp. 143, 251, 260 – 268.

¹⁷ EPA, 2015b. Table 1-1, p. 1-32.

¹⁸ Carlsten et al., 2010. Traffic-related air pollution and incident asthma in a high-risk birth cohort. *Occup Environ Med*, 68, 291-295.

¹⁹ Clougherty et al., 2007. Synergistic effects of traffic-related air pollution and exposure to violence. *Environ Health Persp*, 115(8), 1140-1146.

²⁰ Gehring et al., 2010. Traffic-related air pollution and the development of asthma and allergy during the first 8 years of Life. *Am J Respir Crit Care Med*, 181, 596–603.

²¹ Jerrett et al., 2008. Traffic-related air pollution and asthma onset in children: a prospective cohort study with individual exposure measurement. *Environ Health Perspect*, 116, 1433–1438.

²² EPA, 2015b. Table 6-5, pp. 6-75 to 6-80.

²³ Pujades-Rodriguez et al., 2009. Effect of traffic pollution on respiratory and allergic disease in adults: Cross-sectional and longitudinal analyses. *BMC Pulmon Med*, 9, 42; Rage et al., 2009. Total serum IgE levels are associated with ambient ozone concentration in asthmatic adults. *Allergy*, 64(1), 40-46; Gruzieva et al., 2012. Traffic-related air pollution and development of allergic sensitization in children during the first 8 years of life. *J Allergy Clin Immunol*, 129(1), 240-246; Gehring et al., 2010; Gehring et al., 2013. Air pollution exposure and lung function in children: the ESCAPE project. *Environ Health Perspect*, 121, 1357-1364.

²⁴ Carlsten et al., 2010; Gehring et al., 2010.

Inconsistent Evidence across Disciplines Fails to Provide Biological Plausibility for an Independent Role of Long-Term NO₂ Exposure in Causing Asthma

Both animal toxicology studies and human clinical studies show that single and/or repeated short-term exposures to NO₂ result in increases in immunoglobulins and other proteins that play a role in allergic disease, but only at exposure levels that are much higher than those encountered in the environment. While high-level NO₂ exposure causes allergic responses in laboratory animals and humans, evidence for increases in allergic sensitization at environmentally-relevant NO₂ concentrations is also inconsistent. Similarly, high NO₂ exposures cause airway hyperresponsiveness in laboratory animals but epidemiology studies generally do not report significant associations with biomarkers of airway responsiveness. This inconsistent evidence across disciplines does not describe a coherent and biologically plausible sequence of events by which repeated or prolonged NO₂ exposure at environmentally-relevant concentrations could result in development of asthma.

A “Likely to be Causal Relationship” Determination is Not Supported by the Available Evidence on NO₂-Attributable Long-Term Respiratory Effects

Several problems continue to plague the long-term NO₂ respiratory effect studies. High correlations and differential measurement/estimation errors among the individual constituents in pollutant mixtures has made ascertaining which constituent is responsible for associations difficult. Many studies still do not evaluate the potential for confounding by other co-pollutants and volatile organic compounds are rarely evaluated in the studies, despite the fact that they are important constituents of traffic-related pollution and several short-term studies showed that they are better predictors of health effects than NO₂.²⁵

Based on a thorough review of the scientific evidence for the association between long-term NO₂ exposure and the development of asthma, it is concluded that the evidence is not sufficient to support an upgrade in causal determination to a “likely to be causal relationship.” Although uncertainties may remain in the evidence overall, the ability to demonstrate health effects in studies in which chance, confounding, and other biases can be ruled out with reasonable confidence in at least some studies is a key component of EPA’s weight-of-evidence determination for a “likely to be causal relationship.” However, given that the authors of most of the long-term studies conclude that the effects of NO₂ cannot be disentangled from the effects of other traffic-related pollutants, confounding and other biases cannot be ruled out in these studies. Moreover, given that evidence for an independent role of NO₂ exposure in the development of asthma is still lacking, quantitative risk assessment based on information from these studies would not reduce uncertainties or limitations identified in the assessment conducted during the last review.

²⁵ Greenwald et al., 2013; Smargiassi et al., 2014; Martins et al., 2012.