Ambient ozone exposure and depression:

Epidemiological findings considering long- and short-term exposures to ozone and indicators of depression

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München, February 22, 2021 Tianyu Zhao

Contents

Abbreviations

DALY	disability-adjusted life-year
DesTeen	Depression Screener for Teenagers
FeNO	fractional exhaled nitric oxide
GAM	generalized additive model
GBD	Global Burden of Disease
GINIplus	German Infant study on the influence of a Nutritional Intervention plus environmental and genetic influences on allergy development
hs-CRP	high-sensitivity C-reactive protein
ICD	international statistical classification of diseases and related health problems
IL	interleukin
LISA	influence of Life-style factors on the development of the Immune System and Allergies in East and West Germany
NO_2	nitrogen dioxide
PM	particulate matter
PM_{10}	particulate matter with an aerodynamic diameter < 10 μm
UBA	Umweltbundesamt (German Environment Agency)
WHO	World Health Organization

Summary

Ambient ozone exposure has been hypothesized to be a threat to several aspects of health, but some of them have rarely been investigated. Published studies mainly dealt with respiratory and cardio-cerebrovascular outcomes, and few studies explored the effects of ozone on, for example, mental health, and among this, depressive disorder. Depression is a disorder with complex etiology, and genetic factors, socioeconomic circumstances, and physical environment all play a role. Air pollution, in general, is thought to be among the factors contributing to depression. Nevertheless, only a handful of studies by now have focused on depression and air pollution, especially ozone, as a major component besides particulate matter.

This thesis comprises three original studies that targeted to assess the association between ambient air ozone exposure and depression or its indicators, as well as possible underlying mechanisms.

The first publication investigated the association between both long- and short-term exposure to ambient ozone and symptoms of depression among 2,827 adolescents aged 15 years. Within GINIplus and LISA, two German birth cohorts, depressive symptoms were assessed by questionnaire. Both long-term and short-time ozone exposure were assigned to the participants' residential addresses. The results were inconsistent and were not in line with the hypothesis that exposure to ozone might increase the symptoms of depression in adolescents. This is an important finding, as it narrows, for the first time, the age range of subjects in whom it is sensitive to look for such associations.

The second publication analyzed the association between long-term exposure to ambient ozone and depressive disorders among adults (aged more than 16 years). The outpatient clinical diagnoses of depression were available from a claim database of a statutory health insurance company, while the estimates of ozone exposure were allocated to the residential areas of 1.13 million beneficiaries. The findings supported the assumption that increased ambient ozone levels may correlate with a higher risk of depression diagnosis in a general population. Irrespective of all weaknesses of such an observational study, this result indicates that there could well be direct, e.g., mediated via inflammation or oxidative stress, or indirect links, mediated by life-style or socioeconomic factors, between the clinical diagnosis of depression, as a hard outcome, and ambient ozone exposure.

The third study explored the association between short-term ambient ozone exposure and the variation in the concentrations of inflammatory biomarkers. Overall, 1,330 10-year-old and 1,591 15-year-old participants from the GINIplus and LISA cohorts were

included. As markers of inflammation, the fractional concentration of exhaled nitric oxide (FeNO) and the serum level of the high-sensitivity C-reactive protein (hs-CRP) were used. These indicators were measured at the ages of 10 and 15 years. Additionally, the level of interleukin (IL)-6 was measured at the age of 10 years. Our study revealed a robust association between short-term exposure to ambient ozone and elevated FeNO in 15-year-olds, but not in 10-year-olds. There was a J-shaped relationship between ozone and hs-CRP levels – a lower concentration of ambient ozone was associated with a decreased hs-CRP level, while a higher concentration was associated with an increased hs-CRP level. No association was identified between ozone and IL-6.

In conclusion, these results add to the available epidemiological evidence on ambient ozone exposure and either mental health or local or systemic inflammation. They support the notion that exposure to ambient ozone might be associated with depression in adults, although this was not mirrored in adolescent populations. The results also affirm the association between ozone and inflammation, as one of the potential mechanisms linking ozone to non-respiratory disorder, such as depression. The analyses also demonstrated the need for more well-designed epidemiological and experimental studies to confirm the robustness of the observed associations and to get clues on the underlying pathomechanisms. The association between ozone and depression might provide new insights in terms of city planning and disease prevention.

Zusammenfassung

Es wird häufig angenommen, dass die Ozonbelastung in der Umgebungsluft eine Gefahr für eine Reihe von Aspekten der Gesundheit darstellt, aber diese mögliche Assoziation wird nur in begrenztem Umfang untersucht. Die veröffentlichten Studien befassten sich hauptsächlich mit respiratorischen und kardio-zerebrovaskulären Ergebnissen, und nur wenige Studien untersuchten die Auswirkungen von Ozon auf andere Aspekte wie die psychische Gesundheit, darunter Depression. Dies ist eine Erkrankung mit komplexer Ätiologie, und genetische Faktoren, sozioökonomische Umstände und die physische Umwelt spielen eine Rolle. Immer wieder wird angenommen, dass Luftverschmutzung, darunter Ozon, zu den Verursachern von mentalen Störungen gehört, allerdings konzentrierten sich bisher nur eine Handvoll Studien auf die Assoziation zwischen Ozon und dem Auftreten von Depressionen.

Die vorliegende Arbeit umfasst drei Originalstudien, die den Zusammenhang zwischen Ozonbelastung und Depression oder deren Symptomen, sowie die möglichen zugrunde liegenden Mechanismen untersuchen sollten.

Die erste Publikation untersuchte den Zusammenhang zwischen Ozonbelastung und Symptomen einer Depression bei 2.827 15-jährigen Jugendlichen. Innerhalb der beiden deutschen Geburtskohorten GINIplus und LISA wurden die entsprechenden Symptome mittels Fragebogen erhoben. Jedem der Teilnehmer wurden die jährliche Langzeit-Ozon-Exposition und die Kurzzeit-Exposition zugeordnet. Die Ergebnisse, welche aufgrund des Stichprobenumfangs keine geringe Teststärke (Power) aufwiesen, untermauerten nicht die Hypothese, dass eine lang- oder kurzfristige Ozonbelastung die Prävalenz von Symptomen einer Depression bei Jugendlichen erhöht.

Die zweite Publikation analysierte den Zusammenhang zwischen langfristiger Ozonbelastung und der Diagnose einer Depression bei Erwachsenen (Alter mehr als 16 Jahre). Die ambulant erhobenen klinischen Diagnosen einer Depression waren aus einer Leistungsdatenbank einer gesetzlichen Krankenkasse verfügbar. Schätzwerte der Ozonbelastung wurden den Wohngebieten von 1,13 Millionen Begünstigten zugeordnet. Die Ergebnisse deuteten darauf hin, dass erhöhte Ozonkonzentrationen in der Außenluft mit einem erhöhten Risiko der Diagnose einer Depression in der Allgemeinbevölkerung verbunden sein könnten.

Die dritte Studie untersuchte die Beziehung zwischen kurzfristiger Exposition gegenüber Ozon und den Schwankungen der Konzentrationen von Entzündungsmarkern. Insgesamt 1.330 10-jährige und 1.591 15-jährige Teilnehmer aus den GINIplus- und LISA-Kohorten wurden eingeschlossen. Die fraktionelle Konzentration des ausgeatmeten Stickstoffmonox-

ids (FeNO) und die Serumkonzentration des hochempfindlichen C-reaktives Proteins (hs-CRP) wurden nach 10 und 15 Jahren gemessen, während der Serumspiegel von Interleukin (IL)-6 nur nach 10 Jahren gemessen wurde. Die Studie zeigte eine robuste Assoziation zwischen kurzzeitiger Ozonbelastung und erhöhtem FeNO bei 15-jährigen Jugendlichen, aber nicht bei 10-jährigen. Die Beziehung zwischen hs-CRP-Werten und Ozonkonzentrationen war J-förmig. Relativ niedrige Ozonkonzentrationen waren mit reduzierten hs-CRP-Werten assoziiert, während hohe Konzentrationen tendenziell mit erhöhten hs-CRP-Werten assoziiert waren. Bei Kindern im Alter von 10 Jahren wurde keine Assoziation zwischen Ozon und IL-6 beobachtet.

Diese Ergebnisse fassen die aktuelle epidemiologische Evidenz zur Ozonbelastung und psychischen Gesundheit zusammen und unterstützen die Hypothese, dass die Ozonbelastung mit Depressionen im Erwachsenenalter in Verbindung stehen könnte, wobei sich dieser Zusammenhang im Jugendalter nicht zeigt. Die Ergebnisse bestätigen auch den Zusammenhang zwischen Ozon und lokaler oder systemischer Entzündung, der ein möglicher Mechanismus ist, der Ozon mit nicht-respiratorischen Erkrankungen wie Depressionen in Verbindung bringt. Ein wesentliches Ergebnis der Analysen war, dass künftig besser konzipierte, aufeinander abgestimmt epidemiologische und experimentelle Studien notwendig sind, um die gefundenen Zusammenhänge zu überprüfen und die möglichen zugrundeliegenden Pathomechanismen genauer abzuklären. Der Zusammenhang zwischen Ozon und Depression könnte neue Erkenntnisse in Bezug auf Stadtplanung und Krankheitsprävention liefern.

Publication list

The doctoral thesis is based on the following papers, referred to in the text by numbers (Chapters 7 to 9):

Tianyu Zhao, Iana Markevych, Marie Standl, Gerd Schulte-Koerne, Tamara Schikowski, Dietrich Berdel, Sibylle Koletzko, Carl-Peter Bauer, Andrea von Berg, Dennis Nowak, Joachim Heinrich

Ambient ozone exposure and depressive symptoms in adolescents: Results of the GINIplus and LISA birth cohorts.

Environ Res. 170, 73-81. https://doi.org/10.1016/j.envres.2018.12.014

Tianyu Zhao*, Falko Tesch*, Iana Markevych, Clemens Baumbach, Christian Janßen, Jochen Schmitt, Marcel Romanos, Dennis Nowak, Joachim Heinrich

Depression and anxiety with exposure to ozone and particulate matter: An epidemiological claims data analysis.

Int J Hyg Environ Health. 228, 113562. https://doi.org/10.1016/j.ijheh.2020.113562

* These authors contributed equally to this work

Tianyu Zhao, Iana Markevych, Marie Standl, Tamara Schikowski, Dietrich Berdel, Sibylle Koletzko, Rudolf A. Jörres, Dennis Nowak, Joachim Heinrich

Short-term exposure to ambient ozone and inflammatory biomarkers in cross-sectional studies of children and adolescents: Results of the GINIplus and LISA birth cohorts.

Environ Pollut. 255, 113264. https://doi.org/10.1016/j.envpol.2019.113264

An additional contribution of this thesis is listed in the Appendix (Appendix: Systematic review on ozone and mental health):

Tianyu Zhao, Iana Markevych, Marcel Romanos, Dennis Nowak, Joachim Heinrich

Ambient ozone exposure and mental health: A systematic review of epidemiological studies.

Environ Res. 165, 459-472. https://doi.org/10.1016/j.envres.2018.04.015

Chapter 1

Background

1.1 Ambient ozone exposure and health outcomes

Ambient air pollution has been considered a major cause of the global burden of disease [1]. In accordance with the Global Burden of Disease (GBD) study, in the year 2017, air pollution was estimated to account for 4.9 million deaths and a loss of 147.0 million disability-adjusted life-years (DALYs) [2].

Even though in the GBD study, the majority of the air pollution-related burden is attributed to particulate matter (PM) [2], the estimated burden from ambient ozone is also relevant, and ambient ozone exposure is estimated to have caused 472,000 deaths and 7.4 million DALYs in 2017 [2].

An important point is that according to the evidence rules of the GBD study, only chronic obstructive pulmonary disease (COPD) is currently regarded as an adverse health outcome related to ozone exposure, whereas other health endpoints than COPD were not included in the GBD calculations [2].

The uncertainties regarding potential health outcomes of ambient air ozone exposure are at least partially due to the fact that ozone has been less much investigated than PM, at least in the last two decades: free-term search in PubMed using keywords "ozone" and "particulate matter" yielded 18,586 and 52,760 hits (August 14$^{\text{th}}$, 2020), respectively. Fortunately, the number of original studies dealing with potential associations between exposure to ambient ozone and health effects is growing. Correspondingly, the evidence for the adverse effects of ozone is steadily accumulating. The majority of the studies on both long-term (at least 30 days in duration) [3] and short-term (less than 30 days in period) [4] exposure to ozone focused on respiratory diseases and symptoms [5, 6, 7, 8, 9], which is understandable because of the known irritant effects of ozone. More recent studies have explored possible detrimental effects of ozone on the cardio-cerebrovascular [10, 11] and central nervous system [12, 13]. Ozone may become a more important air pollutant in the future, as it has been hypothesized that its average or peak concentrations will increase in some regions of the world over time, in association with climate change and global warming [14]. This would imply that morbidity and, in particular, mortality [15] attributable to

ozone may increase in the long term.

1.2 Depressive disorder and depressive symptoms

Depression, short for depressive disorder, is a common and, in many instances, severe illness. The World Health Organization (WHO) [16] estimated that 4.4 % of the worldwide population suffered from depression in 2015 – an 18.4 % increase compared with 2005. Looking from a long-term perspective, one study has estimated that the aggregated global lifetime prevalence of depression is 10.8 % [17]. Consequently, major depressive disorder and dysthymia accounted for 43.0 million years lived with disability in 2017, implying that depression ranked as the third-leading cause of GBD [18].

In general, the symptoms of depression include sad, empty, hopeless, or irritable mood, accompanied by somatic and cognitive changes affecting the individual capacity of functioning [19]. Suffering from depression is known to decrease the quality of life [20], and is often correlated with other adverse health outcomes, such as diverse psychiatric disorders [21] and severe somatic diseases [22, 23, 24, 25], as well as self-injury and suicide [26, 27].

Although this complex disorder presents a high prevalence and frequently unfavorable prognosis, its etiology remains unclear. As an example, familial aggregation of depression is considerable [28], while the estimated heritability explains only 37 % to 48 % of the observed cases [29]. This clearly indicates that other etiological factors must be involved. Indeed, beyond genetic and biological influences [30, 31, 32, 33], relevant determinants of depression include sociodemographic and socioeconomic circumstances [34, 35, 36], together with physical environmental factors [37, 38]. Among these factors, exposure to ambient air pollutants has been assumed to increase depression risk [39, 40].

1.3 Ozone exposure and depression

Some studies have explored the association between ozone, which is a powerful oxidant, and depression. Experimental studies showed relatively robust results of relevant effects of ozone on depression-related events or central nervous physiology. Animal studies addressing the neurotoxic effects of ozone inhalation demonstrated that ozone might lead to lipid peroxidation and reduce the number of dopaminergic neurons [41], increase the levels of vascular endothelial growth factor, tumor necrosis factor α and interleukin-6 (IL-6) [42], as well as c-Fos expression in various brain regions [43]. Therefore, it may be reasonably postulated that ozone has an impact on human emotional experience, cognition, or behavior. Following this line of thought, ambient ozone exposure might be seen as a risk factor for depression, acting through the above-mentioned pathomechanisms.

Currently, however, there are only a few epidemiological studies investigating the association between ozone and depression [44, 45, 39, 46, 47, 48, 40], and these studies are difficult to compare, as they are quite heterogeneous regarding their designs of researches, characteristics of participants, assessments of exposure, definitions of outcomes, and the

overall drawn conclusions. This requires a special effort to identify the needs for further research. Specifically, no studies investigated the association among children or adolescents, though childhood or adolescence has been assumed to be a time-window of vulnerability when depression might start developing [49, 50]. Therefore, more well-designed epidemiological studies are warranted to disentangle the association of interest, and the topic of this thesis was to give detailed information on the research needs.

Chapter 2

Specific Aims

The present thesis aimed to elucidate the association between exposure to ambient ozone and depressive symptoms or a diagnosis of depression, and to explore potential underlying mechanisms. This was done by a combination of analysis of the literature and data from observational studies. The specific objectives were:

- To present the up-to-date epidemiological studies that investigated the associations of mental or behavioral disorders, including depression, with exposure to ambient ozone exposure
- To investigate the relationship between exposure to ambient ozone and symptoms of depression among adolescents
- To assess the association between exposure to ambient ozone and the diagnosis of depression among adults
- To explore the variation of levels of local and systemic biomarkers of inflammation within children and adolescents in relation to ambient ozone exposure

This thesis is based on three original papers published in *Environmental Research*, *International Journal of Hygiene and Public Health* and *Environmental Pollution*. In accordance with the regulation of the Elsevier publishing company, as the author of the Elsevier articles, I retain the right to include them in this thesis for non-commercial purposes.

Specifically, as an "introductory remark" for the thesis, the first aim is mainly achieved by a systematic review, an additional contribution (Appendix: Systematic review on ozone and mental health).The second and third ones are covered by two original studies, which investigated exposure to ambient ozone and symptoms of depression as well as the diagnosis of depression in adolescents and in adults, respectively (Chapters 7 and 8). The last aim is addressed by the third original study on exposure to ambient ozone and three biomarkers of inflammation (Chapter 9).

For all of the publications, I am the first (Chapters 7 and 9, and Appendix: Systematic review on ozone and mental health) or co-first author (Chapter 8). I was significantly

included in the conceptualization and data curation. I developed the statistical analysis plans, did statistical modeling (partially for the co-first author publication in Chapter 8), and wrote the first draft of the manuscripts. All comments and suggestions from the coauthors were responded to and incorporated by me. The relatively large number of coauthors is due to the fact that the database of this multicenter study combined data from multiple cohorts, and the cooperation was involved in several organizations and institutes.

Chapter 3

Study Material and Methods

3.1 Ambient ozone exposure and health outcomes

For the first, and the third publications (Chapters 7 and 9), data were derived from the two ongoing population-based birth cohorts, GINIplus (German Infant study on the influence of a Nutritional Intervention plus environmental and genetic influences on allergy development) and LISA (influence of Life-style factors on the development of the Immune System and Allergies in East and West Germany).

Briefly, both cohorts only recruited healthy neonates born at a full term, i.e., gestational age $\geq$ 37 weeks, and with normal birth weight, i.e., body weight $>$ 2500 g. Initially, the GINIplus cohort recruited 2,949 children from Munich and 3042 from Wesel from 1995 to 1998. With a total of 5,991 children, the cohort originally aimed to investigate whether hydrolyzed formulas could prevent the development of allergy. GINIplus has two different arms: the intervention arm included participants with at least one atopic parent or sibling. In contrast, the observation arm included participants without a family history of allergies or those who did not consent for the participation in the intervention arm. Similarly, 3094 participants were recruited into the LISA cohort between 1997 and 1999, with 1,464 of them from Munich, 348 from Wesel, 976 from Leipzig, and 306 from Bad Honnef. Ethical approval of the birth cohorts was acquired accordingly from the local ethics committees (Bavarian Board of Physicians, Board of Physicians of North-Rhine-Westphalia, and University of Leipzig). Besides, written informed consent was acquired from the participants and their legal guardians. Detailed descriptions of these tow birth cohorts can be available elsewhere [51, 52, 53].

Given the ozone exposure metrics cannot be assigned to the participants from Leipzig and Bad Honnef, we restricted our studies to the participants residing in the areas of Munich and Wesel from the 10- and 15-year follow-up visits. The data from the two cohorts were firstly pooled and subsequently stratified by the study area, as has been done in previous analyses of the cohorts [54, 55].

In the second publication (Chapter 8), pseudonymized claims data from a large German statutory health insurance company, Allgemeine Ortskrankenkasse (AOK) PLUS, for the

years from 2005 to 2014 were utilized. Approximately 50 % of the local population from the federal state of Saxony was coded in the database [56]. Overall, the claims data covered information from outpatients on clinical diagnoses, medical procedures, and prescriptions. Age, gender, and residential area of the beneficiaries were included in data as well [57].

3.2 Air Pollution Assessment

Regarding short-term exposure, we obtained hourly or daily concentrations of pollutants from background monitoring sites of the German Environment Agency (UBA, short for Umweltbundesamt).

The concentration of ozone is known to be highly variable; thus we calculated the "maximum of the daily maximum 8-hour average concentration" ($\mu g/m^3$) on the basis of a recommendation of the UBA [58]. For this, we firstly calculated a moving 8-hour (the hour of interest and the preceding 7 hours) average concentration for every hour of the day. Secondly, we selected the maximum of 8-hour average for that day and every day of interest accordingly. The maximum of the daily maximum 8-hour average concentration was selected over 0 (the day of health assessment), and 1, 2, 3, as well as 7 days prior to the assessment of symptoms of depression (lags 0 to 0-7 days) in the first publication (Chapter 7). Likewise, the time window was lag 0 day to lag 0-14 days in the third publication (Chapter 9). For exposures to PM with an aerodynamic diameter < 10 µm (PM_{10}) and nitrogen dioxide (NO_2), we employed 24-hour daily average concentrations ($\mu g/m^3$) within the same time frames as for ozone.

Regarding long-term exposure, estimates of ozone levels were calculated by using data from the UBA in the first and third publications (Chapters 7 and 9). Both annual average concentrations ($\mu g/m^3$) and the "number of days with a maximum 8-hour average concentration exceeding 120 $\mu g/m^3$" [58] were used.

Estimates of PM_{10} and NO_2 levels were modeled for different areas by land-use regression models stemmed from the "European Study of Cohorts for Air Pollution Effects" (ESCAPE) project [59, 60] in the first publication (Chapter 7), and by the UBA-derived data in the second publication (Chapter 8). The long-term exposure estimates of these two pollutants were modeled by annual average concentrations ($\mu g/m^3$).

3.3 Outcome Assessment

Depression symptoms reported in the first publication (Chapter 7) were evaluated by the Depression Screener for Teenagers (DesTeen) [61, 62]. With 14 items, DesTeen is a specific tool used for screening depression among German adolescents [61, 62]. The questionnaire of DesTeen was answered by participants at the age of 15 years, and a total score of more than 12 was regarded as indicating the presence of depressive symptoms [62].

The diagnosis of depression adopted in the second publication (Chapter 8) was determined based on the German modification of ICD, 10^{th} version [63, 64]. Any beneficiaries

who received a diagnosis coded in F32 to F33 of ICD were considered a depression case.

In the work presented in Chapter 9, inflammatory biomarkers were measured during 10- and 15-year follow-up visits. As a local marker of inflammation, FeNO was assessed using the standard method [65] during the two follow-ups. As a systemic marker, hs-CRP concentrations in the serum were measured in line with the standard method [66]. Additionally, serum concentrations of IL-6, as a further systemic marker, were determined in the 10-year-olds [67].

3.4 Analyses

In the first publication (Chapter 7), the associations between exposure to ambient ozone and symptoms of depression at the age of 15 years were analyzed by logistic regression models. This was done because the linearity of the relationship was found according to generalized additive models (GAMs) [68]. Beyond dichotomizing the DesTeen score, we also built a negative binomial regression model using the total score as count data.

All of the models were adjusted for residuals of PM_{10} and NO_2 exposures, and a directed acyclic graph [69, 70, 71] was used to identify further covariates. The analyses were performed for the data from Munich and Wesel area-specifically, and for the combined study population from the two areas. Moreover, analyses were also stratified according to the children's sex.

In the second publication (Chapter 8), generalized estimating equations models [72] were used for the analysis of the longitudinal-structured data, and specifically to reveal the associations between long-term ambient ozone exposure and diagnoses of depression. We built one-pollutant models for ozone as well as two-pollutant models where both ozone and PM_{10} were included. The models were adjusted by covariates that were available from the health insurance company dataset. For sensitivity analysis, we reanalyzed the association by refining individuals diagnosed with depression and without anxiety.

In the third publication (Chapter 9), for normalizing the distributions FeNO concentrations, as common, the data of this local inflammatory biomarker were log (ln)-transformed. The concentrations of hs-CRP and IL-6, as systemic markers of inflammation, were categorized according to their minimal detectable concentrations.

The associations between log-transformed FeNO values or IL-6 concentrations and ozone levels were analyzed by logistic regressions. It turned out that the relationship between ozone and hs-CRP levels was nonlinear. Therefore, ozone exposure was primarily stratified into "low" ($< 120 \mu g/m^3$) and "high" ($\geq 120\ \mu g/m^3$) concentrations and involved as a linear term in the subgroups defined in this manner. Additionally, thin plate regression splines were adopted to model the association between ozone and hs-CRP within GAMs.

The main models were determined after taking into account the residuals of NO_2 and PM_{10} as well as confounders. In addition, two models for sensitivity analyses were built: one excluded the participants with current asthma, one ignored the participants aged 15 years, who smoked cigarettes, or consumed alcohol, or took any medication within the last 7 days before the assessments. All analyses were performed separately for the data from

Munich and Wesel as well as for the two-area combined study population.

Chapter 4

Results

In general, on the basis of the systematic review (Appendix: Systematic review on ozone and mental health), we found six studies [45, 39, 46, 47, 48, 40] that looked into ambient ozone and depression, and the results were mixed. None of these studies investigated the association among children or adolescents. Thus, our first publication (Chapter 7) aimed to fill this gap for the very first time. The overall results from Chapter 7 failed to support the hypothesis that increased ambient ozone levels might increase the prevalence of symptoms of depression in German adolescents aged 15 years. The findings were robust and were not affected by the study area or adolescents' sex.

In addition to children and adolescents, we also investigated the association between long-term ambient ozone exposure and the diagnosis of depression among adults (aged 16 years or older) (Chapter 8). Based on data from 1.13 million individuals, we found that, independently from PM_{10}, long-term exposure to higher ozone levels increased the risk of the diagnosis of depression. These results were statistically significant and consistent across the sequence of models constructed. Nevertheless, our results, although statistically significant, should be considered with some caution as the exposure estimates were semi-individual, and there was a lack of information on some potentially important confounders, such as socioeconomic factors.

Furthermore, potential underlying mechanisms linking ozone exposure to depression were explored. For this purpose, the third publication studied to which degree short-term ambient ozone exposure is related to biomarkers of inflammation (Chapter 9). Overall, there were detrimental associations for the local inflammatory marker, FeNO, among participants aged 15 years, but not at the age of 10 years. This was also insignificant for IL-6 among participants aged 10 years. Importantly, we identified a nonlinear, J-shaped exposure-response relationship between ozone and hs-CRP levels, indicating that relatively low (by German standard, 120 $\mu g/m^3$) concentrations of ambient ozone were correlated with decreased hs-CRP levels, whereas high concentrations were more likely to be related to increased hs-CRP levels in both 10- and 15-year-olds, indicating a possible threshold of ozone action which should be further explored.

Chapter 5

Strengths and limitations

A detailed discussion is included in each publication (Chapters 7 to 8). Here, some main aspects are briefly summarized.

Epidemiologically, a recent systematic review [73] including six previous studies [44, 39, 46, 47, 48, 40], and one of our newly published studies (Chapter 7) failed to uncover a statistically significant association between short-term exposure to ozone and depression. However, when considering this result, one should keep in mind that currently available studies are very heterogeneous with regard to their designs, populations, exposure assessments, and outcome definitions. Last but not least, there are only a few studies on the topic of ozone and depression.

Mechanistically, inflammation and oxidative stress have been hypothesized as mechanisms linking ozone to adverse health effects. Beyond the mechanisms mentioned in Chapter 1.3, experiments in mice found that ozone exposure can disturb regular activity or social behavior of these animals [74]. Moreover, experiments in rats indicated that ozone inhalation might attenuate the effect of antidepressants [75, 76] and that the level of central monoamine, which was compromised, was similar to that observed in depression [76]. Although such experimental studies shed light on the evidence, the ultimate mechanisms behind the association between ozone and depression are not clarified and need further thorough investigations.

The systematic review (Appendix: Systematic review on ozone and mental health) provided, for the first time, a comprehensive picture on how ambient ozone exposure relates to mental health outcomes. Based on the results, depression was selected as the main outcome for the original studies. For all of the three publications (Chapters 7 to 9), large study samples were available from the GINIplus and LISA cohorts as well as the health insurance company dataset, and adequate models adjusted for covariates could be constructed. Additionally, the two separated age groups, adolescents and adults, in two studies (Chapters 7 and 8), were well-suited to stratify and test the effect of age and to deliver combined results with a higher level of certainty.

Even though the longitudinal analysis (Chapter 8) did show the robust result that increased levels of ambient ozone may be linked to an elevated risk of the diagnosis of depression in the general population, causality cannot be inferred, until the possible mech-

anisms have been sufficiently clarified. The two cross-sectional studies (Chapters 7 and 9) naturally cannot be directly interpreted in terms of a causal relationship, but they give hints. Furthermore, estimates of air pollution concentrations only partially reflect individual exposures, particularly for the temporal exposure metrics used in Chapter 9. Some important covariates, such as data on socioeconomic status, were not available in one of our studies (Chapter 8), but this probably can be remedied by using more detailed information from future data sets.

Chapter 6

Conclusion and Outlook

The health effect of exposure to ambient ozone is under-investigated compared to the effects of other common ambient air pollutants such as PM and NO_2 Studies on ozone with an outcome that focused on mental health are even scarcer. The systematic review (Appendix: Systematic review on ozone and mental health) of ambient ozone exposure and mental or behavior disorders provided a comprehensive picture and identified relevant gaps in this area. As a consequence, this thesis, with the aim to elucidate the relationships between exposure to ambient ozone and depressive symptoms or depression, adopted data from the GINIplus and LISA birth cohort to determine the association in adolescents (Chapter 7). The other relevant population comprises adults. In order to address this question, the thesis utilized health insurance company data to analyze the association between adult subjects (Chapter 8). Even though no consistent associations were detected in adolescents (Chapter 7), ambient ozone exposure was found to be significantly associated with the diagnosis of depression in adults (Chapter 8). The next question was for potential mechanisms. For this purpose, we used data from the two birth cohorts to explore local and systemic markers of inflammation as indicators of a potential mechanism linking ozone exposure to adverse health effects. Exposure to high levels of ambient ozone in adolescents was associated linearly with elevated the local marker of inflammation of FeNO, and J-shaped with hs-CRP, but not IL-6 – as two systemic markers (Chapter 9). The studies included in this thesis add to the evidence on adverse health effects of ambient ozone exposure and may increase the interest in this insufficiently investigated research topic.

Taken together, current studies with ambient ozone as exposure and mental health as the outcome are urgently warranted, in light of the predicted increase of ozone concentration and huge burden already attributed to mental health. Although the thesis found different associations in adolescents and adults, the findings on ozone-associated inflammation suggest a possible causal, mechanistic connection between ambient ozone exposure and depression, which might depend on factors linked to age.

Ambient ozone exposure is ubiquitous; thus, a small figure in the health effect estimate is consequently correlated with a considerable burden of disease. The thesis demonstrates the possible association between ozone and depression, providing additional insights for city planning and disease prevention.

Regarding future studies, the relationship between ozone and depression should be tested in other epidemiological researches comprising larger populations, improved exposure assessments, together with better-standardized case definitions.

The hypothesized mechanism of an inflammatory response that might link ozone to depression was supported by the findings (Chapter 9). However, although associations between inflammation and depression have been reviewed and reported [77, 78], the causal relationship cannot be determined based on the limited number of the reviewed longitudinal studies [78] and the cross-sectional nature of some additional studies [79, 80]. Time-series studies on ozone and depression, including a broad panel of markers of inflammation and oxidative stress, would be a promising way to explore the mechanisms.

Except for inflammation and oxidative stress, an increasing number of studies conclude that ozone can affect hormone levels or the endocrine system [81, 82, 83] and metabolism of neurotransmitters [84, 85]. To what extent these phenomena play a role in the physiopathologic process of depression needs to be further investigated. As an instance, puberty is a critical period of life, and early menarche may be associated with depressive symptoms [86]. Air pollution is currently supposed to affect normal pubertal development [87, 88, 89]. Nevertheless, no studies were investigated pubertal development in relation to ambient ozone exposure yet. Therefore, a study on ozone and sex hormones or pubertal development among children and adolescents might provide evidence that allows to better disentangle the association between ozone and depression.

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Chapter 7

Paper 1: Ozone and depressive symptoms in adolescents

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Ambient ozone exposure and depressive symptoms in adolescents: Results of the GINIplus and LISA birth cohorts

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ABSTRACT

Background: Depression has been associated with air pollution, as reported by animal and epidemiological studies. However, the relationship between ozone exposure and depression, especially among adolescents, is scarcely investigated.
Objectives: The study aimed to analyze associations between ozone exposure and depressive symptoms among German adolescents.
Methods: The analyses were based on 2827 adolescents aged 15 from Munich and Wesel areas of the GINIplus and LISA birth cohorts. The depressive symptoms were assessed by the Depression Screener for Teenagers (DesTeen). Long-term ozone exposure was estimated by optimal interpolation techniques and assigned to home addresses. Nitrogen dioxide (NO_2) and particulate matter with an aerodynamic diameter < 10 μm (PM_{10}) were assessed by land use regression models. For short-term exposure, maximum 8-h averages of ozone and daily average concentrations of NO_2 and PM_{10} from the background monitoring sites 0 (same day), 1, 2, 3, and 7 days prior to depressive symptoms assessment were adopted. The cross-sectional analyses were conducted by adjusted logistic regression models controlling for residuals of NO_2 and PM_{10}, and covariates identified by a directed acyclic graph.
Results: The prevalence of depressive symptoms ranged from 10.9% to 13.8% depending on regions. Overall, long- and short-term exposure to ozone were not statistically significantly associated with depressive symptoms. However, subgroup analysis showed inconsistent significant protective associations for short-term exposure to ozone lag 0 day (same day) and depressive symptoms in Wesel (OR = 0.76, 95% CI: (0.59, 0.98)), but not in Munich (OR = 1.00, 95% CI: (0.83, 1.21)).
Conclusions: Our study does not support the hypothesis that ambient ozone exposure might increase the prevalence of depressive symptoms in German adolescents. Nevertheless, due to a lack of similar studies, these results need to be replicated in other samples.

Abbreviations: CI, confidence interval; DAG, directed acyclic graph; DesTeen, Depression Screener for Teenagers; ESCAPE, European Study of Cohorts for Air Pollution Effects; GAM, generalized additive model; GINIplus, German Infant study on the influence of a Nutritional Intervention plus environmental and genetic influences on allergy development; IQR, interquartile range; LISA, influence of Life-style factors on the development of the Immune System and Allergies in East and West Germany; LUR, land use regression; NO_2, nitrogen dioxide; OR, odds ratio; PM_{10}, particulate matter with an aerodynamic diameter < 10 μm; ppb, parts per billion; SES, socio-economic status; UBA, Umweltbundesamt (German Environment Agency); WHO, World Health Organization

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1. Introduction

It is estimated that the proportion of the global population with depression was 4.4% in 2015 and that the number of people living with depression between 2005 and 2015 increased by 18.4% (WHO (World Health Organization), 2017). Given such a high prevalence, depression accounts for a significant proportion of the global burden of disease, leading to over 50 million Years Lived with Disability in 2015 (GBD 2015 Disease and Injury Incidence and Prevalence Collaborators, 2016). Substantially, to scale up effective treatment for depression over the period 2016–2030, the global investment would be 51.9 billion US dollars (Chisholm et al., 2016).

Depressive symptoms include sad, empty, hopeless or irritable mood, accompanied by somatic and cognitive changes that significantly affect the individual capacity of functioning (American Psychiatric Association, 2013). It does not only decrease quality of life (Ruo et al., 2003), but is also associated with several other adverse health outcomes, including other psychiatric (Fergusson and Woodward, 2002) and somatic diseases (Glymour et al., 2010; Hung et al., 2014; O'Neill et al., 2014; Whooley et al., 2008), self-injury (Giletta et al., 2012), and suicide (Dong et al., 2018).

As depression is a complex disorder, its determinants include genetic predisposition (Carver et al., 2011; Milne et al., 2009), as well as neuropsychological and pathophysiological changes (Werner and Covenas, 2010), and socio-economic factors (Arias-de la Torre et al., 2018; Dulaney et al., 2018). Specifically, several recent studies have reported associations between ambient air pollution and depression (Cho et al., 2014; Kim et al., 2016; Pun et al., 2017; Vert et al., 2017). However, the published studies have mainly considered particulate matter (PM) and other gaseous pollutants, such as nitrogen dioxide (NO_2). As summarized by our recent systematic review (Zhao et al., 2018), only a few epidemiological studies (Kioumourtzoglou et al., 2017; Lim et al., 2012; Szyszkowicz, 2007; Szyszkowicz et al., 2016, 2009; Wang et al., 2014) investigated whether such a powerful oxidant and an important air pollutant as ozone - via pathway like dysregulation of inflammatory cytokines (de Prado Bert et al., 2018)- can also increase risk of depression, and found the current evidence is inconclusive.

Depression occurs in children and adolescents as well, and is assumed to start in early childhood (Ignacio et al., 2014). For adolescents aged between 15 and 19 years old, the global prevalence of depressive disorder is about 4.5% in females and 3% in males (WHO, 2017). However, a higher prevalence is also considered. For example, the reported prevalence in German children aged 7–10 years was 10.7% and in children aged 11–17 years it was 11.1% (Ravens-Sieberer et al., 2008). Mojtabai et al. (2016) reported the prevalence of major depressive episodes in American adolescents was 11.3% in 2014.

Therefore, it is of public relevance to investigate whether exposure to higher ozone levels can increase depression risk already in childhood and early adolescence. To our knowledge, there is no such research so far. The present study aimed to investigate the association between long- and short-term ambient ozone exposure and depressive symptoms in 15-year old adolescents residing in two German areas.

2. Materials and methods

2.1. Study population

Data were obtained from the two ongoing population-based German birth cohorts: “German Infant study on the influence of a Nutritional Intervention plus environmental and genetic influences on allergy development” (GINIplus) and “influence of Life-style factors on the development of the Immune System and Allergies in East and West Germany” (LISA). Both cohorts recruited only healthy newborns with a normal birth weight (> 2500 g) at a full term (gestational age ≥ 37 weeks). Briefly, from 1995 to 1998, 5991 children from Munich (N = 2949) and Wesel (N = 3042) were recruited into the GINIplus cohort. There are two different arms in this cohort. The intervention arm allocated participants with at least one atopic parent or sibling to investigate the effect of different hydrolyzed formulas during the first four months of life on later allergy development. The observation study arm included participants who had a negative family history of allergies or whose parents did not consent to participate in the intervention study. LISA recruited 3094 participants from Munich (N = 1464) and Wesel (N = 348), as well as Leipzig (N = 976) and Bad Honnef (N = 306) between 1997 and 1999. Both cohorts were approved by the local ethics committees (Bavarian Board of Physicians, University of Leipzig, and Board of Physicians of North-Rhine-Westphalia) and written consent was obtained from participants’ legal guardians. More details on these two cohorts can be found elsewhere (Heinrich et al., 2002; von Berg et al., 2010; Zutavern et al., 2006).

The present study is restricted to the inhabitants of the cities of Munich and Wesel and their surroundings from the time of recruitment until the time of the 15-year follow-ups because data on NO_2 and PM_{10} could not be assigned to the residents of the Leipzig and Bad Honnef areas. The data from GINIplus and LISA were pooled as the cohorts have very similar design at the later follow-ups, and as this strategy has been widely adopted for our previous analyses (e.g., Fuertes et al., 2016; Markevych et al., 2014). The participants with incomplete outcome, exposure and covariate data were excluded, as well as those who lived in the study area for less than one year (Fig. S1), given the time frame of long-term ozone exposure.

2.2. Ambient ozone, NO_2, and PM_{10} exposure

2.2.1. Long-term exposure assessment

Long-term ambient ozone exposure was estimated at residential addresses at 15 years by using data from the German Environment Agency (Umweltbundesamt, labeled as UBA). The UBA-derived ozone estimates (Flemming et al., 2004; Stern and Flemming, 2004) were modelled at a resolution of two square kilometers for each year from 2005 onwards for Germany using optimal interpolation technique implemented in REM-CALGRID, as well as the measured ozone data from 150 German monitoring stations and meteorological data. Annual average concentrations (μg/m^3) of ozone, and “number of days per year with a maximum 8-h average concentration exceeding 120 μg/m^3” (days/year, as target value for the protection of human health, according to https://www.umweltbundesamt.de/en/topics/air/ozone) were both used. Ozone estimates a year prior to depression symptoms assessment were assigned.

As air pollution is a complex mixture, we considered several of these co-pollutants in our analyses. Annual average concentrations (μg/m^3) of NO_2 and PM with an aerodynamic diameter < 10 μm (PM_{10}) at 15-year residential addresses were estimated by area-specific land use regression (LUR) models originally developed within the “European Study of Cohorts for Air Pollution Effects” (ESCAPE, www.escapeproject.eu) (Beelen et al., 2013; Cyrys et al., 2012; Eeftens et al., 2012a, 2012b). Briefly, NO_2 and PM_{10} were monitored at 20 and 40 air measuring stations, respectively, for three two-week measurement periods between October 2008 and November 2009 in both the Munich and Wesel study areas. The pollutant annual averages at measurement sites were calculated as averages of these three measurements and adjusted for temporal variation derived from a yearly-operating background measuring station. Land use, population and traffic predictor variables were used to generate area-specific LUR models to estimate pollution at each residential address (model explained variance (R^2) of the models ranged from 0.78 to 0.97).

Assignment of air pollution estimates to geocoded residential addresses was done in ArcGIS Geographical Information System (GIS) (version 10.4, ESRI, Redlands, CA).

2.2.2. Short-term exposure assessment

The short-term ozone data were derived from UBA as well. The concentrations were obtained from a background monitoring site (www.env-it.de/stationen/public/station.do) in Munich which is approximately 9 km northeast of city center (Johanneskirchen) and a site which is approximately 2 km northeast the center of Wesel (Feldmark) (Fuertes et al., 2015).

For ozone, according to its high within- and across-day variability, as well as recommendation from the UBA (https://www.umweltbundesamt.de/en/topics/air/ozone), we calculated concentration ($\mu g/m^3$) of moving 8-h average for every hour (7 h before and of the hour of interest) and thereby identified a maximum of 8-h average for every day. The maximum of the daily maximum 8-h average concentration was selected over 0 (same day), and 1, 2, 3, and 7 days prior to the depressive symptoms assessment (lags 0–7 days). Since all of the published prior studies that reported the significant short-term ozone effects may detect the associations within 7 days (reviewed by Zhao et al., 2018), our selected time frames enabled us to detect the possible association between short-term ozone and depressive symptoms.

For NO_2 and PM_{10}, we utilized average of the daily concentrations ($\mu g/m^3$) of 0 (same day), and 1, 2, 3, and 7 days prior to the day that depressive symptoms were evaluated for our analysis (lags 0–7 days, same time frames as in the case of ozone).

2.3. Depressive symptoms

Depressive symptoms were assessed by Depression Screener for Teenagers (DesTeen). DesTeen is a specific validated tool for screening of adolescent depression in Germany. It contains 14 items on four-point scale that focus on cognitive and emotional symptoms for assessing the depressive symptoms over the preceding two weeks (Allgaier et al., 2014; Pietsch et al., 2011). This questionnaire was filled by children at the 15-year follow-up. Presence of depressive symptoms was defined as a total score ≥ 12 (Pietsch et al., 2011) and considered as an outcome in associations with both long- and short-term ozone exposures.

2.4. Covariates

Based on our previous analyses on behavioral problems in GINIplus and LISA cohorts in relation to environmental factors (Fuertes et al., 2016; Markevych et al., 2014; Tiesler et al., 2013), other than co-pollutants, potential covariates included: cohort (GINIplus observation, GINIplus intervention and LISA), exact age of a child at the 15-year follow-up, sex of the child, parental education (based on the highest number of years of school education reported by either parent; low, medium and high were respectively defined as < 10 years, = 10 years, and > 10 years), maternal age at birth (≤ 30 years, 30–35 years, > 35 years), net equivalent household income (area-specific tertiles), single parent family status (yes/no), maternal smoking during pregnancy (yes/no), secondhand smoke exposure at home (never, likely never, or ever from birth until 15 years), time spent in front of a screen (e.g., computer, television; high defined as ≥ 1 h/day in summer or ≥ 2 h/day in winter), and time spent outside (high defined as ≥ 4 h/day in summer or ≥ 2 h/day in winter). Additionally, the Global Severity Index score greater than the 90th percentile based on the Brief Symptom Inventory 18 was used to evaluate parental psychopathology (Derogatis, 2001; Fuertes et al., 2016).

Table 1
Characteristics of the study population.

Variable	Category	Munich n (%)	Wesel n (%)	p-value	All n (%)
Cohort	GINIplus intervention	477 (30.5)	442 (35.0)	< 0.001*	919 (32.5)
	GINIplus observation	510 (32.6)	688 (54.5)		1198 (42.4)
	LISA	578 (36.9)	132 (10.5)		710 (25.1)
Age[a]		15.23 ± 0.29	15.15 ± 0.32	< 0.001*	15.20 ± 0.30
Sex	Female	796 (50.9)	636 (50.4)	0.830	1432 (50.7)
	Male	769 (49.1)	626 (49.6)		1395 (49.3)
Parental education[b]	Low (< 10 years)	147 (9.4)	403 (31.9)	0.003*	550 (19.5)
	Medium (= 10 years)	206 (13.2)	297 (23.5)		503 (17.8)
	High (> 10 years)	1212 (77.4)	562 (44.5)		1774 (62.7)
Maternal age at birth	≤ 30 years	474 (30.3)	624 (49.5)	< 0.001*	1098 (38.8)
	> 30 to ≤ 35 years	749 (47.8)	505 (40.0)		1254 (44.4)
	> 35 years	342 (21.9)	133 (10.5)		475 (16.8)
Income (euro/month)[c]	Low	(134, 1560]	(162, 1070]		–
	Medium	(1560, 2250]	(1070, 1530]		–
	High	(2250, 5130]	(1530, 5130]		–
Parental psychopathology[d]	Abnormal	215 (13.7)	142 (11.3)	0.055	358 (12.7)
Single parent family	Yes	208 (13.3)	137 (10.9)	0.056	345 (12.2)
	Missing	61 (3.9)	45 (3.6)		106 (3.7)
Smoking exposure	During pregnancy	178 (11.4)	175 (13.9)	0.053	353 (12.5)
	between 0 and 15 years	445 (28.4)	671 (53.2)	< 0.001*	1116 (39.5)
Time spent outside[e]	High	148 (9.5)	319 (25.3)	< 0.001*	467 (16.5)
	Low	1377 (88.0)	892 (70.7)		2269 (80.3)
	Missing	40 (2.6)	51 (4.0)		91 (3.2)
Time in front of a screen[f]	High	1267 (81.0)	1105 (87.6)	< 0.001*	2373 (83.9)
	Low	297 (19.0)	157 (12.4)		454 (16.1)
Depressive symptoms (DesTeen)	Normal	1349 (86.2)	1125 (89.1)	0.022*	2474 (87.5)
	Abnormal	216 (13.8)	137 (10.9)		353 (12.5)
Total		1565 (55.4)	1262 (44.6)		2827 (100)

Note:
[a] Mean ± standard deviation.
[b] According to German education system, calculated as the highest number of years of school education for either parent.
[c] Net equivalent household income (euro/month), Min and max of area-specific tertiles.
[d] According to the Global Severity Index score (Derogatis, 2001), subscore is categorized at 90th percentile (Fuertes et al., 2016).
[e] High is defined as ≥4 h per day in summer or ≥2 h in winter.
[f] High is defined as ≥1 h per day in summer or ≥2 h per day in winter.
* Significant difference was detected between participants from Munich and from Wesel in this variable, $p < 0.05$.

2.5. Statistical analysis

The Chi-square test or Student's *t*-test was adopted to examine the differences between the selected analytic and the original population, as well as the differences between the two analytic populations from Munich and from Wesel. The Wilcoxon test was used to examine the differences of pollutants between these two areas. We also calculated Spearman correlation coefficients to assess relationships between different pollutant metrics for area and combined participants of two areas.

Individual associations between each of the ozone exposure variables and depressive symptoms at 15 years were assessed by logistic regression models. Ozone was modelled as continuous variables in regression analyses, because the relationship of ozone with depressive symptoms did not show major deviations from linearity in generalized additive models (GAMs) (Hastie and Tibshirani, 1986). The results are presented as odds ratios (ORs) and 95% confidence intervals (CIs) scaled by specific interquartile range (IQR) increase in ozone values. Furthermore, we also had a model with the DesTeen score as original count data, analyzed by negative binomial regressions. The resulted count ratios and 95% CIs were scaled by IQR of ozone as well.

To reduce multicollinearity, unnecessary adjustment and over-adjustment, we defined a minimal adjustment set of adjustment variables (Rohrig et al., 2014) from the potential covariates by using a directed acyclic graph (DAG) (Greenland et al., 1999) in DAGitty (Textor et al., 2011, 2016). Thus, our main models were adjusted for income, parental education, parental psychopathology, single parent family status, time spent by a child outside, time spent in front of a screen, exact age, and sex of the child (Fig. S2). Since area and cohort are basic design variables, we additionally adopted these two variables in our main models. We also present minimal adjusted models, which considered only area, cohort and sex and models with an adjustment for all covariates mentioned in the subsection 2.4. All the analyses were conducted for Munich and for Wesel separately, as well as for the entire study population. Given the higher prevalence of depression in females (WHO, 2017), we also stratified the analyses by sex of the child.

We adjusted our models for residuals of PM_{10} and NO_2. Briefly, we regressed each of the PM_{10} and NO_2 variables on each of the ozone variables and derived model residuals, which were afterwards included into models in a similar manner to how it was done before (Yang et al., 2018). All analyses were conducted using R 3.4.4 (R Core Team, 2018). GAM models were fitted by *gam* function from the *mgcv* package (Wood, 2011). The negative binomial regression models were fitted by *glm.nb* function from the *MASS* package (Venables and Ripley, 2002).

3. Results

3.1. Characteristics of participants and pollutants

After the selection of subjects with complete data, 1565 15-year-old participants from Munich and 1262 from Wesel were included in this study (Fig. S1). We found that GINIplus intervention children were more likely to be included in our analytic samples ($p < 0.001$), as well as children of parents with high education ($p < 0.001$). The characteristics of participants from both study areas are listed in Table 1. The overall prevalence for depressive symptoms was 12.5%, and it was higher in Munich compared to Wesel (13.8% *vs* 10.9%, $p = 0.022$). The participants from Munich and Wesel differed in nearly all characteristics. Specifically, children from Munich were more likely to have parents with higher education ($p = 0.003$), older mothers at birth ($p < 0.001$), to spend less time in front of a screen ($p < 0.001$), as well as outside ($p < 0.001$), and to be less exposed to passive smoking at home ($p < 0.001$).

The distributions of long- and short-term air pollutants concentrations are presented in Table 2. The median ozone concentrations were 43.3 μg/m^3 in Munich and 42.4 μg/m^3 in Wesel, respectively. The ozone

Table 2
Concentrations of ozone and other air pollutants.

Exposure	Area	Air pollutant	Mean	SD	Min	Max	Median	IQR	p-value
Long-term	Munich	O_3-UBA-annual[a]	43.1	2.9	31.0	48.4	43.3	2.9	–
		O_3-UBA-number of days[b]	14.7	4.7	6.0	26.0	15.0	8.0	–
		NO_2[c]	19.7	5.0	11.5	58.0	18.6	6.4	–
		PM_{10}[c]	20.0	2.3	14.8	32.0	20.4	3.0	–
	Wesel	O_3-UBA-annual[a]	41.1	3.3	30.4	48.4	42.4	3.2	–
		O_3-UBA-number of days[b]	15.6	4.5	6.0	26.0	16.0	7.0	–
		NO_2[c]	23.7	3.3	11.5	59.8	21.8	6.1	–
		PM_{10}[c]	25.5	1.3	14.8	32.7	22.5	4.9	–
	All	O_3-UBA-annual[a]	42.2	3.2	30.4	48.4	42.4	3.2	$p < 0.001$*
		O_3-UBA-number of days[b]	15.1	4.6	6.0	26.0	16.0	7.0	$p < 0.001$†
		NO_2[c]	21.5	4.7	11.5	59.8	21.8	6.1	$p < 0.001$†
		PM_{10}[c]	22.5	3.3	14.8	32.7	22.5	4.9	$p < 0.001$†
Short-term	Munich	Ozone[d]	72.1	25.3	7.9	134.8	76.2	35.6	–
		NO_2[e]	20.7	6.9	10.6	47.5	18.3	8.4	–
		PM_{10}[e]	16.3	8.8	3.9	62.8	14.3	8.4	–
	Wesel	Ozone[d]	62.7	27.7	4.2	135.2	63.0	38.5	–
		NO_2[e]	22.2	8.5	6.2	44.5	20.7	13.3	–
		PM_{10}[e]	23.1	9.2	10.3	52.2	20.7	10.7	–
	All	Ozone[d]	67.9	26.8	4.2	135.2	71.3	39.7	$p < 0.001$*
		NO_2[e]	21.4	7.7	6.2	47.5	19.3	10.6	$p < 0.001$†
		PM_{10}[e]	19.3	9.6	3.9	62.8	16.9	10.9	$p < 0.001$†

Note:
Abbreviation: IQR, interquartile range; SD, standard deviation.
[a] Annual average concentration (μg/m^3), from the German Environment Agency (Umweltbundesamt, UBA, www.umweltbundesamt.de).
[b] Number of days per year with maximum daily 8-h concentration exceeding 120 μg/m^3 (days/year), from the UBA.
[c] Annual average concentration (μg/m^3), from the "European Study of Cohorts for Air Pollution Effects" (ESCAPE, www.escapeproject.eu).
[d] The maximum 8-h (7 h before and the hour of interest) daily average (μg/m^3), 7 days prior to the depressive symptoms assessment from the background monitor stations, from the UBA.
[e] Average of the daily concentration (μg/m^3), 7 days prior to the depressive symptoms assessment, from the UBA.
* This metric was higher in Munich than in Wesel, $p < 0.05$.
† This metric was higher in Wesel than in Munich, $p < 0.05$.

concentrations in Munich were higher than in Wesel ($p < 0.001$). However, the number of days with ozone levels exceeding 120 μg/m^3 in Munich was slightly less than in Wesel (15 days/year *vs* 16 days /year, $p < 0.001$). Unlike ozone, the concentrations of NO_2 and PM_{10} were higher in Wesel compared to Munich ($p < 0.001$).

The short-term ozone exposure ranged widely (Table 2). For example, the daily maximum 8-h average concentration (7 days prior to the day of depressive symptoms assessment) ranged from 4.2 to 135.2 μg/m^3, confirming the notion that single day average of short-term ozone exposure is strongly varying from day to day. The daily maximum 8-h average concentrations were 76.2 μg/m^3 in Munich and 63.0 μg/m^3 in Wesel. Similar to long-term exposure, the short-term ozone level in Munich was higher than in Wesel ($p < 0.001$), while Wesel was more polluted by NO_2 and PM_{10} than Munich ($p < 0.001$).

Additionally, NO_2 and PM_{10} were positively and strongly correlated with each other considering both long-term exposure and short-term exposure. However they were weakly correlated with ozone in both long- and short-term exposure, except that moderate negative correlations between short-term exposures of ozone and NO_2 were detected (Figs. S3 and S4) (Figs. S3 and S4).

3.2. Long-term ambient ozone exposure and depressive symptoms

Table 3 shows the adjusted ORs for long-term exposure to ozone and depressive symptoms (results from different sensitivity analyses can be found in Supplementary, Tables S1–S3). No significant associations were found in main analysis (Table 3). This holds true for the two study areas (Munich, Wesel), and for the two types of exposure metrics (annual means, number of days per year exceeding the limit). For example, an OR = 1.08 (95% CI: (0.92, 1.26)) per IQR increase (2.9 μg/m^3) in annual ozone concentration in Munich, and 0.95 (95% CI: (0.69, 1.32)) per IQR increase (7 days/year) in days with ozone levels exceeding 120 μg/m^3 in Wesel. Similarly, no significantly associations were detected in the models with the DesTeen score as original variable (Table 4). There were also no significant associations between long-term exposure to ozone and depressive symptoms in the sensitivity analyses (Supplementary Tables S1–S3).

3.3. Short-term ambient ozone exposure and depressive symptoms

Overall, the results of short-term ozone exposure and depressive symptoms were mixed and not significant for the entire study population (Table 3). Due to a lack of exposure data across all days of the year, the numbers of participants varied between analyses (Table 3). Within several subgroup analyses, one statistically significant protective association was detected for Wesel for lag 0 day (same day): an IQR increase (38.5 μg/m^3) in ozone concentration decreased the odds of depressive symptoms by 24% in Wesel (OR = 0.76, 95% CI: (0.59, 0.98), p = 0.037). This association was also found in two sensitivity analyses: models adjusted for minimal covariates (OR = 0.77, 95% CI: (0.60, 0.99), p = 0.038, Table S1), and models adjusted for all covariates (OR = 0.74, 95% CI: (0.57, 0.96), p = 0.024, Table S2). No such association was observed for Munich (Table 3 and Tables S1, S2, and S3). Sex-stratified models also identified the same inverse association with short-term ozone exposure in males from Wesel (for lag 0 day, OR = 0.61, 95% CI: (0.39, 0.96), p = 0.033, Table S3), but similar decreased odds ratios were not observed for Munich males (Table S3). Additionally, when considered the score of DesTeen as the original count data, there were no significant associations between ozone exposure and depressive symptoms (Table 4).

4. Discussion

4.1. Main study findings

The overall results of our analyses based on long-term exposure to ozone and depressive symptoms do not support the notion that increased ambient ozone levels increase the prevalence of depressive symptoms in our sample of 15-year-old German adolescents. This finding was robust across different adjustment strategies and statistical approaches, and did not depend on the study area and sex of participant. Even though some statistically significant protective associations were detected for short-term ozone exposure, the results are isolated and inconsistent between the two study areas. Therefore, these results of potential short-term associations should be interpreted with caution.

Table 3
Adjusted associations between ozone exposure and depressive symptoms (Models adjusted for the DAG-identified covariates).

Exposure	Area	Pollutant	DesTeen	
			15-year (OR, 95%CI)	Participants
Long-term	Munich	O_3-UBA-annual[a]	1.08 (0.92, 1.26)	1565/1565
		O_3-UBA-days[b]	1.07 (0.74, 1.55)	1565/1565
	Wesel	O_3-UBA-annual[a]	1.10 (0.91, 1.32)	1262/1262
		O_3-UBA-days[b]	0.95 (0.69, 1.32)	1262/1262
	All	O_3-UBA-annual[a]	1.08 (0.94, 1.23)	2827/2827
		O_3-UBA-days[b]	1.02 (0.81, 1.28)	2827/2827
Short-term	Munich	Lag 0 day[c]	1.00 (0.83, 1.21)	1524/1565
		Lag 0–1 days[d]	0.97 (0.81, 1.15)	1528/1565
		Lag 0–2 days[e]	1.00 (0.84, 1.20)	1535/1565
		Lag 0–3 days[f]	0.99 (0.81, 1.20)	1544/1565
		Lag 0–7 days[g]	0.90 (0.74, 1.10)	1559/1565
	Wesel	Lag 0 day[c]	0.76 (0.59, 0.98)*	1200/1262
		Lag 0–1 days[d]	0.86 (0.67, 1.10)	1201/1262
		Lag 0–2 days[e]	0.88 (0.69, 1.13)	1238/1262
		Lag 0–3 days[f]	0.88 (0.69, 1.13)	1250/1262
		Lag 0–7 days[g]	0.95 (0.76, 1.19)	1262/1262
	All	Lag 0 day[c]	0.90 (0.77, 1.06)	2724/2827
		Lag 0–1 days[d]	0.92 (0.79, 1.08)	2729/2827
		Lag 0–2 days[e]	0.95 (0.82, 1.11)	2773/2827
		Lag 0–3 days[f]	0.94 (0.81, 1.10)	2794/2827
		Lag 0–7 days[g]	0.92 (0.79, 1.07)	2821/2827

Note:
Abbreviation: CI, confidence interval; DesTeen, Depression Screener for Teenagers; OR, odds ratio.

1. ORs and 95% CIs are scaled by an interquartile range increase according to specific areas or metrics (see Table 2).
2. All estimates are from logistic regression models adjusted for PM_{10} and NO_2 residuals, income, parental education, parental psychopathology, single parent family status, time spent outside and time spent in front of a screen, exact age at the 15 year follow-up and sex of the child, cohort and area (only for the area "all").
3. Participants, "sample number analyzed/total number analyzed"; missings are due to a lack of exposure data.

[a] Annual average concentration, from the German Environment Agency (Umweltbundesamt, UBA, www.umweltbundesamt.de).
[b] Number of days per year with maximum 8-h concentration exceeding 120 μg/m^3, from the UBA.
[c] The maximum of the daily maximum 8-h average concentration was selected over 0 days (same day) prior to the depressive symptoms assessment, from the background monitor stations, from the UBA.
[d] The maximum of the daily maximum 8-h average concentration was selected over 1 days prior to the depressive symptoms assessment, from the background monitor stations, from the UBA.
[e] The maximum of the daily maximum 8-h average concentration was selected over 2 days prior to the depressive symptoms assessment, from the background monitor stations, from the UBA.
[f] The maximum of the daily maximum 8-h average concentration was selected over 3 days prior to the depressive symptoms assessment, from the background monitor stations, from the UBA.
[g] The maximum of the daily maximum 8-h average concentration was selected over 7 days prior to the depressive symptoms assessment, from the background monitor stations, from the UBA.
* p = 0.037.

Table 4
Adjusted associations between long-term ozone exposure and depressive symptoms (Models with the DesTeen score as count data, adjusted for the DAG-identified covariates).

Exposure	Area	Pollutant	DesTeen	
			15-year (Count ratio, 95%CI)	Participants
Long-term	Munich	O_3-UBA-annual[a]	0.99 (0.97, 1.03)	1565/1565
		O_3-UBA-days[b]	0.97 (0.89, 1.05)	1565/1565
	Wesel	O_3-UBA-annual[a]	1.01 (0.97, 1.05)	1262/1262
		O_3-UBA-days[b]	0.98 (0.91, 1.05)	1262/1262
	All	O_3-UBA-annual[a]	0.99 (0.97, 1.03)	2827/2827
		O_3-UBA-days[b]	0.98 (0.93, 1.03)	2827/2827
Short-term	Munich	Lag 0 day[c]	0.99 (0.95, 1.03)	1524/1565
		Lag 0–1 days[d]	0.99 (0.95, 1.03)	1528/1565
		Lag 0–2 days[e]	1.00 (0.96, 1.04)	1535/1565
		Lag 0–3 days[f]	0.99 (0.96, 1.04)	1544/1565
		Lag 0–7 days[g]	0.98 (0.94, 1.02)	1559/1565
	Wesel	Lag 0 day[c]	0.95 (0.90, 1.00)	1200/1262
		Lag 0–1 days[d]	0.97 (0.93, 1.03)	1201/1262
		Lag 0–2 days[e]	0.98 (0.93, 1.03)	1238/1262
		Lag 0–3 days[f]	0.98 (0.93, 1.03)	1250/1262
		Lag 0–7 days[g]	0.99 (0.95, 1.05)	1262/1262
	All	Lag 0 day[c]	0.97 (0.93, 1.00)	2724/2827
		Lag 0–1 days[d]	0.98 (0.95, 1.02)	2729/2827
		Lag 0–2 days[e]	0.99 (0.96, 1.03)	2773/2827
		Lag 0–3 days[f]	0.99 (0.96, 1.03)	2794/2827
		Lag 0–7 days[g]	0.99 (0.96, 1.02)	2821/2827

Note:
Abbreviation: CI, confidence interval; DesTeen, Depression Screener for Teenagers; OR, odds ratio.

1. Count ratios and 95% CIs are scaled by an interquartile range increase according to specific areas or metrics (see Table 2).
2. All estimates are from negative binomial regression models adjusted for PM_{10} and NO_2 residuals, income, parental education, parental psychopathology, single parent family status, time spent outside and time spent in front of a screen, exact age at the 15 year follow-up and sex of the child, cohort and area (only for the area "all").
3. Participants, "sample number analyzed/total number analyzed"; missings are due to a lack of exposure data.

[a] Annual average concentration, from the German Environment Agency (Umweltbundesamt, UBA, www.umweltbundesamt.de).

[b] Number of days per year with maximum 8-h concentration exceeding 120 $\mu g/m^3$, from the UBA.

[c] The maximum of the daily maximum 8-h average concentration was selected over 0 days (same day) prior to the depressive symptoms assessment, from the background monitor stations, from the UBA.

[d] The maximum of the daily maximum 8-h average concentration was selected over 1 days prior to the depressive symptoms assessment, from the background monitor stations, from the UBA.

[e] The maximum of the daily maximum 8-h average concentration was selected over 2 days prior to the depressive symptoms assessment, from the background monitor stations, from the UBA.

[f] The maximum of the daily maximum 8-h average concentration was selected over 3 days prior to the depressive symptoms assessment, from the background monitor stations, from the UBA.

[g] The maximum of the daily maximum 8-h average concentration was selected over 7 days prior to the depressive symptoms assessment, from the background monitor stations, from the UBA).

4.2. Interpretations and comparisons with other studies

According to our results, there were no associations between long-term ozone exposure and depressive symptoms in adolescents. Considering the protective effects for short-term exposure in Wesel, and the sex-specific results for males in Wesel and the inconsistency between Wesel and Munich, we interpret the protective effects as chance findings.

Although there are no published studies in adolescents, the conclusion of our recent systematic review of epidemiological studies (Zhao et al., 2018) - the evidence about ozone exposure and depressive disorder is inconclusive - is in line with the interpretations of this study. More specifically, Wang et al. (2014) analyzed data from a cohort of 732 adults (mean age 78.1 years) in USA. They reported no significant associations between short-term changes in ozone over two weeks preceding assessment and depressive symptoms. Furthermore, Szyszkowicz et al. (2009) investigated associations between emergency department visits for depression and short-term air pollution in Canada and found also no statistically significant associations for ozone exposure using data from 27,047 emergency department visits. However, some studies have reported associations between ozone and depression outcomes. Szyszkowicz (2007) found an increased risk for daily emergency department visits for depression and 1-day lagged ground level ozone for females during the warm season based on 15,556 patients. Similarly, Szyszkowicz et al. (2016) also reported that ground on 118,602 patients, emergency department visits for depression were associated with ozone between 1 and 7 days prior to emergency department visit among males, as well as between 1 and 5, and 8 days for females. Kioumourtzoglou et al. (2017) used data from a prospective cohort study with 41,844 women (mean age 66.6 years) in the United States investigated the association between air pollution and oneset of depression defined as doctor's diagnosis or use of antidepression medication. Hazard ratios for both outcomes were reported to be associated with ozone in summer (May to September) ozone. Lim et al. (2012) reported the Korean version of the Geriatric Depression Scale-Short Form scores were positively associated with increases in a 3-day (lag 0–2) moving average of ozone, based on a cohort with 537 participants (mean age 71 years).

There are several potential reasons why our findings might be different from the studies that reported associations of ozone with depression. While our study subjects are adolescents, other studies were mainly conducted in the elderly (Kioumourtzoglou et al., 2017; Lim et al., 2012) who was reported to have a higher prevalence of depressive disorders compared to adolescents (WHO, 2017). Moreover, we used depressive symptoms as outcomes, while other studies used emergency department visits (Szyszkowicz, 2007; Szyszkowicz et al., 2016), which is an acute outcome for patients. Additionally, almost all of the reported direct associations were detected with short-term (i.e. days) exposure (Lim et al., 2012; Szyszkowicz, 2007; Szyszkowicz et al., 2016) instead of long-term (i.e. seasons) exposure (Kioumourtzoglou et al., 2017). The present study appears to be the first study that adopted annual ozone metrics to investigate the association between ozone exposure and the development of depressive symptoms.

A further possible reason to why we failed to uncover positive associations between ozone exposure and depressive symptoms is the low ozone levels in our study. At 1 atm pressure, 25 °C, in case of the long-term exposure, our average concentration was approximately 21.6 parts per billion (ppb) in summer months. For comparison, in a previous study that observed the association of interest, the average long-term concentration of ozone during the summer months was 31.9 ppb (Kioumourtzoglou et al., 2017), which is higher than our ozone levels. Regarding the short-term exposure, apart from the studies conducted in patients which are different from other cohort studies, the reported daily maximum ozone level (metric similar to what we used) associated with depression was 48.1 ppb (Lim et al., 2012), while ours was around 36.4 ppb. On the other hand, the study that observed no associations between ozone and depressive symptoms had generally lower daily ozone level - 23.4 ppb (Wang et al., 2014). Nevertheless, we should be aware that these ozone estimates differed greatly across studies and cannot be compared directly. Additionally, even though there is no definite conclusion about exposure-response relationships between ozone exposure and health (Goodman et al., 2015), the heterogeneous

results on ozone and depressive symptoms suggest that the levels of ozone might critically affect the association. If further studies confirm this assumption, it should be considered when revising air quality guidelines for ozone.

4.3. Potential mechanisms

Although our study did not find associations between ozone exposure and depressive symptoms, it is plausible to consider ozone exposure as a potentially contributing risk factor in increasing depression prevalence (Zhao et al., 2018). Ozone exposure can either provoke the production of pro-inflammatory cytokines which may cross the blood-brain barrier (Dantzer and Kelley, 2007; Dunn and Swiergiel, 1998), or increase vascular endothelial growth factor (VEGF), interleukin-6 (IL-6), tumor necrosis factor α (TNF α) and c-Fos expression in some brain regions (Araneda et al., 2008), and thereby affect normal brain function. Ozone was also reported to have an ability to affect the secretion of hormones (Gonzalez-Pina and Paz, 1997) or the metabolism of neurotransmitters (Odermatt and Gumy, 2008; Thomson et al., 2013), resulting in a pathological process of mental disorder. Some animal studies provide evidence for this speculation. Ozone exposure might perturb normal activity/social behavior of mice (Musi et al., 1994). Rat experiments indicated that ozone inhalation elevated hippocampal superoxide accumulation and lipid peroxidation, as well as attenuated the antidepressant effects of imipramine, desipramine and escitalopram (Mokoena et al., 2010, 2015); in addition, the indicated compromised central monoamine level was similar to that noted in depression (Mokoena et al., 2015).

4.4. Strengths and limitations

There are several strengths of this study, including two different metrics of ozone data for long-term ozone exposure (annual means and number of days per year), comprehensive time frames (annual average of ozone and lag effects for 0–7 days) for long-term and short-term ozone exposure. We could include data from two different areas. Additionally, information on many potential confounders, including air co-pollutants, time spent outdoors and parental psychopathology, were available. Use of such statistical techniques, as DAG and adjustment for residuals of co-pollutants helped us to implement parsimonious and yet minimally biased models. To the best of our knowledge, this is the first study on ambient ozone exposure and depressive symptoms in adolescents.

Our study is not without limitations. Firstly, our analyses were cross-sectional, which cannot infer that the depressive symptoms were caused by ozone exposure. Secondly, we might have neglected some indirect pathways, such as feelings of annoyance from air pollution (Dzhambov et al., 2018b), through which the association of interest might be concealed or cancelled. To better uncover the possible relationships between exposure, outcome, and covariates, including moderation and mediation, more sophisticated techniques like structural equation modeling can be considered in the future studies instead of conventional regression analyses (e.g., Dzhambov et al., 2018a, 2018b). Thirdly, due to selection bias by socio-economic status (SES) that initial under-recruitment and later higher loss to follow-up of participants are from families with low SES, which is also reported by other birth cohorts studies (Bornehag et al., 2012; Jacobsen et al., 2010; MAL-ED Network Investigators, 2017), the external validity of our study is limited, and the generalizability to the general German population of this age is questionable. Fourthly, the depressive symptoms were evaluated by screening questionnaires answered by participants, instead of being clinically diagnosed by medical doctors. Even though the questionnaire-based depressive symptoms prevalence of 12.5% in our study was similar with the prevalence of 11.1% in children aged 11–17 years reported in a previous German study which used the Depression Scale for Children (CES-DC) (Ravens-Sieberer et al., 2008) and the prevalence of 11.3% in the US (Mojtabai et al., 2016), outcome misclassification could be present. Furthermore, the reported depressive symptoms may bias the associations of interest due to recall bias when answering the questionnaire (Kruijshaar et al., 2005). Fifthly, we might neglect other possible variables, like noise exposure (Seidler et al., 2017), or alcohol intake of parents (Pisinger et al., 2016), which may also affect the association. Finally, there is the most important drawback that the relatively coarse spatial resolution of the ozone raster of 2 km limited the precision of the exposure and might have obscured the effect estimates.

5. Conclusions

Our study does not support the hypothesis that long- and short-term ambient ozone exposure might increase the prevalence of depressive symptoms in adolescents. However, since no other studies investigated this association in young-aged populations, our results should be interpreted with caution. Further studies with more precise exposure assessment conducted in various populations and conditions are needed.

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Declarations of interest

None

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.envres.2018.12.014.

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Paper 1: Supplementary Material

Supplementary

Ambient ozone exposure and depressive symptoms in adolescents: Results of the GINIplus and LISA birth cohorts

Content:

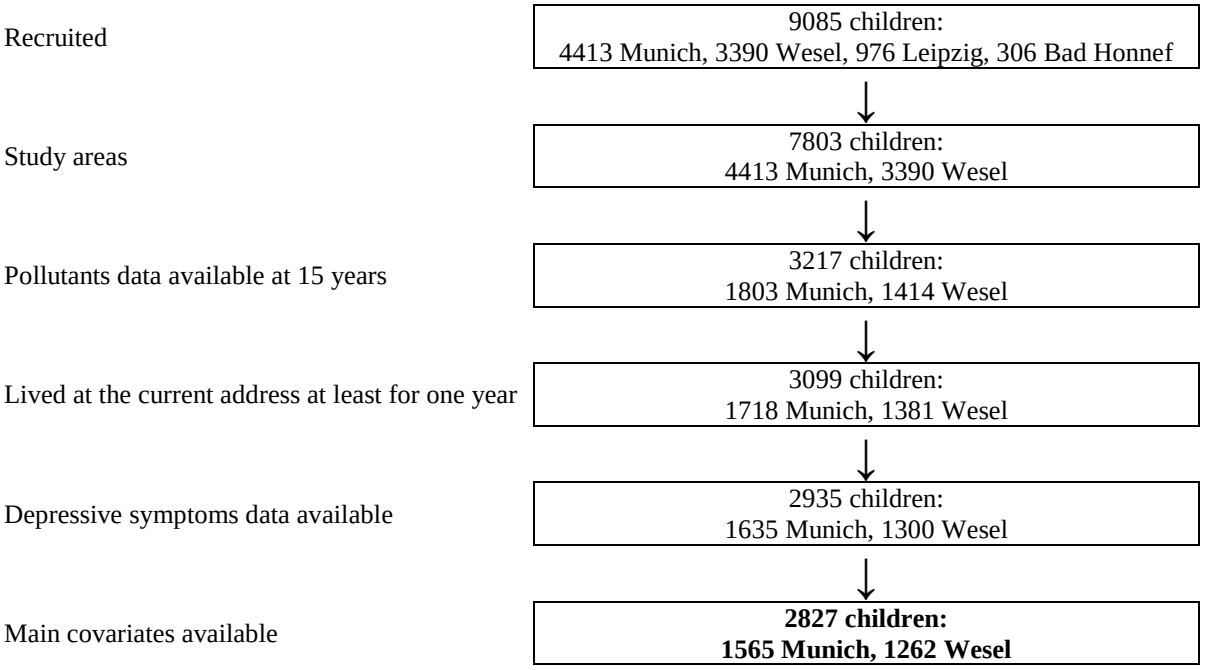

Figure S1. Flow chart for participant selection

Pollutants data available at 15 years: ozone, NO_2 and PM_{10}.
Main covariates include: parental education, parental psychopathology, time spent in front of a screen and smoking status during pregnancy.

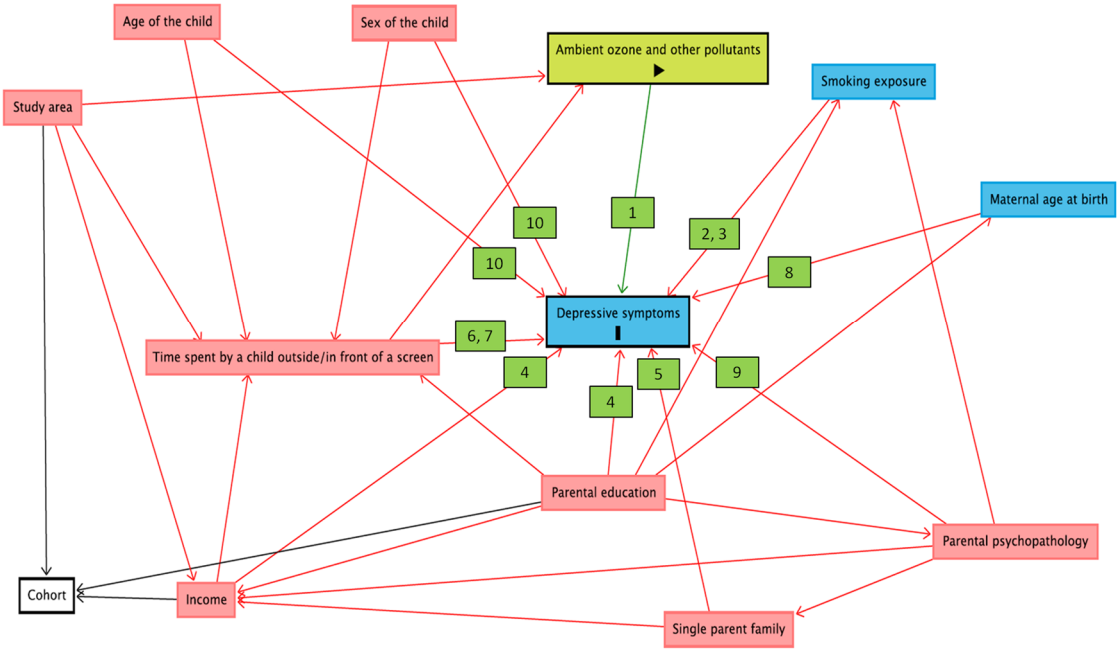

Figure S2. DAG for ozone exposure and depressive symptoms in this study

The DAG is based on published literature and expert knowledge, and consists of nodes and arrows representing variables and the causal associations between them, respectively. The backtracking algorithm enables the identification of minimally sufficient sets, which take into account all important confounders needed for obtaining unbiased estimates.

DAG identified variables: income, parental education, parental psychopathology, single parent family status, time spent outside and time spent in front of a screen, exact age at the 15-year follow-ups and sex of the child.

The node labeling "Ambient ozone and other pollutants" indicates exposure and the one with "Depressive symptom" indicates the outcome. The associations between the possible variables and outcome derived from literature are marked by number, and are listed below:

1. Lim, Y.H., et al., Air pollution and symptoms of depression in elderly adults. Environ Health Perspect, 2012. 120(7): p. 1023-8.
2. Lee, K.J., Current smoking and secondhand smoke exposure and depression among Korean adolescents: analysis of a national cross-sectional survey. BMJ Open, 2014. 4(2): p. e003734.
3. Huang, J., et al., The association between second-hand smoke exposure and depressive symptoms among pregnant women. Psychiatry Res, 2017. 256: p. 469-474.
4. Park, H.Y., et al., Socioeconomic inequalities in adolescent depression in South Korea: a multilevel analysis. PLoS One, 2012. 7(10): p. e47025.
5. Di Manno, L., J.A. Macdonald, and T. Knight, Family dissolution and offspring depression and depressive symptoms: A systematic review of moderation effects. J Affect Disord, 2015. 188: p. 68-79.
6. Lissak, G., Adverse physiological and psychological effects of screen time on children and adolescents: Literature review and case study. Environ Res, 2018. 164: p. 149-157.
7. Uglesic, B., et al., Prevalence of depressive symptoms among college students and the influence of sport activity. Coll Antropol, 2014. 38(1): p. 235-9.
8. Muraca, G.M. and K.S. Joseph, The association between maternal age and depression. J Obstet Gynaecol Can, 2014. 36(9): p. 803-810.
9. Wilkinson, P.O., et al., Associations between adolescent depression and parental mental health, before and after treatment of adolescent depression. Eur Child Adolesc Psychiatry, 2013. 22(1): p. 3-11.
10. Kazem, A.M. and A.S. Alzubaidi, Depression symptoms among Omani children: age and sex differences. Psychol Rep, 2011. 108(3): p. 805-12.

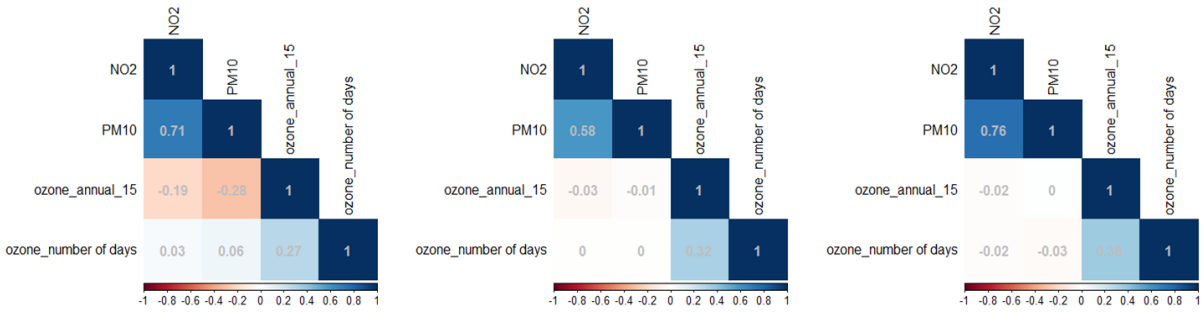

Figure S3. The heatmap of correlations for long-term pollutants

Spearman correlation coefficients for relationships between different pollutants (metrics) in Table 2 (long-term exposure).

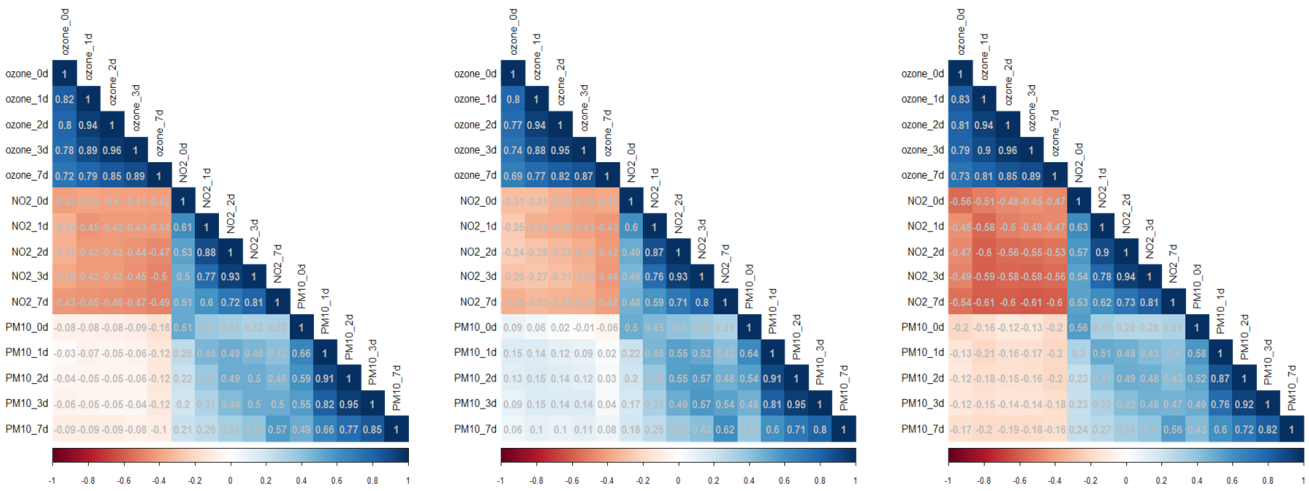

Figure S4. The heatmap of correlations for short-term pollutants

Spearman correlation coefficients for relationships between different short-term pollutants (metrics):

For ozone, we calculated concentration (µg/m³) of moving 8-h average for every hour (7 hours before and of the hour of interest) and thereby identified a maximum 8-h average for every day. The maximum of the daily maximum 8-h average concentration was selected over 0 (same day), 1, 2, 3, and 7 days prior to the depressive symptoms assessment. For NO_2 and PM_{10}, we utilized average of the daily concentrations (µg/m³) of 0 (same day), 1, 2, 3, and 7 days prior to the day that depressive symptoms evaluated for our analysis (same time frame like ozone).

Table S1. Adjusted associations for ambient ozone exposure and depressive symptoms (Models adjusted for minimal covariates)

Exposure	Area	Pollutant	DesTeen	
			15-year (OR, 95%CI)	Participants
Long-term	Munich	O_3-UBA-annual [a]	1.09 (0.93, 1.26)	1565/1565
		O_3-UBA-days [b]	1.04 (0.73, 1.50)	1565/1565
	Wesel	O_3-UBA-annual [a]	1.10 (0.92, 1.32)	1262/1262
		O_3-UBA-days [b]	0.96 (0.70, 1.32)	1262/1262
	All	O_3-UBA-annual [a]	1.07 (0.94, 1.22)	2827/2827
		O_3-UBA-days [b]	1.01 (0.81, 1.27)	2827/2827
Short-term	Munich	Lag 0 day [c]	1.01 (0.84, 1.22)	1524/1564
		Lag 0-1 days [d]	0.95 (0.80, 1.14)	1528/1565
		Lag 0-2 days [e]	0.99 (0.84, 1.189	1535/1565
		Lag 0-3 days [f]	0.99 (0.83, 1.18)	1544/1565
		Lag 0-7 days [g]	0.92 (0.77, 1.10)	1559/1565
	Wesel	Lag 0 day [c]	0.77 (0.60, 0.99) *	1200/1262
		Lag 0-1 days [d]	0.86 (0.68, 1.10)	1201/1262
		Lag 0-2 days [e]	0.88 (0.70, 1.12)	1238/1262
		Lag 0-3 days [f]	0.87 (0.69, 1.10)	1250/1262
		Lag 0-7 days [g]	0.94 (0.76, 1.16)	1262/1262
	All	Lag 0 day [c]	0.90 (0.77, 1.05)	2724/2827
		Lag 0-1 days [d]	0.91 (0.78, 1.06)	2729/2827
		Lag 0-2 days [e]	0.95 (0.82, 1.10)	2773/2827
		Lag 0-3 days [f]	0.94 (0.81, 1.09)	2794/2827
		Lag 0-7 days [g]	0.92 (0.80, 1.07)	2821/2827

Note:

Abbreviation: CI, confidence interval; DesTeen, Depression Screener for Teenagers; OR, odds ratio.

1. ORs and 95% CIs are scaled by an interquartile range increase according to specific areas or metrics (see Table 2).
2. All estimates are from logistic regression models adjusted for PM_{10} and NO_2 residuals, sex of the child, cohort and area (only for the area "all").
3. Participants, "sample number analyzed/total number analyzed"; missings are due to a lack of exposure data.

a. Annual average concentration, from the German Environment Agency (Umweltbundesamt, UBA, www.umweltbundesamt.de).

b. Number of days per year with maximum 8-h concentration exceeding 120 $\mu g/m^3$, from the UBA.

c. The maximum of the daily maximum 8-h average concentration was selected over 0 days prior to the depressive symptoms assessment, from the background monitor stations, from the UBA.

d. The maximum of the daily maximum 8-h average concentration was selected over 1 days prior to the depressive symptoms assessment, from the background monitor stations, from the UBA.

e. The maximum of the daily maximum 8-h average concentration was selected over 2 days prior to the depressive symptoms assessment, from the background monitor stations, from the UBA.

f. The maximum of the daily maximum 8-h average concentration was selected over 3 days prior to the depressive symptoms assessment, from the background monitor stations, from the UBA.

g. The maximum of the daily maximum 8-h average concentration was selected over 7 days prior to the depressive symptoms assessment, from the background monitor stations, from the UBA.

* $p = 0.038$

Table S2. Adjusted associations for ambient ozone exposure and depressive symptoms (Models adjusted for all available covariates)

Exposure	Area	Pollutant	DesTeen	
			15-year (OR, 95%CI)	Participants
Long-term	Munich	O_3-UBA-annual [a]	1.07 (0.91, 1.25)	1565/1565
		O_3-UBA-days [b]	1.04 (0.72, 1.51)	1565/1565
	Wesel	O_3-UBA-annual [a]	1.09 (0.91, 1.31)	1262/1262
		O_3-UBA-days [b]	0.96 (0.69, 1.33)	1262/1262
	All	O_3-UBA-annual [a]	1.07 (0.93, 1.22)	2827/2827
		O_3-UBA-days [b]	1.00 (0.80, 1.26)	2827/2827
Short-term	Munich	Lag 0 day [c]	1.02 (0.84, 1.23)	1524/1564
		Lag 0-1 days [d]	0.97 (0.81, 1.17)	1528/1565
		Lag 0-2 days [e]	1.01 (0.84, 1.21)	1535/1565
		Lag 0-3 days [f]	0.99 (0.81, 1.21)	1544/1565
		Lag 0-7 days [g]	0.91 (0.76, 1.09)	1559/1565
	Wesel	Lag 0 day [c]	0.74 (0.57, 0.96) *	1200/1262
		Lag 0-1 days [d]	0.86 (0.67, 1.10)	1201/1262
		Lag 0-2 days [e]	0.88 (0.69, 1.12)	1238/1262
		Lag 0-3 days [f]	0.88 (0.69, 1.12)	1250/1262
		Lag 0-7 days [g]	0.96 (0.77, 1.19)	1262/1262
	All	Lag 0 day [c]	0.90 (0.77, 1.06)	2724/2827
		Lag 0-1 days [d]	0.92 (0.79, 1.08)	2729/2827
		Lag 0-2 days [e]	0.96 (0.82, 1.11)	2773/2827
		Lag 0-3 days [f]	0.94 (0.81, 1.10)	2794/2827
		Lag 0-7 days [g]	0.92 (0.79, 1.07)	2821/2827

Note:

Abbreviation: CI, confidence interval; DesTeen, Depression Screener for Teenagers; OR, odds ratio.

1. ORs and 95% CIs are scaled by an interquartile range increase according to specific areas or metrics (see Table 2).
2. All estimates are from logistic regression models adjusted for PM_{10} and NO_2 residuals, income, parental education, parental psychopathology, single parent family status, time spent outside and time spent in front of a screen, exact age at the 15 year follow-up and sex of the child, as well as maternal age at birth, smoking status during pregnancy and at home, cohort and area (only for the area "all").
3. Participants, "sample number analyzed/total number analyzed"; missings are due to a lack of exposure data.

a. Annual average concentration, from the German Environment Agency (Umweltbundesamt, UBA, www.umweltbundesamt.de)
b. Number of days per year with maximum 8-h concentration exceeding 120 $\mu g/m^3$, from the UBA
c. The maximum of the daily maximum 8-h average concentration was selected over 0 days prior to the depressive symptoms assessment, from the background monitor stations, from the UBA.
d. The maximum of the daily maximum 8-h average concentration was selected over 1 days prior to the depressive symptoms assessment, from the background monitor stations, from the UBA.
e. The maximum of the daily maximum 8-h average concentration was selected over 2 days prior to the depressive symptoms assessment, from the background monitor stations, from the UBA.
f. The maximum of the daily maximum 8-h average concentration was selected over 3 days prior to the depressive symptoms assessment, from the background monitor stations, from the UBA.
g. The maximum of the daily maximum 8-h average concentration was selected over 7 days prior to the depressive symptoms assessment, from the background monitor stations, from the UBA.
* $p = 0.024$

Table S3. Adjusted associations for ambient ozone exposure and depressive symptoms (Models stratified by sex)

Exposure	Area	Sex	Pollutant	DesTeen	
				15-year (OR, 95%CI)	Participants
Long-term	Munich	Female	O_3-UBA-annual [a]	1.10 (0.91, 1.33)	796/796
			O_3-UBA-days [b]	1.12 (0.72, 1.76)	796/796
		Male	O_3-UBA-annual [a]	1.09 (0.83, 1.43)	769/769
			O_3-UBA-days [b]	0.92 (0.47, 1.82)	769/769
	Wesel	Female	O_3-UBA-annual [a]	1.06 (0.84, 1.34)	636/636
			O_3-UBA-days [b]	0.95 (0.62, 1.46)	636/636
		Male	O_3-UBA-annual [a]	1.19 (0.88, 1.62)	626/626
			O_3-UBA-days [b]	0.96 (0.57, 1.62)	626/626
	All	Female	O_3-UBA-annual [a]	1.13 (0.98, 1.31)	1432/1432
			O_3-UBA-days [b]	0.99 (0.76, 1.29)	1432/1432
		Male	O_3-UBA-annual [a]	1.12 (0.91, 1.37)	1395/1395
			O_3-UBA-days [b]	0.98 (0.69, 1.39)	1395/1395
Short-term	Munich	Female	Lag 0 day [c]	1.02 (0.81, 1.28)	747/796
			Lag 0-1 days [d]	0.95 (0.76, 1.17)	779/796
			Lag 0-2 days [e]	0.98 (0.79, 1.21)	784/796
			Lag 0-3 days [f]	0.96 (0.78, 1.19)	786/796
			Lag 0-7 days [g]	0.90 (0.72, 1.12)	796/796
		Male	Lag 0 day [c]	0.96 (0.71, 1.31)	747/769
			Lag 0-1 days [d]	1.01 (0.72, 1.41)	749/769
			Lag 0-2 days [e]	1.05 (0.75, 1.47)	751/769
			Lag 0-3 days [f]	0.99 (0.74, 1.42)	758/769
			Lag 0-7 days [g]	0.99 (0.67, 1.31)	763/769
	Wesel	Female	Lag 0 day [c]	0.86 (0.62, 1.18)	604/636
			Lag 0-1 days [d]	0.90 (0.65, 1.23)	608/636
			Lag 0-2 days [e]	0.90 (0.66, 1.24)	625/636
			Lag 0-3 days [f]	0.85 (0.62, 1.17)	632/636
			Lag 0-7 days [g]	0.85 (0.63, 1.14)	636/636
		Male	Lag 0 day [c]	0.61 (0.39, 0.96) *	593/626
			Lag 0-1 days [d]	0.79 (0.53, 1.17)	596/626
			Lag 0-2 days [e]	0.83 (0.55, 1.23)	613/626
			Lag 0-3 days [f]	0.90 (0.61, 1.33)	618/626
			Lag 0-7 days [g]	1.11 (0.79, 1.55)	626/626
	All	Female	Lag 0 day [c]	0.96 (0.79, 1.16)	1381/1432
			Lag 0-1 days [d]	0.93 (0.77, 1.12)	1387/1432
			Lag 0-2 days [e]	0.95 (0.99, 1.15)	1409/1432
			Lag 0-3 days [f]	0.92 (0.76, 1.11)	1418/1432
			Lag 0-7 days [g]	0.87 (0.72, 1.04)	1432/1432
		Male	Lag 0 day [c]	0.80 (0.62, 1.05)	1340/1395
			Lag 0-1 days [d]	0.91 (0.70, 1.19)	1345/1395
			Lag 0-2 days [e]	0.96 (0.74, 1.25)	1364/1395
			Lag 0-3 days [f]	0.99 (0.77, 1.30)	1376/1395
			Lag 0-7 days [g]	1.07 (0.82, 1.38)	1389/1395

Note:

Abbreviation: CI, confidence interval; DesTeen, Depression Screener for Teenagers; OR, odds ratio.

1. ORs and 95% CIs are scaled by an interquartile range increase according to specific areas or metrics (see Table 2).
2. All estimates are from logistic regression models adjusted for PM_{10} and NO_2 residuals, income, parental education, parental psychopathology, single parent family status, time spent outside and time spent in front of a screen, exact age at the 15 year follow-up, cohort and area (only for the area "all").
3. Participants, "sample number analyzed/total number analyzed"; missings are due to a lack of exposure data.

a. Annual average concentration, from the German Environment Agency (Umweltbundesamt, UBA, www.umweltbundesamt.de).

b. Number of days per year with maximum 8-h concentration exceeding 120 $\mu g/m^3$, from the UBA.

c. The maximum of the daily maximum 8-h average concentration was selected over 0 days prior to the depressive symptoms assessment, from the background monitor stations, from the UBA.

d. The maximum of the daily maximum 8-h average concentration was selected over 1 days prior to the depressive symptoms assessment, from the background monitor stations, from the UBA.

e. The maximum of the daily maximum 8-h average concentration was selected over 2 days prior to the depressive symptoms assessment, from the background monitor stations, from the UBA.

f. The maximum of the daily maximum 8-h average concentration was selected over 3 days prior to the depressive symptoms assessment, from the background monitor stations, from the UBA.

g. The maximum of the daily maximum 8-h average concentration was selected over 7 days prior to the depressive symptoms assessment, from the background monitor stations, from the UBA.

* $p = 0.033$

Chapter 8

Paper 2: Ozone and depression and anxiety diagnoses in adults

Title of article:	Depression and anxiety with exposure to ozone and particulate matter: An epidemiological claims data analysis
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Depression and anxiety with exposure to ozone and particulate matter: An epidemiological claims data analysis

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ABSTRACT

Background: Depression and anxiety have complex etiologies and are associated with a significant burden of disease. Although air pollution has been hypothesized as a possible risk factor of these disorders, the associations are still under-investigated. We aimed to analyze associations between long-term exposure to ambient ozone and particulate matter with diameter < 10 μm (PM_{10}) and diagnoses of depression and anxiety in a general population.

Methods: We utilized data from a large statutory health insurance company from Saxony, Germany. Information on outpatient clinical diagnoses of depression and anxiety was available for the years 2005–2014. We assigned ambient ozone and PM_{10} estimates to residential districts of 1.13 million individuals aged 16 and older. Depression and anxiety were defined as diagnoses counts. Associations with depression and anxiety were assessed using adjusted generalized estimating equations models.

Results: In the ten-year study period, the observed prevalences of depression and anxiety were 7.40% and 3.82%, respectively. In the two-pollutant model, 10 more days with a maximum 8-h average ozone concentration exceeding 120 μg/m^3 resulted in a relative risk (RR) of 1.010 with 95% confidence interval (CI) (1.005, 1.014) for depression and an RR of 1.007 (95% CI (1.000, 1.014)) for anxiety. The effect estimates of PM_{10} for depression and anxiety were 1.180 (95% CI (1.160, 1.201)) and 1.176 (95% CI (1.148, 1.205)) per 10 μg/m^3 increase in PM_{10} concentration, respectively. Age, sex, and access to healthcare of the individual were also associated with the diagnosis of the disorders. The associations were consistent across one- and two-pollutant models.

Conclusions: Our findings indicate that increased levels of ambient ozone and PM_{10} may elevate the risk of a depression or anxiety diagnosis in the general population. However, given the lack of data on individual air pollutant exposure and socioeconomic status, our results should be interpreted with caution. Further well-designed epidemiological studies should replicate our findings.

1. Introduction

Depressive disorder, hereafter referred to as depression, is a common illness. On a global scale, the aggregated estimated lifetime prevalence of depression is 10.8% (Lim et al., 2018). In 2017, 43.0 million years lived with disability (YLDs) were due to major depressive disorder and dysthymia leaving depression the third leading cause of burden of disease worldwide (GBD 2017 Disease and Injury Incidence and Prevalence Collaborators, 2018). Anxiety, short for anxiety disorders, has a global estimated lifetime prevalence of 12.9% (Steel et al.,

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Abbreviations

CI	confidence interval
ICD	international statistical classification of diseases and related health problems
IQR	interquartile range
$PM_{2.5}$	particulate matter with an aerodynamic diameter <2.5 μm
PM_{10}	particulate matter with an aerodynamic diameter <10 μm
ppb	parts per billion
RR	relative risk
SES	socioeconomic status
UBA	Umweltbundesamt (German Environment Agency)
WHO	World Health Organization
YLD	years lived with disability

2014) and accounted for 27.1 million YLDs in 2017 (GBD 2017 Disease and Injury Incidence and Prevalence Collaborators, 2018). This made it the second largest contributor to the mental disorder-related burden of disease.

While the burden caused by depression and anxiety when occurring in isolation is already significant, these two disorders are frequently comorbid in addition (Gorman, 1996; Kessler et al., 2008; Tiller, 2013). Indeed, it has been estimated that two thirds of depression patients also have an anxiety disorder, and more than one third of patients with panic disorder or generalized anxiety disorder also suffer from depression (Gorman, 1996). A study also indicated that 72% of lifetime anxiety cases had a history of depression, while 48% of lifetime depression cases had anxiety (Moffitt et al., 2007). This comorbidity pattern of depression and anxiety is often associated with more severe symptoms and unfavorable prognosis (Klein Hofmeijer-Sevink et al., 2012). Such a pattern might be a reflection of some common pathophysiological mechanisms (Chen et al., 2017; Cui et al., 2017; Eleonora et al., 2019; Fiksdal et al., 2019; He et al., 2019).

Nevertheless, the etiology of depression and anxiety remains unclear. For instance, familial aggregation of anxiety is substantial (Lawrence et al., 2019); however, heritability accounts only for 30%–50% of observed cases (Shimada-Sugimoto et al., 2015) which indicates that other factors must play a role. Besides genetic and biological influences (Kennis et al., 2019; Purves et al., 2019; Wohleb et al., 2016; Wray et al., 2018), relevant determinants include sociodemographic correlates and socioeconomic circumstances (Gur et al., 2019; Kessler and Bromet, 2013; Rojas-Garcia et al., 2015) as well as physical environmental factors (Pun et al., 2018; van den Bosch and Meyer-Lindenberg, 2019). In particular, exposure to ambient air pollutants is hypothesized to be associated with depression and anxiety (Power et al., 2015; Pun et al., 2017; Zhao et al., 2018).

Although the adverse health effects of particulate matter (PM), as a typical ambient pollutant, have been extensively investigated, associations with depression were surprisingly inconsistent across published studies. The statistically significant associations observed in two systematic reviews (Gu et al., 2019; Zeng et al., 2019) were not replicated by the latest systematic review (Fan et al., 2020), in which the sophisticated inverse variance heterogeneity model (Doi et al., 2015, 2017) was adopted for the meta-analysis. Thus, it is still not clear whether PM increases the risk of depression. Also, considering PM and anxiety, the small number of studies (Brokamp et al., 2019; Jorcano et al., 2019; Power et al., 2015; Pun et al., 2017; Roberts et al., 2019; Sheffield et al., 2018; Vert et al., 2017; Yue et al., 2020) warrants further research.

For ambient ozone, an air pollutant and potent oxidant, the situation is worse: only a handful of published studies investigated the association between ozone and depression or depressive symptoms (Cho et al., 2014; Lim et al., 2012; Szyszkowicz, 2007; Szyszkowicz et al., 2009, 2016; Wang et al., 2014; Zhao et al., 2019b). The existing research is heterogeneous in terms of study designs, study participants, exposure assessment, outcome definitions, and drawn conclusions. Two recent systematic reviews synthesized the relationship between ozone and depression (Fan et al., 2020; Zeng et al., 2019). Meta-analyses of short-term ozone exposure failed to uncover a significant association with depression, and a meta-analysis of long-term exposure scenarios was not possible (Fan et al., 2020; Zeng et al., 2019) because there were only two studies (Kioumourtzoglou et al., 2017; Zhao et al., 2019b). The association between ozone and depression should be further explored in well-designed studies with large populations, improved exposure assessment methods, and standard case definitions. To our knowledge, there have been no studies on ozone and anxiety.

The present study is based on a large proportion of the general population above 16 years of age, residing in Saxony, Germany. We explored the association between long-term exposure to ambient ozone and depression and anxiety and also tested the association between PM and these two disorders.

2. Materials and methods

2.1. Study settings and case definition

We undertook a semi-individual study (Kunzli and Tager, 1997) utilizing pseudonymized claims data for the years from 2005 to 2014 from a large German statutory health insurance company (AOK PLUS) that covers about 50% of the population of the federal state of Saxony, Germany (AOK PLUS). Saxony is in eastern Germany, with an area of 18,415 km^2 and a population of approximately four million. The AOK data include information from outpatient care about diagnoses, medical procedures, and prescriptions, as well as age, sex, and residential district of the beneficiaries (Datzmann et al., 2018). Here, a residential district is defined as the combined region of all five-digit postal code regions with the same four leading digits. There are 186 residential districts with areas ranging from 4.3 to 408.3 km^2 covering all of Saxony (Markevych et al., 2018). The information on outpatients has quarterly resolution, where quarters go from January to March, April to June, July to September and October to December. To ensure that study subjects were at least 16 years old at the study baseline, only individuals that were born before 1990 were included in the study. Those who died, changed insurance company, or moved outside their residential district within the 10-year observation period were excluded.

The study is in accordance with Good Practice in Secondary Data Analysis (Swart et al., 2015). Consent on data transmission and analysis was obtained from the Saxon State Ministry for Social Affairs and Consumer Protection. More details on the AOK PLUS data (Datzmann et al., 2018; Markevych et al., 2018) and German claims databases (Andersohn and Walker, 2016; Pigeot and Ahrens, 2008) in general are available elsewhere.

Diagnoses of depression and anxiety were done according to ICD-10-GM, the German modification of the 10th version of the international statistical classification of diseases and related health problems (World Health Organization, 2007, 2016). Any outpatient who received an F32 or F33 diagnosis from a physician was defined as a depression case in the quarter of the year. Similarly, any outpatient who received an F40 or F41 diagnosis was defined as having anxiety in the given quarter. We excluded individuals who received the same diagnosis of depression or anxiety for every quarter over the entire ten-year period.

2.2. Ambient ozone and PM_{10} exposure

Data on ozone and PM with an aerodynamic diameter <10 μm

(PM_{10}) were provided by the German Environment Agency (Umweltbundesamt, UBA for short). The data were specifically modeled for Germany to a resolution of 2 km^2 by Optimal Interpolation using air pollutant and meteorological measurements from 150 German monitoring stations (Flemming et al., 2004; Stern and Flemming, 2004).

Since ozone concentrations are highly variable, we used a more robust metric – number of days with a maximum 8-h average concentration exceeding 120 μg/m^3. UBA introduces this metric to define a threshold beyond which ozone concentrations are deemed harmful for human health (Umweltbundesamt, 2013). We have employed the same metric in our previous study (Zhao et al., 2019b). The original ozone data had a time resolution of 1 h. In a first step, we computed the 8-h moving average for every hour, i.e., the average of the hour of interest and the preceding 7 h, taken from the previous calendar day if necessary. In a second step, we identified the maximum 8-h average concentration for every calendar day. Finally, we counted, for every quarter, the number of days with a maximum 8-h average concentration exceeding 120 μg/m^3. Since the seasonal fluctuations of PM concentrations are much less pronounced than those of ozone, we used annual average PM_{10} concentrations in μg/m^3 as exposure metric.

The calculation of the quarter ozone metrics from hourly concentrations over the 10-year period was performed in Python 3.4. Mean district-wide values were calculated for both ozone and PM_{10}. The assignment of ozone and PM_{10} estimates to residential districts, i.e., the calculation of district mean values, was performed in ArcGIS Geographical Information System (ArcMap 10.4, ESRI, Redlands, CA).

2.3. Statistical analysis

Since ozone levels are much higher in the warm season than in the cold season, we discarded the cold season and summed the values of the ozone metric over quarters 2 and 3, from April to September, to obtain a single value for every year. This avoids confounding by seasonal effect.

In the same manner, for every outpatient, depression and anxiety diagnosis counts were summed over the same two quarters resulting in a warm season diagnosis count of 0, 1, or 2. Hence, we calculated person-warm-seasons of diagnosis of depression and anxiety for each year from 2005 to 2014.

Considering the longitudinal data structure of this study, we utilized generalized estimating equations (GEE) models (Zeger and Liang, 1986) to analyze the associations between long-term exposure to pollutants and diagnoses counts of depression or anxiety. In the GEE models, we used a Poisson probability distribution with a logarithmic link function, an exchangeable correlation structure, and robust standard errors to compute the confidence intervals (CIs).

We built one-pollutant models incorporating the number of days with ozone levels exceeding 120 μg/m^3 and the concentration of PM_{10}, respectively. Additionally, since the two metrics were not highly correlated (Spearman correlation coefficient 0.279), we included them both to build two-pollutant models. As a sensitivity analysis, we investigated the associations between exposure and diagnosis of depression without anxiety and anxiety without depression by excluding individuals who were diagnosed with both depression and anxiety at the same time.

Due to the protection of personal information in the AOK PLUS data, the number of relevant covariates was limited. We used year of birth, sex, year of observation, and an estimate of individual access to healthcare that was based on a simplification of a standard method (Luo and Wang, 2003). Since we only had information on residential district instead of exact addresses, we assumed that all addresses within a residential district were located at the centroid of the district. Access to healthcare was defined as the ratio of the number of general practitioners over the number of people in a 10-km circular buffer divided by the Saxony-wide general practitioner-over-people ratio, all based on information from 2011(Census year). A more detailed description of our method was published elsewhere (Markevych et al., 2018).

Table 1
Characteristics of the study population.

Variable	Category	Population	Percentage (%)
Year of birth	Before 1930	83,098	7.38
	1930–39	198,538	17.63
	1940–49	182,843	16.24
	1950–59	224,889	19.97
	1960–69	209,741	18.63
	1970–79	121,115	10.76
	1980–89	105,790	9.40
Sex	Female	614,870	54.61
	Male	511,144	45.39
Access to healthcare*	Mean ± SD	1.003 ± 0.110	–
Total		1,126,014	100

Note.
Abbreviation: SD, standard deviation.
*Access to healthcare was defined as the ratio of the number of general practitioners over the number of people in a 10-km circular buffer divided by the Saxony-wide general practitioner-over-people ratio, all based on information from 2011.

Our analysis results are presented as relative risks (RRs) per 10-day increase in the ozone metric or 10-μg/m^3 increase in PM_{10} concentration. All analyses that included claims data were undertaken by the center for evidence-based healthcare, TU Dresden (Technical University Dresden). Data management was done in Microsoft SQL Server 2007. Statistical analyses were performed with Stata (StataCrop. 2015. Stata Statistical Software: Release 15.1 College Station, TX: StataCorp LP). Figures were created using the *ggplot2* package (Wickham, 2016) in R 3.5.2 (R Core Team, 2018).

3. Results

3.1. Characteristics of participants and pollutants

Our analytic sample included 1,126,014 individuals. The characterization of the sample is given in Table 1. The study population included 10% more females than males and more older individuals with 61.2% being older than 46 years in 2005. In total, there were 11,260,140 person-warm-seasons (Table 2), 7.4% with a diagnosis of depression and 3.8% with a diagnosis of anxiety.

The characteristics of air pollutants are presented in Table 3. The 10-year average number of days with a maximum 8-h average ozone concentration exceeding 120 μg/m^3 in the warm season (quarters 2 and 3, April to September) was 16 days in Saxony. The average concentration of ozone during the same quarters was 61.1 μg/m^3. The 10-year mean of annual average PM_{10} concentrations was 19.9 μg/m^3.

Table 2
10-year total person-warm-seasons of diagnoses of depression and anxiety.

Diagnosis	Count *	Person-warm-seasons	Percentage (%)
Depression	0	1,0426,832	92.60
	1	323,132	2.87
	2	510,176	4.53
Anxiety	0	10,829,823	96.18
	1	213,599	1.90
	2	216,718	1.92
Total		11,260,140	100

Note.
* Any outpatient who received the diagnosis of depression or anxiety was defined as a case in the quarter of the year. Depression and anxiety diagnosis counts were summed over the two quarters of the warm season (April to September) resulting in a diagnosis count of 0, 1, or 2, for each patient.

Table 3
Descriptions of ozone and PM_{10} over the 10-year study period.

Air pollutant	Mean	SD	Min	Max	Median	IQR
Ozone-number of days [a]	16.024	6.987	0	40.250	15.667	9.333
PM_{10}-concentration [b]	19.999	2.718	12.533	30.450	19.800	3.480

Note.
Abbreviation: IQR, interquartile range; SD, standard deviation.
[a] 10-year average of number of days with maximum daily 8-h concentration exceeding 120 μg/m^3 in the warm season (quarters 2 and 3, April to September).
[b] 10-year average of annual average concentration (μg/m^3).

3.2. Associations of ozone and PM_{10} with depression and anxiety

Table 4 shows results from GEE models presented as RRs with 95% CIs. We observed an association between ozone and depression that was consistent across one-pollutant and two-pollutant models. In the two-pollutant model, 10 more days with a maximum 8-h average ozone concentration exceeding 120 μg/m^3 increased the RR of diagnosis of depression by 1% (RR = 1.010, 95% CI (1.005, 1.014)). A similarly consistent association was found between ozone and anxiety with a RR of 1.007 in the two-pollutant model (95% CI (1.000, 1.014)).

The associations between PM_{10} and depression and anxiety were in the same direction as with ozone (Table 4). The effect estimates from two-pollutant models for depression and anxiety were 1.180 (95% CI (1.160, 1.201)) and 1.176 (95% CI (1.148, 1.205)) per 10-μg/m^3 increase in PM_{10} concentration, respectively.

The results of sensitivity analyses are illustrated in Fig. 1 and Table S1. The above associations with both ozone and PM_{10} persisted when we refined the outcomes and used depression without anxiety and anxiety without depression.

3.3. Associations of covariates with depression and anxiety

We found a clear trend that all individuals born after 1930 had fewer diagnoses of depression compared to the reference group of individuals born before 1930, with the youngest generation having the fewest diagnoses (Table 4). There were more diagnoses of depression in the more recent years of observation. Males were 54% less likely to be diagnosed with depression. We saw no association with access to healthcare.

Interestingly, the abovementioned associations were slightly different for anxiety. As with depression, younger generations had fewer diagnoses of anxiety. However, this trend was reversed for individuals born between 1930 and 1959 who had a higher risk of anxiety diagnoses than the reference category of individuals born before 1930. Associations of year of observation and sex with anxiety were in line with the ones found in depression. Unlike for depression, we found that individuals with better access to healthcare were more likely to get diagnosed with anxiety.

4. Discussion

4.1. Main study findings

The results of our analyses based on 1.13 million individuals from the general population support the notion that long-term elevated ozone and PM_{10} levels increase the risk of depression and anxiety independently from each other. The findings were robust across different models. However, they should be interpreted with caution because we used semi-individual data and because we lacked information on other potential confounders, e.g., socioeconomic status (SES).

4.2. Interpretation and comparison with other studies

Our observed associations between ozone and depression mirror some previous results on long-term (Kioumourtzoglou et al., 2017) and short-term ozone exposure (Cho et al., 2014; Lim et al., 2012; Szyszkowicz, 2007; Szyszkowicz et al., 2016). For instance, Kioumourtzoglou et al. (2017) found that increased ozone concentrations from May to September were positively associated with depression onset in the United States. The study was based on 41,844 women with an average age of 67 years. Depression was defined as use of antidepressant medication or report of doctor's diagnosis.

Other studies, mainly on short-term ozone exposures, did not find any associations with depression. Szyszkowicz et al. (2009) investigated associations between emergency department visits for depression in relation to air pollution in Canada and found no relationship for short-term ozone exposure using data on 27,047 emergency department visits. Wang et al. (2014) analyzed data from an American cohort of 732 adults with a mean age of 78.1 years. They reported no significant associations between short-term exposure to ozone and depressive symptoms measured by questionnaires. Based on data from 2827 German adolescents aged 15 years, Zhao et al. (2019b) found no associations between short- or long-term exposure to ozone and questionnaire-based depressive symptoms.

Besides different study settings, different study populations, and different outcome definitions, different levels of ozone concentration across studies might explain the previous mixed results (Zhao et al., 2019b). For comparison, we consider that a volumetric ozone concentration of 1 part per billion (ppb) is equivalent to a gravimetric concentration of 2 μg/m^3. In our study, the average ozone concentration was 30.5 ppb. Kioumourtzoglou et al. (2017) reported that their average ozone concentration was 31.9 ppb. In contrast, the study that observed no associations had an average ozone concentration of 21.6 ppb (Zhao et al., 2019b). We should be aware that various ozone metrics were used in different studies and a direct comparison might be inappropriate, especially when taking into account the possible non-linear, threshold-like, or hormesis-like relationships between ozone exposure and health effects (Zhao et al., 2019a).

To the best of our knowledge, there are no studies on exposure to ambient ozone and anxiety. Therefore, we cannot compare our findings with others. Nevertheless, two controlled exposure studies (Fiedler et al., 2005, 2008) partially and indirectly investigated the association of our interest. They explored the effect between different exposures to stress, mixtures of indoor air volatile organic compounds and their ozone oxidation products on anxiety symptoms of participants. The studies found that low negative affect subjects reported more severe anxiety when exposed to volatile organic compounds in combination with ozone (Fiedler et al., 2008).

There are several published studies on PM and depression, yet no clear conclusion can be drawn from them — even different systematic reviews generated inconsistent results (Fan et al., 2020; Gu et al., 2019; Zeng et al., 2019). Due to its large sample size and standardized diagnosis, the present study adds to the available evidence, and a further meta-analysis involving this study might change the current inconsistent results.

Few studies investigated the association between exposure to PM and anxiety. Five studies were in line with us and found that higher levels of PM increase the risk of anxiety (Power et al., 2015; Pun et al., 2017; Roberts et al., 2019; Vert et al., 2017; Yue et al., 2020) while three other studies did not find any association (Brokamp et al., 2019; Jorcano et al., 2019; Sheffield et al., 2018). The current association between PM and anxiety is unresolved, although a systematic review (Braithwaite et al., 2019) included two studies (Power et al., 2015; Pun et al., 2017) concluded the positive associations between $PM_{2.5}$ and anxiety symptoms clinically relevant.

Despite the small effect estimates, air pollution leads to a high burden of disease due to its ubiquitous nature, which makes it a serious

Table 4
Adjusted associations of ozone and PM_{10} exposures with depression and anxiety.

Diagnosis	One-pollutant model			One-pollutant model		P-value	Two-pollutant model	RR (95% CI)	P-value
		RR (95% CI)	P-value		RR (95% CI)				
Depression	Ozone-number of days	1.010 (1.005, 1.014)	0.000	–	–	–	Ozone-number of days	1.010 (1.005, 1.014)	0.000
	–	–	–	PM_{10}-concentration	1.180 (1.160–1.200)	0.000	PM_{10}-concentration	1.180 (1.160, 1.201)	0.000
	Year of birth			Year of birth			Year of birth		
	Before 1930 (ref)	1	–	Before 1930 (ref)	1	–	Before 1930 (ref)	1	–
	1930–39	0.952 (0.932, 0.973)	0.000	1930–39	0.954 (0.933–0.974)	0.000	1930–39	0.954 (0.933, 0.974)	0.000
	1940–49	0.826 (0.808, 0.845)	0.000	1940–49	0.828 (0.809–0.847)	0.000	1940–49	0.828 (0.810, 0.847)	0.000
	1950–59	0.801 (0.784, 0.818)	0.000	1950–59	0.804 (0.787–0.822)	0.000	1950–59	0.804 (0.787, 0.822)	0.000
	1960–69	0.605 (0.591, 0.619)	0.000	1960–69	0.607 (0.593–0.621)	0.000	1960–69	0.607 (0.593, 0.621)	0.000
	1970–79	0.442 (0.430, 0.454)	0.000	1970–79	0.443 (0.431–0.456)	0.000	1970–79	0.443 (0.431, 0.456)	0.000
	1980–89	0.309 (0.300, 0.319)	0.000	1980–89	0.310 (0.300–0.320)	0.000	1980–89	0.310 (0.301, 0.320)	0.000
	Year of observation			Year of observation			Year of observation		
	2005 (ref)	1	–	2005 (ref)	1	–	2005 (ref)	1	–
	2006	1.069 (1.061, 1.077)	0.000	2006	1.084 (1.077–1.092)	0.000	2006	1.077 (1.069, 1.085)	0.000
	2007	1.162 (1.154, 1.171)	0.000	2007	1.236 (1.223–1.248)	0.000	2007	1.234 (1.222, 1.246)	0.000
	2008	1.275 (1.265, 1.285)	0.000	2008	1.373 (1.357–1.388)	0.000	2008	1.377 (1.361, 1.392)	0.000
	2009	1.450 (1.436, 1.465)	0.000	2009	1.495 (1.481–1.509)	0.000	2009	1.515 (1.498, 1.531)	0.000
	2010	1.562 (1.550, 1.575)	0.000	2010	1.619 (1.605–1.634)	0.000	2010	1.622 (1.607, 1.636)	0.000
	2011	1.642 (1.628, 1.657)	0.000	2011	1.728 (1.710–1.746)	0.000	2011	1.736 (1.718, 1.755)	0.000
	2012	1.775 (1.759, 1.793)	0.000	2012	1.916 (1.892–1.940)	0.000	2012	1.933 (1.908, 1.958)	0.000
	2013	1.886 (1.870, 1.903)	0.000	2013	2.024 (2.000–2.238)	0.000	2013	2.032 (2.009, 2.057)	0.000
	2014	2.070 (2.051, 2.089)	0.000	2014	2.212 (2.187–2.238)	0.000	2014	2.224 (2.198, 2.251)	0.000
	Sex			Sex			Sex		
	Female (ref)	1	–	Female (ref)	1	–	Female (ref)	1	–
	Male	0.456 (0.300, 0.462)	0.000	Male	0.456 (0.445–0.462)	0.000	Male	0.456 (0.450, 0.462)	0.000
	Access to healthcare	0.972 (0.923, 1.023)	0.278	Access to healthcare	0.971 (0.921–1.023)	0.265	Access to healthcare	0.971 (0.921, 1.022)	0.262
Anxiety	Ozone-number of days	1.008 (1.001, 1.015)	0.023	–	–	–	Ozone-number of days	1.007 (1.000, 1.014)	0.037
	–	–	–	PM_{10}-concentration	1.177 (1.148–1.206)	0.000	PM_{10}-concentration	1.176 (1.148, 1.205)	0.000
	Year of birth			Year of birth			Year of birth		
	Before 1930 (ref)	1	–	Before 1930 (ref)	1	–	Before 1930 (ref)	1	–
	1930–39	1.150 (1.112, 1.190)	0.000	1930–39	1.153 (1.114–1.193)	0.000	1930–39	1.153 (1.114, 1.193)	0.000
	1940–49	1.123 (1.084, 1.162)	0.000	1940–49	1.126 (1.088–1.166)	0.000	1940–49	1.126 (1.088, 1.170)	0.000
	1950–59	1.086 (1.050, 1.123)	0.000	1950–59	1.092 (1.055–1.129)	0.000	1950–59	1.092 (1.055, 1.129)	0.000
	1960–69	0.956 (0.924, 0.989)	0.000	1960–69	0.959 (0.927–0.993)	0.018	1960–69	0.960 (0.927, 0.993)	0.000
	1970–79	0.859 (0.827, 0.892)	0.000	1970–79	0.861 (0.829–0.895)	0.000	1970–79	0.861 (0.829, 0.895)	0.000
	1980–89	0.770 (0.741, 0.800)	0.000	1980–89	0.772 (0.743–0.802)	0.000	1980–89	0.772 (0.743, 0.803)	0.000
	Year of observation			Year of observation			Year of observation		
	2005 (ref)	1	–	2005 (ref)	1	–	2005 (ref)	1	–
	2006	1.027 (1.015, 1.038)	0.000	2006	1.040 (1.029–1.051)	0.000	2006	1.035 (1.023, 1.047)	0.000
	2007	1.087 (1.074, 1.099)	0.000	2007	1.153 (1.137–1.170)	0.000	2007	1.152 (1.135, 1.169)	0.000
	2008	1.182 (1.168, 1.197)	0.000	2008	1.271 (1.250–1.292)	0.000	2008	1.274 (1.253, 1.295)	0.000
	2009	1.361 (1.340, 1.381)	0.000	2009	1.405 (1.385–1.424)	0.000	2009	1.418 (1.395, 1.441)	0.000
	2010	1.506 (1.488, 1.525)	0.000	2010	1.560 (1.540–1.581)	0.000	2010	1.561 (1.540, 1.582)	0.000
	2011	1.589 (1.568, 1.610)	0.000	2011	1.670 (1.645–1.696)	0.000	2011	1.676 (1.651, 1.703)	0.000
	2012	1.753 (1.729, 1.778)	0.000	2012	1.891 (1.857–1.925)	0.000	2012	1.903 (1.868, 1.939)	0.000
	2013	1.862 (1.838, 1.886)	0.000	2013	1.995 (1.962–2.029)	0.000	2013	2.001 (1.968, 2.036)	0.000

(*continued on next page*)

Table 4 (*continued*)

Diagnosis	One-pollutant model				Two-pollutant model			
		RR (95% CI)	P-value		RR (95% CI)	P-value	RR (95% CI)	P-value
	2014	2.042 (2.016, 2.070)	0.000	2014	2.180 (2.145–2.217)	0.000	2.189 (2.152, 2.227)	0.000
	Sex			Sex				
	Female (ref)	1	–	Female (ref)	1	–	1	–
	Male	0.417 (0.410, 0.425)	0.000	Male	0.418	0.000	0.418 (0.410, 0.425)	0.000
	Access to healthcare	1.186 (1.107, 1.271)	0.000	Access to healthcare	1.194 (1.113–1.281)	0.000	1.193 (1.112, 1.280)	0.000

Note.
Abbreviation: CI, confidence interval; RR, relative risk; ref, reference category.
1. RRs and 95% CIs are scaled by 10-unit increase in specific metrics (days or μg/m^3).
2. Ozone-number of days: Number of days with maximum daily 8-h concentration exceeding 120 μg/m^3 in the warm season (April to September).
3. PM_{10}-concentration: Annual average concentration (μg/m^3).

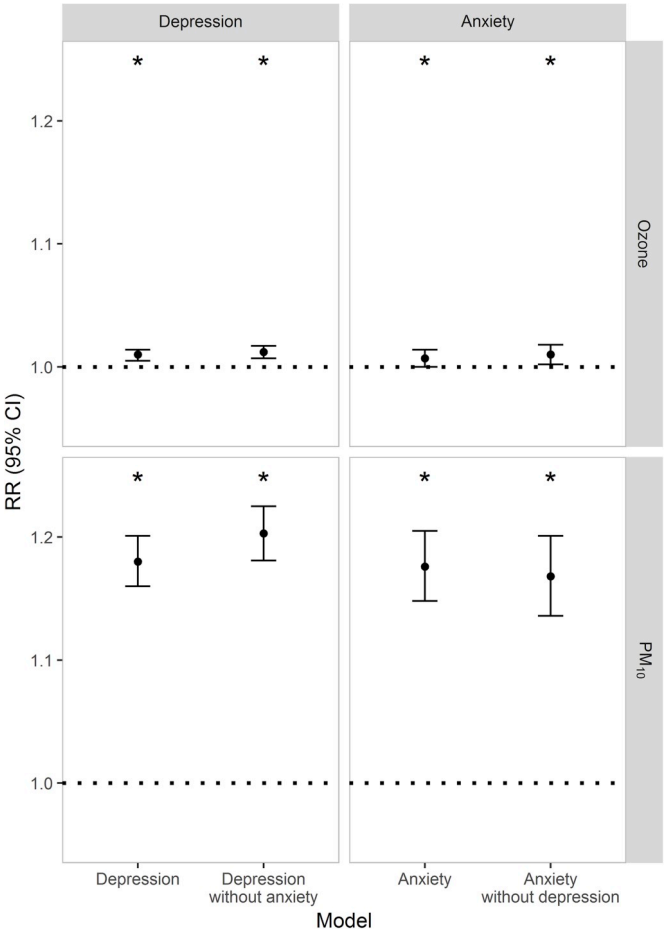

Fig. 1. Adjusted associations per 10-day increase in the number of days with maximum daily 8-h concentration exceeding 120 μg/m^3 during the warm season (April to September) or per 10-μg/m^3 increase in annual PM_{10} concentration; asterisks indicate P-values < 0.05.

health concern all over the world. Our results indicate that increased levels of air pollutants may increase the risks of depression and anxiety in the general population exacerbating the already massive air pollution-related burden of disease (GBD 2017 Risk Factor Collaborators, 2018).

Additionally, we observed other associations with depression and anxiety. The effect of year of birth and year of observation on depression are in line with the "from early childhood to late in life" prevalence of depression (Ferrari et al., 2013). The lifetime prevalence of anxiety has a different pattern. Some phobias, especially social phobia and separation anxiety disorder, have an early age-of-onset pattern. Generalized anxiety disorder and some severe disorders like panic disorder have later ages of onset (Cia et al., 2018; Kessler et al., 2007). The distinct age-of-onset distributions of the diverse anxiety disorders may explain the effects of year of birth, i.e., age. The higher risk of anxiety diagnoses in those born between 1930 and 1959 compared to the individuals born before 1930 might be because the former were more likely to experience the late age-of-onset anxiety disorders than any other generations. The younger generations were off-peak of the early age-of-onset disorders. The growing awareness of mental disorders over the years (Kessler et al., 2005) explains why the number of diagnoses increases with year of observation. This study also confirmed that women have a higher risk of depression and anxiety (Craske et al., 2017; Kuehner, 2017; McLean et al., 2011). Access to healthcare was associated with anxiety but not with depression. This could be due to the fact that, compared to depression, anxiety disorders are more often under-diagnosed, misdiagnosed, and inappropriately treated (Kasper,

2006; Kroenke et al., 2007). It often requires a doctor specialized in mental health to diagnose an anxiety disorder. Individuals with access to more doctors are more likely to see a mental health specialist and thus the awareness, diagnosis, and treatment rate of anxiety can be higher in districts with more doctors.

We observed a higher prevalence of depression than of anxiety. The observed prevalence of anxiety in our study might be underestimated (Wittchen et al., 2011) due to the fact that many cases remain undetected by healthcare systems (Kasper, 2006; Kroenke et al., 2007). Since both depression and anxiety are socially stigmatized, claims data are likely to underestimate the actual prevalence (Kane et al., 2019; Mackenzie et al., 2014). Therefore, our results should be cautiously interpreted.

4.3. Potential mechanisms

The suggested mechanisms linking ozone to mental disorders include the occurrence of oxidative stress or inflammation (Araneda et al., 2008; Chounlamountry et al., 2015), the dysregulation of the endocrine system or metabolic processes (Miller et al., 2016; Thomson, 2019; Thomson et al., 2018), and the disturbance of neurotransmitters (Gonzalez-Pina and Paz, 1997). Rat experiments showed that ozone inhalation could induce depression-like effects and attenuate the antidepressant effects of antidepressant medications (Mokoena et al., 2015).

We assume that the abovementioned mechanisms might also play a role in the association between ozone and anxiety because the etiologies of depression and anxiety are related in terms of genetic predisposition (Demirkan et al., 2011), neuroinflammation (Gallagher et al., 2019), and endocrine function (Asselmann et al., 2019). In the same animal study, rats showed anxiety-like effects after chronically inhaling ozone (Mokoena et al., 2015).

The mechanisms linking PM to depression are mostly related to inflammation and hormonal changes (Fan et al., 2020; Gu et al., 2019; Thomson, 2019; Zeng et al., 2019). They may also contribute to the pathophysiology of anxiety. Mice models have demonstrated that exposure of dim light at night and $PM_{2.5}$ can upregulate neuroinflammatory cytokines, alter the hippocampal structure, and induce depressive-like responses (Hogan et al., 2015). Exposure to $PM_{2.5}$ can cause cell apoptosis perturbing the development of the cerebral cortex and provoking anxious and depressive behavior in mice offspring (Zhang et al., 2018).

4.4. Strengths and limitations

Our study should be understood in the context of its limitations. Since we did not have house addresses, estimates of air pollution concentrations were assigned to residential districts and do not reflect individual exposure. Also, our claims data might not provide accurate prevalences or incidences of depression and anxiety due to underdiagnosis (Allan et al., 2014; Kroenke et al., 2007) and stigmatization (Kane et al., 2019; Mackenzie et al., 2014) of both diseases, and characteristics of the data source (Frank, 2016; Grobe et al., 2019). Given detailed personal data were unavailable due to data protection, we could adjust our analyses only for relatively few covariates. Residual confounding, especially by SES, cannot be ruled out. Another AOK database based study faced the same challenge (Gomm et al., 2016). Furthermore, our study population was restricted to individuals who were alive and never changed their place of residence throughout the study period. This limits the generalizability of our results.

Our study has several strengths. First, we had a large number of subjects and multiple observations per subject. This gave us enough statistical power to detect even small effect sizes. Second, our results are less affected by information and selection bias since the claims data covers half the local population and medical data were collected in an indirect and automated fashion. We furthermore standardized our outcome definitions by using doctor diagnoses instead of questionnaire-based symptoms. Assessment exclusively based on questionnaires would likely have exaggerated disease prevalences (Levis et al., 2019). Finally, two-pollutant models enabled us to conclude that both ozone and PM_{10} exposure can increase the risk of depression and anxiety independently from each other.

5. Conclusions

Our findings indicate that increased levels of ambient ozone and PM_{10} may elevate the risk of a depression or anxiety diagnosis in the general population. However, given the lack of data on individual air pollutant exposure and SES, our results should be interpreted with caution. Further well-designed epidemiological studies on the subject matter should replicate our findings. If confirmed, the clinical relevance of the observed associations needs to be determined in studies with clinical practice data.

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Authors' contributions

JH, IM, FT, and TZ conceived the study. JS and FT contributed to the AOK PLUS data management. IM and CB contributed to the exposure data calculation. FT analyzed the data. TZ wrote the first draft of the manuscript. All authors involved in the result interpretation and text revision. All authors approved the final manuscript.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Appendix A. Supplementary data

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Paper 2: Supplementary Material

Supplementary

Depression and anxiety with exposure to ozone and particulate matter: an epidemiological claims data analysis

Contents

Table S1 Sensitivity analysis of 10-year total person-warm-seasons for individuals having only the diagnosis of depression or the diagnosis of anxiety

Diagnosis	Count *	Person-warm-seasons	Percentage (%)
Depression without anxiety	0	10,567,641	93.85
	1	269,117	2.39
	2	423,381	3.76
Anxiety without depression	0	10,962,872	97.36
	1	154,263	1.37
	2	143,003	1.27
Total		11,260,140	100

Note:

* Any outpatient who received the diagnosis of depression or anxiety was defined as a case in the quarter of the year. Depression and anxiety diagnosis counts were summed over the two quarters of the warm season (April to September) resulting in a diagnosis count of 0, 1, or 2, for each patient.

Chapter 9

Paper 3: Oozone on inflammatory markers in children and adolescents

Title of article:	Short-term exposure to ambient ozone and inflammatory biomarkers in cross-sectional studies of children and adolescents: Results of the GINIplus and LISA birth cohorts
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Short-term exposure to ambient ozone and inflammatory biomarkers in cross-sectional studies of children and adolescents: Results of the GINIplus and LISA birth cohorts☆

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ABSTRACT

Background: While exposure to ambient particulate matter (PM) and nitrogen dioxide (NO_2) is thought to be associated with diseases via inflammatory response, the association between exposure to ozone, an oxidative pollutant, and inflammation has been less investigated.
Aim: We analyzed associations between short-term exposure to ozone and three inflammatory biomarkers among children and adolescents.
Methods: These cross-sectional analyses were based on two follow-ups of the GINIplus and LISA German birth cohorts. We included 1330 10-year-old and 1591 15-year-old participants. Fractional exhaled nitric oxide (FeNO) and high-sensitivity C-reactive protein (hs-CRP) were available for both age groups while interleukin (IL)-6 was measured at 10 years only. Maximum 8-h averages of ozone and daily average concentrations of NO_2 and PM with an aerodynamic diameter <10 μm (PM_{10}) were adopted from two background monitoring stations 0 (same day), 1, 2, 3, 5, 7, 10 and 14 days prior to the FeNO measurement or blood sampling. To assess associations, we utilized linear regression models for FeNO, and logistic regressions for IL-6 and hs-CRP, adjusting for potential covariates and co-pollutants NO_2 and PM_{10}.
Results: We found that short-term ozone exposure was robustly associated with higher FeNO in adolescents at age 15, but not at age 10. No consistent associations were observed between ozone and IL-6 in children aged 10 years. The relationship between hs-CRP levels and ozone was J-shaped. Relatively low ozone concentrations (e.g., <120 μg/m^3) were associated with reduced hs-CRP levels, while high concentrations (e.g., ≥120 μg/m^3) tended to be associated with elevated levels for both 10- and 15-year-old participants.
Conclusions: Our study demonstrates significant associations between short-term ozone exposure and FeNO at 15 years of age and a J-shaped relationship between ozone and hs-CRP. The finding indicates that high ozone exposure may favor inflammatory responses in adolescents, especially regarding airway inflammation.

1. Introduction

Increasing evidence suggests associations between ambient air pollution, especially particulate matter (PM) and nitric oxides, and

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Abbreviations

BMI	body mass index
CI	confidence interval
FeNO	fractional exhaled nitric oxide
GAM	generalized additive model
GINIplus	German Infant study on the influence of a Nutritional Intervention plus environmental and genetic influences on allergy development
hs-CRP	high-sensitivity C-reactive protein
IL	interleukin
IQR	interquartile range
LISA	influence of Life-style factors on the development of the Immune System and Allergies in East and West Germany
MARS	multivariate adaptive regression splines
NO	nitric oxide
NO_2	nitrogen dioxide
NOS	nitric oxide synthases
OR	odds ratio
PM	particulate matter
PM_{10}	particulate matter with an aerodynamic diameter $<10\,\mu m$
ppb	parts per billion
SD	standard deviation
UBA	Umweltbundesamt (German Environment Agency)

the onset of adverse health conditions (Buoli et al., 2018; Guan et al., 2016; Hassoun et al., 2019; Rajagopalan et al., 2018). Ozone, as a major photochemical pollutant and a powerful oxidant, has not yet equally attracted research attention. However, results from recent epidemiological studies indicated that ozone might not only affect the respiratory system (Nhung et al., 2017; Zu et al., 2018), but also influence the cardio-cerebrovascular (Shah et al., 2013; Shah et al., 2015), central nervous system (Croze and Zimmer, 2018; Kasdagli et al., 2019) or mental health (Zhao et al., 2018).

Although the picture remains vague, oxidative stress and inflammation are postulated as mechanisms linking air pollutants exposure with health effect outcomes. Exposure to PM, nitrogen dioxide (NO_2) or ozone has been associated with inflammatory response in animal studies (Ji et al., 2015; Martin et al., 2013; Mishra et al., 2016; Wang et al., 2015; Wilson et al., 2010; Yoshizaki et al., 2017), and in epidemiological studies (Delfino et al., 2010; Liu et al., 2014; Mirowsky et al., 2017; Perret et al., 2017; Ruckerl et al., 2016; Shi et al., 2016). Nevertheless, the majority of epidemiological studies on ozone (e.g., Barraza-Villarreal et al., 2008; Lee et al., 2018; Liu et al., 2009) either had relatively small sample sizes or addressed potentially susceptible population groups, such as the elderly or patients, who are partially predisposed because of risk factors (e.g., age, lifestyle, smoking, diet) or morbidities, yielding overall limited and heterogeneous results. In comparison, studies in general populations, particularly at a young age, appear critical to assess whether ambient ozone exposure can cause local or systemic inflammation at an early stage of life possibly favoring the development of diseases.

The present study aimed to investigate associations between short-term ozone exposure and three inflammatory biomarkers among 10- and 15-year-old children and adolescents residing in two German areas. The markers were fractional exhaled nitric oxide (FeNO), as a noninvasive marker of respiratory inflammation, and interleukin (IL)-6 as well as high-sensitivity C-reactive protein (hs-CRP), as systemic markers.

2. Material and methods

2.1. Study population

The study populations originated from two population-based German birth cohorts "German Infant study on the influence of a Nutritional Intervention plus environmental and genetic influences on allergy development" (GINIplus) and "influence of Life-style factors on the development of the Immune System and Allergies in East and West Germany" (LISA). Both cohorts recruited healthy newborns with a full gestational age (≥37 weeks) and a normal birth weight (>2500 g) from 1995 to 1999. For the GINIplus cohort, 2949 participants from Munich and 3042 participants from Wesel were enrolled in two different arms. The intervention arm, investigating associations between the development of allergy and different hydrolyzed formulas given in the first four months of life, selected participants with at least one atopic parent or sibling. The observation arm selected participants without a family history of allergies or a consent about participating in the intervention from a legal guardian. For the LISA cohort, 1464 participants were recruited from Munich and 348 from Wesel, 976 from Leipzig and 306 from Bad Honnef. All of the subjects had physical examinations including FeNO measurement and blood sampling between the year 2005 to 2009 for the 10-year, and 2010 to 2014 for 15-year follow-ups. Ethical approval of GINIplus and LISA was acquired from the local ethics committees (Bavarian Board of Physicians, University of Leipzig, and Board of Physicians of North-Rhine-Westphalia), and written informed consent was obtained from the legal guardians of participants as well as from the participants themselves. Details on the two cohorts can be acquired elsewhere (Heinrich et al., 2002; von Berg et al., 2010; Zutavern et al., 2006).

We primarily restricted this analysis to participants with complete information on exposure and outcome from the follow-ups at 10 and 15 years residing in Munich and Wesel. Subjects with self-reported infections during the week before the FeNO measurement or blood sampling (863 participants) were excluded (Fig. S1). The data from the two cohorts were pooled and stratified by area as we did for previous analyses (Liu et al., 2014; Zhao et al., 2019).

2.2. Measurements of inflammatory biomarkers

2.2.1. Measurements of FeNO

FeNO was measured at both 10- and 15-year follow-ups using the device NIOX MINO® (Aerocrine) in accordance with guidelines (Maestrelli et al., 2007). Before FeNO measurements, participants refrained from eating or drinking for at least 1 h, from having nitrite-rich food intake (e.g., green vegetables or fruits, and smoked meats) for at least 4 h, and from taking any anti-asthmatic or anti-inflammatory medication for at least 4 h. While in a standing position, the participants were asked to inhale nitric oxide (NO)-free air quickly to total lung capacity through the mouthpiece of the NIOX MINO® and then exhale slowly and evenly for at least 6 s through the mouthpiece at a flow rate of 50 ± 5 mL/s. A nose clip was used to avoid nasal inspiration. The device automatically controlled the quality of the FeNO measurement, and repeated tests were taken until a value of acceptable quality was displayed (Liu et al., 2014).

2.2.2. Measurements of IL-6 and hs-CRP

During both 10- and 15-year follow-up visits, venous blood was sampled into serum separator tubes and centrifuged. The serum was stored at −80 °C. Concentrations of IL-6 were measured in the serum of the 10-year-olds only by flow cytometry using a

cytometric bead array (BD™ CBA Human Soluble Flex Set system, Becton Dickinson, Heidelberg, Germany) as previously described (Herberth et al., 2009). Concentrations of hs-CRP were determined in the serum of both the 10- and 15-year-olds using the Tina-quant® CRP (latex) high-sensitive assay (Roche, Mannheim, Germany) in one single lab, according to the standard method described in the in manufacturer's instruction (Harris et al., 2017).

2.3. Assessment of ambient ozone, and other pollutants

Data on ozone, NO_2, and PM with an aerodynamic diameter <10 μm (PM_{10}) of the Munich and Wesel areas were obtained from the German Environment Agency (Umweltbundesamt, labeled as UBA, https://www.umweltbundesamt.de/en), were measured by background monitoring stations, which can present the typical air quality in the city (UBA, 2017), following standard methods: ozone was measured by ultraviolet photometry, NO_2 by chemiluminescence and PM_{10} by the gravimetric measurement method. One monitoring station is about 9 km northeast of the center of Munich (Johanneskirchen), and one is approximately 2 km northeast of the center of Wesel (Feldmark) (Fuertes et al., 2015; Zhao et al., 2019).

Because ozone concentrations are highly variable, we computed a "maximum of the daily maximum 8-h average concentration (μg/m^3)" as recommended by UBA (UBA, 2013), which has been used in our previous study (Zhao et al., 2019). We initially calculated a moving 8-h (7 h before the hour of interest and the hour itself) average concentration for each hour of the day and subsequently identified the maximum of 8-h average for each day. In terms of NO_2 and PM_{10}, we adopted 24-h daily average concentrations (μg/m^3).

We utilized a broad time frame for this study. For ozone exposure, the maximum of the daily maximum 8-h average concentration (μg/m^3) was selected over day 0 (same day), and the period between day 0 and the time points of 1, 2, 3, 5, 7, 10 and 14 days prior to the FeNO measurement or blood sampling (lag 0-day to lag 0–14 days). Regarding the average values of the daily concentrations (μg/m^3), the same time frame of lag 0-day to lag 0–14 days was used for NO_2 and PM_{10}.

2.4. Covariates

Based on our published studies on inflammatory biomarkers in GINIplus and LISA cohorts (Liu et al., 2014; Yang et al., 2019), we considered a number of covariates for the present study apart from co-pollutants. These included basic information on area (Munich, Wesel) and study (GINIplus observation, GINIplus intervention, and LISA), as well as participants related factors such as sex (female, male), exact age at each follow-up visit (days expressed in years), body mass index (BMI, kg/m^2), onset of puberty (for the 10-year follow-up, based on hormone measurements (Harris et al., 2017): estradiol > 18.4 pmol/L in females; testosterone > 0.09 nmol/L in males; for the 15-year follow-up, based on questionnaire (Petersen et al., 1988): prepubertal, early pubertal, midpubertal, late pubertal, postpubertal), secondhand smoke exposure at home (never or ever from birth until 10 or 15 years), time spent in front of a screen (e.g., computer, television; high was defined as ≥ 1 h/day in summer or ≥ 2 h/day in winter), time spent outside (high was defined as ≥ 4 h/day in summer or ≥ 2 h/day in winter), physical activity level (low, medium and high were defined as moderate physical activity < 7 h per week, moderate physical activity ≥ 7 h and <10.5 h per week, moderate physical activity ≥ 10.5 h per week, alternatively vigorous physical activity ≥ 3.5 h per week, respectively (Janssen, 2007)), current asthmatic status (as ever doctor-diagnosed asthma from three years onwards and use of asthma medication in the last 12 months, or asthma symptoms in the last 12 months). We also considered factors related to the FeNO measurement or blood sampling: season (warm: April to October; cold: November to March), day time (8:00–11:00, 11.01–14:00, 14:01–19:00), fasting state (yes, no). Family-related factors were involved: maternal smoking during pregnancy (yes/no), maternal age at birth (≤30 years, 30–35 years, > 35 years), parental education (based on the highest number of years of school education reported by either parent; low, medium and high were respectively defined as < 10 years, = 10 years, and >10 years), single-parent family status (yes, no) and net equivalent household income (area-specific tertiles).

Additionally, for the 15-year follow-up, data about smoking (as ever smoking), alcohol consumption (as ever drinking), and medication (as ever taking any medication during the last seven days), were available.

2.5. Statistical analysis

The Chi-square test and Student's t-test were adopted to examine the differences between the selected analytic samples and the original population, as well as the differences between the two analytic samples from Munich and Wesel. The Wilcoxon test was used to examine the differences between pollutants. We also calculated Spearman correlation coefficients to assess correlations between different pollutant metrics.

The concentrations of FeNO were log (ln)-transformed to normalize their distributions. No outliers, as defined as more than quadplex standard deviations (SD) from the mean, were detected. The majority of concentrations of hs-CRP and IL-6 were below the detection limit of the instruments, and no outlier was identified under the definitions hs-CRP > 1 mg/dL and IL-6 > 20 pg/mL, respectively. We thus categorized the concentrations of these two systemic biomarkers into two levels. IL-6 was categorized with reference to the minimal detectable concentration (limit of detection, 1.5 pg/mL): undetectable, IL-6 ≤1.5 pg/mL; detectable, IL-6 > 1.5 pg/mL. Likewise, hs-CRP was categorized, based on the limit of quantification, as following: undetectable, hs-CRP < detection limit (0.020 mg/dL at 10 years and 0.016 mg/dL at 15 years due to modified assays); detectable, hs-CRP ≥ detection limit.

Since there was only a partial overlap of analytic samples and other differences in data across 10- and 15-year follow-ups, particularly the pubertal development, we analyzed associations between short-term ozone and inflammatory markers for each age group separately. The presence of linearity in the associations between the ozone metrics and inflammatory biomarkers was tested by generalized additive models (GAMs, Hastie and Tibshirani, 1986). The relationship between ln-transformed FeNO and ozone did not deviate from linearity, thereby ozone entered the GAMs as a linear term and fitted linear regression models for analyzing FeNO. Similarly, logistic regression with ozone as a linear term was adopted for IL-6, given the linearity of their relationships. However, ozone and hs-CRP showed a nonlinear exposure-response function (Figs. S2 and S3). Therefore, we primarily stratified ozone exposure into "low" and "high" concentrations and treated ozone as a linear term in both. Two different cut-offs were used: first, 120 μg/m^3 as the maximum daily 8-h mean concentration as a target value for the protection of human health recommended by the UBA (UBA, 2013) and second, 110 μg/m^3 as an average value of each lag's hinge point as calculated by multivariate adaptive regression splines (MARS (Hastie et al., 2009), they were utilized for identifying the optimal hinge points for interpreting the non-linear associations between ozone and hs-CRP). Furthermore, ozone was additionally modeled using thin plate regression splines in GAMs.

The main model was determined after selecting confounders

among the aforementioned covariates (subsection 2.4.). A confounder was traditionally defined as a correlate related to both the exposure and the outcome (VanderWeele and Shpitser, 2011). Based on this, our main adjustment set contained exact age at each follow-up, sex, time spent outside, physical activity level, season and day time of the FeNO measurement or blood sampling, and net equivalent household income. The set additionally included the two basic design variables area and study. To separate potential associations with ozone from those of other air pollutants, we also adjusted the models for the residuals of NO_2 and PM_{10}: we regressed each of the NO_2 and PM_{10} variables on each of the ozone metrics and derived model residuals, which were afterward included into the models (Yang et al., 2018; Zhao et al., 2019). We also present models with an adjustment for all covariates mentioned in subsection 2.4. (fully adjusted model). In addition, we built two models for sensitivity analyses considering the main adjustment set: (1) excluding participants with current asthma; (2) excluding 15-year-old participants who ever smoked, consumed alcohol, or took any medication in the last seven days. All the analyses were conducted for Munich and Wesel separately, and for the combined study populations from two areas. We further specifically analyzed the interaction between area (Munich versus Wesel) and ozone in the children aged 10 years by adding an interaction term in the main model.

The results of our analyses are presented as back ln-transformed percent changes for FeNO, and odds ratios (ORs) for IL-6 and hs-CRP, with 95% confidence intervals (CIs) scaled by specific interquartile range (IQR) increase in ozone. R 3.5.2 (R Core Team, 2018) was utilized. GAMs were fitted by *gam* function from the *mgcv* package (Wood, 2011). MARS were fitted by *earth* function from the *earth* package (Milborrow, 2019). We considered the significant level as 0.05 in our analyses.

3. Results

3.1. Characteristics of participants

Our analytic samples included 1330 participants aged 10 years and 1591 participants aged 15 years (Fig. S1, Table 1). We found that the GINIplus intervention children were more likely to be included in our analytic samples, and the children of parents with high education. The results were in line with our previous findings (Markevych et al., 2019; Zhao et al., 2019).

Almost all characteristics differed between participants from Munich and Wesel. Specifically, children from Munich were more likely to have a lower BMI, to spend less time outside, to have less physical activity, to be not exposed to passive smoking at home, and to have parents with higher education levels. However, for the 10 years old children, the difference on the season of the FeNO measurement or blood sampling was not statistically significant between Munich and Wesel; for the 15 years old adolescents, the data disruptions of pubertal development and alcohol consumption state were similar.

The children aged 10 years from Munich had a higher level of FeNO compared with the participants from Wesel. Nevertheless, the children from the two areas had similar levels of IL-6 and hs-CRP. Around 80% of 10-year-old children had a low IL-6 level, and more than 50% had a low hs-CRP level. Regarding the adolescents aged 15 years, the levels of FeNO and hs-CRP were higher among the participants from Munich.

3.2. Characteristics of ozone and other air pollutants

According to Table 2, lag 0–14 days averages of the daily maximum 8-h ozone concentrations were 69.73 $\mu g/m^3$ in Munich and 69.85 $\mu g/m^3$ in Wesel at 10 years, while the numbers were 73.28 and 68.48 $\mu g/m^3$ at 15 years (detailed concentrations for each lag are listed in Tables S1 and S2). Though the difference between the two areas was not significant at 10 years, Munich had a higher concentration of ozone than Wesel at 15 years. Besides, considering both 10 and 15 years, ozone concentrations were higher at 15 years than they were at 10 years. The NO_2 levels in Munich were higher than in Wesel, while Wesel was more polluted by PM_{10} than Munich.

Additionally, NO_2 and PM_{10} were strongly positively correlated with each other. PM_{10} was only weakly correlated with ozone, while the correlation between NO_2 and ozone was moderately negative (Fig. S4).

3.3. Associations between ozone and inflammatory biomarkers

The results of associations between short-term ambient ozone and inflammatory biomarkers are separately presented for FeNO, IL-6, and hs-CRP in Tables 3–6, Tables S3–S13, and Figs. S5–S6. Due to missing values in air pollution data for part of the days, the number of participants varied across different lags.

3.3.1. Ozone and FeNO

We observed significant positive associations between ozone and FeNO in adolescents aged 15 years (Table 3), with stronger effects for the shorter lags, and the most significant effect for the combined population was lag 0–2 days (percent change = 7.78, 95% CI: (2.76, 13.05)). No consistent associations were found in 10-year-old subjects. Additionally, there was no significant interaction between area and ozone, although the direction of effect was opposite in the two areas for the 10-year follow-up (Tables 3 and S3).

Similar associations were observed in models adjusted for all variables (Table S4). In addition, the models in which asthmatic patients were excluded showed similar effects, indicating that the observed effect estimates were not restricted to asthmatics (Table S5). After excluding smokers, and those who reported consumed alcohol or took medication, the positive associations remained, but the effect estimates were slightly reduced compared to the main models (Table S6).

3.3.2. Ozone and IL-6

We found no significant associations between ozone and IL-6 in children aged 10 years in neither area nor in the combined populations (Table 4). Likewise, the fully adjusted models indicated no association (Table S7). Excluding the currently asthmatic participant did not change the results (Table S8).

3.3.3. Ozone and hs-CRP

Overall, the relationship between hs-CRP levels and ozone was J-shaped (Figs. S2, S3, S5, and S6). The results stratified by ozone level <120 versus ≥ 120 $\mu g/m^3$ are shown in Tables 5 and 6. We identified that a reduced hs-CRP level was correlated with ozone exposure for the subgroup below 120 $\mu g/m^3$ (German standard), especially for the combined populations of Munich and Wesel, and in adolescents in Wesel aged 15 years. In the subgroup with high ozone concentration, no such effects were observed neither in the children nor in the adolescents (Tables 5 and 6).

The results from the mains model and the fully adjusted models were similar as well (Tables S9 and S10). When asthmatic patients were excluded, the formally protective effect for the ozone subgroup below 120 $\mu g/m^3$ remained, and the effect estimates for the high ozone subgroup did not change substantially (Table S11). When adolescents smoked, consumed alcohol, and those with medication intakes were dropped, the formally protective effects for the ozone subgroup below 120 $\mu g/m^3$ were slightly attenuated

Table 1
Characteristics of study populations

Variable	Category	10 years			15 years		
		Munich n (%)	Wesel n (%)	All n (%)	Munich n (%)	Wesel n (%)	All n (%)
Study	GINIplus observation	243 (28.83)	235 (48.25)	478 (35.94)	273 (29.61)	327 (48.88)	600 (37.71)
	GINIplus intervention	330 (39.15)	185 (37.99)	515 (38.72)	341 (36.98)	269 (40.21)	610 (38.34)
	LISA	270 (32.03)	67 (13.76)	337 (25.34)	308 (33.41)	73 (10.91)	381 (23.95)
Age	Mean ± SD	10.03 ± 0.19	10.03 ± 0.10	10.04 ± 0.16	15.20 ± 0.28	15.12 ± 0.30	15.17 ± 0.29
Sex	Female	392 (46.50)	232 (47.64)	624 (46.92)	470 (50.98)	366 (54.71)	836 (52.55)
	Male	451 (53.50)	255 (52.36)	706 (53.08)	452 (49.02)	303 (45.29)	755 (47.45)
BMI	Mean ± SD	16.92 ± 2.10	17.87 ± 2.63	17.27 ± 2.35	20.47 ± 2.95	21.03 ± 3.30	20.12 ± 3.47
Time spent outside [a]	High	119 (14.12)	139 (28.54)	258 (19.40)	82 (8.89)	181 (27.06)	263 (16.53)
	Low	724 (85.88)	348 (71.46)	1072 (80.60)	840 (91.11)	488 (72.94)	1328 (83.47)
Time in front of a screen [b]	High	208 (24.67)	205 (42.09)	413 (31.05)	745 (80.80)	588 (87.89)	1333 (83.78)
	Low	626 (74.26)	281 (57.70)	907 (68.20)	169 (1.88)	77 (11.51)	246 (15.46)
	Missing	9 (1.07)	1 (0.21)	10 (0.75)	8 (0.87)	4 (0.60)	12 (0.75)
Physical activity [c]	High	270 (30.03)	199 (40.86)	469 (35.26)	200 (21.69)	225 (33.63)	425 (26.71)
	Medium	230 (27.28)	123 (25.26)	353 (26.54)	233 (25.27)	175 (26.16)	408 (25.64)
	Low	220 (26.10)	85 (17.45)	305 (22.93)	329 (35.68)	151 (22.57)	480 (30.17)
	Missing	123 (14.59)	80 (16.43)	203 (15.26)	160 (17.35)	118 (17.64)	278 (17.47)
Puberty (10 years) [d]	Yes	421 (49.94)	206 (42.30)	627 (47.14)	-	-	-
	No	412 (48.87)	252 (51.75)	664 (49.92)	-	-	-
	Missing	10 (1.19)	29 (5.95)	39 (2.93)	-	-	-
Puberty (15 years) [e]	Prepubertal	-	-	-	2 (0.22)	3 (0.45)	5 (0.31)
	Early pubertal	-	-	-	19 (2.06)	14 (2.09)	33 (2.07)
	Midpubertal	-	-	-	155 (16.81)	118 (17.64)	273 (17.16)
	Late pubertal	-	-	-	558 (60.82)	385 (57.55)	943 (59.27)
	Postpubertal	-	-	-	76 (8.24)	45 (6.73)	121 (7.61)
	Missing	-	-	-	112 (12.15)	104 (15.55)	216 (13.58)
Parental education [f]	Low (< 10 years)	85 (10.08)	157 (32.24)	242 (18.19)	72 (7.81)	202 (30.19)	274 (17.22)
	Medium (= 10 years)	118 (14.00)	105 (21.56)	223 (16.77)	140 (15.18)	156 (23.32)	296 (18.60)
	High (> 10 years)	638 (75.68)	224 (46.00)	862 (64.81)	709 (76.90)	310 (46.34)	1019 (64.05)
	Missing	2 (0.24)	1 (0.21)	3 (0.23)	1 (0.11)	1 (0.15)	2 (0.13)
Maternal age at birth	≤ 30 years	278 (32.98)	237 (48.67)	515 (38.72)	299 (32.43)	319 (47.68)	618 (38.84)
	> 30 to ≤ 35 years	387 (45.91)	197 (40.45)	584 (43.91)	433 (46.96)	281 (41.00)	714 (44.88)
	> 35 years	178 (21.12)	53 (10.88)	231 (17.37)	190 (20.61)	69 (10.31)	259 (16.28)
Single parent family	Yes	98 (11.63)	32 (6.57)	130 (9.77)	120 (13.02)	84 (12.56)	204 (12.82)
	No	731 (86.71)	452 (92.81)	1183 (88.95)	764 (82.86)	566 (84.60)	1330 (83.60)
	Missing	14 (1.66)	3 (0.62)	17 (1.28)	38 (4.12)	19 (2.84)	57 (3.58)
Smoking exposure	During pregnancy	97 (11.51)	67 (13.76)	164 (12.33)	94 (10.20)	103 (15.40)	197 (12.38)
	between 0 and 10/15 years	259 (30.72)	240 (49.44)	499(37.52)	257 (27.87)	351 (52.47)	608 (38.21)
Income (euro/month) [g]	Low	244 (28.94)	138 (28.34)	382 (28.72)	277 (30.04)	178 (26.61)	455 (28.60)
	Medium	295 (34.99)	155 (31.83)	450 (33.83)	279 (30.26)	199 (29.75)	478 (30.04)
	High	249 (29.54)	153 (31.42)	402 (30.23)	270 (29.28)	208 (31.09)	478 (30.04)
	Missing	55 (6.52)	41 (8.24)	96 (7.22)	96 (10.41)	84 (12.56)	180 (11.31)
Season [h]	Warm	560 (66.43)	341 (70.02)	901 (67.74)	715 (77.55)	485 (72.50)	1200 (75.42)
	Cold	283 (33.57)	146 (29.98)	429 (32.26)	207 (22.45)	184 (27.50)	391 (24.58)
Time	8:00-11:00	305 (36.18)	95 (19.51)	400 (30.08)	415 (45.01)	284 (42.45)	699 (43.93)
	11:01-14:00	118 (14.00)	39 (8.01)	157 (11.80)	172 (18.66)	62 (9.27)	234 (14.71)
	14:01-19:00	420 (49.82)	353 (72.48)	773 (58.12)	335 (36.33)	323 (48.28)	658 (41.36)
Fasting state of blood sample	Yes	192 (22.78)	43 (8.83)	235 (17.67)	95 (10.30)	17 (2.54)	112 (7.04)
	No	651 (77.22)	439 (90.14)	1090 (81.95)	550 (59.65)	350 (52.32)	900 (56.57)
	Missing	0 (0.00)	5 (1.03)	5 (0.38)	277 (30.04)	302 (45.14)	579 (36.39)
Participant smoking	Yes	-	-	-	71 (8.16)	28 (4.19)	99 (6.22)
	No	-	-	-	839 (91.84)	633 (94.62)	1472 (92.52)
	Missing	-	-	-	12 (1.30)	8 (1.20)	20 (1.26)
Participant consumed alcohol	Yes	-	-	-	146 (15.84)	102 (15.25)	248 (15.59)
	No	-	-	-	745 (80.80)	535 (79.97)	1280 (80.45)
	Missing	-	-	-	31 (3.36)	32 (4.78)	63 (3.96)
Medication intake last 7 days	Yes	-	-	-	263 (28.52)	43 (6.43)	306 (19.23)
	No	-	-	-	659 (71.48)	626 (93.57)	1285 (80.77)
Current asthma [i]	Yes	44 (5.22)	33 (6.78)	77 (5.79)	56 (6.07)	42 (6.28)	98 (6.16)
	No	787 (93.36)	447 (91.79)	1234 (92.78)	848 (91.97)	612 (91.48)	1460 (91.77)
	Missing	12 (1.42)	7 (1.44)	19 (1.43)	18 (1.95)	15 (2.24)	33 (2.07)
FeNO	ppb (median; IQR)	13; 11	11; 8	12; 10	18; 12	14; 10	16; 12
hs-CRP [j]	Undetectable	452 (53.62)	244 (50.10)	696 (52.33)	65 (7.05)	221 (33.03)	286 (17.98)
	Detectable	391 (46.38)	243 (49.90)	634 (46.67)	857 (92.95)	448 (66.97)	1305 (82.02)
IL-6 [k]	Undetectable	704 (83.51)	387 (79.47)	1091 (82.03)	-	-	-
	Detectable	139 (16.49)	100 (20.53)	239 (17.97)	-	-	-
Total		843 (63.38)	487 (36.62)	1330 (100.00)	922 (57.95)	669 (42.05)	1591 (100.00)

Note:
Abbreviations: BMI, body mass index; FeNO, fractional concentration of exhaled nitric oxide; IL-6, interleukin-6; hs-CRP, high sensitivity-C reactive protein; SD, standard deviation;

[a] High is defined as ≥ 4 hours per day in summer or ≥ 2 hours in winter

[b] High is defined as ≥ 1 hour per day in summer or ≥ 2 hours per day in winter

[c] Low, moderate physical activity < 7 h per week; medium, moderate physical activity ≥ 7 h and < 10.5 h per week; high, moderate physical activity ≥ 10.5 h per week or vigorous physical activity ≥ 3.5 h per week

[d] Puberty onset, females: estradiol > 18.4 pmol/L, males: testosterone > 0.09 nmol/L
[e] Puberty stage, according to puberty category scores from Puberty Development Scale (Petrersen et al., 1988)
[f] Highest number of years of school education for either parent was calculated, based on the German education system,
[g] Net equivalent household income (euro/month), according to area-specific tertiles
[h] Warm, April to October; cold, November to March
[i] Ever doctor-diagnosed asthma from three years onwards, use of asthma medication in the last 12 months or asthma symptoms last 12 months
[j] Due to modified assays, 10 year, detection limit was 0.020 mg/dL; 15 year, detection limit was 0.016 mg/dL
[k] IL-6, detection limit was 1.5 pg/mL

Table 2
Averaged concentrations of ozone and other air pollutants for lag 0-14 days

Area	Air pollutant	Mean	SD	Min	Max	Median	IQR	Mean	SD	Min	Max	Median	IQR
		10 years						15 years					
Munich	Ozone [a]	69.73	27.23	16.41	137.78	77.12	43.04	73.28	24.72	12.12	117.46	79.10	40.27
	NO_2 [b]	29.10	8.27	16.59	73.19	27.57	10.77	20.58	5.74	12.21	40.00	19.14	6.85
	PM_{10} [b]	21.03	9.82	9.09	91.84	18.49	8.76	17.09	5.69	8.24	46.30	16.01	5.47
Wesel	Ozone [a]	69.85	31.31	14.53	160.38	66.30	43.58	68.48	23.31	6.93	118.96	70.92	35.86
	NO_2 [b]	24.72	8.10	10.40	53.25	23.51	11.33	19.88	6.85	8.04	41.84	18.29	10.21
	PM_{10} [b]	24.77	7.73	11.47	68.16	23.25	8.91	21.64	7.29	11.98	45.25	19.31	9.00
All	Ozone [a]	69.78	28.78	14.53	160.38	73.43	43.14	71.26	24.24	6.93	118.96	75.85	38.56
	NO_2 [b]	27.50	8.47	10.40	73.19	25.57	10.51	20.29	6.24	8.04	41.84	19.04	8.05
	PM_{10} [b]	22.40	9.28	9.09	91.84	20.28	9.31	19.00	6.79	8.24	46.30	17.22	7.16

Note:
Abbreviation: SD, standard deviation; IQR, interquartile range
[a] The maximum 8-hour (7 hours before and the hour of interest) daily average (μg/m^3), 14 days prior to the FeNO measurement or blood sampling, from the background monitor stations of UBA. The detailed concentrations for each lag are listed in Tables S1 and S2
[b] Average of the daily concentration (μg/m^3), 14 days prior to the FeNO measurement or blood sampling, from the background monitor stations of UBA. The detailed concentrations for each lag are listed in Tables S1 and S2

Table 3
Adjusted associations between short-term ozone and FeNO at the ages of 10 and 15 years.

Area	Pollutant	10 years			15 years		
		Main model (Percent change, 95% CI)	p value	Participants	Main model (Percent change, 95% CI)	p value	Participants
Munich	Lag 0-day [a]	−4.13 (−11.40, 3.72)	0.293	835/843	4.92 (−1.19, 11.40)	0.117	911/922
	Lag 0–1 days [b]	−4.61 (−12.38, 3.85)	0.276	830/843	**7.73 (0.67, 15.28)**	**0.031**	921/922
	Lag 0–2 days [c]	−3.53 (−11.82, 5.53)	0.432	834/843	7.16 (−0.01, 14.85)	0.050	922/922
	Lag 0–3 days [d]	−5.18 (−13.41, 3.83)	0.250	837/843	3.47 (−3.40, 10.83)	0.330	922/922
	Lag 0–5 days [e]	−5.14 (−13.08, 3.53)	0.237	842/843	0.93 (−5.78, 8.12)	0.791	922/922
	Lag 0–7 days [f]	−5.60 (−13.62, 3.16)	0.203	842/843	3.39 (−3.61, 10.90)	0.351	922/922
	Lag 0–10 days [g]	−8.89 (−17.51, 0.62)	0.066	842/843	0.19 (−7.11, 8.07)	0.960	922/922
	Lag 0–14 days [h]	**−10.37 (-19.64–0.03)**	**0.049**	843/843	−0.27 (−7.90, 7.99)	0.946	922/922
Wesel	Lag 0-day [a]	1.40 (−7.32, 10.94)	0.762	406/487	6.63 (−0.10, 13.82)	0.054	622/669
	Lag 0–1 days [b]	3.00 (−6.44, 13.39)	0.547	397/487	**8.40 (1.20, 16.10)**	**0.021**	626/669
	Lag 0–2 days [c]	4.40 (−5.03, 14.77)	0.373	425/487	**9.68 (2.54, 17.32)**	**0.007**	648/669
	Lag 0–3 days [d]	3.71 (−5.24, 13.50)	0.429	447/487	**9.40 (2.21, 17.09)**	**0.009**	658/669
	Lag 0–5 days [e]	5.41 (−2.91, 14.45)	0.209	469/487	**7.66 (0.70, 15.10)**	**0.030**	669/669
	Lag 0–7 days [f]	6.00 (−2.12, 14.80)	0.152	479/487	6.02 (−0.56, 13.04)	0.074	669/669
	Lag 0–10 days [g]	5.15 (−2.54, 13.44)	0.195	483/487	6.45 (−0.45, 13.83)	0.067	669/669
	Lag 0–14 days [h]	5.30 (−2.44, 13.64)	0.185	484/487	6.34 (−0.47, 13.62)	0.069	669/669
All	Lag 0-day [a]	−2.28 (−7.91, 3.68)	0.445	1241/1330	**5.69 (1.22, 10.34)**	**0.012**	1533/1591
	Lag 0–1 days [b]	−2.17 (−8.21, 4.27)	0.500	1227/1330	**7.34 (2.39, 12.54)**	**0.003**	1547/1591
	Lag 0–2 days [c]	−0.59 (−6.86, 6.10)	0.859	1259/1330	**7.78 (2.76, 13.05)**	**0.002**	1570/1591
	Lag 0–3 days [d]	−1.96 (−8.06, 4.55)	0.547	1284/1330	**6.04 (1.06, 11.25)**	**0.016**	1580/1591
	Lag 0–5 days [e]	−0.80 (−6.63, 5.39)	0.794	1311/1330	4.04 (−0.83, 9.16)	0.105	1591/1591
	Lag 0–7 days [f]	−0.80 (−6.58, 5.34)	0.793	1321/1330	4.82 (−0.08, 9.95)	0.054	1591/1591
	Lag 0–10 days [g]	−1.74 (−7.62, 4.50)	0.575	1325/1330	3.65 (−1.45, 9.01)	0.163	1591/1591
	Lag 0–14 days [h]	−1.83 (−7.91, 4.66)	0.572	1327/1330	3.87 (−1.32, 9.33)	0.147	1591/1591

Note:
Abbreviation: FeNO, fractional concentration of exhaled nitric oxide; CI, confidence interval.
1. All estimates were scaled by an interquartile range increase according to specific areas (see Table 2). Percent change was back transformed from the ln-transformed FeNO.
2. Main model: all estimates were adjusted for the exact age, sex, time spent outside, physical activity level, season and time of the FeNO measurement or blood sampling, net equivalent household income, cohort, and area (only for "all").
3. Participants, "sample number analyzed/total number analyzed"; missing values were due to a lack of exposure data.
a. h. The maximum of the daily maximum 8-h average concentration was selected over 0, and the period between 0 day and the days of 1, 2, 3, 5, 7, 10 and 14 days prior to the FeNO measurement or blood sampling, from the background monitor stations.

(Table S12).

As an additional subanalysis, we used a cutoff of 110 μg/m^3 ozone because it was a hinge point according to the results of MARS analyses. The results based on this cutoff are presented in Table S13. Comparing to the cutoff of 120 μg/m^3, we could find an attenuated formally protective effect estimate in the relatively lower ozone concentration subgroup, and an increased estimate pointing towards adverse effects in the high ozone subgroup.

Table 4
Adjusted associations between short-term ozone and IL-6 at the age of 10 years.

Area	Pollutant	Main model (OR, 95% CI)	p value	Participants
Munich	Lag 0-day [a]	1.12 (0.84, 1.49)	0.424	835/843
	Lag 0–1 days [b]	1.22 (0.90, 1.65)	0.208	830/843
	Lag 0–2 days [c]	1.14 (0.82, 1.56)	0.437	834/843
	Lag 0–3 days [d]	1.18 (0.85, 1.63)	0.324	837/843
	Lag 0–5 days [e]	1.18 (0.86, 1.61)	0.299	842/843
	Lag 0–7 days [f]	1.31 (0.95, 1.80)	0.096	842/843
	Lag 0–10 days [g]	1.36 (0.95, 1.94)	0.094	842/843
	Lag 0–14 days [h]	1.35 (0.92, 1.99)	0.127	843/843
Wesel	Lag 0-day [a]	1.07 (0.75, 1.52)	0.713	406/487
	Lag 0–1 days [b]	0.93 (0.64, 1.35)	0.689	397/487
	Lag 0–2 days [c]	1.09 (0.77, 1.55)	0.616	425/487
	Lag 0–3 days [d]	1.06 (0.76, 1.48)	0.727	447/487
	Lag 0–5 days [e]	1.09 (0.81, 1.47)	0.576	469/487
	Lag 0–7 days [f]	1.06 (0.79, 1.43)	0.695	479/487
	Lag 0–10 days [g]	1.04 (0.78, 1.38)	0.796	483/487
	Lag 0–14 days [h]	1.06 (0.79, 1.42)	0.686	484/487
All	Lag 0-day [a]	1.12 (0.90, 1.38)	0.310	1241/1330
	Lag 0–1 days [b]	1.13 (0.90, 1.41)	0.303	1227/1330
	Lag 0–2 days [c]	1.14 (0.91, 1.43)	0.265	1259/1330
	Lag 0–3 days [d]	1.14 (0.91, 1.43)	0.240	1284/1330
	Lag 0–5 days [e]	1.15 (0.93, 1.42)	0.199	1311/1330
	Lag 0–7 days [f]	1.18 (0.96, 1.46)	0.121	1321/1330
	Lag 0–10 days [g]	1.16 (0.94, 1.44)	0.173	1325/1330
	Lag 0–14 days [h]	1.17 (0.94, 1.47)	0.162	1327/1330

Note:
Abbreviation: IL-6, interleukin-6; CI, confidence interval; OR, odds ratio.
1. All estimates were scaled by an interquartile range increase according to specific areas (see Table 2).
2. Main model: all estimates were adjusted for the exact age, sex, time spent outside, physical activity level, season and time of the FeNO measurement or blood sampling, net equivalent household income, cohort, and area (only for "all").
3. Participants, "sample number analyzed/total number analyzed"; missing values were due to a lack of exposure data.
a. h. The maximum of the daily maximum 8-h average concentration was selected over 0, and the period between 0 day and the days of 1, 2, 3, 5, 7, 10 and 14 days prior to the FeNO measurement or blood sampling, from the background monitor stations.

Generally, the results from GAMs (Figs. S5–S6) supported the results from our subgroup-approach and indicated that medium- and low-level ozone might either be not associated with hs-CRP or be associated with the reduced hs-CRP level, while high-level ozone could be associated with the elevated hs-CRP level.

4. Discussion

4.1. Main study findings

Overall, based on short-term exposure to ozone, we observed positive associations for FeNO among adolescents aged 15 years and no association for FeNO and IL-6 among children at the age of 10 years. Remarkably, a nonlinear J-shaped relationship between ozone and hs-CRP levels was identified, indicating that the below German standard ozone concentrations might be related to the reduced hs-CRP levels, whereas high concentrations tended to be associated with the elevated hs-CRP level in both 10- and 15-year-old participants.

4.2. Interpretations and comparisons with other studies

For the purpose of comparison, we consider a volumetric ozone concentration of 1 ppb equivalent to a gravimetric concentration of 2 $\mu g/m^3$. The following concentrations and effect estimates were accordingly transformed if needed.

4.2.1. Ozone and FeNO

Catalyzed primarily by the inducible nitric oxide synthase (NOS), NO is formed in the airways when L-arginine oxidizes to L-citrulline (Pijnenburg and De Jongste, 2008). FeNO is recommended by the European Respiratory Society (Horvath et al., 2017) as a marker of Th-2 related airway inflammation and is widely used in studies on respiratory health, especially asthma and allergies.

Several studies investigated ozone exposure versus FeNO, but few of them were conducted among healthy children. Barraza-Villarreal et al. (2008) observed a positive association between ozone exposure and FeNO (per 44 $\mu g/m^3$ for ozone, 1.23 (95% CI: 0.85, 1.77)) in a longitudinal study of 50 Mexican non-asthmatic children (aged 7.9–11.5 years), and the similar positive association in 158 asthmatic children (aged 7.9–11.5 years), based on fixed-site monitoring (8-h moving average concentration ranging from 9.8 to 172.6 $\mu g/m^3$). Karakatsani et al. (2017) conducted a panel study among 188, 10- to 11-year-old Greek children. The researchers used weekly personal ozone exposure (24-h average concentration ranged from 4.7 to 10.8 $\mu g/m^3$; meanwhile the daily concentration at fixed monitor sites ranged from 24.6 to 63.8 $\mu g/m^3$), and observed that a 10 $\mu g/m^3$ increase in ozone was associated with an 11.10% (95% CI: 4.23, 18.43) increase in FeNO. Likewise, Nickmilder et al. (2007) also reported a significant increase in FeNO in a panel study with 72 participants aged 6.5–15 years, at an ambient 1-h ozone level of 167 $\mu g/m^3$ (concentration ranging from 48 to 221 $\mu g/m^3$).

However, this observed ozone-FeNO association might be sensitive to the range or the level of ozone concentration. Different from the findings mentioned above, based on data from 2240 8- to 9-year-old school children from the USA, Berhane et al. (2011) observed a longer lag structure, as over 1–23 days 8-h cumulative average values of ozone were associated with higher FeNO levels. The reported ozone concentrations were mainly lower than 120 $\mu g/m^3$ (detailed numbers were not reported). Moreover, ground on data from 605 children 9–13 years old from Turkey, Altug et al. (2014) did not find a significant change in FeNO levels when the weekly ozone concentration ranged from 26.4 to 133.3 $\mu g/m^3$. It had been hypothesized that there was a threshold effect for the ozone-induced increase in FeNO: Nickmilder et al. (2007) considered the threshold of 135 $\mu g/m^3$ for 1-h exposure and of 110 $\mu g/m^3$ for 8-h exposure. Even though the different ozone metrics are incomparable across studies, the above two studies (Altug et al., 2014; Berhane et al., 2011) with possibly lower ozone concentrations observed no short-term effects.

Four human exposure studies (Barath et al., 2013; Nightingale et al., 1999; Nightingale et al., 2000; Olin et al., 2001) investigated the effects of a single time high concentration ozone exposure (exposure concentration ranging from 400 to 800 $\mu g/m^3$, exposure time ranging from 75 min to 4 h) on repeatedly assessed FeNO levels in adults. These studies did not observe that ozone affected FeNO. Thus, they do not support the findings of our epidemiological study in adolescents. The difference between experimental studies and epidemiological studies might be attributed to characteristics of the participants, in particular age, co-pollutants and the effect of single, relatively short-term exposures.

Overall, studies on ozone exposure versus FeNO in children or adolescents, therefore, have yielded different results. Our study with the null finding in children and positive associations in adolescents adds to the current knowledge, as it has a large sample size, and because we analyzed children and adolescents separately while the other studies mixed them or were conducted only in children. Our results show that even a small difference in age might affect the susceptibility to ozone; thus, this factor should be cautiously considered.

Table 5
Adjusted associations between ozone and hs-CRP at the age of 10 years with ozone stratified by < 120 versus ≥120 μg/m^3.

Area	Pollutant	Main model <120 μg/m^3 (OR, 95% CI)	p value	Participants	Main model ≥ 120 μg/m^3 (OR, 95% CI)	p value	Participants
Munich	Lag 0-day [a]	0.97 (0.92, 1.03)	0.385	775/843	1.03 (0.45, 2.39)	0.938	60/843
	Lag 0–1 days [b]	0.96 (0.91, 1.03)	0.249	785/843	1.61 (0.72, 3.61)	0.253	45/843
	Lag 0–2 days [c]	0.97 (0.91, 1.04)	0.382	775/843	1.79 (0.92, 3.49)	0.092	59/843
	Lag 0–3 days [d]	0.96 (0.90, 1.03)	0.274	771/843	1.65 (0.95, 2.88)	0.083	66/843
	Lag 0–5 days [e]	**0.93 (0.87, 0.99)**	**0.032**	724/843	1.34 (0.98, 1.82)	0.067	118/843
	Lag 0–7 days [f]	**0.91 (0.85, 0.98)**	**0.014**	708/843	1.22 (0.94, 1.58)	0.131	134/843
	Lag 0–10 days [g]	0.94 (0.86, 1.02)	0.122	679/843	1.26 (0.98, 1.62)	0.069	163/843
	Lag 0–14 days [h]	0.93 (0.84, 1.03)	0.146	647/843	1.13 (0.90, 1.42)	0.290	196/843
Wesel	Lag 0-day [a]	1.00 (0.91, 1.09)	0.937	373/487	0.67 (0.36, 1.25)	0.226	33/487
	Lag 0–1 days [b]	0.99 (0.90, 1.09)	0.782	354/487	0.82 (0.57, 1.19)	0.309	43/487
	Lag 0–2 days [c]	0.99 (0.90, 1.09)	0.844	374/487	0.86 (0.62, 1.18)	0.357	51/487
	Lag 0–3 days [d]	0.97 (0.87, 1.08)	0.595	376/487	0.92 (0.68, 1.24)	0.573	71/487
	Lag 0–5 days [e]	0.94 (0.83, 1.05)	0.276	373/487	1.12 (0.91, 1.38)	0.287	96/487
	Lag 0–7 days [f]	0.92 (0.81, 1.04)	0.180	363/487	1.08 (0.88, 1.33)	0.445	116/487
	Lag 0–10 days [g]	0.91 (0.79, 1.04)	0.181	348/487	1.00 (0.82, 1.21)	0.962	135/487
	Lag 0–14 days [h]	0.89 (0.76, 1.03)	0.111	330/487	1.00 (0.82, 1.23)	0.984	154/487
All	Lag 0-day [a]	0.98 (0.93, 1.03)	0.410	1241/1330	1.24 (0.80, 1.92)	0.334	93/1330
	Lag 0–1 days [b]	0.97 (0.92, 1.02)	0.257	1139/1330	0.91 (0.66, 1.25)	0.561	88/1330
	Lag 0–2 days [c]	0.97 (0.92, 1.03)	0.329	1149/1330	1.05 (0.80, 1.39)	0.712	110/1330
	Lag 0–3 days [d]	0.96 (0.91, 1.02)	0.155	1147/1330	1.15 (0.91, 1.47)	0.241	137/1330
	Lag 0–5 days [e]	**0.92 (0.87, 0.98)**	**0.007**	1097/1330	**1.22 (1.04, 1.43)**	**0.018**	214/1330
	Lag 0–7 days [f]	**0.91 (0.85, 0.96)**	**0.001**	1071/1330	1.15 (0.98, 1.34)	0.079	250/1330
	Lag 0–10 days [g]	**0.92 (0.86, 0.98)**	**0.012**	1027/1330	1.09 (0.94, 1.26)	0.235	298/1330
	Lag 0–14 days [h]	**0.90 (0.83, 0.98)**	**0.011**	977/1330	1.04 (0.90, 1.20)	0.584	350/1330

Note:
Abbreviation: hs-CRP, high sensitivity-C reactive protein; CI, confidence interval; OR, odds ratio.
1. All estimates were scaled by an interquartile range increase according to specific areas (see Table 2).
2. Main model: all estimates were adjusted for the exact age, sex, time spent outside, physical activity level, season and time of the FeNO measurement or blood sampling, net equivalent household income, cohort, and area (only for "all").
3. Participants, "sample number analyzed/total number analyzed"; missing values were due to a lack of exposure data.
a. h. The maximum of the daily maximum 8-h average concentration was selected over 0, and the period between 0 day and the days of 1, 2, 3, 5, 7, 10 and 14 days prior to the FeNO measurement or blood sampling, from the background monitor stations.

Table 6
Adjusted associations between ozone and hs-CRP at the age of 15 years with ozone stratified by < 120 versus ≥120 μg/m^3.

Area	Pollutant	Main model <120 μg/m^3 (OR, 95% CI)	p value	Participants	Main model ≥ 120 μg/m^3 (OR, 95% CI)	p value	Participants
Munich	Lag 0-day [a]	0.98 (0.96, 1.01)	0.294	825/922	0.97 (0.60, 1.56)	0.891	59/922
	Lag 0–1 days [b]	1.01 (0.97, 1.04)	0.742	888/922	1.00 (0.71, 1.41)	0.999	33/922
	Lag 0–2 days [c]	1.01 (0.98, 1.04)	0.574	862/922	0.88 (0.59, 1.31)	0.531	66/922
	Lag 0–3 days [d]	1.01 (0.98, 1.05)	0.499	819/922	0.91 (0.73, 1.14)	0.413	103/922
	Lag 0–5 days [e]	1.00 (0.96, 1.03)	0.875	742/922	1.01 (0.85, 1.20)	0.938	180/922
	Lag 0–7 days [f]	1.00 (0.95, 1.04)	0.827	672/922	0.95 (0.82, 1.10)	0.473	250/922
	Lag 0–10 days [g]	0.98 (0.93, 1.03)	0.403	634/922	0.98 (0.85, 1.15)	0.837	288/922
	Lag 0–14 days [h]	0.98 (0.93, 1.03)	0.403	615/922	0.97 (0.83, 1.14)	0.723	307/922
Wesel	Lag 0-day [a]	0.99 (0.93, 1.05)	0.669	590/669	1.44 (0.88, 2.37)	0.165	32/669
	Lag 0–1 days [b]	**0.93 (0.87, 0.99)**	**0.018**	593/669	1.30 (0.70, 2.39)	0.415	33/669
	Lag 0–2 days [c]	**0.91 (0.85, 0.98)**	**0.011**	582/669	0.99 (0.71, 1.40)	0.976	66/669
	Lag 0–3 days [d]	**0.90 (0.84, 0.97)**	**0.008**	577/669	0.81 (0.63, 1.05)	0.117	81/669
	Lag 0–5 days [e]	**0.89 (0.82, 0.96)**	**0.003**	560/669	0.93 (0.76, 1.14)	0.501	109/669
	Lag 0–7 days [f]	**0.88 (0.81, 0.95)**	**0.002**	542/669	0.96 (0.81, 1.13)	0.591	127/669
	Lag 0–10 days [g]	**0.85 (0.78, 0.94)**	**0.001**	517/669	1.04 (0.89, 1.22)	0.630	152/669
	Lag 0–14 days [h]	0.91 (0.83, 1.01)	0.078	491/669	1.00 (0.87, 1.15)	0.985	178/669
All	Lag 0-day [a]	0.98 (0.95, 1.01)	0.222	1442/1591	1.27 (0.95, 1.69)	0.108	91/1591
	Lag 0–1 days [b]	0.98 (0.95, 1.01)	0.126	1481/1591	1.24 (0.90, 1.71)	0.191	66/1591
	Lag 0–2 days [c]	0.97 (0.94, 1.01)	0.102	1444/1591	1.02 (0.81, 1.29)	0.836	126/1591
	Lag 0–3 days [d]	**0.96 (0.93, 0.99)**	**0.049**	1396/1591	0.94 (0.80, 1.10)	0.417	184/1591
	Lag 0–5 days [e]	**0.95 (0.91, 0.98)**	**0.003**	1302/1591	1.03 (0.91, 1.17)	0.626	289/1591
	Lag 0–7 days [f]	**0.94 (0.90, 0.98)**	**0.003**	1214/1591	1.05 (0.95, 1.16)	0.350	377/1591
	Lag 0–10 days [g]	**0.92 (0.88, 0.96)**	**< 0.001**	1151/1591	1.09 (0.99, 1.21)	0.071	440/1591
	Lag 0–14 days [h]	**0.93 (0.89, 0.98)**	**0.006**	1106/1591	1.06 (0.97, 1.16)	0.228	485/1591

Note:
Abbreviation: hs-CRP, high sensitivity-C reactive protein; CI, confidence interval; OR, odds ratio.
1. All estimates were scaled by an interquartile range increase according to specific areas (see Table 2).
2. Main model: all estimates were adjusted for the exact age, sex, time spent outside, physical activity level, season and time of the FeNO measurement or blood sampling, net equivalent household income, cohort, and area (only for "all").
3. Participants, "sample number analyzed/total number analyzed"; missing values were due to a lack of exposure data.
a. h. The maximum of the daily maximum 8-h average concentration was selected over 0, and the period between 0 day and the days of 1, 2, 3, 5, 7, 10 and 14 days prior to the FeNO measurement or blood sampling, from the background monitor stations.

4.2.2. Ozone and IL-6 and hs-CRP

IL-6 and CRP have complex biological effects, being considered as typical biomarkers of systemic inflammation. IL-6 can function as an inflammatory cytokine and an anti-inflammatory myokine; CRP is mainly produced in the liver and secreted into the circulation, in response to IL-6, IL-1 or tumor necrosis factor-α (Del Giudice and Gangestad, 2018).

Studies on ozone exposure versus IL-6 have been rarely conducted in children. The result from a long-term pilot study (Calderon-Garciduenas et al., 2013) included 35 clinically healthy Mexican children (mean age 6.2 years) indicated significantly higher systemic levels of IL-6 after a lifetime exposure to ozone. The observed fourth-highest daily maximum 8-h average ozone concentrations were 240 μg/m^3, 250 μg/m^3 and 244 μg/m^3 in the year from 2007 to 2009, respectively. The studies performed in asthmatic children generated controversial results. An intervention study performed in Mexico (Sienra-Monge et al., 2004) with 117 (mean age 9.0 years) children with asthma observed increased IL-6 levels in nasal lavage fluid related to ozone exposure (8-h moving ozone average ranging from 22.2 to 285.0 μg/m^3). Liu et al. (2009) studied 182, 9- to 14-year-old asthmatic children in Italy and found that IL-6 in breath condensate was not associated with ozone (3-day average concentration ranging (5th to 95th percentile) from 15 to 42 μg/m^3).

In general, the evidence from epidemiological studies regarding IL-6 was inconsistent, possibly due to the diverse study designs, sample sizes, participants' characteristics, and ozone levels. However, results from the human exposure studies showed inconsistency as well. Devlin et al. (1991) found that exposure of 28 volunteers (18–35 years of age) to 160 μg/m^3 for 6.6 h was sufficient to initiate an increase of IL-6 in the bronchoalveolar lavage fluid. Similarly, Torres et al. (1997) also observed the positive ozone-associated (440 μg/m^3, 4 h) increase IL-6 in the bronchoalveolar lavage and alveolar lavage fluids among 38 participants age 18–40 years. Furthermore, a controlled exposure study (Bennett et al., 2016) with 40 women aged 18–35 years found increased plasma IL-6 after exposure to an 800 μg/m^3 level, which is a high concentration even among experimental studies, for 2 h. Nevertheless, under the same exposure condition (800 μg/m^3, 2 h), Fahy et al. (1995) found non-significantly higher IL-6 levels in the induced sputum, based on a small sample of 10 subjects (mean age 30.0 years). Urch et al. (2010) conducted a study with 23 participants aged 21–40 years and did not found IL-6 response in the induced sputum nor in blood in relation to 240 μg/m^3, 2-h ozone exposure. Similarly, the result from Arjomandi et al. (2018) was that 240 μg/m^3, 3 h of ozone exposure did not significantly affect the IL-6 in the sputum supernatants. However, Jörres et al. (2000) pointed out that a repeated ozone exposure (400 μg/m^3 ozone over 4 h of intermittent exercise on each of 4 consecutive days) was associated with an increase in IL-6 in bronchoalveolar lavage fluid assessed on the fifth day, as compared to a single-day ozone exposure. It may be assumed that though ozone-induced inflammation might initially occur in the respiratory system, the local or systemic IL-6 levels could not be visible after a single, relatively short-term, low concentration exposure to ozone.

The knowledge about associations between ozone exposure and hs-CRP or CRP is currently limited and inconsistent, with data mainly derived from studies in adults. Some positive associations were reported. A panel study (Chuang et al., 2007) with 76 students aged 18–25 years reported an increase in hs-CRP in association with an increase in ozone (3-day average concentration ranging from 45.0 to 96.6 μg/m^3); but this association disappeared in two-pollutant models. A cross-sectional study (Michikawa et al., 2016) conducted with 2360 participants aged more than 20 years observed positive associations with ozone (mean concentration on the day of blood draw was 69.2 μg/m^3). However, most studies found no associations (Forbes et al., 2009; Huang et al., 2014; Lee et al., 2018; Li et al., 2017b; Steinvil et al., 2008). Notably, the majority of the reported associations were formally protective although not statistically significant (Forbes et al., 2009; Huang et al., 2014; Lee et al., 2018; Steinvil et al., 2008). Considering only the ozone levels, the data from short-term studies (Steinvil et al., 2008) and long-term studies (Forbes et al., 2009; Huang et al., 2014; Lee et al., 2018) were mainly less than 120 μg/m^3 and are comparable with our finding regarding the below German standard ozone concentration condition, where lower ozone levels were associated with the reduced hs-CRP level. While considering the exposure-response relationship, Pilz et al. (2018) reported a non-linear, negative ozone-CRP association with an annual average ozone range of 31.5–45.8 μg/m^3. Michikawa et al. (2016) adopted logistic regression models and observed positive associations between hs-CRP and ozone, although they found no statistical evidence for a linear trend in the associations. In contrast, other studies adopted linear models but reported no results of linearity test (Chuang et al., 2007; Forbes et al., 2009; Huang et al., 2014; Lee et al., 2018; Steinvil et al., 2008).

Given that there are no similar studies about ozone and hs-CRP conducted in children or adolescents, we cannot directly compare our results with those of other studies. However, the above-mentioned epidemiological studies support that the ozone concentration below the German standard might be related to the reduced hs-CRP level, underlying the nonlinearity of the response. Our results suggest that the associations in this manner might be highly dose-dependent. Irrespective of whether ozone levels were stratified according to the previously chosen cutoff (120 μg/m^3) or statistically identified hinge point (110 μg/m^3), the absence of the formally protective effect was consistent for ozone exposures at the relatively lower concentrations. Thereby, the distribution of ozone, especially the “distance” between a specific concentration and the threshold level (e.g., 120 or 110 μg/m^3 in the present study) would be critically related to the ozone-induced variation of hs-CRP. The J-shaped, threshold-like or hormesis-like relationship would be important for explaining the association between ozone exposure and hs-CRP level, in accordance with the results by Nickmilder et al. (2007) who reported threshold effects for ozone exposure regarding FeNO, i.e. 135 μg/m^3 for 1-h exposure and 110 μg/m^3 for 8-h exposure.

4.3. Possible mechanisms

The mechanism of ozone-induced variations in inflammatory biomarkers is not clear yet. Generally, lipid peroxidation is considered to be one of the inducers of ozone-related inflammation; and surface macrophages and epithelial cells are involved in the generation of pro-inflammatory mediators (Bromberg, 2016). Dysfunctions of purine metabolites (Esther et al., 2011) or hormones (Henriquez et al., 2018) might also play a role in response to ozone.

Few animal studies investigated the relationship between ozone exposure and FeNO. Recent data from Niu et al. (2018) indicated that ozone could result in a decrease in *NOS2A* methylation and an increase in inducible NOS expression, suggesting that ozone inhalation may affect DNA methyltransferases. Elevated FeNO levels were also hypothesized to be associated with decreased arginase and elevated arginase-2 methylation (Niu et al., 2018).

Most of the animal or cell studies demonstrated positive associations between ozone exposure and IL-6 (Arsalane et al., 1995; Bhalla et al., 2002; Gonzalez-Guevara et al., 2014; Yu et al., 2002), while few studies investigating CRP had mixed results (Jakubowski et al., 2004; Song et al., 2018). The inconsistent results on IL-6 or

CRP across different studies might be additionally attributed to the presence of a threshold effect or hormesis with respect to ozone and inflammation, or attributed to the presence of ozone-induced inflammation in the airways may be more visible.

The evidence regarding ozone versus inflammation is currently scarce, which results in difficulties when interpreting the observed effect, especially the formally protective effect for the below German standard ozone concentration and hs-CRP. However, several animal studies (Chang et al., 2005; Kaya et al., 2017; Wei et al., 2018) and a clinical trial (Niu et al., 2018) reported that ozone therapy (perfusion or injection with ozone or ozone-absorbed liquid) was associated with a reduction in cytokines levels. It has been assumed that the toll-like receptor 4 (Chen et al., 2016) and the nuclear factor - κB pathway (Yu et al., 2016), which mediate the immune responses to lipopolysaccharide, could be suppressed by ozone, accompanied by a reduction of inflammatory cytokines levels. This possible hormetic dose-response relationship of ozone is already observed in different, non-epidemiological studies (Bocci et al., 2011; Martinez-Sanchez et al., 2010).

4.4. Limitations and strengths

Our study has several limitations. The ozone concentrations were measured at a single background monitoring station per area, and the outcome variables IL-6 and hs-CRP had to be dichotomized due to skewed distributions. These factors may have decreased statistical power and affected our results towards the null. Multiple comparison problems stemmed from analyses among several divided groups would be another limitation for statistical power. Further, since the selection by socioeconomic status resulted in initial under-recruitment and in subsequent higher loss to follow-up of participants from families with low socioeconomic status (also reported for other birth cohort studies (Bornehag et al., 2012; MAL-ED Network Investigators, 2017)), the external validity of our study might be limited. In addition, we might have missed some indirect pathways or other possible variables, which may also affect the associations of interest, like temperature (Li et al., 2017a) although we have adjusted the season for the long-term, and the daytime for intraday temperature variance; or humidity (Bind et al., 2014). Finally, a significant limitation is that our analyses were cross-sectional, an approach that cannot confirm the causality of associations. A panel study with repeated measurements of inflammatory markers might have served as a more robust design.

There are also several strengths of the present study. Firstly, we had a relatively large and comparable study sample, especially in terms of the separated age groups (10 and 15 years of age), from two population-based cohorts. Secondly, we adopted a broad time frame (lag 0-day to lag 0–14 days) to detect as many as possible time-dependent associations. Thirdly, a number of data on potentially relevant covariates, including infections, time spent outdoors, physical activity, smoking, drinking, and intake of medication were available. Fourthly, the check for non-linearity which we conducted could be used as a guide for future analysis methods in case of hs-CRP. Finally, we considered two major co-pollutants while analyzing the effects of ozone; thus, we can conclude with more certainty that the observed effects were due to ozone and not to residual confounding by other pollutants.

5. Conclusions

We observed that short-term ambient ozone exposure was associated with elevated levels of FeNO, but not related to systemic levels of IL-6. Moreover, a J-shaped relationship between ozone exposure and systemic hs-CRP was identified. Our findings indicate that acute ozone exposure may cause inflammation, which is most pronounced for airway inflammation in adolescents. No definite conclusion can be drawn currently for systemic inflammation.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2019.113264.

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Paper 3: Supplementary Material

Supplementary

Short-term exposure to ambient ozone and inflammatory biomarkers in cross-sectional studies of children and adolescents: Results of the GINIplus and LISA birth cohorts

Contents

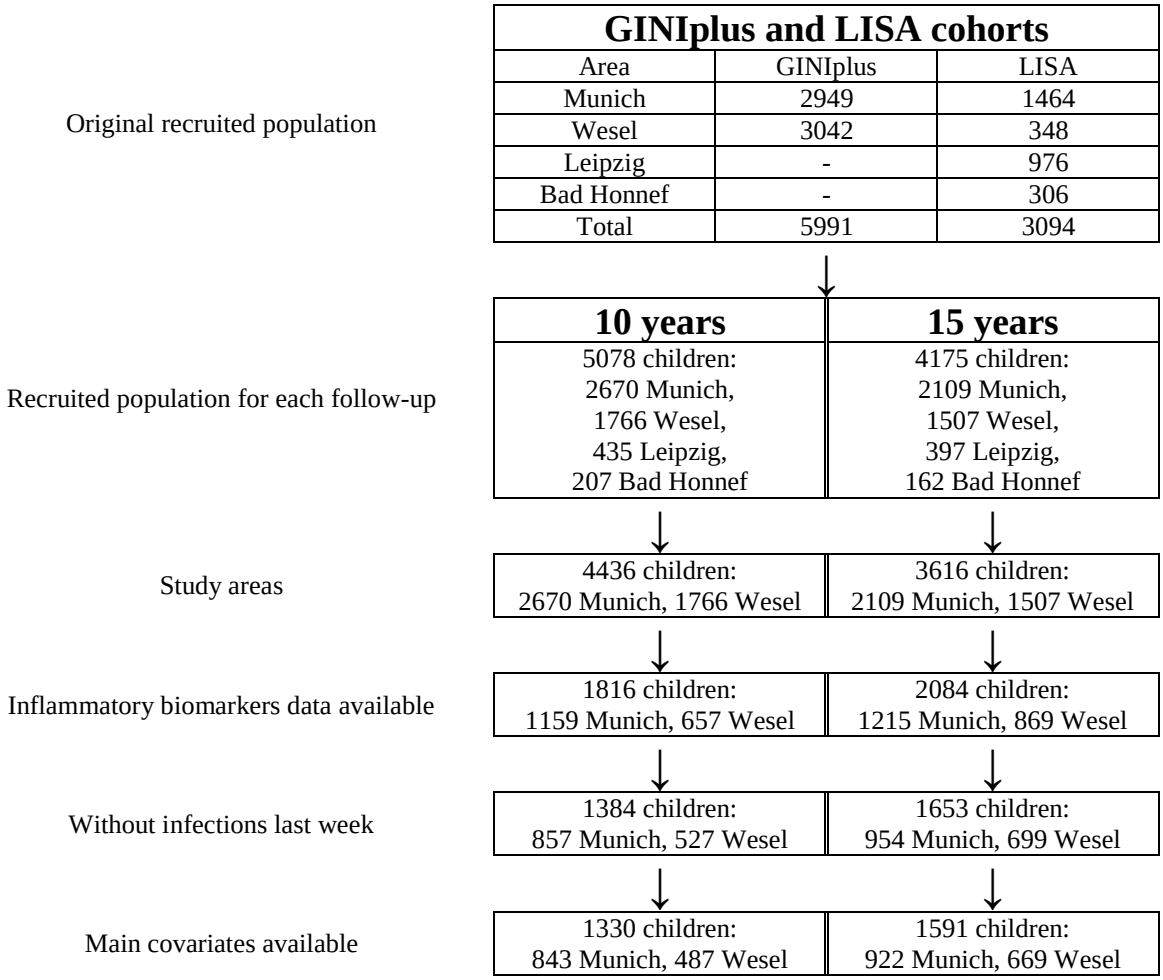

Figure S1. Flow chart for participant selection

Note:

Main covariates available, the selected confounders with less than 30 missing values (not applicable, N/A). For the present study, they were time spent by a child outside and time of the FeNO measurement or blood sampling

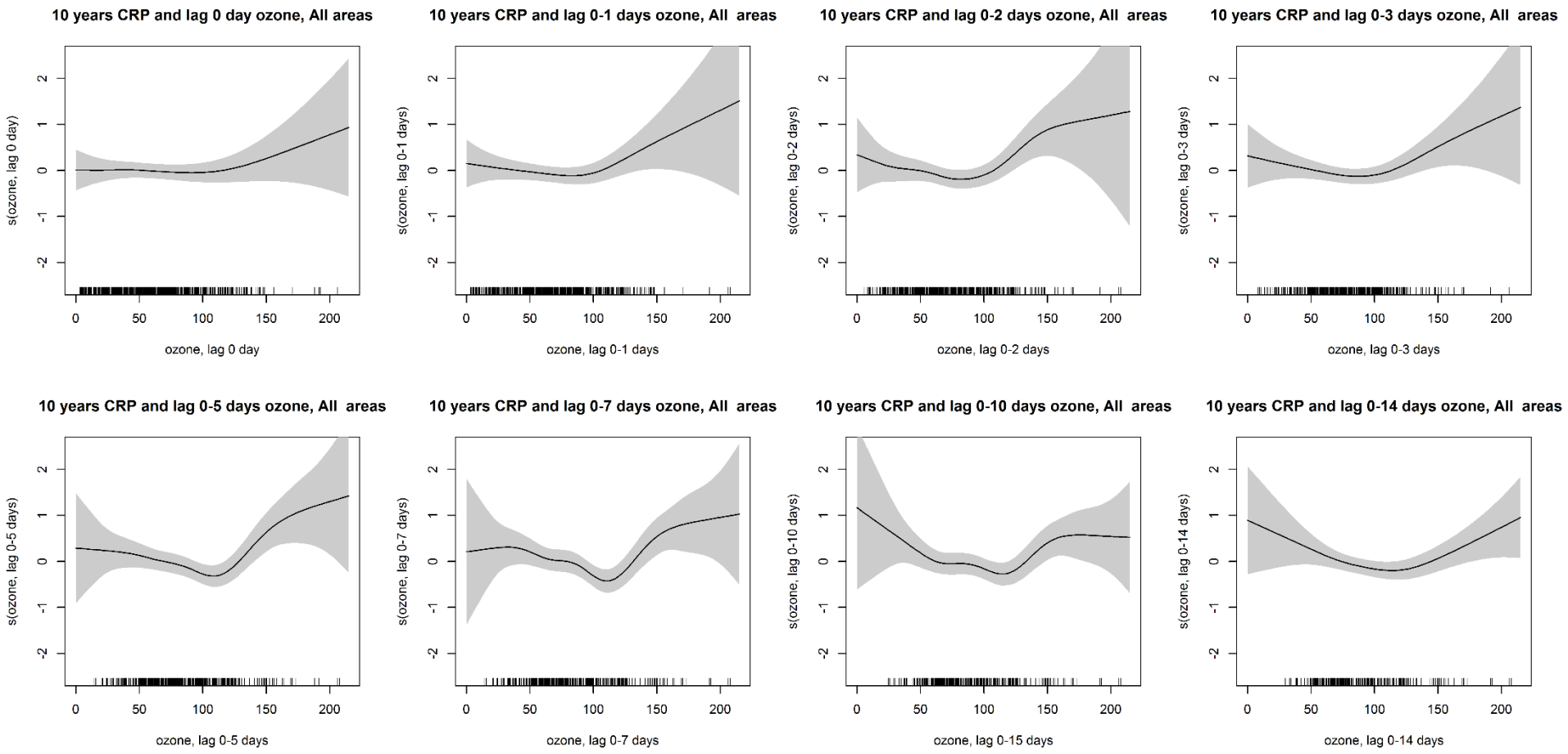

Figure S2. Generalized additive model plots between short-term ozone exposure (lag 0 day to lag 0-14 days) and hs-CRP at the age of 10 years

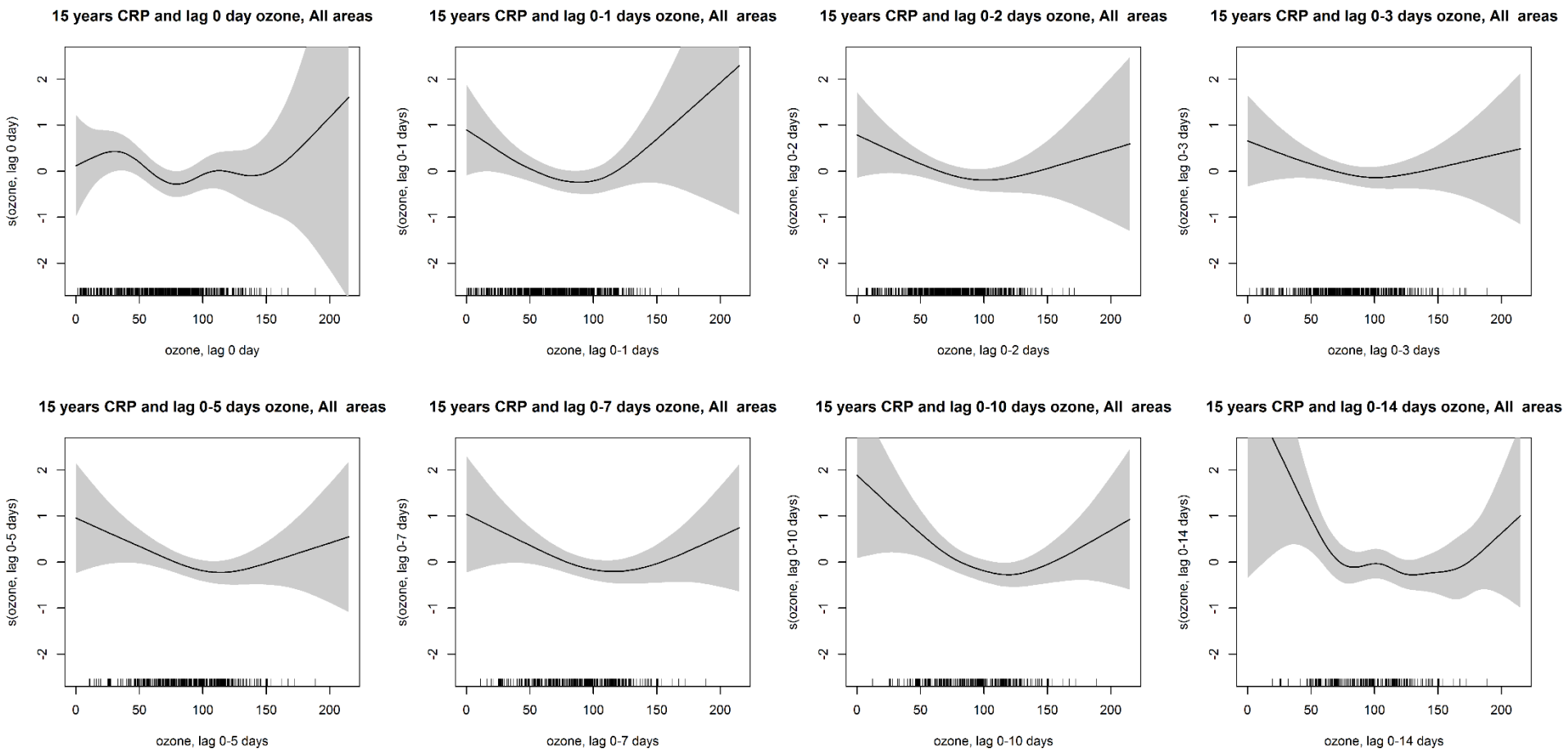

Figure S3. Generalized additive model plots between short-term ozone exposure (lag 0 day to lag 0-14 days) and hs-CRP at the age of 15 years

Table S1. Concentrations of ozone and other air pollutants for each lag at the age of 10 years

All (Combined populations)

lag 0 day

variable	mean	sd	min	max	median	IQR
ozone	65.55	34.74	3.00	205.94	64.50	45.69
NO2	30.85	12.50	6.50	94.65	29.75	16.21
PM10	23.23	13.62	2.75	110.19	20.16	16.11

lags 0-1 days

variable	mean	sd	min	max	median	IQR
ozone	67.78	33.35	3.12	207.69	66.88	41.82
NO2	28.19	13.38	5.00	94.65	25.77	17.58
PM10	22.05	13.75	2.75	140.40	19.94	15.53

lags 0-2 days

variable	mean	sd	min	max	median	IQR
ozone	75.23	32.45	5.75	207.69	72.38	44.38
NO2	26.78	11.70	5.00	86.49	25.21	14.65
PM10	21.89	13.18	3.61	143.08	18.82	13.77

lags 0-3 days

variable	mean	sd	min	max	median	IQR
ozone	80.69	32.53	8.06	207.69	81.06	44.59
NO2	26.06	10.38	2.99	78.78	24.19	12.90
PM10	22.03	12.12	5.53	119.65	19.16	13.95

lags 0-5 days

variable	mean	sd	min	max	median	IQR
ozone	87.41	34.89	14.19	207.69	88.62	48.28
NO2	26.40	9.30	9.00	79.89	24.73	11.82
PM10	22.52	10.87	6.06	110.18	20.96	12.35

lags 0-7 days

variable	mean	sd	min	max	median	IQR
ozone	90.52	35.99	14.19	207.69	90.00	52.38
NO2	27.37	9.24	9.52	92.47	25.59	10.85
PM10	22.74	10.45	6.76	111.46	20.56	11.20

lags 0-10 days

variable	mean	sd	min	max	median	IQR
ozone	95.70	35.84	24.59	207.69	94.69	49.38
NO2	27.06	8.89	10.03	78.83	25.20	10.94
PM10	22.37	9.91	7.46	105.43	20.48	9.94

lags 0-14 days

variable	mean	sd	min	max	median	IQR
ozone	100.12	36.28	29.52	207.69	97.62	53.78
NO2	27.50	8.47	10.40	73.19	25.57	10.51
PM10	22.41	9.29	9.09	91.84	20.28	9.33

Munich

lag 0 day

variable	mean	sd	min	max	median	IQR
ozone	66.28	33.08	3.00	147.44	65.81	46.81
NO2	32.96	12.26	12.21	94.65	31.25	14.30
PM10	22.91	14.24	2.75	110.19	20.04	18.67

lags 0-1 days

variable	mean	sd	min	max	median	IQR
ozone	67.24	31.33	3.12	146.62	67.56	41.38
NO2	30.47	13.56	11.33	94.65	27.90	15.79
PM10	21.36	14.53	2.75	140.40	18.87	17.44

lags 0-2 days

variable	mean	sd	min	max	median	IQR
ozone	74.66	30.05	8.06	146.62	73.81	43.00
NO2	28.86	11.75	10.79	86.49	26.41	13.87
PM10	21.02	13.56	3.61	130.38	17.84	14.56

lags 0-3 days

variable	mean	sd	min	max	median	IQR
ozone	79.94	29.98	8.06	152.62	81.56	43.25
NO2	27.71	10.33	9.72	78.78	24.99	12.01
PM10	20.95	12.46	5.53	119.65	18.13	14.99

lags 0-5 days

variable	mean	sd	min	max	median	IQR
ozone	85.84	31.91	15.50	173.06	89.75	47.88
NO2	27.95	9.19	13.25	79.89	25.56	11.37
PM10	21.30	11.08	6.06	110.18	19.91	12.04

lags 0-7 days

variable	mean	sd	min	max	median	IQR
ozone	88.91	32.54	15.50	173.06	94.62	48.47
NO2	29.06	9.16	15.29	92.47	27.07	10.72
PM10	21.59	10.79	6.76	111.46	19.73	11.09

lags 0-10 days

variable	mean	sd	min	max	median	IQR
ozone	93.64	30.72	28.69	173.06	99.50	47.62
NO2	28.73	8.72	16.84	78.83	27.15	10.54
PM10	21.06	10.24	7.46	105.43	18.82	8.86

lags 0-14 days

variable	mean	sd	min	max	median	IQR
ozone	97.80	30.06	32.94	173.06	102.50	50.38
NO2	29.06	8.28	16.59	73.19	27.53	10.77
PM10	21.07	9.83	9.09	91.84	18.49	8.83

Wesel

lag 0 day

variable	mean	sd	min	max	median	IQR
ozone	64.04	37.92	3.93	205.94	59.62	47.09
NO2	26.51	11.87	6.50	62.55	24.36	19.27
PM10	23.88	12.23	6.45	103.66	20.36	13.36

lags 0-1 days

variable	mean	sd	min	max	median	IQR
ozone	68.91	37.23	3.64	207.69	65.29	42.00
NO2	23.41	11.63	5.00	59.54	22.28	17.35
PM10	23.50	11.84	7.16	116.23	20.85	13.59

lags 0-2 days

variable	mean	sd	min	max	median	IQR
ozone	76.36	36.71	5.75	207.69	69.62	43.69
NO2	22.72	10.50	5.00	52.87	19.70	13.71
PM10	23.61	12.24	7.44	143.08	21.39	13.94

lags 0-3 days

variable	mean	sd	min	max	median	IQR
ozone	82.09	36.81	14.19	207.69	77.81	43.19
NO2	22.97	9.76	2.99	53.25	20.97	13.29
PM10	24.07	11.18	8.30	105.05	21.91	14.34

lags 0-5 days

variable	mean	sd	min	max	median	IQR
ozone	90.22	39.56	14.19	207.69	84.25	48.82
NO2	23.63	8.85	9.00	56.00	22.29	12.71
PM10	24.69	10.14	9.14	97.34	22.32	12.65

lags 0-7 days

variable	mean	sd	min	max	median	IQR
ozone	93.37	41.26	14.19	207.69	84.31	53.06
NO2	24.40	8.61	9.52	53.25	23.61	11.97
PM10	24.77	9.50	9.39	74.98	23.09	11.77

lags 0-10 days

variable	mean	sd	min	max	median	IQR
ozone	99.29	43.14	24.59	207.69	88.62	65.13
NO2	24.16	8.42	10.03	53.25	22.52	12.14
PM10	24.67	8.85	7.71	67.74	22.79	10.01

lags 0-14 days

variable	mean	sd	min	max	median	IQR
ozone	104.14	44.84	29.52	207.69	90.44	82.03
NO2	24.80	8.12	10.40	53.25	23.58	11.51
PM10	24.73	7.75	10.93	68.16	23.28	8.90

Table S2. Concentrations of ozone and other air pollutants for each lag at the age of 15 years

All (combined populations)

lag 0 day

variable	mean	sd	min	max	median	IQR
ozone	70.44	32.37	1.41	188.81	70.06	43.58
NO2	22.43	10.03	3.72	61.85	20.67	13.22
PM10	19.16	11.15	2.59	88.04	16.73	10.92

lags 0-1 days

variable	mean	sd	min	max	median	IQR
ozone	71.43	31.19	0.50	167.30	71.77	42.34
NO2	20.75	10.79	0.38	65.30	18.96	12.33
PM10	19.29	14.05	3.24	126.66	16.25	11.94

lags 0-2 days

variable	mean	sd	min	max	median	IQR
ozone	78.00	31.70	0.94	171.18	77.84	43.37
NO2	20.37	9.92	0.90	64.75	18.44	11.51
PM10	19.11	11.57	3.64	73.86	16.13	11.33

lags 0-3 days

variable	mean	sd	min	max	median	IQR
ozone	83.54	31.43	1.68	188.81	84.41	45.77
NO2	19.96	8.90	1.84	63.18	18.29	10.13
PM10	18.91	10.29	4.28	67.50	16.47	10.67

lags 0-5 days

variable	mean	sd	min	max	median	IQR
ozone	90.01	31.79	10.22	188.81	89.91	44.98
NO2	19.65	7.45	3.30	54.78	18.10	8.69
PM10	18.82	8.75	4.92	68.99	16.71	9.27

lags 0-7 days

variable	mean	sd	min	max	median	IQR
ozone	93.81	32.43	11.10	188.81	93.70	50.87
NO2	20.22	6.80	6.23	47.02	19.21	8.43
PM10	19.18	8.08	6.07	61.86	17.48	8.12

lags 0-10 days

variable	mean	sd	min	max	median	IQR
ozone	98.14	31.33	12.13	188.81	98.31	51.48
NO2	20.15	6.42	7.11	45.12	19.03	7.72
PM10	19.06	7.28	5.68	53.07	17.50	7.37

lags 0-14 days

variable	mean	sd	min	max	median	IQR
ozone	101.65	31.06	19.43	188.81	105.05	52.38
NO2	20.29	6.24	8.04	41.84	19.04	8.05
PM10	19.00	6.79	8.24	46.30	17.22	7.16

Munich

lag 0 day

variable	mean	sd	min	max	median	IQR
ozone	72.23	33.72	3.67	153.37	71.50	42.77
NO2	22.97	9.18	7.64	56.75	20.97	12.32
PM10	16.98	10.40	2.59	76.52	14.92	11.23

lags 0-1 days

variable	mean	sd	min	max	median	IQR
ozone	72.75	32.52	2.12	153.37	73.85	45.53
NO2	21.36	10.66	5.14	65.30	19.82	10.28
PM10	17.56	14.84	3.24	126.66	13.70	11.31

lags 0-2 days

variable	mean	sd	min	max	median	IQR
ozone	78.81	32.59	7.07	153.37	83.43	46.22
NO2	20.80	9.74	7.58	64.75	18.52	10.79
PM10	17.16	11.12	3.64	69.83	15.23	11.07

lags 0-3 days

variable	mean	sd	min	max	median	IQR
ozone	84.83	31.77	7.08	153.37	86.45	43.28
NO2	20.31	8.65	8.05	63.18	18.29	8.97
PM10	16.85	9.32	4.28	62.86	14.78	9.36

lags 0-5 days

variable	mean	sd	min	max	median	IQR
ozone	91.59	31.61	10.22	153.37	93.70	46.87
NO2	19.89	6.87	9.70	54.78	18.57	7.79
PM10	16.74	7.37	4.92	68.99	15.61	8.22

lags 0-7 days

variable	mean	sd	min	max	median	IQR
ozone	95.48	31.55	16.25	153.37	97.87	49.74
NO2	20.42	6.12	10.60	47.02	19.27	6.66
PM10	17.10	6.62	6.07	61.86	16.32	7.97

lags 0-10 days

variable	mean	sd	min	max	median	IQR
ozone	99.70	29.78	25.34	153.37	106.34	54.19
NO2	20.39	5.84	11.53	45.12	19.23	6.73
PM10	17.11	6.00	5.68	53.07	15.98	6.93

lags 0-14 days

variable	mean	sd	min	max	median	IQR
ozone	102.69	28.36	25.34	153.37	108.48	46.32
NO2	20.58	5.74	12.21	40.00	19.14	6.85
PM10	17.09	5.69	8.24	46.30	16.01	5.47

Wesel

lag 0 day

variable	mean	sd	min	max	median	IQR
ozone	67.82	30.12	1.41	188.81	66.04	37.24
NO2	21.64	11.11	3.72	61.85	19.16	15.64
PM10	22.34	11.46	6.23	88.04	19.55	13.10

lags 0-1 days

variable	mean	sd	min	max	median	IQR
ozone	69.50	29.04	0.50	167.30	69.08	37.02
NO2	19.86	10.92	0.38	61.85	17.76	15.66
PM10	21.83	12.38	7.16	88.04	18.19	14.31

lags 0-2 days

variable	mean	sd	min	max	median	IQR
ozone	76.84	30.39	0.94	171.18	75.36	42.02
NO2	19.75	10.16	0.90	57.95	18.05	13.50
PM10	21.89	11.65	7.60	73.86	18.21	12.07

lags 0-3 days

variable	mean	sd	min	max	median	IQR
ozone	81.74	30.87	1.68	188.81	80.58	38.97
NO2	19.48	9.23	1.84	51.37	17.94	12.59
PM10	21.80	10.87	8.05	67.50	18.67	12.63

lags 0-5 days

variable	mean	sd	min	max	median	IQR
ozone	87.83	31.93	11.10	188.81	85.47	45.31
NO2	19.32	8.17	3.30	45.57	17.62	10.90
PM10	21.69	9.65	9.56	63.66	18.68	11.09

lags 0-7 days

variable	mean	sd	min	max	median	IQR
ozone	91.51	33.49	11.10	188.81	87.91	45.08
NO2	19.95	7.64	6.23	44.47	18.60	10.78
PM10	22.04	8.98	10.35	52.16	19.56	10.60

lags 0-10 days

variable	mean	sd	min	max	median	IQR
ozone	95.98	33.25	12.13	188.81	94.11	48.89
NO2	19.82	7.12	7.11	42.34	18.70	10.44
PM10	21.75	8.01	11.15	46.33	19.50	9.03

lags 0-14 days

variable	mean	sd	min	max	median	IQR
ozone	100.21	34.41	19.43	188.81	99.91	52.75
NO2	19.88	6.85	8.04	41.84	18.29	10.21
PM10	21.64	7.29	11.98	45.25	19.31	9.00

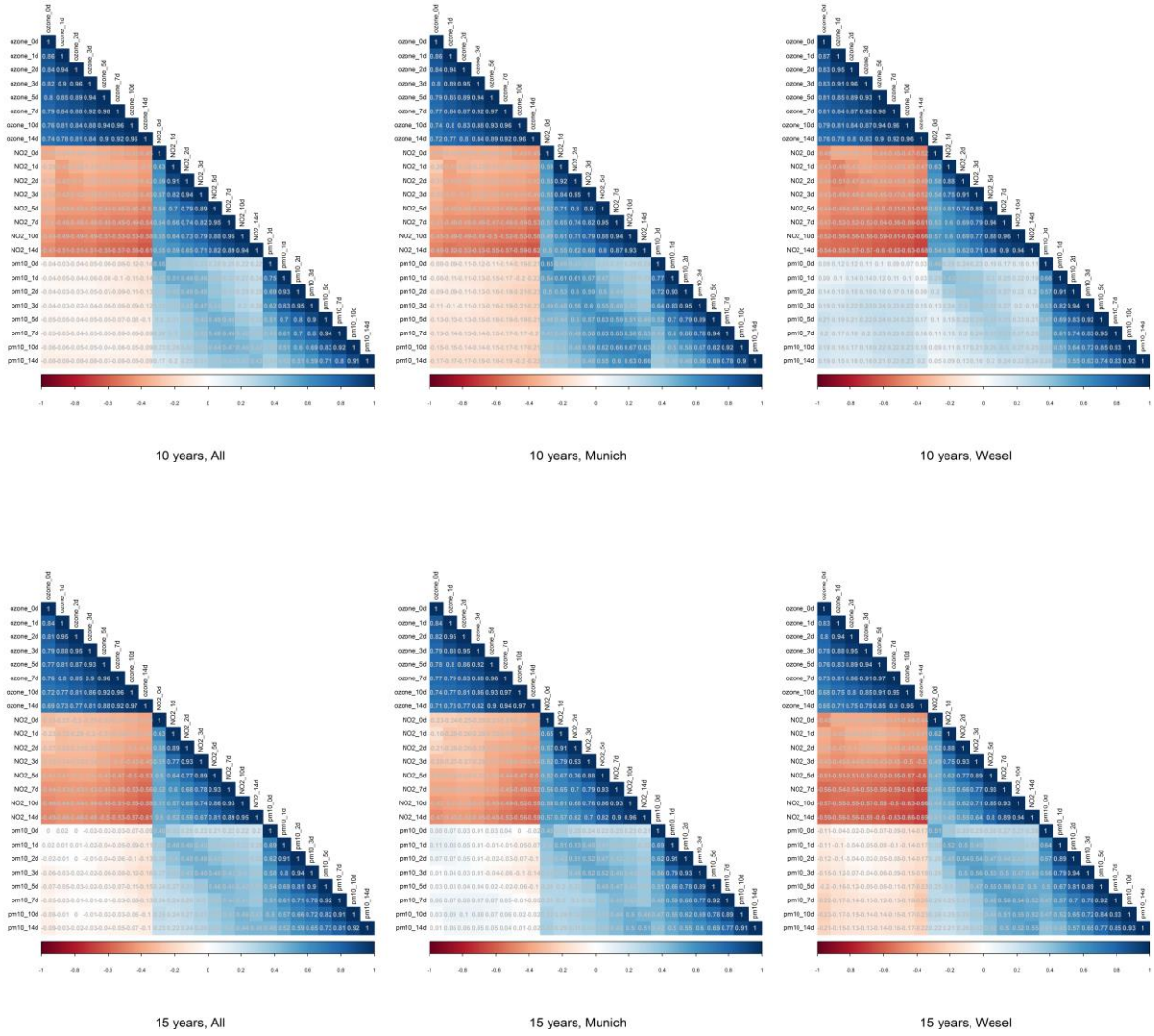

Figure S4. The heatmap of correlations for pollutants

Note:

Spearman correlation coefficients for relationships between different short-term pollutants (metrics). For ozone, we calculated concentration (µg/m³) of moving 8-h average for every hour (7 hours before and of the hour of interest) and thereby identified a maximum 8-h average for every day. The maximum of the daily maximum 8-h average concentration was selected over 0 (same day), 1, 2, 3, 5, 7, 10 and 14 days prior to the FeNO measurement or blood sampling. For NO_2 and PM_{10}, we utilized averages of the daily concentrations (µg/m³) of 0 (same day), 1, 2, 3, 5, 7, 10 and 14 days prior to the FeNO measurement or blood sampling (same time frame as ozone).

Table S3. Adjusted associations between short-term ozone and FeNO with interaction term area×ozone (Munich versus Wesel), at the age of 10 years

Area	Pollutant	10 years		10 years		10 years		
		Main model (ozone) (Percent change, 95% CI)	p value	Main model (area) (Percent change, 95% CI)	p value	Main model (area×ozone) (Percent change, 95% CI)	p value	Participants
All	Lag 0-day [a]	-4.17 (-10.95, 3.12)	0.255	-2.77 (-19.13, 16.89)	0.765	4.89 (-5.63, 16.59)	0.376	1241/1330
	Lag 0-1 days [b]	-4.54 (-11.81, 3.33)	0.251	-7.29 (-23.93, 12.98)	0.453	5.96 (-5.19, 18.42)	0.308	1227/1330
	Lag 0-2 days [c]	-3.77 (-11.50, 4.62)	0.367	-12.41 (-29.37, 8.64)	0.228	7.30 (-4.22, 20.21)	0.224	1259/1330
	Lag 0-3 days [d]	-4.74 (-12.46, 3.65)	0.260	-13.00 (-30.56, 9.00)	0.226	6.12 (-5.21, 18.81)	0.303	1284/1330
	Lag 0-5 days [e]	-4.31 (-11.70, 3.69)	0.282	-16.52 (-33.22, 4.34)	0.113	7.28 (-3.23, 18.94)	0.182	1311/1330
	Lag 0-7 days [f]	-5.07 (-12.41, 2.87)	0.205	-18.09 (-34.34, 2.18)	0.077	8.49 (-1.73, 19.77)	0.107	1321/1330
	Lag 0-10 days [g]	-7.50 (-15.28, 1.00)	0.082	**-22.14 (-38.40, -1.59)**	**0.037**	10.13 (-0.38, 21.74)	0.060	1325/1330
	Lag 0-14 days [h]	-8.30 (-16.53, 0.75)	0.071	-21.57 (-38.62, 0.23)	0.052	10.52 (-0.12, 22.28)	0.053	1327/1330

Note:

Abbreviation: FeNO, fractional concentration of exhaled nitric oxide; CI, confidence interval

1. All estimates were scaled by an interquartile range increase according to specific areas (see Table 2), except the model for area (Main model (area)). Percent change was back transformed from ln-transformed FeNO
2. Main model: all estimates were adjusted for the interaction term area×ozone, exact age, sex, time spent outside, physical activity level, season and time of the FeNO measurement or blood sampling, net equivalent household income, cohort, and area
3. Participants, "sample number analyzed/total number analyzed"; missing values were due to a lack of exposure data

a. *h.* The maximum of the daily maximum 8-hour average concentration was selected over 0, and the period between 0 day and the days of 1, 2, 3, 5, 7, 10 and 14 days prior to the FeNO measurement, from the background monitor stations

Table S4. Fully adjusted associations between short-term ozone and FeNO at the ages of 10 and 15 years

Area	Pollutant	10 years			15 years		
		Fully adjusted model (Percent change, 95% CI)	p value	Participants	Fully adjusted model Percent change, 95% CI	p value	Participants
Munich	Lag 0-day [a]	-4.85 (-12.17, 3.07)	0.223	835/843	5.36 (-0.13, 12.60)	0.055	911/922
	Lag 0-1 days [b]	-4.60 (-12.48, 3.99)	0.284	830/843	**6.92 (0.68, 15.42)**	**0.031**	921/922
	Lag 0-2 days [c]	-3.30 (-11.70, 5.90)	0.469	834/843	6.05 (-0.37, 14.51)	0.063	922/922
	Lag 0-3 days [d]	-5.14 (-13.44, 3.949	0.258	837/843	2.96 (-3.55, 10.69)	0.351	922/922
	Lag 0-5 days [e]	-5.07 (-13.09, 3.69)	0.248	842/843	1.03 (-5.59, 8.37)	0.744	922/922
	Lag 0-7 days [f]	-5.52 (-13.63, 3.35)	0.215	842/843	3.04 (-3.58, 10.92)	0.347	922/922
	Lag 0-10 days [g]	-9.31 (-17.96, 0.259	0.056	842/843	0.04 (-7.23, 7.89)	0.990	922/922
	Lag 0-14 days [h]	-10.41 (-19.76, 0.04)	0.051	843/843	-0.79 (-8.44, 7.30)	0.826	922/922
Wesel	Lag 0-day [a]	0.30 (-8.49, 9.93)	0.948	406/487	6.76 (-0.11, 14.11)	0.054	622/669
	Lag 0-1 days [b]	2.13 (-7.53, 12.81)	0.677	397/487	**7.80 (0.46, 15.68)**	**0.037**	626/669
	Lag 0-2 days [c]	3.21 (-6.42, 13.83)	0.527	425/487	**9.71 (2.42, 17.52)**	**0.008**	648/669
	Lag 0-3 days [d]	2.05 (-6.97, 11.96)	0.667	447/487	**9.11 (1.84, 16.90)**	**0.013**	658/669
	Lag 0-5 days [e]	3.67 (-4.70, 12.78)	0.401	469/487	6.42 (-0.52, 13.84)	0.071	669/669
	Lag 0-7 days [f]	4.65 (-3.54, 13.53)	0.275	479/487	4.92 (-1.69, 11.98)	0.148	669/669
	Lag 0-10 days [g]	3.96 (-3.76, 12.30)	0.323	483/487	5.98 (-0.98, 13.44)	0.094	669/669
	Lag 0-14 days [h]	3.68 (-4.08, 12.06)	0.363	484/487	6.48 (-0.42, 13.85)	0.066	669/669
All	Lag 0-day [a]	-2.89 (-8.53, 3.09)	0.335	1241/1330	**6.13 (2.11, 11.30)**	**0.003**	1533/1591
	Lag 0-1 days [b]	-1.50 (-7.67, 5.08)	0.646	1227/1330	**6.96 (2.51, 12.73)**	**0.002**	1547/1591
	Lag 0-2 days [c]	0.07 (-6.33, 6.91)	0.982	1259/1330	**7.55 (3.09, 13.44)**	**0.001**	1570/1591
	Lag 0-3 days [d]	-1.74 (-7.92, 4.86)	0.597	1284/1330	**5.80 (1.29, 11.47)**	**0.013**	1580/1591
	Lag 0-5 days [e]	-0.42 (-6.32, 5.85)	0.891	1311/1330	3.94 (-0.62, 9.34)	0.088	1591/1591
	Lag 0-7 days [f]	-0.15 (-6.02, 6.09)	0.962	1321/1330	4.37 (-0.15, 9.81)	0.057	1591/1591
	Lag 0-10 days [g]	-1.23 (-7.16, 5.09)	0.695	1325/1330	3.55 (-1.27, 9.17)	0.144	1591/1591
	Lag 0-14 days [h]	-1.74 (-7.89, 4.83)	0.595	1327/1330	3.69 (-1.20, 9.42)	0.134	1591/1591

Note:

Abbreviation: FeNO, fractional concentration of exhaled nitric oxide; CI, confidence interval

1. All estimates were scaled by an interquartile range increase according to specific areas (see Table 2). Percent change was back transformed from ln-transformed FeNO
2. Fully adjusted model: all estimates were adjusted for sex, exact age, BMI, onset of puberty, secondhand smoke exposure at home, time spent in front of a screen, time spent outside, physical activity level, season and day time of the FeNO measurement or blood sampling, fasting state, parental education, maternal age at birth, net equivalent household income, single-parent family status, maternal smoking during pregnancy, allergy history of parents, cohort, and area (only for "all")
3. Participants, "sample number analyzed/total number analyzed"; missing values were due to a lack of exposure data

b. *h.* The maximum of the daily maximum 8-hour average concentration was selected over 0, and the period between 0 day and the days of 1, 2, 3, 5, 7, 10 and 14 days prior to the FeNO measurement or blood sampling, from the background monitor stations

Table S5. Fully adjusted associations between short-term ozone and FeNO at the ages of 10 and 15 years (excluded participants with current asthma)

Area	Pollutant	10 years			15 years		
		Main model (Percent change, 95% CI)	p value	Participants	Main model (Percent change, 95% CI)	p value	Participants
Munich	Lag 0-day [a]	-5.30 (-12.54, 2.55)	0.180	779/843	3.96 (-1.64, 10.94)	0.156	839/922
	Lag 0-1 days [b]	-4.65 (-12.48, 3.87)	0.275	774/843	5.41 (-0.85, 13.51)	0.087	847/922
	Lag 0-2 days [c]	-3.07 (-11.47, 6.14)	0.501	778/843	4.54 (-1.92, 12.63)	0.158	848/922
	Lag 0-3 days [d]	-5.68 (-13.94, 3.38)	0.211	781/843	1.25 (-5.31, 8.60)	0.689	848/922
	Lag 0-5 days [e]	-6.26 (-14.20, 2.41)	0.152	786/843	0.55 (-6.07, 7.78)	0.860	848/922
	Lag 0-7 days [f]	-6.40 (-14.46, 2.43)	0.150	786/843	2.70 (-3.91, 10.49)	0.400	848/922
	Lag 0-10 days [g]	**-9.62 (-18.29, -0.03)**	**0.049**	786/843	-0.43 (-7.73, 7.33)	0.900	848/922
	Lag 0-14 days [h]	**-11.49 (-20.83, -1.07)**	**0.032**	787/843	-0.96 (-8.64, 7.12)	0.790	848/922
Wesel	Lag 0-day [a]	2.47 (-6.28, 12.03)	0.592	373/487	5.65 (-1.05, 12.80)	0.100	568/669
	Lag 0-1 days [b]	4.83 (-4.68, 15.29)	0.331	364/487	**8.71 (1.43, 16.53)**	**0.018**	573/669
	Lag 0-2 days [c]	6.76 (-2.85, 17.31)	0.175	387/487	**9.37 (2.17, 17.09)**	**0.010**	593/669
	Lag 0-3 days [d]	5.97 (-3.12, 15.90)	0.206	408/487	**8.28 (1.13, 15.94)**	**0.022**	601/669
	Lag 0-5 days [e]	7.22 (-1.13, 16.27)	0.092	430/487	5.61 (-1.33, 13.04)	0.115	612/669
	Lag 0-7 days [f]	7.88 (-0.26, 16.69)	0.058	440/487	4.34 (-2.33, 11.46)	0.208	612/669
	Lag 0-10 days [g]	7.58 (-0.17, 15.94)	0.056	444/487	4.85 (-2.16, 12.35)	0.180	612/669
	Lag 0-14 days [h]	7.11 (-0.75, 15.60)	0.078	444/487	5.33 (-1.55, 12.70)	0.132	612/669
All	Lag 0-day [a]	-2.71 (-8.33, 3.26)	0.365	1152/1330	**4.32 (0.22, 9.28)**	**0.039**	1533/1591
	Lag 0-1 days [b]	-1.58 (-7.66, 4.90)	0.624	1138/1330	**5.68 (1.21, 11.27)**	**0.014**	1547/1591
	Lag 0-2 days [c]	0.45 (-5.91, 7.24)	0.893	1165/1330	**5.80 (1.26, 11.47)**	**0.013**	1570/1591
	Lag 0-3 days [d]	-1.41 (-7.56, 5.15)	0.665	1189/1330	3.86 (-0.73, 9.29)	0.097	1580/1591
	Lag 0-5 days [e]	-0.53 (-6.38, 5.68)	0.863	1216/1330	2.40 (-2.25, 7.65)	0.299	1591/1591
	Lag 0-7 days [f]	-0.11 (-5.95, 6.08)	0.970	1226/1330	3.18 (-1.43, 8.53)	0.169	1591/1591
	Lag 0-10 days [g]	-0.52 (-6.48, 5.82)	0.868	1230/1330	2.00 (-2.92, 7.50)	0.413	1591/1591
	Lag 0-14 days [h]	-1.17 (-7.38, 5.45)	0.722	1231/1330	2.48 (-2.50, 8.11)	0.318	1591/1591

Note:

Abbreviation: FeNO, fractional concentration of exhaled nitric oxide; CI, confidence interval

1. All estimates were scaled by an interquartile range increase according to specific areas (see Table 2). Percent change was back transformed from ln-transformed FeNO
2. Main model: all estimates were adjusted for the exact age, sex, time spent outside, physical activity level, season and day time of the FeNO measurement or blood sampling, net equivalent household income, cohort, and area (only for "all")
3. Participants, "sample number analyzed/total number analyzed"; missing values were due to a lack of exposure data

c. *h.* The maximum of the daily maximum 8-hour average concentration was selected over 0, and the period between 0 day and the days of 1, 2, 3, 5, 7, 10 and 14 days prior to the FeNO test or blood sampling, from the background monitor stations

Table S6. Adjusted associations between short-term ozone and FeNO at the age of 15 years (excluded participants who ever smoked, consumed alcohol or took any medication seven days prior to the FeNO measurement or blood sampling)

Area	Pollutant	15 years		
		Main model (Percent change, 95% CI)	p value	Participants
Munich	Lag 0-day [a]	3.96 (-3.28, 12.82)	0.267	567/922
	Lag 0-1 days [b]	6.70 (-1.52, 17.48)	0.106	573/922
	Lag 0-2 days [c]	6.42 (-1.88, 17.19)	0.123	574/922
	Lag 0-3 days [d]	5.07 (-3.37, 15.64)	0.225	574/922
	Lag 0-5 days [e]	2.60 (-5.85, 12.51)	0.526	574/922
	Lag 0-7 days [f]	4.06 (-4.40, 14.39)	0.329	574/922
	Lag 0-10 days [g]	2.86 (-6.22, 13.61)	0.517	574/922
	Lag 0-14 days [h]	0.99 (-8.60, 11.85)	0.830	574/922
Wesel	Lag 0-day [a]	6.70 (-1.13, 15.15)	0.095	484/669
	Lag 0-1 days [b]	7.39 (-0.82, 6.27)	0.079	495/669
	Lag 0-2 days [c]	**8.57 (0.54, 17.25)**	**0.036**	510/669
	Lag 0-3 days [d]	8.04 (-0.07, 16.81)	0.052	517/669
	Lag 0-5 days [e]	5.05 (-2.77, 13.51)	0.212	524/669
	Lag 0-7 days [f]	3.77 (-3.63, 11.75)	0.327	524/669
	Lag 0-10 days [g]	4.14 (-3.54, 12.44)	0.300	524/669
	Lag 0-14 days [h]	4.37 (-3.27, 12.62)	0.270	524/669
All	Lag 0-day [a]	4.83 (-0.26, 10.96)	0.062	1051/1591
	Lag 0-1 days [b]	**6.31 (0.78, 13.17)**	**0.026**	1068/1591
	Lag 0-2 days [c]	**6.95 (1.48, 13.86)**	**0.014**	1084/1591
	Lag 0-3 days [d]	**6.24 (0.64, 13.17)**	**0.029**	1091/1591
	Lag 0-5 days [e]	3.52 (-2.14, 10.07)	0.215	1098/1591
	Lag 0-7 days [f]	3.68 (-1.87, 10.13)	0.187	1098/1591
	Lag 0-10 days [g]	3.46 (-2.35, 10.18)	0.235	1098/1591
	Lag 0-14 days [h]	3.10 (-2.81, 9.87)	0.293	1098/1591

Note:

Abbreviation: FeNO, fractional concentration of exhaled nitric oxide; CI, confidence interval

1. All estimates were scaled by an interquartile range increase according to specific areas (see Table 2). Percent change was back transformed from ln-transformed FeNO
2. Main model: all estimates were adjusted for the exact age, sex, time spent outside, physical activity level, season and day time of the FeNO measurement or blood sampling, net equivalent household income, cohort, and area (only for "all")
3. Participants, "sample number analyzed/total number analyzed"; missing values were due to a lack of exposure data

a. *h.* The maximum of the daily maximum 8-hour average concentration was selected over 0, and the period between 0 day and the days of 1, 2, 3, 5, 7, 10 and 14 days prior to the FeNO test or blood sampling, from the background monitor stations

Table S7. Fully adjusted associations between short-term ozone and IL-6 at the age of 10 years

Area	Pollutant	Fully adjusted model (OR, 95% CI)	p value	Participants
Munich	Lag 0-day [a]	1.12 (0.83, 1.51)	0.446	835/843
	Lag 0-1 days [b]	1.24 (0.90, 1.71)	0.183	830/843
	Lag 0-2 days [c]	1.14 (0.82, 1.59)	0.441	834/843
	Lag 0-3 days [d]	1.16 (0.83, 1.62)	0.396	837/843
	Lag 0-5 days [e]	1.14 (0.82, 1.58)	0.434	842/843
	Lag 0-7 days [f]	1.27 (0.91, 1.77)	0.160	842/843
	Lag 0-10 days [g]	1.33 (0.92, 1.93)	0.132	842/843
	Lag 0-14 days [h]	1.32 (0.88, 1.98)	0.179	843/843
Wesel	Lag 0-day [a]	1.21 (0.82, 1.79)	0.341	406/487
	Lag 0-1 days [b]	1.01 (0.66, 1.55)	0.952	397/487
	Lag 0-2 days [c]	1.24 (0.84, 1.83)	0.276	425/487
	Lag 0-3 days [d]	1.15 (0.80, 1.66)	0.460	447/487
	Lag 0-5 days [e]	1.13 (0.81, 1.57)	0.467	469/487
	Lag 0-7 days [f]	1.09 (0.79, 1.50)	0.600	479/487
	Lag 0-10 days [g]	1.06 (0.78, 1.44)	0.709	483/487
	Lag 0-14 days [h]	1.09 (0.79, 1.49)	0.596	484/487
All	Lag 0-day [a]	1.15 (0.93, 1.44)	0.199	1241/1330
	Lag 0-1 days [b]	1.18 (0.93, 1.49)	0.171	1227/1330
	Lag 0-2 days [c]	1.20 (0.94, 1.52)	0.139	1259/1330
	Lag 0-3 days [d]	1.18 (0.94, 1.49)	0.153	1284/1330
	Lag 0-5 days [e]	1.16 (0.94, 1.44)	0.175	1311/1330
	Lag 0-7 days [f]	1.19 (0.96, 1.48)	0.111	1321/1330
	Lag 0-10 days [g]	1.18 (0.94, 1.47)	0.151	1325/1330
	Lag 0-14 days [h]	1.18 (0.94, 1.49)	0.159	1327/1330

Note:

Abbreviation: IL-6, interleukin-6; CI, confidence interval; OR, odds ratio

1. All estimates were scaled by an interquartile range increase according to specific areas (see Table 2)
2. Fully adjusted model: all estimates were adjusted for sex, exact age, BMI, onset of puberty, secondhand smoke exposure at home, time spent in front of a screen, time spent outside, physical activity level, season and day time of the FeNO measurement or blood sampling, fasting state, parental education, maternal age at birth, net equivalent household income, single-parent family status, maternal smoking during pregnancy, allergy history of parents, cohort, and area (only for "all")
3. Participants, "sample number analyzed/total number analyzed"; missing values were due to a lack of exposure data

a. *h.* The maximum of the daily maximum 8-hour average concentration was selected over 0, and the period between 0 day and the days of 1, 2, 3, 5, 7, 10 and 14 days prior to the FeNO measurement or blood sampling, from the background monitor stations

Table S8. Adjusted associations between ozone exposure and IL-6 at the age of 10 years (excluded participants with current asthma)

Area	Pollutant	IL-6		
		Main model (OR, 95% CI)	p value	Participants
Munich	Lag 0-day [a]	1.02 (0.76, 1.37)	0.901	779/843
	Lag 0-1 days [b]	1.17 (0.85, 1.61)	0.346	774/843
	Lag 0-2 days [c]	1.06 (0.76, 1.48)	0.732	778/843
	Lag 0-3 days [d]	1.11 (0.79, 1.56)	0.538	781/843
	Lag 0-5 days [e]	1.09 (0.79, 1.52)	0.592	786/843
	Lag 0-7 days [f]	1.17 (0.84, 1.63)	0.360	786/843
	Lag 0-10 days [g]	1.20 (0.82, 1.74)	0.345	786/843
	Lag 0-14 days [h]	1.09 (0.72, 1.64)	0.681	787/843
Wesel	Lag 0-day [a]	0.98 (0.68, 1.41)	0.904	373/487
	Lag 0-1 days [b]	0.85 (0.58, 1.25)	0.412	364/487
	Lag 0-2 days [c]	1.06 (0.74, 1.53)	0.747	387/487
	Lag 0-3 days [d]	1.04 (0.74, 1.47)	0.816	408/487
	Lag 0-5 days [e]	1.08 (0.79, 1.47)	0.627	430/487
	Lag 0-7 days [f]	1.06 (0.78, 1.43)	0.724	440/487
	Lag 0-10 days [g]	1.05 (0.78, 1.41)	0.758	444/487
	Lag 0-14 days [h]	1.10 (0.81, 1.49)	0.536	444/487
All	Lag 0-day [a]	1.02 (0.82, 1.28)	0.857	1152/1330
	Lag 0-1 days [b]	1.06 (0.84, 1.34)	0.633	1138/1330
	Lag 0-2 days [c]	1.08 (0.85, 1.37)	0.508	1165/1330
	Lag 0-3 days [d]	1.10 (0.87, 1.39)	0.419	1189/1330
	Lag 0-5 days [e]	1.10 (0.89, 1.37)	0.385	1216/1330
	Lag 0-7 days [f]	1.12 (0.90, 1.39)	0.314	1226/1330
	Lag 0-10 days [g]	1.12 (0.89, 1.39)	0.338	1230/1330
	Lag 0-14 days [h]	1.12 (0.89, 1.42)	0.333	1231/1330

Note:
Abbreviation: IL-6, interleukin-6; CI, confidence interval; OR, odds ratio

1. All estimates were scaled by an interquartile range increase according to specific areas (see Table 2)
2. Main model: all estimates were adjusted for the exact age, sex, time spent outside, physical activity level, season and day time of the FeNO measurement or blood sampling, net equivalent household income, cohort, and area (only for "all")
3. Participants, "sample number analyzed/total number analyzed"; missing values were due to a lack of exposure data

a. *h.* The maximum of the daily maximum 8-hour average concentration was selected over 0, and the period between 0 day and the days of 1, 2, 3, 5, 7, 10 and 14 days prior to the FeNO test or blood sampling, from the background monitor stations

Table S9. Fully adjusted associations between ozone and hs-CRP at the age of 10 years with ozone stratified by < 120 versus ≥ 120 µg/m³

Area	Pollutant	Fully adjusted model < 120 µg/m³ (OR, 95% CI)	p value	Participants	Fully adjusted model ≥ 120 µg/m³ (OR, 95% CI)	p value	Participants
Munich	Lag 0-day [a]	0.99 (0.93, 1.05)	0.644	775/843	0.97 (0.36, 2.64)	0.950	60/843
	Lag 0-1 days [b]	0.99 (0.93, 1.05)	0.638	785/843	1.67 (0.62, 4.49)	0.324	45/843
	Lag 0-2 days [c]	0.99 (0.93, 1.06)	0.848	775/843	2.15 (0.93, 4.94)	0.083	59/843
	Lag 0-3 days [d]	0.98 (0.92, 1.05)	0.544	771/843	1.63 (0.89, 3.01)	0.123	66/843
	Lag 0-5 days [e]	0.94 (0.88, 1.00)	0.065	724/843	1.30 (0.93, 1.81)	0.135	118/843
	Lag 0-7 days [f]	**0.93 (0.87, 0.99)**	**0.045**	708/843	1.24 (0.94, 1.63)	0.124	134/843
	Lag 0-10 days [g]	0.96 (0.88, 1.04)	0.284	679/843	1.22 (0.94, 1.58)	0.136	163/843
	Lag 0-14 days [h]	0.95 (0.86, 1.04)	0.282	647/843	1.11 (0.88, 1.40)	0.395	196/843
Wesel	Lag 0-day [a]	0.96 (0.88, 1.05)	0.388	373/487	0.40 (0.17, 0.91)	0.095	33/487
	Lag 0-1 days [b]	0.97 (0.88, 1.06)	0.493	354/487	0.85 (0.42, 1.73)	0.666	43/487
	Lag 0-2 days [c]	0.98 (0.88, 1.07)	0.609	374/487	0.92 (0.58, 1.47)	0.744	51/487
	Lag 0-3 days [d]	0.94 (0.85, 1.05)	0.287	376/487	1.05 (0.72, 1.53)	0.797	71/487
	Lag 0-5 days [e]	0.92 (0.82, 1.04)	0.180	373/487	1.15 (0.92, 1.44)	0.217	96/487
	Lag 0-7 days [f]	0.89 (0.79, 1.00)	0.060	363/487	1.01 (0.82, 1.26)	0.897	116/487
	Lag 0-10 days [g]	0.88 (0.77, 1.00)	0.055	348/487	1.02 (0.84, 1.22)	0.875	135/487
	Lag 0-14 days [h]	0.88 (0.76, 1.01)	0.075	330/487	1.09 (0.89, 1.34)	0.389	154/487
All	Lag 0-day [a]	0.98 (0.94, 1.03)	0.480	1241/1330	1.22 (0.76, 1.98)	0.413	93/1330
	Lag 0-1 days [b]	0.98 (0.93, 1.03)	0.461	1139/1330	0.94 (0.61, 1.45)	0.777	88/1330
	Lag 0-2 days [c]	0.99 (0.94, 1.04)	0.624	1149/1330	1.08 (0.78, 1.49)	0.652	110/1330
	Lag 0-3 days [d]	0.97 (0.92, 1.02)	0.288	1147/1330	1.19 (0.92, 1.55)	0.191	137/1330
	Lag 0-5 days [e]	**0.93 (0.88, 0.99)**	**0.014**	1097/1330	**1.24 (1.06, 1.45)**	**0.009**	214/1330
	Lag 0-7 days [f]	**0.92 (0.86, 0.97)**	**0.003**	1071/1330	1.14 (0.98, 1.32)	0.096	250/1330
	Lag 0-10 days [g]	**0.93 (0.87, 0.87)**	**0.028**	1027/1330	1.09 (0.95, 1.26)	0.220	298/1330
	Lag 0-14 days [h]	**0.92 (0.85, 0.99)**	**0.035**	977/1330	1.07 (0.93, 1.23)	0.373	350/1330

Note:

Abbreviation: hs-CRP, high sensitivity-C reactive protein; CI, confidence interval; OR, odds ratio

1. All estimates were scaled by an interquartile range increase according to specific areas (see Table 2)
2. Fully adjusted model: all estimates were adjusted for sex, exact age, BMI, onset of puberty, secondhand smoke exposure at home, time spent in front of a screen, time spent outside, physical activity level, season and day time of the FeNO measurement or blood sampling, fasting state, parental education, maternal age at birth, net equivalent household income, single-parent family status, maternal smoking during pregnancy, allergy history of parents, cohort, and area (only for "all")
3. Participants, "sample number analyzed/total number analyzed"; missing values were due to a lack of exposure data

a. *h.* The maximum of the daily maximum 8-hour average concentration was selected over 0, and the period between 0 day and the days of 1, 2, 3, 5, 7, 10 and 14 days prior to the FeNO measurement or blood sampling, from the background monitor stations

Table S10. Fully adjusted associations between ozone and hs-CRP at the age of 15 years with ozone stratified by < 120 versus ≥ 120 μg/m³

Area	Pollutant	Fully adjusted model < 120 μg/m³ (OR, 95% CI)	p value	Participants	Fully adjusted model ≥ 120 μg/m³ (OR, 95% CI)	p value	Participants
Munich	Lag 0-day [a]	0.98 (0.95, 1.01)	0.200	825/922	0.88 (0.46, 1.68)	0.698	59/922
	Lag 0-1 days [b]	1.01 (0.98, 1.04)	0.711	888/922	0.74 (0.36, 1.52)	0.460	33/922
	Lag 0-2 days [c]	1.01 (0.98, 1.04)	0.541	862/922	1.06 (0.70, 1.59)	0.794	66/922
	Lag 0-3 days [d]	1.01 (0.98, 1.05)	0.547	819/922	0.96 (0.78, 1.19)	0.717	103/922
	Lag 0-5 days [e]	1.00 (0.96, 1.03)	0.818	742/922	0.94 (0.79, 1.12)	0.477	180/922
	Lag 0-7 days [f]	0.99 (0.95, 1.03)	0.687	672/922	0.89 (0.77, 1.03)	0.117	250/922
	Lag 0-10 days [g]	0.97 (0.92, 1.02)	0.298	634/922	0.94 (0.81, 1.09)	0.434	288/922
	Lag 0-14 days [h]	0.97 (0.92, 1.03)	0.285	615/922	0.93 (0.80, 1.08)	0.349	307/922
Wesel	Lag 0-day [a]	0.99 (0.93, 1.05)	0.654	590/669	1.41 (0.82, 2.42)	0.283	32/669
	Lag 0-1 days [b]	**0.93 (0.87, 0.99)**	**0.021**	593/669	1.96 (0.75, 5.13)	0.241	33/669
	Lag 0-2 days [c]	**0.91 (0.85, 0.98)**	**0.012**	582/669	0.83 (0.55, 1.24)	0.365	66/669
	Lag 0-3 days [d]	**0.92 (0.85, 0.99)**	**0.031**	577/669	0.75 (0.57, 0.98)	0.038	81/669
	Lag 0-5 days [e]	**0.91 (0.84, 0.99)**	**0.024**	560/669	0.87 (0.70, 1.08)	0.211	109/669
	Lag 0-7 days [f]	**0.90 (0.83, 0.98)**	**0.017**	542/669	0.94 (0.79, 1.12)	0.489	127/669
	Lag 0-10 days [g]	**0.89 (0.81, 0.97)**	**0.011**	517/669	0.98 (0.83, 1.15)	0.776	152/669
	Lag 0-14 days [h]	0.95 (0.86, 1.05)	0.307	491/669	0.98 (0.85, 1.13)	0.772	178/669
All	Lag 0-day [a]	0.98 (0.95, 1.01)	0.189	1442/1591	1.21 (0.91, 1.62)	0.201	91/1591
	Lag 0-1 days [b]	0.98 (0.95, 1.01)	0.164	1481/1591	1.46 (0.98, 2.16)	0.070	66/1591
	Lag 0-2 days [c]	0.98 (0.94, 1.01)	0.160	1444/1591	0.96 (0.75, 1.23)	0.742	126/1591
	Lag 0-3 days [d]	0.97 (0.94, 1.01)	0.109	1396/1591	0.92 (0.79, 1.08)	0.316	184/1591
	Lag 0-5 days [e]	**0.96 (0.92, 0.99)**	**0.023**	1302/1591	0.98 (0.87, 1.11)	0.731	289/1591
	Lag 0-7 days [f]	**0.95 (0.91, 0.99)**	**0.012**	1214/1591	1.02 (0.93, 1.13)	0.640	377/1591
	Lag 0-10 days [g]	**0.93 (0.88, 0.97)**	**0.002**	1151/1591	1.05 (0.95, 1.16)	0.311	440/1591
	Lag 0-14 days [h]	**0.94 (0.90, 0.99)**	**0.020**	1106/1591	1.03 (0.95, 1.13)	0.452	485/1591

Note:

Abbreviation: hs-CRP, high sensitivity-C reactive protein; CI, confidence interval; OR, odds ratio

1. All estimates were scaled by an interquartile range increase according to specific areas (see Table 2)
2. Fully adjusted model: all estimates were adjusted for sex, exact age, BMI, onset of puberty, secondhand smoke exposure at home, time spent in front of a screen, time spent outside, physical activity level, season and day time of the FeNO measurement or blood sampling, fasting state, parental education, maternal age at birth, net equivalent household income, single-parent family status, maternal smoking during pregnancy, allergy history of parents, cohort, and area (only for "all")
3. Participants, "sample number analyzed/total number analyzed"; missing values were due to a lack of exposure data

a. *h.* The maximum of the daily maximum 8-hour average concentration was selected over 0, and the period between 0 day and the days of 1, 2, 3, 5, 7, 10 and 14 days prior to the FeNO measurement or blood sampling, from the background monitor station

Table S11. Adjusted associations between ozone exposure and hs-CRP at the ages of 10 and 15 years (excluded participants with current asthma)

Area	Pollutant	10 years hs-CRP						15 years hs-CRP					
		Main model < 120 μg/m^3 (OR, 95% CI)	p value	Participants	Main model ≥ 120 μg/m^3 (OR, 95% CI)	p value	Participants	Main model < 120 μg/m^3 (OR, 95% CI)	p value	Participants	Main model ≥ 120 μg/m^3 (OR, 95% CI)	p value	Participants
Munich	Lag 0-day [a]	0.97 (0.91, 1.03)	0.346	724/843	1.45 (0.57, 3.68)	0.440	55/843	0.98 (0.96, 1.01)	0.300	59/922	0.93 (0.55, 1.57)	0.785	59/922
	Lag 0-1 days [b]	0.95 (0.90, 1.02)	0.206	732/843	2.03 (0.86, 4.78)	0.115	42/843	1.01 (0.98, 1.04)	0.693	33/922	1.02 (0.71, 1.47)	0.902	33/922
	Lag 0-2 days [c]	0.96 (0.89, 1.02)	0.219	723/843	1.97 (0.95, 4.09)	0.074	55/843	1.01 (0.98, 1.05)	0.478	66/922	0.85 (0.57, 1.27)	0.431	66/922
	Lag 0-3 days [d]	0.95 (0.89, 1.01)	0.079	719/843	**1.92 (1.05, 3.54)**	**0.040**	62/843	1.01 (0.98, 1.05)	0.496	103/922	0.96 (0.76, 1.22)	0.744	103/922
	Lag 0-5 days [e]	**0.91 (0.85, 0.98)**	**0.002**	675/843	**1.47 (1.06, 2.04)**	**0.023**	111/843	1.00 (0.97, 1.04)	0.848	180/922	1.03 (0.86, 1.24)	0.715	180/922
	Lag 0-7 days [f]	**0.90 (0.83, 0.97)**	**< 0.001**	660/843	1.30 (0.99, 1.71)	0.057	126/843	1.00 (0.96, 1.05)	0.947	250/922	0.95 (0.82, 1.11)	0.522	250/922
	Lag 0-10 days [g]	**0.91 (0.84, 0.99)**	**0.003**	634/843	**1.34 (1.03, 1.73)**	**0.029**	152/843	0.99 (0.94, 1.04)	0.591	288/922	0.99 (0.85, 1.15)	0.892	288/922
	Lag 0-14 days [h]	**0.89 (0.81, 0.99)**	**0.002**	606/843	1.19 (0.93, 1.50)	0.162	181/843	0.98 (0.92, 1.03)	0.418	307/922	0.97 (0.83, 1.13)	0.656	307/922
Wesel	Lag 0-day [a]	1.00 (0.91, 1.10)	0.971	342/487	0.64 (0.34, 1.22)	0.195	373/487	0.98 (0.91, 1.05)	0.528	32/669	1.37 (0.83, 2.26)	0.236	32/669
	Lag 0-1 days [b]	0.99 (0.90, 1.10)	0.874	322/487	0.85 (0.58, 1.23)	0.391	354/487	**0.93 (0.87, 0.99)**	**0.023**	33/669	1.16 (0.62, 2.17)	0.655	33/669
	Lag 0-2 days [c]	1.00 (0.90, 1.11)	0.986	337/487	0.92 (0.66, 1.28)	0.618	374/487	**0.92 (0.85, 0.99)**	**0.031**	66/669	1.06 (0.75, 1.49)	0.738	66/669
	Lag 0-3 days [d]	0.97 (0.87, 1.09)	0.610	339/487	0.90 (0.67, 1.22)	0.514	376/487	**0.90 (0.84, 0.98)**	**0.015**	81/669	0.85 (0.65, 1.10)	0.217	81/669
	Lag 0-5 days [e]	0.94 (0.83, 1.06)	0.305	336/487	1.06 (0.86, 1.32)	0.587	373/487	**0.88 (0.81, 0.95)**	**0.002**	109/669	0.95 (0.76, 1.18)	0.658	109/669
	Lag 0-7 days [f]	0.91 (0.80, 1.04)	0.153	327/487	1.03 (0.84, 1.28)	0.754	363/487	**0.87 (0.80, 0.95)**	**0.002**	127/669	0.95 (0.80, 1.14)	0.598	127/669
	Lag 0-10 days [g]	0.90 (0.78, 1.04)	0.147	315/487	0.98 (0.81, 1.19)	0.843	348/487	**0.85 (0.77, 0.94)**	**0.001**	152/669	1.02 (0.86, 1.21)	0.833	152/669
	Lag 0-14 days [h]	0.88 (0.75, 1.03)	0.109	300/487	0.96 (0.77, 1.18)	0.677	330/487	0.91 (0.82, 1.02)	0.094	178/669	0.97 (0.84, 1.12)	0.691	178/669
All	Lag 0-day [a]	0.98 (0.93, 1.03)	0.253	1066/1330	1.25 (0.79, 1.99)	0.346	86/1330	0.98 (0.95, 1.01)	0.252	91/1591	1.24 (0.92, 1.66)	0.158	91/1591
	Lag 0-1 days [b]	0.97 (0.92, 1.02)	0.142	1054/1330	0.96 (0.69, 1.34)	0.817	84/1330	0.98 (0.95, 1.01)	0.212	66/1591	1.19 (0.87, 1.62)	0.289	66/1591
	Lag 0-2 days [c]	0.97 (0.91, 1.02)	0.198	1060/1330	1.12 (0.84, 1.49)	0.441	105/1330	0.98 (0.95, 1.02)	0.301	126/1591	1.06 (0.84, 1.34)	0.613	126/1591
	Lag 0-3 days [d]	0.95 (0.90, 1.01)	0.125	1058/1330	1.19 (0.93, 1.53)	0.172	131/1330	0.97 (0.94, 1.01)	0.109	184/1591	0.96 (0.82, 1.12)	0.587	184/1591
	Lag 0-5 days [e]	**0.91 (0.86, 0.97)**	**0.010**	1011/1330	**1.22 (1.03, 1.44)**	**0.022**	205/1330	**0.95 (0.91, 0.99)**	**0.009**	289/1591	1.05 (0.92, 1.19)	0.499	289/1591
	Lag 0-7 days [f]	**0.89 (0.84, 0.95)**	**0.004**	987/1330	1.15 (0.98, 1.34)	0.090	239/1330	**0.94 (0.90, 0.99)**	**0.009**	377/1591	1.05 (0.94, 1.16)	0.376	377/1591
	Lag 0-10 days [g]	**0.90 (0.84, 0.96)**	**0.036**	949/1330	1.10 (0.94, 1.28)	0.231	281/1330	**0.92 (0.88, 0.97)**	**0.002**	440/1591	1.08 (0.97, 1.19)	0.164	440/1591
	Lag 0-14 days [h]	**0.88 (0.81, 0.96)**	**0.031**	906/1330	1.04 (0.90, 1.22)	0.583	325/1330	**0.93 (0.89, 0.99)**	**0.013**	485/1591	1.03 (0.94, 1.13)	0.529	485/1591

Note:

Abbreviation: hs-CRP, high sensitivity-C reactive protein; CI, confidence interval; OR, odds ratio

1. All estimates were scaled by an interquartile range increase according to specific areas (see Table 2)
2. Main model: all estimates were adjusted for the exact age, sex, time spent outside, physical activity level, season and day time of the FeNO measurement or blood sampling, net equivalent household income, cohort, and area (only for "all")
3. Participants, "sample number analyzed/total number analyzed"; missing values were due to a lack of exposure data

b. *h.* The maximum of the daily maximum 8-hour average concentration was selected over 0, and the period between 0 day and the days of 1, 2, 3, 5, 7, 10 and 14 days prior to the FeNO test or blood sampling, from the background monitor stations

Table S12. Adjusted associations between ozone exposure and hs-CRP at the age of 15 years (excluded participants who ever smoked, consumed alcohol or took any medication seven days prior to the FeNO measurement or blood sampling)

Area	Pollutant	15 years hs-CRP					
		Main model < 120 µg/m^3 (OR, 95% CI)	p value	Participants	Main model ≥ 120 µg/m^3 (OR, 95% CI)	p value	Participants
Munich	Lag 0-day [c]	0.99 (0.95, 1.02)	0.427	553/922	0.98 (0.57, 1.69)	0.953	34/922
	Lag 0-1 days [d]	1.00 (0.97, 1.05)	0.807	558/922	0.40 (0.00, 3230.18)	0.873	15/922
	Lag 0-2 days [e]	1.01 (0.97, 1.06)	0.543	540/922	1.06 (0.49, 2.29)	0.888	34/922
	Lag 0-3 days [f]	1.02 (0.98, 1.07)	0.364	517/922	0.96 (0.69, 1.35)	0.834	57/922
	Lag 0-5 days	1.02 (0.97, 1.07)	0.455	464/922	0.99 (0.76, 1.30)	0.954	110/922
	Lag 0-7 days [g]	1.02 (0.97, 1.08)	0.435	514/922	0.90 (0.73, 1.11)	0.322	159/922
	Lag 0-10 days	1.01 (0.94, 1.07)	0.872	389/922	0.97 (0.78, 1.19)	0.758	185/922
	Lag 0-14 days	1.01 (0.94, 1.08)	0.823	377/922	0.92 (0.75, 1.13)	0.432	197/922
Wesel	Lag 0-day [c]	1.02 (0.95, 1.10)	0.615	457/669	1.75 (1.08, 2.82)	0.041	27/669
	Lag 0-1 days [d]	0.94 (0.87, 1.01)	0.077	471/669	1.25 (0.66, 2.38)	0.505	24/669
	Lag 0-2 days [e]	0.93 (0.86, 1.01)	0.079	457/669	0.98 (0.69, 1.40)	0.915	53/669
	Lag 0-3 days [f]	**0.92 (0.84, 0.99)**	**0.048**	456/669	0.79 (0.59, 1.06)	0.124	61/669
	Lag 0-5 days	**0.91 (0.83, 0.99)**	**0.042**	441/669	0.98 (0.77, 1.24)	0.838	83/669
	Lag 0-7 days [g]	**0.89 (0.81, 0.98)**	**0.020**	425/669	0.97 (0.80, 1.17)	0.738	99/669
	Lag 0-10 days	**0.88 (0.79, 0.98)**	**0.019**	406/669	1.06 (0.89, 1.27)	0.499	118/669
	Lag 0-14 days	0.95 (0.84, 1.07)	0.389	382/669	1.01 (0.86, 1.18)	0.932	142/669
All	Lag 0-day [c]	1.00 (0.96, 1.03)	0.818	990/1591	1.30 (0.93, 1.83)	0.130	61/1591
	Lag 0-1 days [d]	0.99 (0.95, 1.03)	0.475	1029/1591	1.17 (0.76, 1.78)	0.484	39/1591
	Lag 0-2 days [e]	0.99 (0.95, 1.03)	0.561	997/1591	0.95 (0.72, 1.26)	0.741	87/1591
	Lag 0-3 days [f]	0.98 (0.93, 1.02)	0.326	973/1591	0.87 80.71, 1.06)	0.181	118/1591
	Lag 0-5 days	0.96 (0.92, 1.01)	0.125	905/1591	1.02 (0.86, 1.20)	0.849	193/1591
	Lag 0-7 days [g]	0.95 (0.90, 1.01)	0.080	840/1591	1.04 (0.92, 1.18)	0.524	258/1591
	Lag 0-10 days	**0.94 (0.88, 0.99)**	**0.035**	795/1591	1.08 (0.96, 1.22)	0.196	303/1591
	Lag 0-14 days	0.96 (0.90, 1.02)	0.186	759/1591	1.03 (0.92, 1.15)	0.600	339/1591

Note:

Abbreviation: hs-CRP, high sensitivity-C reactive protein; CI, confidence interval; OR, odds ratio

1. All estimates were scaled by an interquartile range increase according to specific areas (see Table 2)
2. Main model: all estimates were adjusted for the exact age, sex, time spent outside, physical activity level, season and day time of the FeNO measurement or blood sampling, net equivalent household income, cohort, and area (only for "all")
3. Participants, "sample number analyzed/total number analyzed"; missing values were due to a lack of exposure data

b. *h.* The maximum of the daily maximum 8-hour average concentration was selected over 0, and the period between 0 day and the days of 1, 2, 3, 5, 7, 10 and 14 days prior to the FeNO test or blood sampling, from the background monitor stations

Table S13. Adjusted associations between ozone and hs-CRP at the age of 10 years with ozone stratified by < 110 versus ≥ 110 µg/m³

Area	Pollutant	10 years C hs-RP						15 years hs-CRP					
		Main model < 110 µg/m³ (OR, 95% CI)	p value	Participants	Main model ≥ 110 µg/m³ (OR, 95% CI)	p value	Participants	Main model < 110 µg/m³ (OR, 95% CI)	p value	Participants	Main model ≥ 110 µg/m³ (OR, 95% CI)	p value	Participants
Munich	Lag 0-day [c]	0.98 (0.92, 1.04)	0.474	743/843	1.65 (0.99, 2.71)	0.052	92/843	0.98 (0.95, 1.01)	0.258	781/922	1.02 (0.84, 1.24)	0.860	130/922
	Lag 0-1 days [d]	0.95 (0.89, 1.01)	0.107	754/843	0.97 (0.54, 1.76)	0.931	76/843	0.99 (0.96, 1.03)	0.758	796/922	1.09 (0.90, 1.31)	0.373	125/922
	Lag 0-2 days [e]	0.96 (0.90, 1.03)	0.275	743/843	1.44 (0.86, 2.40)	0.166	91/843	1.00 (0.96, 1.04)	0.963	748/922	1.05 (0.89, 1.24)	0.557	174/922
	Lag 0-3 days [f]	0.94 (0.88, 1.01)	0.096	710/843	1.17 (0.77, 1.78)	0.458	127/843	1.00 (0.96, 1.04)	0.945	693/922	1.06 (0.93, 1.20)	0.405	229/922
	Lag 0-5 days	**0.92 (0.85, 0.99)**	**0.046**	627/843	**1.45 (1.14, 1.83)**	**0.002**	215/843	0.99 (0.95, 1.04)	0.798	618/922	1.06 (0.94, 1.21)	0.338	304/922
	Lag 0-7 days [g]	**0.90 (0.83, 0.98)**	**0.019**	595/843	**1.31 (1.07, 1.61)**	**0.008**	247/843	0.99 (0.95, 1.04)	0.817	563/922	1.02 (0.91, 1.15)	0.682	359/922
	Lag 0-10 days	0.96 (0.87, 1.06)	0.394	559/843	**1.32 (1.09, 1.60)**	**0.004**	283/843	1.00 (0.94, 1.05)	0.865	528/922	1.08 (0.96, 1.21)	0.191	394/922
	Lag 0-14 days	0.96 (0.85, 1.09)	0.573	523/843	1.16 (0.97, 1.38)	0.100	320/843	0.99 (0.92, 1.05)	0.684	479/922	1.07 (0.96, 1.18)	0.221	443/922
Wesel	Lag 0-day [c]	1.01 (0.91, 1.11)	0.898	363/487	0.82 (0.54, 1.24)	0.347	43/487	0.97 (0.90, 1.03)	0.326	573/669	1.06 (0.75, 1.48)	0.751	49/669
	Lag 0-1 days [d]	0.98 (0.88, 1.09)	0.724	338/487	0.74 (0.55, 1.00)	0.051	59/487	**0.93 (0.86, 0.99)**	**0.037**	564/669	1.33 (0.94, 1.87)	0.109	62/669
	Lag 0-2 days [e]	0.99 (0.88, 1.10)	0.809	256/487	0.98 (0.98, 1.29)	0.888	69/487	**0.90 (0.83, 0.97)**	**0.009**	553/669	1.08 (0.84, 1.40)	0.543	95/669
	Lag 0-3 days [f]	0.97 (0.86, 1.09)	0.575	360/487	1.00 (0.78, 1.28)	0.989	87/487	**0.89 (0.82, 0.97)**	**0.007**	540/669	0.94 (0.77, 1.14)	0.515	118/669
	Lag 0-5 days	0.97 (0.85, 1.10)	0.607	351/487	1.16 (0.97, 1.39)	0.107	118/487	**0.89 80.82, 0.97)**	**0.011**	523/669	1.02 (0.87, 1.20)	0.778	146/669
	Lag 0-7 days [g]	0.96 (0.83, 1.10)	0.523	346/487	1.09 (0.92, 1.29)	0.334	133/487	**0.89 (0.81, 0.98)**	**0.015**	491/669	1.04 (0.91, 1.20)	0.547	178/669
	Lag 0-10 days	1.00 (0.85, 1.17)	0.989	325/487	1.05 (0.89, 1.23)	0.564	158/487	**0.90 (0.80, 0.99)**	**0.044**	447/669	1.06 (0.92, 1.22)	0.408	220/669
	Lag 0-14 days	0.96 (0.80, 1.15)	0.642	304/487	1.06 (0.91, 1.22)	0.474	180/487	0.97 (0.86, 1.08)	0.557	409/669	0.98 (0.87, 1.11)	0.760	260/669
All	Lag 0-day [c]	0.99 (0.94, 1.04)	0.656	1106/1330	**1.36 (1.02, 1.82)**	**0.039**	135/1330	0.97 (0.94, 1.00)	0.091	1354/1591	1.05 (0.90, 1.23)	0.538	179/1591
	Lag 0-1 days [d]	0.96 (0.91, 1.01)	0.114	1092/1330	0.89 (0.69, 1.16)	0.384	135/1330	**0.96 (0.93, 0.99)**	**0.043**	1360/1591	**1.26 (1.05, 1.50)**	**0.012**	187/1591
	Lag 0-2 days [e]	0.96 (0.91, 1.02)	0.226	1099/1330	1.11 (0.89, 1.40)	0.360	160/1330	**0.96 (0.92, 0.99)**	**0.032**	1301/1591	1.07 (0.93, 1.24)	0.335	269/1591
	Lag 0-3 days [f]	0.94 (0.89, 1.00)	0.063	1070/1330	1.14 (0.93, 1.40)	0.201	214/1330	**0.95 (0.91, 0.99)**	**0.015**	1233/1591	1.01 (0.90, 1.12)	0.891	347/1591
	Lag 0-5 days	**0.93 (0.87, 0.99)**	**0.036**	978/1330	**1.28 (1.12, 1.47)**	**< 0.001**	333/1330	**0.94 (0.90, 0.98)**	**0.005**	1141/1591	1.06 (0.97, 1.17)	0.207	450/1591
	Lag 0-7 days [g]	**0.91 (0.85, 0.98)**	**0.009**	941/1330	**1.22 (1.08, 1.39)**	**0.001**	380/1330	**0.94 (0.90, 0.99)**	**0.014**	1054/1591	1.08 (0.99, 1.17)	0.063	537/1591
	Lag 0-10 days	0.95 (0.87, 1.03)	0.224	884/1330	**1.17 (1.04, 1.32)**	**0.010**	441/1330	**0.94 (0.89, 0.99)**	**0.021**	975/1591	**1.11 (1.02, 1.21)**	**0.013**	616/1591
	Lag 0-14 days	0.94 (0.85, 1.03)	0.200	827/1330	1.10 (0.99, 1.23)	0.085	500/1330	**0.94 (0.89, 0.99)**	**0.043**	888/1591	1.05 (0.97, 1.13)	0.210	703/1591

Note:

Abbreviation: hs-CRP, high sensitivity-C reactive protein; CI, confidence interval; OR, odds ratio

1. All estimates were scaled by an interquartile range increase according to specific areas (see Table 2)
2. Main model: all estimates were adjusted for the exact age, sex, time spent outside, physical activity level, season and day time of the FeNO measurement or blood sampling, net equivalent household income, cohort, and area (only for "all")
3. Participants, "sample number analyzed/total number analyzed"; missing values were due to a lack of exposure data

a. *h.* The maximum of the daily maximum 8-hour average concentration was selected over 0, and the period between 0 day and the days of 1, 2, 3, 5, 7, 10 and 14 days prior to the FeNO test or blood sampling, from the background monitor stations

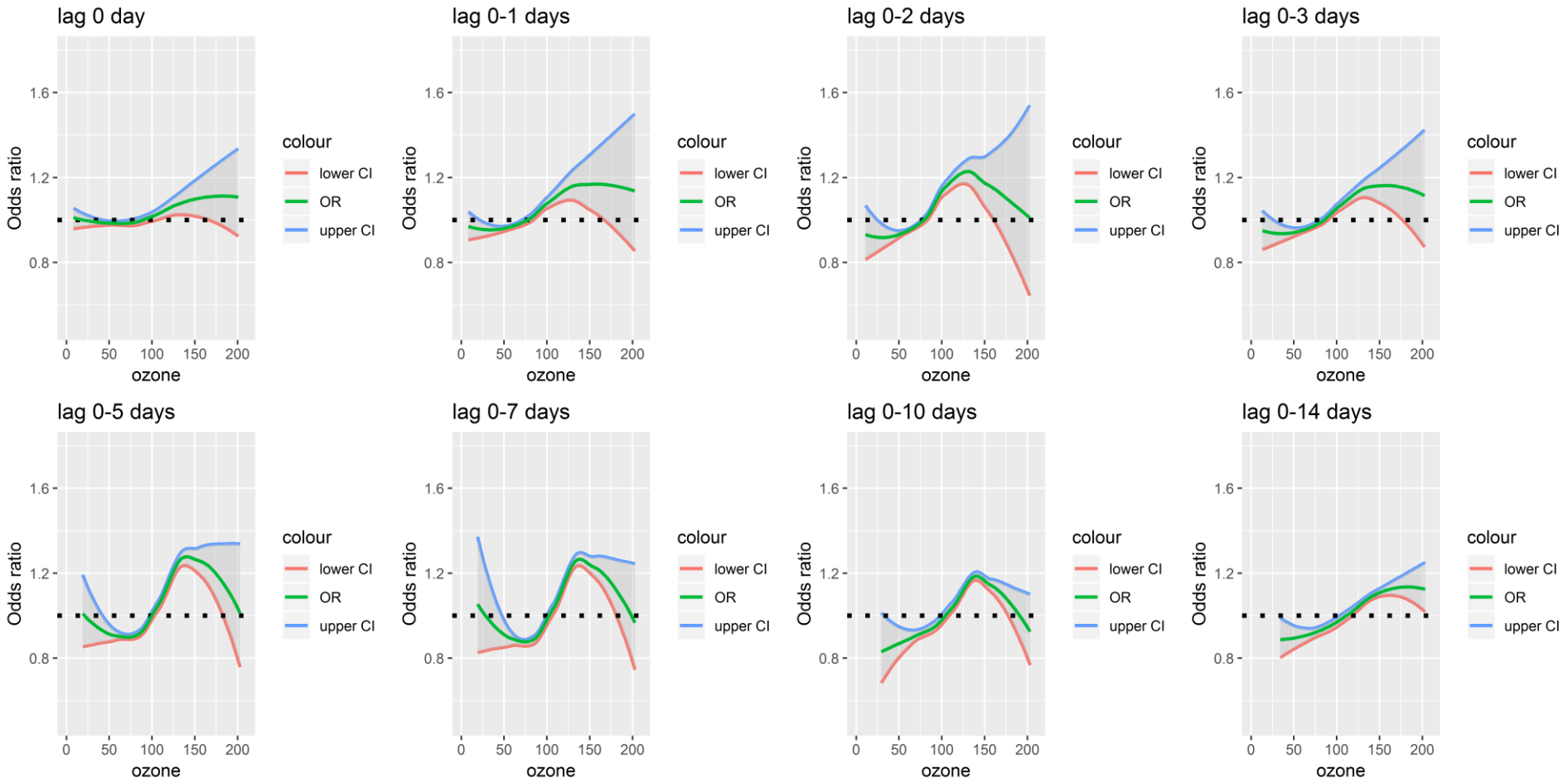

Figure S5. Odds ratios for a 10 µg/m³ increase in short-term ozone exposure (lag 0 day to lag 0-14 days) and hs-CRP at the age of 10 years in the combined populations (area "All") based on main model

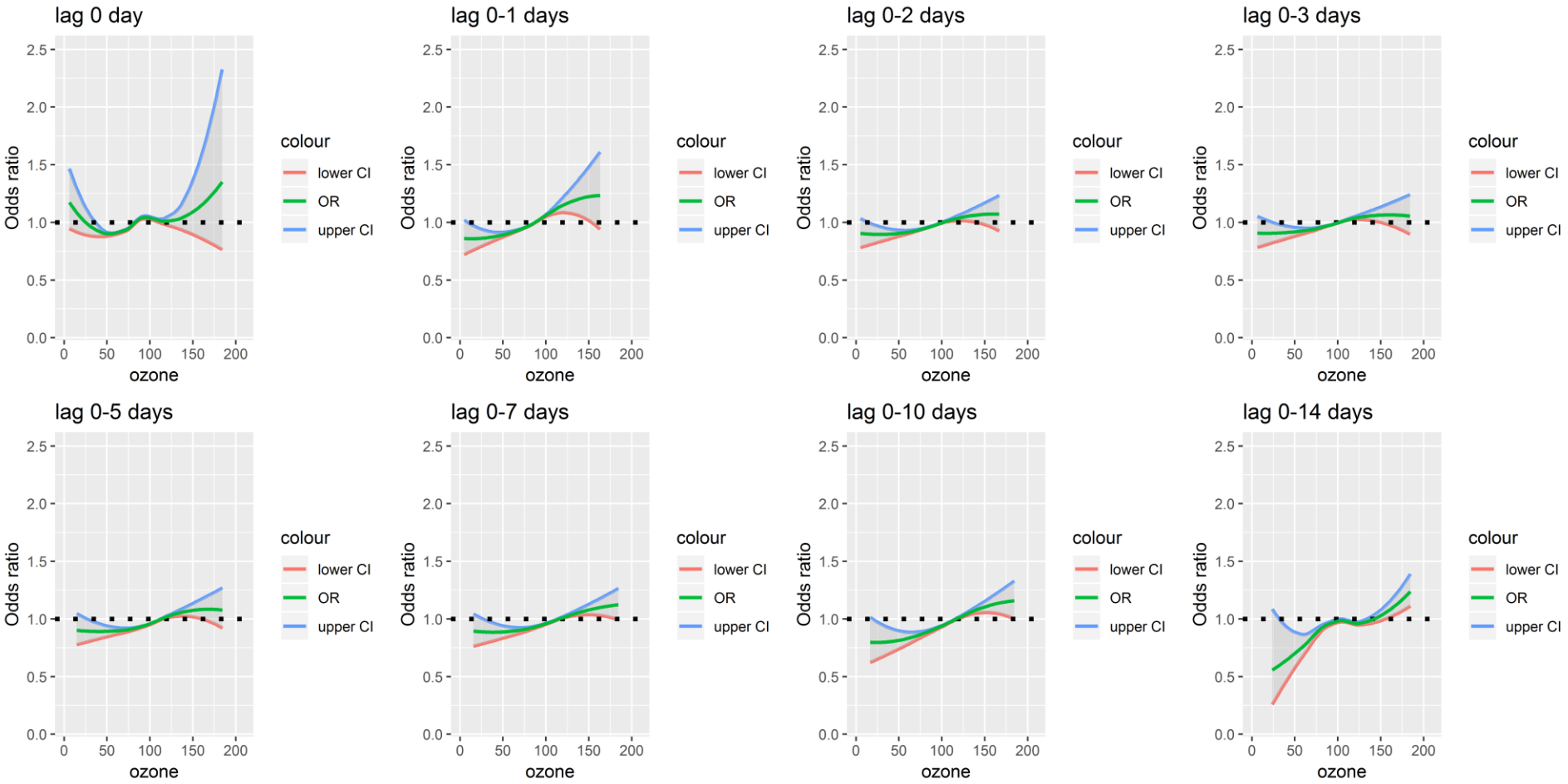

Figure S6. Odds ratios for a 10 µg/m³ increase in short-term ozone exposure (lag 0 day to lag 0-14 days) and hs-CRP at the age of 15 years in the combined populations (area "All") based on main model

Appendix: Systematic review on ozone and mental health

Title of article:	Ambient ozone exposure and mental health: A systematic review of epidemiological studies
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Review article

Ambient ozone exposure and mental health: A systematic review of epidemiological studies

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ABSTRACT

Background: An increasing number of studies have suggested adverse effects of air pollution on mental health. Given the potentially negative impacts of ozone exposure on the immune and nervous system driven from animal experiments, ozone might also affect mental health. However, no systematic synthesis of the relevant literature has been conducted yet. This paper reviews the studies that assessed the link between ozone exposure and mental health thus far.

Methods: We followed the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA). PubMed, Web of Science, and EMBASE were systematically searched for epidemiological studies on ambient ozone exposure and mental or behavioral disorders according to the International Classification of Disease. The period was from January 1st, 1960 to December 14st, 2017. We evaluated the risk of bias by the Office of Health Assessment and Translation (OHAT) Approach and Navigation Guide for each included study.

Results: The keyword search yielded 567 results. 31 papers met the selection criteria and were included in the review. We found only inconclusive evidence that ozone affects autism spectrum disorders, impairment of cognitive functions and dementia, depression, and suicide. The large heterogeneity of study designs, outcome definitions and study quality in general prevented us from conducting meta-analyses.

Conclusions: Current evidence for an association between ambient ozone exposure and mental health outcomes is inconclusive and further high quality studies are needed to assess any potential links given the strong biologic plausibility.

1. Introduction

More than a decade ago, it was proposed that the central nervous system (CNS) may be subject to detrimental effects from exposure to particulate matter as found in air pollution (Oberdorster and Utell, 2002). At present, increasing evidence from experimental, clinical and epidemiological studies suggests that certain neurological diseases, such as Alzheimer's (Block and Calderon-Garciduenas, 2009; Calderon-Garciduenas et al., 2002) and Parkinson's disease (Kremens et al., 2014; Ritz et al., 2016), may be associated with ambient air pollution.

Mechanistically, air pollution may affect the CNS through a variety of molecular and cellular pathways that either directly damage brain tissue or lead to a predisposition to neurological diseases (Genc et al., 2012). Possible adverse effects are related to the physical and chemical characteristics of the pollutants themselves (Kremens et al., 2014). Although the exact mechanisms of air-pollutant induced brain pathology are not fully understood, recent evidence points toward neuroinflammation, oxidative stress, and disturbance of neurotransmitter systems (Block and Calderon-Garciduenas, 2009; Oberdorster and Utell, 2002) as possible pathways.

Ozone is one of the most important air pollutants in terms of its chemical characteristics as a powerful oxidant (Lauer, 2010). Animal studies that investigated the neurotoxic effects of ozone inhalation in various experimental settings indicate that ozone exposure may increase lipid peroxidation (Pereyra-Munoz et al., 2006), reduce the dopaminergic neurons (Pereyra-Munoz et al., 2006), increase vascular endothelial growth factor (VEGF), interleukin-6 (IL-6), tumor necrosis factor α (TNF α) (Araneda et al., 2008), and c-Fos expression in different brain regions (Gackiere et al., 2011). These findings suggest that ozone may significantly interfere with central nervous physiology, and thus, one may reasonably hypothesize that ozone may have relevant impact on human behavior, cognitive processes and emotion. In this line of thought, ozone may be a potential environmental risk factor for impaired mental health mediated by the above mentioned suggestive

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pathomechanisms.

In the absence of any synthesis of the relevant literature on this topic, here we aim to systematically review the epidemiological studies on ambient ozone exposure and mental or behavioral disorders to describe consistent associations as they exist or identify gaps in our current knowledge.

2. Methods

For the systematic review, we followed the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) (Moher et al., 2015). A complete PRISMA checklist can be found in the Supplementary A.

The work was conducted by one reviewer (TZ) and in case of indetermination a second reviewer (JH) checked.

The overall Population-Exposure-Comparator-Outcome (PECO) statement is as follow, Participants: Humans; Exposures: ambient ozone; Comparisons: comparison group is varied with studies. We are investigating whether exposure to higher concentrations of ambient ozone is associated with mental and behavioral disorders; Outcomes: any mental and behavioral disorder. Study design: observational epidemiological studies

2.1. Search strategy

A systematic literature search was conducted in three different electronic databases: PubMed, Web of Science and EMBASE, for publication dates between January 1, 1960 and December 14, 2017. In accordance with the terminology in “Mental and behavioural disorders (F00–F99)”, International Classification of Disease-10 (ICD-10) (WHO, 2016), combinations of both Mesh headings and free terms connected with ozone and different mental or behavioral disorders were used for the search. In addition, we also manually searched the reference lists of included studies and other related review articles. A more detailed account of the different search strategies is provided in the Supplementary B.

2.2. Studies selection

The search results were filtered and only epidemiological studies that were written in English and investigated the relationship between ambient ozone exposure and mental or behavioral disorders were included. Reviews, letters to the editor, clinical research studies, animal experiments and studies concerned with indoor or occupational exposure to ozone were not considered.

2.3. Data extraction

For each study, information on paper (author and publication time), study location, study design, participants, exposure assessment, outcomes, covariates, and results was extracted. Furthermore, a detailed account of each study's PECO statement is provided in the Supplementary C.

2.4. Assessment of studies

2.4.1. Quality assessment

The Newcastle-Ottawa scale (Wells et al., 2013) was adopted in this review to evaluate the quality of cohort and case-control studies. It contains eight items grouped into three dimensions. Items can be scored with 0 or 1 star except for one item that can be scored with 0–2 stars resulting in a maximum score of 9 stars. The total score is meant to be an indication of the overall quality of a study: 0–5 stars indicate low quality while 6–9 stars are typically taken to indicate high quality.

In addition, we used the criterion from Mustafić (Mustafic et al., 2012) to rate the quality of time-series and case-crossover studies. This criterion consists of three dimensions: exposure (scores between 0 and 1), outcome (0–1) and confounders (0–3). Studies that achieved a total combined score of 5 are regarded as being of high quality while studies that scored 0 in any of the three dimensions are judged to be of low quality. Studies reaching any intermediate score are classified as medium quality.

We did not perform any quality evaluation on cross sectional studies and ecological studies.

2.4.2. Risk of bias assessment

Assessment of risk of bias is related to but distinguished from assessment of methodological quality (OHAT, 2015). Thereby risk of bias assessment was also conducted. Given no established tool for time series and case-crossover study (Achilleos et al., 2017), we evaluated the risk of bias on the Office of Health Assessment and Translation (OHAT) tool by the National Institutes of Environmental Health Sciences National Toxicology Program (OHAT, 2015) and Navigation Guide by the University of California (Lam et al., 2016; Woodruff and Sutton, 2014) for each included study.

We assessed our studies for key criteria (Exposure assessment, Outcome assessment, Confounding bias) and Other Criteria (Selection bias, Attrition/exclusion bias, Selective reporting bias, Conflict of interest, Other source of bias). Each of above domain is evaluated as “low”, “probably low”, “probably high”, or “high” risk according to specific criteria. The criteria of risk of bias assessment is provided in the Supplementary D.

According to OHAT Approach (OHAT, 2015) studies for which the key criteria and most of the other criteria are characterized as “high” or “probably high” risk are recommended to remove.

3. Results

3.1. Search results

The flowchart in Fig. 1 illustrates the selection process for inclusion of studies in the present review. The database search yielded 567 unique hits, 43 of which passed a first selection based on the title and abstract only. These 43 articles underwent a full text evaluation which brought the total number down to 31 published articles that met our inclusion criteria.

The study characteristics of the 31 selected publications are summarized in Table 1 ordered by outcomes, date of publication and results. Seven studies investigated autism or autism spectrum disorder (ASD), two looked into impairment of cognitive functions, five addressed dementia, six researched depression, and five examined suicide. The remaining studies assessed disorders of sex preference, mental disorders, neurobehavioral disorders, panic attacks, psychiatric emergencies and sexual dysfunction (one paper per outcome).

Among the 31 articles, there were seven cohort, six case-control, four case-crossover, six time-series, six cross-sectional and two ecological studies. Additionally, between these 31 studies, 16 focused on long-term exposure and the other 15 on short-term exposure. These details can be checked in the Table 1, column “exposure assessment” as well.

3.2. Assessment of studies

All selected cohort studies received at least 7 stars on the Newcastle–Ottawa scale, and five of the six case-control studies received more than 5 stars. They can thus all be regarded as high quality studies. Two of the selected case-crossover studies and three time-series studies reached at least 3 points according to the Mustafić’s criterion (Mustafic et al., 2012) and are therefore considered to be of medium to high quality. A more detailed account of each study's quality assessment is provided in the Supplementary C

Based on the risk of bias assessment, none of these 31 articles was

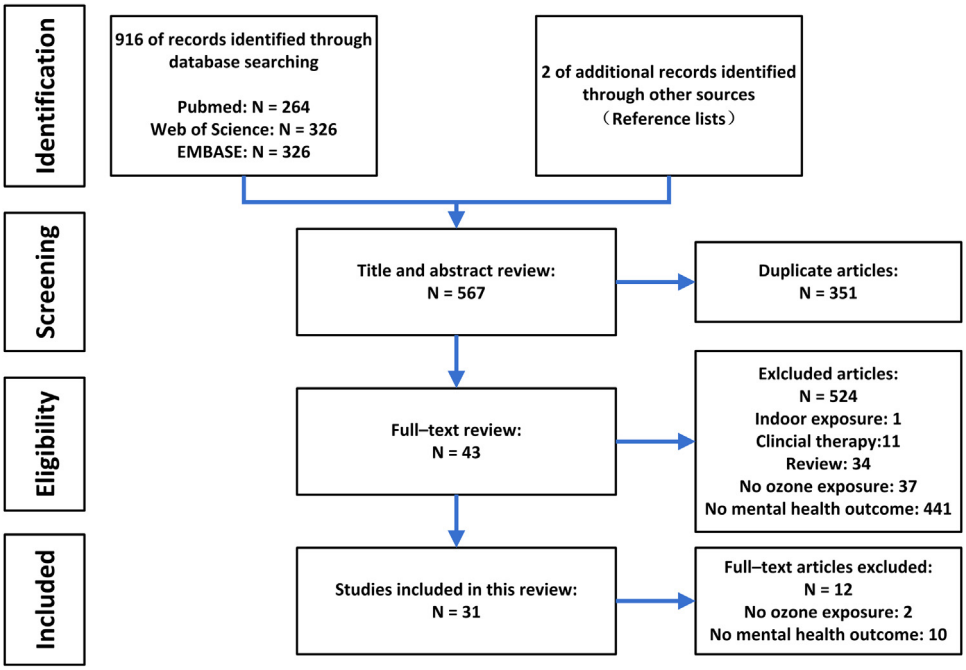

Fig. 1. Flow chart illustrating the literature search and subsequent study selection process.

excluded for being assessed as high risk of bias. However, two studies (Biermann et al., 2009; Oudin et al., 2018) might be regarded as "nearly excluded" as they got one "High" and one "Probably high" risk of bias evaluation within the three key criteria. The heat map illustrating this rating process is provided in Table 2. The detailed account of each study's risk of bias assessment is listed in the Supplementary C.

3.3. Autism and Autism spectrum disorder

Seven articles evaluated the association between ozone and autism spectrum disorder (ASD) or autism (Becerra et al., 2013; Goodrich et al., 2017; Jung et al., 2013; Kerin et al., 2017; Kim et al., 2017; Volk et al., 2014, 2013) but only two studies reported an increase in incidence risk. In particular, Becerra et al. (Becerra et al., 2013) investigated the associations of air pollution during pregnancy on the development of ASD among children aged 3–5 years. Prenatal exposure to an ozone concentration increase of 11.54 parts per billion (ppb) was associated with a 12% higher probability of developing ASD. Another study by Jung et al. (Jung et al., 2013) with children below 3 years of age found that each 10 ppb increase in ambient ozone concentration in the preceding 1 year to 4years may increase the risk of developing ASD by 59%.

Five studies (Goodrich et al., 2017; Kerin et al., 2017; Kim et al., 2017; Volk et al., 2014, 2013) were conducted within the same population as part of the Childhood Autism Risks from Genetics and Environment programme in California. They reported no direct association between ozone and ASD or autism per se, but saw some association modifications by folic acid intake and by genotype. Goodrich et al. (2017) illustrated joint associations of prenatal air pollution exposure and maternal folic acid (FA) supplementation. Children of mothers who were exposed to higher concentrations of ozone (33.41 $\mu g/m^3$) during the first trimester of pregnancy and who reported low FA intake were at a 19% higher risk of developing ASD compared to children of mothers who were exposed to lower levels of the same air pollutant and who reported high first month FA intake. Kim, (2017) reported a gene-environment interaction between ozone and autism in subjects with different copy number variations. The study indicated that a 1-standard-deviation (SD) increase in duplication burden (1,356,513 base pairs) combined with a 1-SD increase in ozone exposure (6.2 ppb) was associated with elevated odds of autism (odds ratio (OR) = 3.4, $P < 0.005$). The latter were much greater than the increased odds of either genomic duplication (OR = 1.85, 95% confidence interval (CI) = 1.25–2.73) or elevated ozone exposure (OR = 1.20, 95% CI = 0.93–1.54) alone. However, Volk et al. (2013) found no statistically significant correlation between autism form and ozone exposure, or *MET* genotype. Subjects with both *MET* rs1858830 CC genotype and high air pollution exposure were at an increased risk of autism compared to subjects who had both the CG/GG genotype and a lower air pollution exposure. Another study (Volk et al., 2014) also reported no statistically significant correlation between continuous regional ozone exposure and ASD. The ecological study conducted by Kerin et al. (2017) reported no statistically significant correlation between ozone and autism severity or functioning.

Although two high quality articles (Becerra et al., 2013; Jung et al., 2013) point toward a positive association between ozone exposure and ASD or autism, this correlation was not confirmed by the other studies included in this review and the association should thus be regarded as unclear.

3.4. Impairment of cognitive functions and dementia

Two cross-sectional studies (Chen et al., 2009; Gatto et al., 2014) from the USA found a correlation between ozone exposure and impairment of cognitive functions. Chen et al. (2009) indicated that each 10 ppb increase in the annual averaged ozone concentration was associated with a cognitive impairment leading to lower test scores in the symbol-digit substitution and serial-digital learning tests by 0.16 and 0.56, respectively. Gatto et al. (2014) reported that exposure to ozone concentrations above 49 ppb was associated with a lower executive function ($\beta = -0.66$).

Five of the selected articles (Calderon-Garciduenas et al., 2015; Chen et al., 2017; Cleary et al., 2018; Linares et al., 2017; Wu et al., 2015) investigated the correlation between ozone exposure and dementia. The case-control study by Wu et al. (2015) observed increased odds of Alzheimer's disease (highest vs lowest tertiles in ozone exposure: OR = 2.00) and of vascular dementia (OR = 2.09). A high quality cohort study by Cleary et al. (2018) investigated ozone exposure, *APOE* genotype and cognitive function. They found a

Table 1
Description of the 31 selected studies on ozone exposure and mental and behavioral disorders. (Ordered by outcomes, paper publication time and results).

Paper	Study location	Study design	Participants	Exposure assessment	Outcomes	Covariates	Results	Quality assessment
Autism spectrum disorder (ASD) or Autism								
1. Becerra et al., 2013	Los Angeles, California, USA	Case-control study	7594 cases and 75635 controls (aged 3-14 years)	Ozone data from nearest monitoring stations; hourly measurements (1000 – 1800 hours) were averaged for each day, daily average exposure for the entire pregnancy and specific pregnancy periods; short-term exposure	Children with ASD were identified by Department of Developmental Services between 36 and 71 months of age	Maternal age, education, race, maternal place of birth, type of birth, parity, insurance type, gestational weeks at birth	Per 11.54 parts per billion (ppb) increase in ozone, a 12 -15 % relative increase in odds, odds ratio (OR) = 1.12, 95% confidence interval (CI): 1.06 - 1.19.	8/9[§]
2. Jung et al., 2013	Taiwan	Cohort study (Longitudinal health insurance database 2000)	49073 children aged less than 3 years in 2000, followed up from 2000 through 2010	Ozone data from three nearest monitoring stations within 25 km combined with inverse distance weighting method (100 m resolution); yearly mean concentration (monthly average of daily maximum value, post-code level address); short-term exposure	342 cases of ASD diagnosed by doctors from January 1st, 2000 to December 31st, 2010	Age, anxiety, sex, intellectual disabilities, preterm, municipal-level socioeconomic status	Per 10 ppb increase in ozone, a 59% risk was increased, adjusted hazard ratio (HR) = 1.59, 95% CI: 1.42 - 1.78.	8/9[§]
3. Kim et al., 2017	California, USA	Case-control study (Childhood Autism Risks from Genetics and Environment study, CHARGE study)	158 cases and 147 controls (aged 24 – 60 months at the time of recruitment)	Ozone data from up to four monitoring stations within 50 km of each residence (if one or more stations were located within 5 km, only data from these were used) combined with inverse distance-squared weighting; average range of ozone measurements from 1000 to 1800 hour (reflecting the high 8-hr daytime exposure); long-term exposure	Children with ASD were identified by Department of Developmental Services (children were born between 1999 and 2008)	Maximum education level of parent, child's sex, child's ethnicity	Per 1356513 base pair increase in duplication burden combined with a 6.2 ppb increase in ozone exposure was associated with an elevated autism risk, OR = 3.4, $P < 0.005$; genomic duplication alone: OR = 1.85, 95% CI: 1.25 – 2.73; ozone alone: OR = 1.20, 95% CI: 0.93 – 1.54.	6/9[§]
4. Volk et al., 2013	California, USA	Case-control study (Childhood Autism Risks from Genetics and Environment study, CHARGE study)	279 cases and 245 controls (aged 24 – 60 months at the time of recruitment)	Ozone data from up to four monitoring stations within 50 km of each residence (if one or more stations were located within 5 km, only data from these were used) combined with inverse distance-squared weighting; average range of ozone measurements from 1000 to 1800 hour (reflecting the high 8-hr daytime exposure); long-term exposure	Children with ASD were identified by Department of Developmental Services	Sex, child ethnicity, maximum education of parents, maternal age, prenatal smoking	No statistically significant association. Suggested increase in odds for autism with ozone exposure in different periods (e.g. first year, OR = 1.15, 95% CI: 0.72 – 1.86, all pregnancy OR = 1.09, 95 % CI: 0.76 – 1.55, per increase of 16.1 ppb ozone).	6/9[§]
5. Volk et al., 2014	California, USA	Case-control study (Childhood Autism Risks from Genetics and Environment study, CHARGE study)	252 cases and 156 controls (aged 24 – 60 months at the time of recruitment)	Ozone data from up to four monitoring stations within 50 km of each residence (if one or more stations were located within 5 km, only data from these were used) combined with inverse distance-squared weighting; average range of ozone measurements from 1000 to 1800 hour (reflecting the high 8-hr daytime exposure); long-term exposure	Children with ASD were identified by Department of Developmental Services	Sex, child ethnicity, maximum education of parents, maternal age, prenatal smoking, home ownership	No statistically significant correlation between *MET* rs1858830 CC genotype and ozone (ozone concentration ≥ 41.8 ppb, with CC *Met* genotype, OR = 0.95, 95% CI: 0.42 – 2.2).	6/9[§]
6. Goodrich et al., 2017	California, USA	Case-control study (Childhood Autism Risks from Genetics and Environment study, CHARGE study)	346 cases and 260 controls (aged 24 – 60 months at the time of recruitment)		Children with ASD were identified by Department of Developmental Services	Self-reported financial hardship between 3 months before pregnancy to time of interview, child's year of birth, vitamin A and zinc intake during the first month of pregnancy		6/9[§]

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Table 1 (*continued*)

Paper	Study location	Study design	Participants	Exposure assessment	Outcomes	Covariates	Results	Quality assessment
				Ozone data from up to four monitoring stations within 50 km of each residence (if one or more stations were located within 5 km, only data from these were used) combined with inverse distance-squared weighting; average range of ozone measurements from 1000 to 1800 hour (reflecting the high 8-hr daytime exposure); long-term exposure			No statistically significant association. FA intake is dichotomized at 800 μg, median ozone = 33.41 μg/m^3, OR (high ozone and low FA) = 1.08, 95% CI: 0.56 - 2.08; OR (low ozone and low FA) = 1.19, 95% CI: 0.61 - 2.30.	
7. Kerin et al., 2017	California, USA	Ecological study (Childhood Autism Risks from Genetics and Environment study, CHARGE study)	325 children with ASD (aged 24 – 60 months at the time of recruitment)	Ozone data from up to four monitoring stations within 50 km of each residence (if one or more stations were located within 5 km, only data from these were used) combined with inverse distance-squared weighting; average range of ozone measurements from 1000 to 1800 hour (reflecting the high 8-hr daytime exposure); long-term exposure	Severity score calibrated by the Mullen Scales of Early Learning (MSEL), the Vineland Adaptive Behavior Scales (VABS), the Autism Diagnostic Observation Schedule (ADOS)	Sex, max education in the home, referral center, race, mother's age, prenatal smoking, season of conception, home ownership	No statistically significant association between ozone and autism severity or functioning (P > 0.05, per 11.1 ppb increase of ozone, Prenatal: VABS composite score – 0.91 %, 95 % CI: - 8.74 % – 6.98 %; MSEL composite development quotient – 0.06, 95 % CI: - 2.78 - 2.66; Year 1: VABS composite score 0.91 %, 95 % CI: - 11.74 % – 13.4 %; MSEL composite development quotient 1.43, 95 % CI: - 2.58 – 5.71).	
Impairment of cognitive functions								
8. Chen et al., 2009	USA	Cross- sectional study (the Third National Health and Nutrition Examination Survey, NHANES III)	1764 adult subjects (age 37.5 ± 10.9) from the Third National Health and Nutrition Examination Survey in 1988 - 1991	Ozone data from Environmental Protection Agency and combined with inverse distance weighting; annual ozone at geocoding residential information; long-term exposure	Scores of simple reaction time test (SRTT), symbol – digit substitution test (SDST), serial – digital learning test (SDLT)	Age, sex, race, demographics, socioeconomic status, lifestyle, household and neighborhood characteristics, cardiovascular risk factors	A per 10 ppb increase in annual ozone prior to testing was associated with increased SDST and SDLT scores (regression coefficient β, 0.16, 95% CI: 0.01 – 0.23 and 0.56, 95% CI: 0.07 – 1.05, respectively).	
9. Gatto et al., 2014	Los Angeles, USA	Cross- sectional study	1496 healthy, cognitively intact adult participants (age 60.5 ± 8.1) enrolled during 2000-2006	Ozone from monitoring station (one for station located within 5 km, otherwise 3 closest ones for located within 100 km) and combined with inverse distance weighting; annual average ozone (8h maximum concentration for daily ozone, geocoded residence address); long-term exposure	Cognitive tests (executive function, verbal learning, logical memory, visual processing, visual episodic memory, semantic memory) conducted by psychometrist	Age, gender, race, education, income, study, mood	Exposure above 49 ppb ozone was associated with lower executive function (beta coefficient β = -0.66, 95% CI: - 1.35, 0.03; P = 0.059).	

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Table 1 (*continued*)

Paper	Study location	Study design	Participants	Exposure assessment	Outcomes	Covariates	Results	Quality assessment
Dementia								
10. Calderon-Garciduenas et al., 2015	Mexico and Polotitlán,Mexico	Cross- sectional study	57 right – handed children (age 12.45 ± 3.4) and their 48 right – handed parents (age 37.5 ± 6.77) from Mexico City; 9 control children (age 9.77 ± 0.83) and their 7 control parents (age 34.57 ± 6.02) from Polotitlán	Ozone in Mexico City (high ozone) and Polotitlán (control, low ozone); long-term exposure	NNA/Cr, Cho/Cr and mI/Cr ratios [N–acetylaspartate (NAA), choline, creatine (Cr) and myoinositol (mI)]	Age, gender, body mass index, apolipoprotein E (*APOE*) genotype	The right hippocampus NAA/Cr ratio was significantly different between control (P = 0.007). *APOE* ε4 carriers are at higher risk.	
11. Wu et al., 2015	Taiwan	Case-Control study	249 Alzheimer's disease (AD) patients, 125 vascular dementia (VaD) patients and 497 controls from 2007 to 2010 (aged ≥ 60)	Ozone data from Environmental Protection Administration combined with Bayesian maximum entropy method; annual average exposure (residential place); long-term exposure	AD or VaD was diagnosed based on criteria	Age, sex, *APOE* ε4 status, PM_{10} level, education years, alcohol consumption	Increased risk observed for dementia with ozone exposure, for AD (highest vs. lowest tertile: aOR = 2.00, 95% CI: 1.14 – 3.50) and for VaD (highest vs. lowest tertile: aOR = 2.09, 95% CI: 1.01 – 4.33). Ozone exposure: lowest tertile, < 20.22 ppb, highest tertile > 21.56 ppb.	5/9[§]
12. Linares et al., 2017	Madrid, Spain	Time-series study	1175 dementia-related emergency from January1st 2001 to December 31st 2009	Ozone data from the Madrid Municipal Air Quality Monitoring Grid; daily mean concentration; short-term exposure	1175 dementia – related emergencies (ICD – 10 codes 290.0 – 290.2, 290.4 – 290.9, 294.1 - 294)	Day of week	An increase of 10 μg/m^3 in ozone, RR = 1.09, 95% CI: 1.04 – 1.15, (lag 5). And a higher RR can be observed when daily ozone concertation surpass a threshold of 45 μg/m^3.	2/5[§§]
13. Cleary et al., 2018	USA	Cohort study (National Alzheimer's Disease Center program)	5419 participants aged 60 or more, with a baseline Mini – Mental Status Examination score > 0 and a diagnosis of cognitive impairment in at least on follow – up visit	Ozone data from Environmental Protection Agency combined with space – time Hierarchical Bayesian Model (12 km ×12 km resolution covering the east and 36 km ×36 km resolution across USA) and inverse distance weighting; yearly ozone (average the 8-h maximum over year, ZIP code residence address); long-term exposure	3624 participants with normal cognition, 1492 participants with cognitive impairment, diagnosed based on examination	Age, sex, education, race, apolipoprotein E (*APOE*) genotype, smoking status, vitamin B12 deficiency, population density,	Baseline cognitive performance was significantly reduced by highest (> 40 ppb) versus lowest level (< 36.7 ppb) of ozone for assessing both the Mini - Mental State Examination (MMSE) (β - coefficient = 0; β - coefficient = 0.83, 95% CI: 0.5 - 1.2) and Cognitive Dementia Rating - Sum of Boxes (CDR - SB) (β - coefficient = 0; β - coefficient = -0.60, 95% CI: - 0.8 - -0.3). *APOE* ε 4 alleles exhibited a faster rate of cognitive decline.	8/9[§]
14. Chen et al., 2017	Ontario, Canada	Cohort study (Ontario Population Health and Environment Cohort)	2066639 individuals (were 55 – 85 years old on 1st April 2001), resided in Ontario for > 5 years, Canadian-born, free of physician-diagnosed dementia. Follow-up extended till 31st March 2013	Ozone data from optimal interpolation technique; annual exposure (postal code residence address); long-term exposure	257816 incident cases of dementia in 2001 - 2013	Age, sex, pre-existing comorbidities (diabetes, hypertension, coronary heart disease, stroke, congestive heart failure, arrhythmia, traumatic brain injury), income quintile, urban residency, north/south indicator, unemployment rate education, immigrants,	No statistically significant association was found for ozone and dementia. Hazard ratios was 0.98, 95% CI: 0.96 – 1.00 (per 6.3 ppb increase for ozone).	7/9[§]

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Table 1 (*continued*)

Paper	Study location	Study design	Participants	Exposure assessment	Outcomes	Covariates	Results	Quality assessment
Depression disorder								
15. Szysykowicz et al., 2007	Edmonton, Canada	Time-series study	15556 emergency department visit for depression between 1992 and 2002 (70.9 & aged between 20 and 50 years)	Ozone data from fixed monitoring stations; averaged hourly data over 24-h periods; short-term exposure	Emergency department visits for depression	Temperature, relative humidity, sex, season	An increase 14.0 ppb in ozone, an increment in daily depression-related emergency department visits could be noted: 6.9% (95% CI: 0.6 - 13.6) for ground level ozone (1-day lagged) for female patients in warm season could be noted.	2/5[§§]
16. Lim et al., 2012	Seoul, South Korea	Cohort study	537 participants (averaged age 71 ± 5) performed from 2008 to 2010	Ozone data from the nearest monitoring site to residential address; moving average of daily maximum values between 09:00 and 18:00 hours; short-term exposure	Depression diagnosed by the Korean version of the Geriatric Depression Scale-Short Form	Age, sex, number of school, body mass index, alcohol consumption, regular exercise, creatinine-adjusted urine cotinine level, systolic blood pressure, triglyceride, daily mean temperature, follow-up time, day of week	Per 37 ppb increase in ozone (3 - day moving average) was associated with depressive symptomatology (43.7%, 95% CI: 11.5 - 85.2). Per 37 ppb increase for ozone (28 - day moving average) emotional symptoms (emotional symptoms: 132.5%, 95% CI: 32.0 – 309.3).	7/9[§]
17. Szyszkowicz et al., 2016	Canada	Case-crossover study	118602 emergency department visits for depression from April 2004 to December 2011	Ozone data from National Air Pollution Surveillance stations within 35 km of each patient's postal code; averaged hourly data over 24-h periods; short-term exposure	Emergency department visits for depression	Sex, day of week,	Per increase 14.5 ppb in ozone was associated with increased risk of an emergency department visit for depression: for females, between 1 and 7 days after exposure, ORs ranging between 1.02 and 1.03; for males, was between 1 and 5, and 8 days, ORs ranging between 1.02 and 1.03.	2/5[§§]
18. Kioumourtzoglou et al., 2017	USA	Cohort study (Nurses' Health Study)	41844 women (averaged age 66.6 ± 7.6) followed from 1996 to 2006	Ozone data from up to 5 monitors and at least 1 monitor within 50 km to participant's house, using the squares of the distances as weights; monthly averaged ozone concentrations (residence address, May – September); long-term exposure	Defined as first report of either a physician diagnosis or use of antidepressant medication	Calendar year and month at questionnaire return, census region, living in a metropolitan statistical area, race, physical activity, body mass index, pack-years of smoking, smoking status, dietary habits, participation in social groups, baseline abbreviated Mental Health Inventory score, educational level, parental education, marital status, husband's education, tract-level median income, house value, population density	Per 10 ppb increase in ozone, hazard ratio (HR) = 1.06, 95% CI: 1.00 – 1.12; associations were stronger when only antidepressant use to define cases (for ozone, HR = 1.08, 95% CI: 1.02 – 1.14).	8/9[§]
19. Szyszkowicz et al., 2009	Canada	Time-series study	27047 emergency department visit for depression; Starting date: April; Study period: 13709 days	Ozone data from National Air Pollution Surveillance system; averaged hourly data over 24-h periods; short-term exposure	Emergency department visits for depression	Season (period)	No positively association between emergency department visits for depression disorder and ozone, RR% (worm period) = -1.1, 95% CI: - 5.9 - 3.9, in relation to an increase of 18.9 ppb ozone.	2/5[§§]
20. Wang et al., 2014	Boston, USA	Cohort study (MOBILIZE Boston Study)	732 Boston-area adults ≥ 65 years of age (78.1 ± 5.5) recruited between 2005 and 2008	Ozone data from a single monitoring site within 20 km radius to participant's home; moving average of daily mean value form hourly data; short-term exposure	Depressive symptoms by 20-item Revised Centre for Epidemiological Studies Depression Scale (CESD-R)	Age, sex, race/ethnic, visit, ambient and dew point temperatures, barometric pressure, day of week, season, long -term temporal trends		7/9[§]

(*continued on next page*)

Table 1 (*continued*)

Paper	Study location	Study design	Participants	Exposure assessment	Outcomes	Covariates	Results	Quality assessment
							No evidence of a positive association between depressive symptom short-term changes in pollution levels. OR of CESD-R = 0.71, 95% CI: 0.46 - 1.09 (13.45 ppb for an interquartile range).	
Suicide								
21. Biermann et al., 2009	Bavaria, Germany	Cross-sectional study	1008 suicides and 917 suicide attempts from 2004 to 2007	Ozone data from the Institute of Chemical Analysis of the City of Nuremberg; daily average value for ozone; short-term exposure	1008 suicides as well as 917 suicide attempts leading to police procedures from register of suicides	Not reported	The ozone levels differed statistically significant (T = -0.25; p = 0.014) between days where one or no suicide were observed (mean ozone: 79.8 μg/m^3; SD: 36.3) and days with two more suicides (mean ozone: 86.4 μg/m^3; SD: 39.4). No association between ozone levels and suicide attempts.	
22. Yang et al., 2011	Taiwan	Ecological study	4857 deaths by suicide from January 1st 1991 to 31st December 2008	Ozone from Environmental Protection Administration; monthly average; long-term exposure	4857 deaths by suicide, average counts 22.5 ± 9.6 cases, range = 6 -59 cases from Department of Health	Age, gender, means of suicide (violent, non - violent)	Ozone was particularly associated with suicide (for violent, r = 0.231, p = 0.002; for male, r = 0.213, p = 0.004; for female, r = 0.202, p = 0.006; for age 20 -65, r= 0.194, p value was not reported; for age > 65, r = 0.312, p <0.001) and total, r = 0.244, p=0.001); for 119.1 month/cycle (intrinsic mode function), r = 0.338, p <0.001.	
23. Kim et al., 2015	Korea	Time-series study	The suicide rate per 10 million persons in the 16 administrative regions from January 1st 2006 to December 31st 2011	Ozone data from the Korea Ministry of Environment, ; daily concentration (averaged value for each region); short-term exposure	The variation of weekly suicide rate from the Korea National Statistical Office	Celebrity suicides, meteorological variables (sunlight hours and temperature), economic data, the regional weekly suicide rate, the average national monthly suicide number for the past 5 years	Extending back to 4 weeks, over the range of 2 standard deviations (0.016 ppm) around the annual mean ozone concentration, the adjusted suicide rate increased by an estimated 7.8 % of the annual mean rate(29.1 per 100000 persons per year).	3/5[§§]
24. Casas et al., 2017	Belgium	Case-crossover study	Suicide deaths registered between January 1st 2002 and December 31st 2011	Ozone data from monitoring stations and satellite images, in kriging interpolation model (4 × 4 km grid); 8-h average ozone concentrations; short-term exposure	20533 suicide deaths, aged from 5 to more than 85 years old from the National Population Register	Season, age, sex, the method to commit suicide (non – violent, violent); day of week, duration of sunshine	Per 10 μg/m^3 increase in ozone was associated with suicide mortality in all seasons except winter (P < 0.05 for lags 0 – 2 and 0 – 6, OR ranging from 1.02 to 1.07); 1 -2 % increase in the odds of suicide mortality for all the suicide lags among the adult population; an 8% increase odds of suicide (95% CI: 1 – 16%) among adolescents (in lag 0 -1 ozone); and individuals committed suicide using violent methods. No association between sex and ozone.	3/5[§§]
25. Szyszkowicz et al., 2010	Vancouver, Canada	Case-crossover study				Daily temperature and relative humidity, sex, season		2/5[§§]

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Table 1 (*continued*)

Paper	Study location	Study design	Participants	Exposure assessment	Outcomes	Covariates	Results	Quality assessment
			Emergency department visits with suicide attempt / ideation from July 8,1999 to February 28, 2003	Ozone data from fixed monitoring stations; daily shared exposure (daily mean value form hourly data and an average among monitors); short-term exposure	1605 emergency department visits with suicide attempt / ideation in hospital		No statistically significant association between ozone and suicide attempts ($P > 0.05$, the effect size is not given), the highest positive value was obtained for ozone lagged by 1 day (per 0.9 ppb increase in ozone).	
Disorders of sex preference								
26. Rotton, 1993	Dayton, Ohio, USA	Cross-sectional study	584 reports of rape, 674 complaints about obscene phone calls, 288 calls about indecent exposure and 547 more complaints within 731 days (January 1st, 1975 to December 31st, 1976)	Ozone data from Environmental Protection Agency; average ozone (24-hour readings on 712 days); long-term exposure	Sex crimes reported by police department	Series for 731-day long term trend, season, day of week, holidays	Ozone was associated with complains about obscene phone call (regression coefficients = 0.003, $P < 0.01$)	
Mental disorders (hospital admissions)								
27. Chen et al., 2018	Shanghai, China	Time-series study	Cases of hospital admissions for mental disorder (10^{th} version of the international classification of diseases, F01- F99) identified during January 1st, 2013 to December 31st, 2015	Ozone data from the Shanghai Environmental Monitoring Center; maximum 8-h average ozone; short-term exposure	39143 cases of daily hospital admissions for mental disorder (maniac episode, depressive disorder and others)	Long-term and season trends, temperature, humidity, day of work, holiday	No statistically significant associations. Per 10 $\mu g/m^3$ increase in ozone (lag 01 day) was associated with increment of 0.34%, 95% CI: - 1.08 – 1.75.	4/5[§§]
Neurobehavioral disorder								
28. Lin et al., 2014	Taiwan	Cross-sectional study (Taiwan Birth Cohort Pilot Study, TBCS-q)	533 mother – infant pairs from 11 towns in Taiwan, babies born between October 2003 and January 2004	Ozone data from the Taiwan Air Quality Monitoring Network; daytime (7 a.m. to 7 p.m.) average level (monitoring stations of town); short-time exposure	The 6- and 18-month scales (the Bayley Scales of Infant Development, consists of: gross motor, fine motor, language / communication, social / self – care abilities)	Maternal education level, maternal nationality, gestational age, infant sex, breastfeeding, environmental tobacco smoke exposure, nursery type	No statistically significant association between ozone exposure and subclinical neurodevelopment in early childhood ($P > 0.05$, six months of age, total for 18 months of age, 1^{st} trimester: $\beta = -0.026$, $SE = 0.093$; 2^{nd} and 3^{rd} trimester: $\beta = -0.140$, $SE = 0.137$; birth – 12 month: $\beta = -0.102$, $SE = 0.101$).	
Panic attacks								
29. Cho et al., 2015	Seoul, South Korea	Time-series study	Individuals who visited the emergency department with panic attack (F 41.0) from 2005 - 2009	Ozone data from the Ministry of Environment;; daily average (an average of hourly measurements from 27 monitoring stations); short-term exposure	2320 emergency department visits for panic attacks (F41.0)	Date of the visit, day of week, national holiday, daily mean temperature and relative humidity	Per increment for 10.04 ppb ozone, the adjusted RR of emergency department visits for panic attacks was 1.051 (95% CI: 1.014 – 1.090) for the same – day exposure to ozone; and 1.059 (1.021 – 1.099) in lag 0 – 1, 1.068 (1.029 – 1.107) in lag 0 – 2 and 1.074 (1.035 – 1.114) in lag 0 – 3.	3/5[§§]

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Table 1 (*continued*)

Paper	Study location	Study design	Participants	Exposure assessment	Outcomes	Covariates	Results	Quality assessment
Psychiatric emergency								
30. Oudin et al., 2018	Gothenburg, Sweden	Case-cross over study	Psychiatric emergency visits (PEV) data from July 1st 2012 to November 24st 2016	Ozone data form a measuring station; average ozone; long-term exposure	Number of PEV was 27 ± 6	Daily mean temperature and public holiday	No clear association between outcome and ozone. Per 10 μg/m^3 increase in ozone, change PEV 0.1%, 95% CI: - 0.6 – 0.9; in the three-pollutant models ($PM_{2.5}$, NO_2, O_3) the increase was 3.3%, 95% CI: -0.2 – 6.9.	2/5[§§]
Sexual dysfunction								
31. Tallon et al., 2017	USA	Cohort study (National Social Life, Health, and Aging Project)	412 household-resident older adults aged 57 – 85, conducted from July 2005 to March 2006 and August 2010 to May 2011	Ozone data from the nearest monitor stations (within 60 km of the participants' home); 1 -7 year average exposure based on warm season (April – September); long-term exposure	Erectile dysfunction (ED) status obtained through self- reported questionnaire: 132 men with ED, 280 men without ED	Age, geographic region, ethnic group, education, current smoking status, obesity, diabetes, depression, season, median household income	No association between ozone exposure and odds in incident ED. ORs for 1 and 7 years moving average equaled 1.16 (95% CI: 0.87 – 1.55; IQR = 8.21 ppb) and 1.16 (95% CI: 0.92 – 1.46; IQR = 6.81 ppb).	7/9[§]

Note:

§ The Newcastle-Ottawa scale (Wells G, 2013) was adopted in this review to evaluate the quality of cohort studies and case-control studies. The Newcastle-Ottawa scale contains eight items grouped into three dimensions. Items can be scored with 0 or 1 star except for one item that can be scored with 0 to 2 stars resulting in a maximum score of 9 stars. The total score is meant to be an indication of the overall quality of a study: 0 to 5 stars indicate low quality while 6 to 9 stars are typically taken to indicate high quality.

§§ The criterion from Mustafić's study (Mustafic et al., 2012) was used to evaluate the quality of time-series and case-crossover studies. The evaluation is based on three dimensions that can reach a combined top score of 5. The dimensions are exposure (score of 0 to 1), outcome (0 to 1) and confounders (0 to 3). The studies reaching a total score of 5 were regarded as being of high quality while studies that scored 0 in any one dimension were judged as being of low quality. All remaining studies were regarded as being of medium quality.

The cross-sectional and ecological studies were not given any quality evaluation

Table 2
Heat map of risk of bias rating for 31studies.

Study		Key Criteria			Other Criteria				
		Exposure assessment	Outcome assessment	Confounding bias	Selection bias	Attrition/ exclusion bias	Selective reporting bias	Conflict of interest	Other sources of bias
ASD or Autism	1. Becerra et al., 2013								
	2. Jung et al., 2013								
	3. Kim and Volk et al., 2017								
	4. Volk et al., 2013								
	5. Volk et al., 2014								
	6. Goodrich et al., 2017								
	7. Kerin et al., 2017								
Impairment of cognitive functions	8. Chen et al., 2009								
	9. Gatto et al., 2014								
Dementia	10. Calderón-Garcidueñas et al., 2015								
	11. Wu et al., 2015								
	12. Linares et al., 2017								
	13. Cleary et al., 2018								
	14. Chen et al., 2017								
Depression disorder	15. Szysykowicy et al., 2007								
	16. Lim et al., 2012								
	17. Szysykowicy et al., 2016								
	18. Kioumourtzoglou et al., 2017								
	19. Szysykowicy et al., 2007								
	20. Wang et al., 2014								
Suicide	21. Biermann et al., 2008								
	22. Yang et al., 2010								
	23. Kim et al., 2015								
	24. Casas et al., 2017								
	25. Szysykowicy et al., 2010								
Disorders of sex preference	26. Rotton, 1993								
Mental disorders	27. Chen et al., 2017								
Neurobehavioral disorder	28. Lin et al., 2014								
Panic attacks	29. Cho et al., 2015								
Psychiatric emergency	30. Oudin et al., 2018								
Sexual dysfunction	31. Tallon et al., 2017								
Risk of bias rating		Low		Probably low		Probably high		High	

Note:
Risk of bias assessment was conducted on the Office of Health Assessment and Translation (OHAT) Approach by the National Institutes of Environmental Health Sciences National Toxicology Program (OHAT, 2015) and Navigation Guide by the University of California (Lam et al., 2016; Woodruff and Sutton, 2014) for each included study. We assessed our studies for key criteria (Exposure assessment, Outcome assessment, Confounding bias) and Other Criteria (Selection bias, Attrition/ exclusion bias, Selective reporting bias, Conflict of interest, Other source of bias). Each of above domain is evaluated as “low”, “probably low”, “probably high”, or “high” risk according to specific criteria. The criteria and a detailed account of each study’s risk of bias assessment is provided in the Supplementary D. According to OHAT Approach (OHAT, 2015) studies for which the key criteria and most of the other criteria are characterized as “high” or “probably high” risk are recommended to remove.

significantly reduced baseline cognitive performance for that part of the cohort that was exposed to the highest ozone concentration of more than 40 ppb compared to the lowest concentration of less than 36.7 ppb using both the Mini-Mental State Examination and Cognitive Dementia Rating-Sum of Boxes for the assessment. In addition, they pointed out that *APOE ε4* alleles exhibited a faster rate of cognitive decline.

A cross sectional study by Calderon-Garciduenas et al. (2015) investigated the same gene-environment interaction. Using brain MRI scans they found that chronic overexposure to ozone may lead to neurodegenerative processes that already start in childhood, with *APOE ε4* carriers being at a particularly high risk. However, the study could not distinguish between effects from ozone and fine particulate matter. Linares et al. (2017) observed that ozone might exacerbate the risk of developing dementia symptoms by a factor of 1.09 in an ecological study analyzing dementia-related emergencies in Madrid.

However, a high quality cohort study in Ontario by Chen et al. (2017)) reported no statistically significant association for ozone and dementia.

In summary, we found one high quality cohort study (Cleary et al., 2018), one low quality case-control study (Wu et al., 2015), one low quality time-series study (Linares et al., 2017) and three cross-sectional studies (Calderon-Garciduenas et al., 2015; Chen et al., 2009; Gatto et al., 2014) that reported an association between ozone exposure and cognition impairment. This suggests that ozone exposure might be a possible cause of cognition impairment or even dementia. However, the verdict on a possible association between ozone exposure and dementia is not unanimous due to the heterogeneity in study design and quality.

3.5. Depression disorder

Two cohort (Kioumourtzoglou et al., 2017; Lim et al., 2012) studies demonstrated that an increase in ozone was associated with an increase in depression disorder diagnoses. The high quality study by Lim et al. (2012) showed that for elderly adults in Seoul the depressive symptomatology was positively associated with the increase in ozone. Another high quality study by Kioumourtzoglou et al. (2017) showed a hazard ratio increase by a factor of 1.06 per 10 ppb in ozone increase among middle-aged and older women.

Szysykowicz et al. conducted three studies in Canada (Szyszkowicz, 2007; Szyszkowicz et al., 2016, 2009). One low quality time-series study (Szysykowicz et al., 2007) showed a positive association between ozone and emergency department visits for depression disorder by female patients. One low quality case-crossover study (Szyszkowicz et al., 2016) examining emergency department visits for depression disorder demonstrated a positive association with odds ratios ranging from 1.02 to 1.03 per interquartile range for a daily mean ozone concentration of 14.5 ppb. Nevertheless, another time-series study (Szyszkowicz et al., 2009) examining the same association between ozone and emergency

department visits for depression disorder did not find any significant correlation.

The high quality cohort study by Wang et al. (2014) found no evidence of an association between depressive symptom and short-term changes in pollution levels among older adults.

Based on the heterogeneous study designs and quality, no clear association between ozone and depression can be postulated.

3.6. Suicide

Of the two medium to low quality case-crossover studies (Casas et al., 2017; Szyszkowicz et al., 2010), one medium quality time-series study (Kim et al., 2015), one cross-sectional studies (Biermann et al., 2009) and one ecological study (Yang et al., 2011) on ozone in relation to suicide outcomes, only one (Szyszkowicz et al., 2010) failed to report positive associations. However, the outcome “Emergency department visits with suicide attempt/ideation” (Szyszkowicz et al., 2010) is different and unique from other studies, and this study observed no association.

The time-series study by Kim et al. (2015) found that a 0.016 ppm increase in the average ozone concentrations during the previous 4 weeks (equivalent to 2 standard deviations) led to an increase in the weekly suicide rate in Korea by 7.8% which corresponds to 29.1 additional suicides per 100,000 persons per year. One case-crossover study by Casas et al. (2017) observed that ambient ozone concentrations were associated with suicide mortality in Belgium during all seasons except winter, producing a 1–2% increase in the odds of suicide mortality among the adult population and an 8% increase in the odds among adolescents.

An ecological study conducted by Biermann et al. (2009) found a statistically significant difference in ozone levels between days where one or no suicide occurred (mean ozone level: 79.8 $\mu g/m^3$; SD: 36.3) and days with two or more suicides (mean ozone level: 86.4 $\mu g/m^3$; SD: 39.4). Yang et al. (2011) reported that ozone was correlated with suicide rate ($P < 0.001$; total, $r = 0.244$).

Even though the majority of studies points towards positive association between elevated ozone levels and increased suicide rates, the low quality in relevant studies precludes us from drawing a definitive conclusion.

3.7. Other mental and behavioral disorder

One publication was identified for each of these five outcomes: disorders of sex performance, mental disorders with hospital admission, neurobehavioral disorders, panic attacks, psychiatric emergencies and sexual dysfunctions. Already in 1993, the cross-sectional study by James et al. (Rotton, 1993) reported that higher ozone levels were associated with complaints about obscene phone calls ($\beta = 0.003$, $P < 0.01$).

A time-series study from Shanghai (Chen et al., 2018) reported no association between ozone and cases of hospital admission for mental disorders (maniac episodes, depressive disorders and others). Similarly, a case-crossover study from Sweden by Oudin et al. (2018) found no association between ozone and psychiatric emergencies.

A cross-sectional study from Taiwan (Lin et al., 2014) found no association between ozone exposure and subclinical neurodevelopment in early childhood.

The time-series study by Cho et al. (2015) demonstrated that for each 10.04 ppb increase in the ambient ozone concentration the adjusted relative risks of emergency department visits for panic attacks were between 1.051 and 1.074 for different lags in ozone exposure.

The cohort study by Lindsay et al. (Tallon et al., 2017) found no association between ozone exposure and the odds of incident erectile dysfunction.

Due to the insufficient number of studies for each outcome, no final conclusions can be draw at this point.

4. Discussion

To the best of our knowledge, the present study provides the first systematic literature review on possible associations between ozone and mental or behavioral outcomes such as ASD, impairment of cognitive functions and dementia, depression and suicide. We conducted a broad literature search and selected a total of 31 studies that met our selection criteria for inclusion in this review. All 31 studies exhibited very heterogeneous study designs, sample sizes, outcomes, exposure assessment methods and qualities making meta-analyses impossible.

ASD is a complex developmental disorder characterized by impairments in social interaction, abnormalities in verbal and nonverbal communication and deficits in behavioral flexibility (Bhat et al., 2014). Our analyses did not provide evidence for a conclusive association between ambient ozone exposure and ASD or autism although our literature search delivered some high quality studies (Becerra et al., 2013; Goodrich et al., 2017; Jung et al., 2013; Kim et al., 2017; Volk et al., 2014, 2013). The gene-environmental interaction between ozone exposure and ASD was addressed in studies (Kim et al., 2017; Volk et al., 2014) that found that ozone exposure was only associated with autism risk when accompanied by a high number of genetic copy number aberrations (Kim et al., 2017). Although further research on the complex interactions of heterogeneous genetic predisposition with environmental modifiers is warranted, these findings also suggest that some subpopulations affected by psychiatric morbidity may be more susceptible to ozone exposure than others (Dales and Cakmak, 2016). Furthermore, it may indicate that associations of ozone exposure may not necessarily map to specific disorders per se, but rather impact underlying pathophysiological mechanisms related to them. Hence, genotypic variation should be considered in more detail in future studies.

The dementia, even caused by Alzheimer's disease or Parkinson's disease is categorized as a type of mental health disorder (F00, F02) in ICD-10 (WHO, 2016). A positive association between air pollution and Alzheimer's disease (Block and Calderon-Garciduenas, 2009; Calderon-Garciduenas et al., 2002) or Parkinson's disease (Kremens et al., 2014; Ritz et al., 2016) has already been reported. However, we focus here on the association between ozone and dementia, as this association is less-reported and Alzheimer's disease or Parkinson's disease is more typically categorized as a type of “Diseases of the nervous system” in ICD-10 (WHO, 2016). As a precursor of dementia, impairment of cognitive functions, also cataloged in “Mental and behavioural disorder” ICD-10 (WHO, 2016), may deteriorate into dementia which can in turn result in a three-fold increase in the number of dementia patients by 2050 compared to an already high number of 47 million cases in 2015 (WHO, 2015). While no explicit association was found in this review, ozone as a possible cause for cognition impairment and dementia should be studied in a more systematic manner.

Although suicide (for example X60-X84) is not included in the official ICD-10 F00.0–F99.9 codes (WHO, 2016), it is regarded as a severe consequence of mental disorders such as depression (Draper, 2014; Miret et al., 2013). Therefore, we decided to include studies on ozone and suicide in this review. However, given the heterogeneous study designs and low quality no conclusive results can be derived.

The complex and heterogeneous etiology of mental health problems is still under-investigated and mechanistic models of basically all disorders are either lacking or hypothetical at best. Ozone exposure, a so far scarcely considered environmental risk factor, may be another piece in this puzzle. Based on results from existing animal studies, there are at least five possible mechanisms to explain associations between ozone exposure and mental health. Firstly, ozone is a strong irritant that can result in headache, dizziness, nausea and feelings of ill health (Kleno and Wolkoff, 2004), thereby affecting mental states (Petersen, 2010; Russell, 2017; Walker, 2017). Secondly, inhalation of ozone can provoke inflammatory effects resulting in the production of pro-inflammatory cytokines that are capable of crossing the blood-brain barrier and thereby affect brain function (Dantzer and Kelley, 2007;

Dunn and Swiergiel, 1998), ozone is furthermore known to increase VEGF, IL-6 and TNF α (Araneda et al., 2008) expression in some brain regions. Thirdly, ozone may reduce dopaminergic neurons in CNS (Pereyra-Munoz et al., 2006). Fourthly, ozone can activate the hypothalamo-pituitary-adrenal axis function. Dysregulated hypothalamo-pituitary-adrenal axes with abnormal secretion of hormones take part in the pathological process of mental disorder (Gonzalez-Pina and Paz, 1997). Finally, ozone or its reaction products can affect the metabolism of neurotransmitters like serotonin thereby influencing the function of the nervous system (Odermatt and Gumy, 2008; Thomson et al., 2013).

Although several studies linked ozone exposure to adverse mental outcomes in our review, the evidence presented to date is limited.

According to our quality and risk of bias assessment, controlling for confounding factors is necessary for an accurate estimation of the associations of ozone on mental health. Most of the selected studies already involved several confounders but more covariates should be considered in the future, in particular, meteorological factors. Increasing evidence indicates the importance of gene-environment interactions in associations with ozone (Kim et al., 2017; Volk et al., 2014). Therefore, confounders and effect modifiers, including correlated genotypes, may allow for the derivation of more accurate associations.

Assessment of ozone exposure is another concern. In all selected studies, exposure estimates were simply assigned from monitor stations alone or interpolated with geographic information system techniques such as the inverse-squared weighting method. These techniques are better for estimating long-term exposure but might underestimate spatial contrasts (Brauer et al., 2007) and cannot be used to accurately gauge the exposure of an individual for whom personal monitoring (Choi et al., 2006) or biomonitoring (Autrup et al., 1999) may be more suitable. Furthermore, the definitions of outcomes often represent significant challenges. Future studies should preferentially be based on diagnosis standard criteria for defining the outcome.

Although we attempted to include all published studies on ozone and mental health outcomes, there is a possibility that some published articles were accidentally neglected. Since the studies on each mental and behavioral disorder are few (less than ten), the publication bias and selective reporting are inevitable (Sterne et al., 2011). Additionally, studies with negative results are typically less likely to be published (Siddiqi, 2011; Song et al., 2010) and studies not written in English were excluded from this review. Publication bias is thus likely but cannot be quantified due to the small overall number of studies.

Nevertheless, OHAT approach (OHAT, 2015) extend existing systematic review methods to integrate data from human studies, animal studies and mechanistic studies. A comprehensive review that involve well-designed and result-reasonable animal studies and mechanistic studies would better reveal the association between ozone and mental health.

5. Conclusion

Overall, this review could showcase the large heterogeneity encountered in published studies on ozone exposure and mental health outcomes. Although results from animal models support the notion of adverse effects of ozone on mental health, the little epidemiological evidence we found to date is often inconclusive and does not permit a final verdict. Further high quality studies with more accurate exposure measurements and holistic covariates are warranted.

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Declarations of interest

None.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2018.04.015.

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Appendix: Supplementary Material

Supplementary A. PRISMA checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	NA*
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	4
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	5 (Supplementary B)
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	5
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	5
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	5 (Supplementary B)
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	6

Section/topic	#	Checklist item	Reported on page #
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	NA*
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	12
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	NA
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	6
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	6 (Table. 1)
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	6 (Table. 1)
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	6 (Table. 1) Supplementary C
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	NA*
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	12
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	NA*
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	10
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	11
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	12
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	12

* We did not conduct a meta-analysis.

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

Ambient ozone exposure and mental health: a systematic review of epidemiological studies

Supplementary B. Search strategies

Web of Science

#1 Exposure	(**TI**=("ozone" OR "O3") ***OR*** **TS**=("ozone" OR "O3")) ***AND*** **LANGUAGE:** (English) *AND* **DOCUMENT TYPES:** (Article)
#2 Outcome	(**TI**=("dementia" OR "amnesic syndrome" OR "amnestic" OR "delirium" OR "hallucinosis" OR "catatonic disorder" OR "catatonic" OR "emotionally labile disorder" OR "labile" OR "cognition" OR "cognitive disorder" OR "cognitive" OR "epileptic psychosis" OR "brain syndrome" OR "mental disorder" OR "personality disorder" OR "personality" OR "postconcussional syndrome" OR "postconcussional" OR "psychosyndrome" OR "psychosis" OR "schizophrenia" OR "schizotypal disorders" OR "schizophrenic reaction" OR "delusional disorder" OR "delusional" OR "paranoia" OR "paranoid" OR "paraphrenia" OR "psychotic disorder" OR "psychotic" OR "schizoaffective disorder" OR "schizoaffective" OR "manic episode" OR "manic" OR "hypomania" OR "bipolar disorder" OR "bipolar" OR "depressive episode" OR "depressive" OR "depression" OR "mood disorder" OR "mood" OR "cyclothymia" OR "dysthymia" OR "phobic anxiety disorder" OR "phobia" OR "agoraphobia" OR "anxiety disorder" OR "anxiety" OR "panic disorder" OR "panic" OR "obsessive compulsive disorder" OR "OCD" OR "obsessional" OR "compulsive" OR "stress" OR "post–traumatic stress disorder" OR "PTSD" OR "adjustment disorder" OR "adjustment" OR "dissociative disorders" OR "dissociative" OR "trance and possession disorders" OR "somatoform disorder" OR "somatoform" OR "hypochondriacal disorder" OR "hypochondriacal" OR "neurasthenia" OR "depersonalization-derealization syndrome" OR "Dhat syndrome" OR "neurosis" OR "eating disorder" OR "anorexia nervosa" OR "bulimia nervosa" OR "overeating" OR "vomiting" OR "nonorganic sleep disorder" OR "insomnia" OR "hypersomnia" OR "sleep - wake" OR "sleepwalking" OR "sleep terror" OR "nightmare" OR "sexual dysfunction" OR "sexual desire" OR "sexual aversion" OR "sexual arousal disorder" OR "sexual" OR "erectile disorder" OR "premature ejaculation" OR "puerperium" OR "abuse" OR "habit and impulse disorder" OR "pathological gambling" OR "pathological fire-setting" OR "pathological stealing" OR "trichotillomania" OR "fetishism" OR "exhibitionism" OR "voyeurism" OR "paedophilia" OR "sadomasochism" OR "sexual maturation disorder" OR "sexual maturation disorder" OR "transsexualism" OR "Dual-role transvestism" OR "gender identity disorder" OR "mental retardation" OR "speech articulation disorder" OR "expressive language disorder" OR "receptive language disorder" OR "scholastic skills" OR "motor function" OR "developmental disorders" OR "autism" OR "autism spectrum disorder" OR "ASD" OR "disintegrative disorder" OR "Rett syndrome" OR "Asperger syndrome" OR "hyperkinetic disorder" OR "hyperkinetic" OR "attention deficit hyperactivity disorder" OR "ADHD" OR "attention deficit" OR "conduct disorder" OR "emotional disorder" OR "emotional" OR "mutism" OR "reactive attachment disorder" OR "suicide" OR "suicides") ***OR*** **TS**= ("dementia" OR "amnesic syndrome" OR "amnestic" OR "delirium" OR "hallucinosis" OR "catatonic disorder" OR "catatonic" OR "emotionally labile disorder" OR "labile" OR "cognition" OR "cognitive disorder" OR "cognitive" OR "epileptic psychosis" OR "brain syndrome" OR "mental disorder" OR "personality disorder" OR "personality" OR "postconcussional syndrome" OR "postconcussional" OR "psychosyndrome" OR "psychosis" OR "schizophrenia" OR "schizotypal disorders" OR "schizophrenic reaction" OR "delusional disorder" OR "delusional" OR "paranoia" OR "paranoid" OR "paraphrenia" OR "psychotic disorder" OR "psychotic" OR "schizoaffective disorder" OR "schizoaffective" OR "manic episode" OR "manic" OR "hypomania" OR "bipolar disorder" OR "bipolar" OR "depressive episode" OR "depressive" OR "depression" OR "mood disorder" OR "mood" OR "cyclothymia" OR "dysthymia" OR "phobic anxiety disorder" OR "phobia" OR "agoraphobia" OR "anxiety disorder" OR "anxiety" OR "panic disorder" OR "panic" OR "obsessive compulsive disorder" OR "OCD" OR "obsessional" OR "compulsive" OR "stress" OR "post – traumatic stress disorder" OR "PTSD" OR "adjustment disorder" OR "adjustment" OR "dissociative disorders" OR "dissociative" OR "Trance and possession disorders" OR "somatoform disorder" OR "somatoform" OR "hypochondriacal disorder" OR "hypochondriacal" OR "neurasthenia" OR "depersonalization-derealization syndrome" OR "Dhat syndrome" OR "neurosis" OR "eating disorder" OR "anorexia nervosa" OR "bulimia nervosa" OR "overeating" OR "vomiting" OR "nonorganic sleep disorder" OR "insomnia" OR "hypersomnia" OR "sleep - wake" OR "sleepwalking" OR "sleep terror" OR "nightmare" OR "sexual dysfunction" OR "sexual desire" OR "sexual aversion" OR "sexual arousal disorder" OR "sexual" OR "erectile disorder" OR "premature ejaculation" OR "puerperium" OR "abuse" OR "habit and impulse disorder" OR "pathological gambling" OR "pathological fire-setting" OR "pathological stealing" OR

	"trichotillomania" OR "fetishism" OR "exhibitionism" OR "voyeurism" OR "paedophilia" OR "sadomasochism" OR "sexual maturation disorder" OR "sexual maturation disorder" OR "transsexualism" OR "Dual-role transvestism" OR "gender identity disorder" OR "mental retardation" OR "speech articulation disorder" OR "expressive language disorder" OR "receptive language disorder" OR "scholastic skills" OR "motor function" OR "developmental disorders" OR "autism" OR "autism spectrum disorder" OR "ASD" OR "disintegrative disorder" OR "Rett syndrome" OR "Asperger syndrome" OR "hyperkinetic disorder" OR "hyperkinetic" OR "attention deficit hyperactivity disorder" OR "ADHD" OR "attention deficit" OR "conduct disorder" OR "emotional disorder" OR "emotional" OR "mutism" OR "reactive attachment disorder" OR "suicide" OR "suicides") ***AND*** **LANGUAGE**: (English) ***AND*** **DOCUMENT TYPES**: (Article)
#3 Method	(**TI**=("intervention study" OR "clinical trials" OR "cohort studies" OR "longitudinal studies" OR "case-control studies" OR "health Surveys" OR "cohort" OR "case control" OR "case-control" OR "clinical trial" OR "controlled trial" OR "intervention study" OR "intervention studies" OR "cross-sectional" OR "regression" OR "association") ***OR*** TS=("intervention studies" OR "clinical trials" OR "cohort studies" OR "longitudinal studies" OR "case-control studies" OR "health Surveys" OR "cohort" OR "case control" OR "case-control" OR "clinical trial" OR "controlled trial" OR "intervention study" OR "intervention studies" OR "cross-sectional" OR "regression" OR "association")) ***AND*** **LANGUAGE:** (English) ***AND*** **DOCUMENT TYPES:** (Article)
#4 Exclusion	(**TS**=("mouse" OR "mice" OR "rat" OR "rats" OR "cat" OR "dog" OR "cell" OR "cells" OR "in vivo" OR "in vitro" OR "therapy") ***OR*** **TI**=("mouse" OR "mice" OR "rat" OR "rats" OR "cat" OR "dog" OR "cell" OR "cells" OR "in vivo" OR "in vitro" OR "therapy")) ***AND*** **LANGUAGE:** (English) ***AND*** **DOCUMENT TYPES:** (Article)
Strategy	#1 ***AND*** #2 ***AND*** #3 ***NOT*** #4

EMBASE

#1 Exposure	(ozone or O3).ab.
#2 Outcome	(dementia or amnesic syndrome or amnestic or delirium or hallucinosis or catatonic disorder or catatonic or emotionally labile disorder or labile or cognition or cognitive disorder or cognitive or epileptic psychosis or brain syndrome or mental disorder or personality disorder or personality or postconcussional syndrome or postconcussional or psychosyndrome or psychosis or schizophrenia or schizotypal disorders or schizophrenic reaction or delusional disorder or delusional or paranoia or paranoid or paraphrenia or psychotic disorder or psychotic or schizoaffective disorder or schizoaffective or manic episode or manic or hypomania or bipolar disorder or bipolar or depressive episode or depressive or depression or mood disorder or mood or cyclothymia or dysthymia or phobic anxiety disorder or phobia or agoraphobia or anxiety disorder or anxiety or panic disorder or panic or obsessive compulsive disorder or OCD or obsessional or compulsive or stress or post-traumatic stress disorder or PTSD or adjustment disorder or adjustment or dissociative disorders or dissociative or somatoform disorder or somatoform or hypochondriacal disorder or hypochondriacal or neurasthenia or depersonalization-derealization syndrome or Dhat syndrome or neurosis or eating disorder or anorexia nervosa or bulimia nervosa or overeating or vomiting or nonorganic sleep disorder or insomnia or hypersomnia or sleep - wake or sleepwalking or sleep terror or nightmare or sexual dysfunction or sexual desire or sexual aversion or sexual arousal disorder or sexual or erectile disorder or premature ejaculation or puerperium or abuse or pathological gambling or pathological fire-setting or pathological stealing or trichotillomania or fetishism or exhibitionism or voyeurism or paedophilia or sadomasochism or sexual maturation disorder or sexual maturation disorder or transsexualism or Dual-role transvestism or gender identity disorder or mental retardation or speech articulation disorder or expressive language disorder or receptive language disorder or scholastic skills or motor function or developmental disorders or autism or autism spectrum disorder or ASD or disintegrative disorder or Rett syndrome or Asperger syndrome or hyperkinetic disorder or hyperkinetic or attention deficit hyperactivity disorder or ADHD or attention deficit or conduct disorder or emotional disorder or emotional or mutism or reactive attachment disorder or suicide or suicides).ab.
#3 Method	(intervention studies or cohort studies or longitudinal studies or case-control studies or health Surveys or cohort or case control or case-control or clinical trial or controlled trial or intervention study or intervention studies or cross-sectional or regression or association).ab.
#4 Exclusion	(mouse OR mice OR rat OR rats OR cat OR dog OR cell OR cells OR in vivo OR in vitro OR therapy).ab.
Strategy	1 and #2 and #3 not #4

Pubmed

#1 Exposure	"ozone"[mesh] OR "ozone"[tiab] OR "O3"[tiab]
#2 Outcome	"dementia"[mesh] OR "amnesic syndrome"[mesh] OR "delirium"[mesh] OR "catatonic disorder"[mesh] OR "emotionally labile disorder"[mesh] OR "cognition"[mesh] OR "cognitive disorder"[mesh] OR "mental disorder"[mesh] OR "personality disorder"[mesh] OR "personality"[mesh] OR "postconcussional syndrome"[mesh] OR"schizophrenia"[mesh] OR "schizotypal disorder"[mesh] OR "schizophrenic reaction"[mesh] OR "delusional disorder"[mesh] OR "psychotic disorder"[mesh] OR "psychotic"[mesh] OR "Schizoaffective disorder"[mesh] OR "manic episode"[mesh] OR "hypomania"[mesh] OR "bipolar disorder"[mesh] OR "depressive episode"[mesh] OR "depression"[mesh] OR "mood disorder"[mesh] OR "cyclothymia"[mesh] OR "dysthymia"[mesh] OR "phobic anxiety disorder"[mesh] OR "phobia"[mesh] OR "agoraphobia"[mesh] OR "anxiety disorder"[mesh] OR "panic disorder"[mesh] OR "obsessive compulsive disorder"[mesh] OR "stress"[mesh] OR "post – traumatic stress disorder"[mesh] OR "adjustment disorder"[mesh] OR "dissociative disorder"[mesh] OR "Trance and possession disorder"[mesh] OR "somatoform disorders"[mesh] OR "Hypochondriacal disorder"[mesh] OR "neurasthenia"[mesh] OR "Depersonalization-derealization syndrome"[mesh] OR "Dhat syndrome" [mesh] OR "neurosis"[mesh] OR "eating disorder"[mesh] OR "anorexia nervosa"[mesh] OR "bulimia nervosa"[mesh] OR "Nonorganic sleep disorder"[mesh] OR "insomnia"[mesh] OR "hypersomnia"[mesh] OR "sleep terror"[mesh] OR "nightmare"[mesh] OR "sexual dysfunction"[mesh] OR "sexual desire"[mesh] OR "sexual aversion"[mesh] OR "sexual arousal disorder"[mesh] OR "erectile disorder"[mesh] OR "premature ejaculation"[mesh] OR "puerperium"[mesh] OR "habit and impulse disorder"[mesh] OR "pathological gambling"[mesh] OR "pathological fire-setting"[mesh] OR "pathological stealing"[mesh] OR "trichotillomania"[mesh] OR "fetishism"[mesh] OR "exhibitionism"[mesh] OR "voyeurism"[mesh] OR "paedophilia"[mesh] OR "sadomasochism"[mesh] OR "sexual maturation disorder"[mesh] OR "sexual maturation disorder"[mesh] OR "transsexualism"[mesh] OR "Dual-role transvestism"[mesh] OR "gender identity disorder"[mesh] OR "mental retardation"[mesh] OR "speech articulation disorder"[mesh] OR "expressive language disorder"[mesh] OR "receptive language disorder"[mesh] OR "scholastic skill"[mesh] OR "motor function"[mesh] OR "developmental disorder"[mesh] OR "autism"[mesh] OR "autism spectrum disorder"[mesh] OR "disintegrative disorder"[mesh] OR "Rett syndrome"[mesh] OR "Asperger syndrome"[mesh] OR "hyperkinetic disorder"[mesh] OR "Attention deficit"[mesh] OR "Conduct disorder"[mesh] OR "Emotional disorder"[mesh] OR "Reactive attachment disorder"[mesh] OR "attention deficit hyperactivity disorder"[mesh] OR "suicide" [mesh] OR "dementia"[tiab] OR "amnesic syndrome"[tiab] OR "amnestic"[tiab] OR "delirium"[tiab] OR "hallucinosis"[tiab] OR "catatonic disorder"[tiab] OR "catatonic"[tiab] OR "emotionally labile disorder"[tiab] OR "labile"[tiab] OR "cognition"[tiab] OR "cognitive disorder"[tiab] OR "cognitive"[tiab] OR "Epileptic psychosis"[tiab] OR "brain syndrome"[tiab] OR "mental disorder"[tiab] OR "personality disorder"[tiab] OR "personality"[tiab] OR "postconcussional syndrome"[tiab] OR "postconcussional"[tiab] OR "psychosyndrome"[tiab] OR "psychosis"[tiab] OR "schizophrenia"[tiab] OR "schizotypal disorder"[tiab] OR "schizophrenic reaction"[tiab] OR "delusional disorder"[tiab] OR "delusional"[tiab] OR "paranoia"[tiab] OR "paranoid"[tiab] OR "paraphrenia"[tiab] OR "psychotic disorder"[tiab] OR "psychotic"[tiab] OR "Schizoaffective disorder"[tiab] OR "Schizoaffective"[tiab] OR "manic episode"[tiab] OR "manic"[tiab] OR "hypomania"[tiab] OR "bipolar disorder"[tiab] OR "bipolar"[tiab] OR "depressive episode"[tiab] OR "depressive"[tiab] OR "depression"[tiab] OR "mood disorder"[tiab] OR "mood"[tiab] OR "cyclothymia"[tiab] OR "dysthymia"[tiab] OR "phobic anxiety disorder"[tiab] OR "phobia"[tiab] OR "agoraphobia"[tiab] OR "anxiety disorder"[tiab] OR "anxiety"[tiab] OR "panic disorder"[tiab] OR "panic"[tiab] OR "obsessive compulsive disorder"[tiab] OR "OCD"[tiab] OR "obsessional"[tiab] OR "compulsive"[tiab] OR "stress"[tiab] OR "post – traumatic stress disorder"[tiab] OR "PTSD"[tiab] OR "adjustment disorder"[tiab] OR "adjustment"[tiab] OR "dissociative disorder"[tiab] OR "dissociative"[tiab] OR "trance and possession disorders"[tiab] OR "somatoform disorder"[tiab] OR "somatoform"[tiab] OR "hypochondriacal disorder"[tiab] OR "hypochondriacal"[tiab] OR "neurasthenia"[tiab] OR "depersonalization-derealization syndrome"[tiab] OR "Dhat syndrome" [tiab] OR "neurosis"[tiab] OR "eating disorder"[tiab] OR "anorexia nervosa"[tiab] OR "bulimi a nervosa"[tiab] OR "overeating"[tiab] OR "vomiting"[tiab] OR "Nonorganic sleep disorder"[tiab] OR "insomnia"[tiab] OR "hypersomnia"[tiab] OR "sleep - wake"[tiab] OR "sleepwalking"[tiab] OR "sleep terror"[tiab] OR "nightmare"[tiab] OR "sexual dysfunction"[tiab] OR "sexual desire"[tiab] OR "sexual aversion"[tiab] OR "sexual arousal disorder"[tiab] OR "sexual"[tiab] OR "erectile disorder"[tiab] OR "premature ejaculation"[tiab] OR "puerperium"[tiab] OR "abuse"[tiab] OR "habit and impulse disorder"[tiab] OR "pathological gambling"[tiab] OR "pathological fire-setting"[tiab] OR "pathological stealing"[tiab] OR "trichotillomania"[tiab] OR "fetishism"[tiab] OR

	"exhibitionism"[tiab] OR "voyeurism"[tiab] OR "paedophilia"[tiab] OR "sadomasochism"[tiab] OR "sexual maturation disorder"[tiab] OR "sexual maturation disorder"[tiab] OR "transsexualism"[tiab] OR "Dual-role transvestism"[tiab] OR "gender identity disorder"[tiab] OR "mental retardation"[tiab] OR "speech articulation disorder"[tiab] OR "expressive language disorder"[tiab] OR "receptive language disorder"[tiab] OR "scholastic skills"[tiab] OR "motor function"[tiab] OR "developmental disorders"[tiab] OR "autism"[tiab] OR "autism spectrum disorder"[tiab] OR "ASD"[tiab] OR "disintegrative disorder"[tiab] OR "Rett syndrome"[tiab] OR "Asperger syndrome"[tiab] OR "hyperkinetic disorder"[tiab] OR "hyperkinetic"[tiab] OR "ADHD"[tiab] OR "Attention deficit"[tiab] OR "Conduct disorder"[tiab] OR "Emotional disorder"[tiab] OR "emotional"[tiab] OR "mutism"[tiab] OR "Reactive attachment disorder"[tiab] OR "attention deficit hyperactivity disorder"[tiab] OR "suicide" [tiab] OR "suicide" [tiab]
#3 Method	"intervention studies"[mesh:noexp] OR "clinical trials"[mesh] OR "cohort studies"[mesh:noexp] OR "longitudinal studies"[mesh] OR "case-control studies"[mesh:noexp] OR "Health Surveys"[Mesh:noexp] OR "cohort"[tiab] OR "case control"[tiab] OR "case-control"[tiab] OR "clinical trial"[tiab] OR "controlled trial"[tiab] OR "cross-sectional"[tiab] OR "regression"[tiab] OR "association"[tiab])
#4 Exclusion	"mice"[tiab] OR "mouse"[tiab] OR "rat"[tiab] OR "rats"[tiab] "cat"[tiab]OR "dog"[tiab] OR"cells"[tiab] OR "in vivo"[tiab] OR "in vitro"[tiab] OR "therapy"[ti]
Strategy	1 AND #2 AND #3 NOT #4

Ambient ozone exposure and mental health: a systematic review of epidemiological studies

Supplementary C. PECO statement, quality and risk of bias assessment for each study

The Newcastle-Ottawa scale (Wells G 2013) or criterion from Mustafić (Mustaficet al. 2012) was adopted in this review to evaluate the quality of each study respectively.

The Office of Health Assessment and Translation (OHAT) by the National Institutes of Environmental Health Sciences National Toxicology Program (NEHS-NTP) (OHAT 2015) and Navigation Guide by the University of California (Lamet al. 2016; Woodruff and Sutton 2014) was adopted to evaluate risk of bias for each included study.

Lam, J.; Sutton, P.; Kalkbrenner, A.; Windham, G.; Halladay, A.; Koustas, E.; Lawler, C.; Davidson, L.; Daniels, N.; Newschaffer, C.; Woodruff, T. A Systematic Review and Meta-Analysis of Multiple Airborne Pollutants and Autism Spectrum Disorder. PloS one 2016;11:e0161851

Mustafic, H.; Jabre, P.; Caussin, C.; Murad, M.H.; Escolano, S.; Tafflet, M.; Perier, M.C.; Marijon, E.; Vernerey, D.; Empana, J.P.; Jouven, X. Main air pollutants and myocardial infarction: a systematic review and meta-analysis. Jama 2012;307:713-721

OHAT. Handbook for Conducting Systematic Reviews. Office of Health Assessment and Translation (OHAT) Division of the National Toxicology Program National Institute of Environmental Health Sciences; 2015

Wells G, S.B., O'Connell D, Peterson J, Welch V, Losos M, Tugwell P. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp; 2013

Woodruff, T.J.; Sutton, P. The Navigation Guide systematic review methodology: a rigorous and transparent method for translating environmental health science into better health outcomes. Environ Health Perspect 2014;122:1007-1014

1. Becerra et al. 2013		
Design	Case-control study	
Participants	Human, aged 3-14 years	
Exposure	CO, NO_2, NO, O_3, PM_{10}, $PM_{2.5}$	
Comparison	7594 cases and 75635 controls	
Outcomes	Autism spectrum disorder	
Quality Assessment		
Newcastle-Ottawa Quality Assessment Scale-Case Control Study		**Author's judgement**
Selection	Is the case definition adequate	*
	Representativeness of the case	*
	Selection of controls	*
	Definition of controls	*
Comparability	Comparability of case and controls on the basis of the design or analysis	**
Exposure	Ascertainment of exposure	
	Same method of ascertainment for case and controls	*
	Non-response rate	
Risk of Bias Assessment		
Bias Domain		**Author's judgement**
Key criteria	Detection bias, exposure assessment	Probably high
	Detection bias, outcome assessment	Low
	Confounding bias	Probably low
Other criteria	Selection bias	Low
	Attrition/exclusion bias	Low
	Selective reporting bias	Low
	Conflict of interest	Low
	Other sources of bias	Low

2. Jung et al. 2013		
Design	Cohort study	
Participants	Human, aged less than 3 years	
Exposure	CO, NO_2, O_3, SO_2, PM_{10}	
Comparison	49073 children followed up from 2000 through 2010	
Outcomes	Autism spectrum disorder	
Quality Assessment		
Newcastle-Ottawa Quality Assessment Scale-Cohort Study		**Author's judgement**
Selection	Representative of the exposed cohort	*
	Selection of the non-exposed cohort	*
	Ascertainment of exposure	
	Demonstration that outcome of interest was not present at start of study	*
Comparability	Comparability of cohorts on the basis of the design of analysis	**
Outcome	Assessment of outcome	*
	Was follow-up long enough for outcome to occur	*
	Adequate of follow up of cohorts	*
Risk of Bias Assessment		
Bias Domain		**Author's judgement**
Key criteria	Detection bias, exposure assessment	Probably low
	Detection bias, outcome assessment	Low
	Confounding bias	Probably low
Other criteria	Selection bias	Low
	Attrition/exclusion bias	Low
	Selective reporting bias	Low
	Conflict of interest	Low
	Other sources of bias	Low

3. Kim and Volk et al. 2017		
Design	Case-control study	
Participants	Human, aged 24 – 60 months	
Exposure	$PM_{2.5}$, PM_{10}, O_3, NO_2	
Comparison	158 cases and 147 controls	
Outcomes	Autism spectrum disorder	
Quality Assessment		
Newcastle-Ottawa Quality Assessment Scale-Case Control Study		**Author's judgement**
Selection	Is the case definition adequate	*
	Representativeness of the case	
	Selection of controls	*
	Definition of controls	*
Comparability	Comparability of case and controls on the basis of the design or analysis	**
Exposure	Ascertainment of exposure	
	Same method of ascertainment for case and controls	*
	Non-response rate	
Risk of Bias Assessment		
Bias Domain		**Author's judgement**
Key criteria	Detection bias, exposure assessment	Probably low
	Detection bias, outcome assessment	Low
	Confounding bias	Probably high
Other criteria	Selection bias	Low
	Attrition/exclusion bias	Low
	Selective reporting bias	Probably low
	Conflict of interest	Low
	Other sources of bias	Low

4. Volk et al. 2013		
Design	Case-control study	
Participants	Human, aged 24 – 60 months	
Exposure	$PM_{2.5}$, PM_{10}, O_3, NO_2	
Comparison	279 cases and 245 controls	
Outcomes	Autism spectrum disorder	
Quality Assessment		
Newcastle-Ottawa Quality Assessment Scale-Case Control Study		**Author's judgement**
Selection	Is the case definition adequate	*
	Representativeness of the case	
	Selection of controls	*
	Definition of controls	*
Comparability	Comparability of case and controls on the basis of the design or analysis	**
Exposure	Ascertainment of exposure	
	Same method of ascertainment for case and controls	*
	Non-response rate	
Risk of Bias Assessment		
Bias Domain		**Author's judgement**
Key criteria	Detection bias, exposure assessment	Probably low
	Detection bias, outcome assessment	Low
	Confounding bias	Probably low
Other criteria	Selection bias	Low
	Attrition/exclusion bias	Low
	Selective reporting bias	Low
	Conflict of interest	Low
	Other sources of bias	Low

5. Volk et al. 2014	
Design	Case-control study

Participants	Human, aged 24 – 60 months	
Exposure	$PM_{2.5}$, PM_{10}, O_3, NO_2	
Comparison	252 cases and 156 controls	
Outcomes	Autism spectrum disorder	
Quality Assessment		
Newcastle-Ottawa Quality Assessment Scale-Case Control Study		**Author's judgement**
Selection	Is the case definition adequate	*
	Representativeness of the case	
	Selection of controls	*
	Definition of controls	*
Comparability	Comparability of case and controls on the basis of the design or analysis	**
Exposure	Ascertainment of exposure	
	Same method of ascertainment for case and controls	*
	Non-response rate	
Risk of Bias Assessment		
Bias Domain		**Author's judgement**
Key criteria	Detection bias, exposure assessment	Probably low
	Detection bias, outcome assessment	Low
	Confounding bias	Probably low
Other criteria	Selection bias	Low
	Attrition/exclusion bias	Low
	Selective reporting bias	Low
	Conflict of interest	Low
	Other sources of bias	Low

6. Goodrich et al. 2017		
Design	Case-control study	
Participants	Human, aged 24 – 60 months	
Exposure	$PM_{2.5}$, PM_{10}, O_3, NO_2	
Comparison	346 cases and 260 controls	
Outcomes	Autism spectrum disorder	
Quality Assessment		
Newcastle-Ottawa Quality Assessment Scale-Case Control Study		**Author's judgement**
Selection	Is the case definition adequate	*
	Representativeness of the case	
	Selection of controls	*
	Definition of controls	*
Comparability	Comparability of case and controls on the basis of the design or analysis	**
Exposure	Ascertainment of exposure	
	Same method of ascertainment for case and controls	*
	Non-response rate	
Risk of Bias Assessment		
Bias Domain		**Author's judgement**
Key criteria	Detection bias, exposure assessment	Probably low
	Detection bias, outcome assessment	Low
	Confounding bias	Probably low
Other criteria	Selection bias	Low
	Attrition/exclusion bias	Low
	Selective reporting bias	Low
	Conflict of interest	Low
	Other sources of bias	Low

7. Kerin et al. 2017	
Design	Ecological study
Participants	Human, children with ASD (aged 24 – 60 months)
Exposure	$PM_{2.5}$, PM_{10}, O_3, NO_2
Comparison	

Outcomes	Severity score calibrated by the Mullen Scales of Early Learning (MSEL), the Vineland Adaptive Behavior Scales (VABS), the Autism Diagnostic Observation Schedule (ADOS)	
Risk of Bias Assessment		
Bias Domain		**Author's judgement**
Key criteria	Detection bias, exposure assessment	Probably low
	Detection bias, outcome assessment	Probably low
	Confounding bias	Probably low
Other criteria	Selection bias	Probably low
	Attrition/exclusion bias	Low
	Selective reporting bias	Low
	Conflict of interest	Low
	Other sources of bias	Low

8. Chen et al. 2009		
Design	Cross-sectional study	
Participants	Human, adult (age 37.5 ± 10.9)	
Exposure	PM_{10}, O_3	
Comparison		
Outcomes	Scores of simple reaction time test (SRTT), symbol – digit substitution test (SDST), serial – digital learning test (SDLT)	
Risk of Bias Assessment		
Bias Domain		**Author's judgement**
Key criteria	Detection bias, exposure assessment	Probably low
	Detection bias, outcome assessment	Probably low
	Confounding bias	Probably low
Other criteria	Selection bias	Probably low
	Attrition/exclusion bias	Low
	Selective reporting bias	Low
	Conflict of interest	Low
	Other sources of bias	Low

9. Gatto et al. 2014		
Design	Cross-sectional study	
Participants	Human, adult participants (age 60.5 ± 8.1)	
Exposure	O_3, NO_2, $PM_{2.5}$	
Comparison		
Outcomes	Cognitive tests (executive function, verbal learning, logical memory, visual processing, visual episodic memory, semantic memory)	
Risk of Bias Assessment		
Bias Domain		**Author's judgement**
Key criteria	Detection bias, exposure assessment	Probably low
	Detection bias, outcome assessment	Probably low
	Confounding bias	Probably low
Other criteria	Selection bias	Probably low
	Attrition/exclusion bias	Low
	Selective reporting bias	Low
	Conflict of interest	Low
	Other sources of bias	Low

10. Calderón-Garcidueñas et al. 2015		
Design	Cross-sectional study	
Participants	Human, children (age 12.45 ± 3.4) and their parents (age 37.5 ± 6.77) from Mexico City;	
Exposure	$PM_{2.5}$, O_3	
Comparison	children (age 9.77 ± 0.83) and their parents (age 34.57 ± 6.02) from Polotitlán	
Outcomes	NNA/Cr, Cho/Cr and mI/Cr ratios [N–acetylaspartate (NAA), choline, creatine (Cr) and myoinositol (mI)]	
Risk of Bias Assessment		
Bias Domain		**Author's judgement**
Key criteria	Detection bias, exposure assessment	Probably high
	Detection bias, outcome assessment	Probably high
	Confounding bias	Probably low
Other criteria	Selection bias	Probably low
	Attrition/exclusion bias	Low
	Selective reporting bias	Probably low
	Conflict of interest	Low
	Other sources of bias	Low

11. Wu et al. 2015		
Design	Case-control study	
Participants	Human, Alzheimer's disease (AD) patients and vascular dementia, aged ≥ 60	
Exposure	PM_{10},ozone	
Comparison	497 controls	
Outcomes	AD or VaD diagnosed	
Quality Assessment		
Newcastle-Ottawa Quality Assessment Scale-Case Control Study		**Author's judgement**
Selection	Is the case definition adequate	*
	Representativeness of the case	
	Selection of controls	
	Definition of controls	*
Comparability	Comparability of case and controls on the basis of the design or analysis	**
Exposure	Ascertainment of exposure	
	Same method of ascertainment for case and controls	*
	Non-response rate	
Risk of Bias Assessment		
Bias Domain		**Author's judgement**
Key criteria	Detection bias, exposure assessment	Probably low
	Detection bias, outcome assessment	Probably low
	Confounding bias	Probably low
Other criteria	Selection bias	Low
	Attrition/exclusion bias	Low
	Selective reporting bias	Low
	Conflict of interest	Low
	Other sources of bias	Low

12. Linares et al. 2017		
Design	Time-series study	
Participants	Human, 1175 dementia-related emergency	
Exposure	$PM_{10,}$ $PM_{2.5}$, O_3, NO_2	
Comparison		
Outcomes	AD or VaD diagnosed	
Quality Assessment		
Mustafić's criterion		**Author's judgement**
Exposure	ICD or triad of clinical and laboratory criteria	*
Outcome	Air pollutant measurements frequency and missing data	*
Confounders	Long-term trends, seasonality, temperature and more confounders	
Risk of Bias Assessment		
Bias Domain		**Author's judgement**
Key criteria	Detection bias, exposure assessment	Probably low
	Detection bias, outcome assessment	Probably low
	Confounding bias	High
Other criteria	Selection bias	Probably low
	Attrition/exclusion bias	Low
	Selective reporting bias	Low
	Conflict of interest	Low
	Other sources of bias	Low

13. Cleary et al. 2018		
Design	Cohort study	
Participants	Human, participants aged 60 or more with a baseline Mini-Mental Status Examination score > 0 and a diagnosis of cognitive impairment in at least on follow-up visit	
Exposure	Ozone, $PM_{2.5}$	
Comparison	3624 participants with normal cognition, 1492 participants with cognitive impairment, diagnosed based on examination	
Outcomes	Cognitive impairment	
Quality Assessment		
Newcastle-Ottawa Quality Assessment Scale-Cohort Study		**Author's judgement**
Selection	Representative of the exposed cohort	*
	Selection of the non-exposed cohort	*
	Ascertainment of exposure	
	Demonstration that outcome of interest was not present at start of study	*
Comparability	Comparability of cohorts on the basis of the design of analysis	**
Outcome	Assessment of outcome	
	Was follow-up long enough for outcome to occur	*
	Adequate of follow up of cohorts	*
Risk of Bias Assessment		
Bias Domain		**Author's judgement**
Key criteria	Detection bias, exposure assessment	Probably low
	Detection bias, outcome assessment	Low
	Confounding bias	Probably low
Other criteria	Selection bias	Low
	Attrition/exclusion bias	Low
	Selective reporting bias	Probably low
	Conflict of interest	Low
	Other sources of bias	Low

14. Chen et al. 2017		
Design	Cohort study	
Participants	Human, 2066639 individuals	
Exposure	$PM_{2.5}$, NO_2, O_3,	
Comparison	257816 incident cases of dementia	
Outcomes	Dementia	
Quality Assessment		
Newcastle-Ottawa Quality Assessment Scale-Cohort Study		**Author's judgement**
Selection	Representative of the exposed cohort	*
	Selection of the non-exposed cohort	*
	Ascertainment of exposure	
	Demonstration that outcome of interest was not present at start of study	
Comparability	Comparability of cohorts on the basis of the design of analysis	**
Outcome	Assessment of outcome	*
	Was follow-up long enough for outcome to occur	*
	Adequate of follow up of cohorts	*
Risk of Bias Assessment		
Bias Domain		**Author's judgement**
Key criteria	Detection bias, exposure assessment	Probably low
	Detection bias, outcome assessment	Probably low
	Confounding bias	Low
Other criteria	Selection bias	Low
	Attrition/exclusion bias	Low
	Selective reporting bias	Low
	Conflict of interest	Low
	Other sources of bias	Low

15. Szysykowicy et al. 2007		
Design	Time-series study	
Participants	Human, 15556 emergency department visits	
Exposure	CO, NO_2, SO_2 , O_3, PM_{10}, $PM_{2.5}$	
Comparison		
Outcomes	Emergency department visits for depression	
Quality Assessment		
Mustafić's criterion		**Author's judgement**
Exposure	ICD or triad of clinical and laboratory criteria	*
Outcome	Air pollutant measurements frequency and missing data	*
Confounders	Long-term trends, seasonality, temperature and more confounders	
Risk of Bias Assessment		
Bias Domain		**Author's judgement**
Key criteria	Detection bias, exposure assessment	Probably low
	Detection bias, outcome assessment	Probably low
	Confounding bias	Probably high
Other criteria	Selection bias	Probably low
	Attrition/exclusion bias	Low
	Selective reporting bias	Low
	Conflict of interest	Low
	Other sources of bias	Low

16. Lim et al. 2012		
Design	Cohort study	
Participants	Human, 537 participants (averaged age 71 ± 5)	
Exposure	$PM_{2.5}$, NO_2, O_3,	
Comparison	537 participants performed from 2008 to 2010	
Outcomes	Depression	
Quality Assessment		
Newcastle-Ottawa Quality Assessment Scale-Cohort Study		**Author's judgement**
Selection	Representative of the exposed cohort	*
	Selection of the non-exposed cohort	*
	Ascertainment of exposure	
	Demonstration that outcome of interest was not present at start of study	*
Comparability	Comparability of cohorts on the basis of the design of analysis	**
Outcome	Assessment of outcome	
	Was follow-up long enough for outcome to occur	*
	Adequate of follow up of cohorts	*
Risk of Bias Assessment		
Bias Domain		**Author's judgement**
Key criteria	Detection bias, exposure assessment	Probably low
	Detection bias, outcome assessment	Probably low
	Confounding bias	Low
Other criteria	Selection bias	Low
	Attrition/exclusion bias	Low
	Selective reporting bias	Low
	Conflict of interest	Low
	Other sources of bias	Low

17. Szysykowicy et al. 2016		
Design	Case-crossover study	
Participants	Human, 118602 emergency department visits	
Exposure	PM_{10}, NO_2, O_3, CO, SO_2	
Comparison		
Outcomes	Depression	
Quality Assessment		
Mustafić's criterion		**Author's judgement**
Exposure	ICD or triad of clinical and laboratory criteria	*
Outcome	Air pollutant measurements frequency and missing data	*
Confounders	Long-term trends, seasonality, temperature and more confounders	
Risk of Bias Assessment		
Bias Domain		**Author's judgement**
Key criteria	Detection bias, exposure assessment	Probably low
	Detection bias, outcome assessment	Probably low
	Confounding bias	High
Other criteria	Selection bias	Probably low
	Attrition/exclusion bias	Low
	Selective reporting bias	Low
	Conflict of interest	Low
	Other sources of bias	Low

18. Kioumourtzoglou et al. 2017		
Design	Cohort study	
Participants	Human, women, averaged age 66.6 ± 7.6	
Exposure	O_3, $PM_{2.5}$	
Comparison	41844 women followed from 1996 to 2006	
Outcomes	Depression	
Quality Assessment		
Newcastle-Ottawa Quality Assessment Scale-Cohort Study		**Author's judgement**
Selection	Representative of the exposed cohort	*
	Selection of the non-exposed cohort	*
	Ascertainment of exposure	
	Demonstration that outcome of interest was not present at start of study	*
Comparability	Comparability of cohorts on the basis of the design of analysis	**
Outcome	Assessment of outcome	
	Was follow-up long enough for outcome to occur	*
	Adequate of follow up of cohorts	*
Risk of Bias Assessment		
Bias Domain		**Author's judgement**
Key criteria	Detection bias, exposure assessment	Low
	Detection bias, outcome assessment	Probably high
	Confounding bias	Low
Other criteria	Selection bias	Low
	Attrition/exclusion bias	Low
	Selective reporting bias	Low
	Conflict of interest	Low
	Other sources of bias	Low

19. Szysykowicy et al. 2007		
Design	Time-series study	
Participants	Human, 27047 emergency department visits	
Exposure	CO, NO_2, SO_2 , O_3, PM_{10}, $PM_{2.5}$	
Comparison		
Outcomes	Emergency department visits for depression	
Quality Assessment		
Mustafić's criterion		**Author's judgement**
Exposure	ICD or triad of clinical and laboratory criteria	*
Outcome	Air pollutant measurements frequency and missing data	*
Confounders	Long-term trends, seasonality, temperature and more confounders	
Risk of Bias Assessment		
Bias Domain		**Author's judgement**
Key criteria	Detection bias, exposure assessment	Probably low
	Detection bias, outcome assessment	Probably low
	Confounding bias	Probably high
Other criteria	Selection bias	Probably low
	Attrition/exclusion bias	Low
	Selective reporting bias	Low
	Conflict of interest	Low
	Other sources of bias	Low

20. Wang et al. 2014		
Design	Cohort study	
Participants	Human, adults ≥ 65 years of age (78.1 ± 5.5)	
Exposure	O_3, CO, NO_2, NO	
Comparison	732 Boston-area adults ≥ 65 years of age recruited between 2005 and 2008	
Outcomes	Depressive symptoms by 20-item Revised Centre for Epidemiological Studies Depression Scale (CESD-R)	
Quality Assessment		
Newcastle-Ottawa Quality Assessment Scale-Cohort Study		**Author's judgement**
Selection	Representative of the exposed cohort	*
	Selection of the non-exposed cohort	*
	Ascertainment of exposure	
	Demonstration that outcome of interest was not present at start of study	*
Comparability	Comparability of cohorts on the basis of the design of analysis	**
Outcome	Assessment of outcome	
	Was follow-up long enough for outcome to occur	*
	Adequate of follow up of cohorts	*
Risk of Bias Assessment		
Bias Domain		**Author's judgement**
Key criteria	Detection bias, exposure assessment	Probably high
	Detection bias, outcome assessment	Probably low
	Confounding bias	Low
Other criteria	Selection bias	Low
	Attrition/exclusion bias	Low
	Selective reporting bias	Low
	Conflict of interest	Low
	Other sources of bias	Low

21. Biermann et al. 2008		
Design	Cross-sectional study	
Participants	Human, 1008 suicides and 917 suicide attempts	
Exposure	O_3	
Comparison		
Outcomes	1008 suicides as well as 917 suicide attempts	
Risk of Bias Assessment		
Bias Domain		**Author's judgement**
Key criteria	Detection bias, exposure assessment	Probably high
	Detection bias, outcome assessment	Probably low
	Confounding bias	high
Other criteria	Selection bias	Probably low
	Attrition/exclusion bias	Low
	Selective reporting bias	Low
	Conflict of interest	Low
	Other sources of bias	Low

22. Yang et al. 2010		
Design	Ecological study	
Participants	Human, 4857 deaths by suicide	
Exposure	SO_2, NO_x, O_3, CO, PM_{10}	
Comparison		
Outcomes	4857 deaths by suicide, average counts 22.5 ± 9.6 cases, range = 6 -59 cases	
Risk of Bias Assessment		
Bias Domain		**Author's judgement**
Key criteria	Detection bias, exposure assessment	Probably high
	Detection bias, outcome assessment	Probably low
	Confounding bias	Probably high
Other criteria	Selection bias	Probably low
	Attrition/exclusion bias	Low
	Selective reporting bias	Low
	Conflict of interest	Low
	Other sources of bias	Low

23. Kim et al. 2015		
Design	Time-series study	
Participants	Human, the suicide rate per 10 million persons	
Exposure	O_3, PM_{10}, NO_2, CO, SO_2	
Comparison		
Outcomes	The variation of weekly suicide rate	
Quality Assessment		
Mustafić's criterion		**Author's judgement**
Exposure	ICD or triad of clinical and laboratory criteria	*
Outcome	Air pollutant measurements frequency and missing data	*
Confounders	Long-term trends, seasonality, temperature and more confounders	*
Risk of Bias Assessment		
Bias Domain		**Author's judgement**
Key criteria	Detection bias, exposure assessment	Probably high
	Detection bias, outcome assessment	Probably low
	Confounding bias	Low
Other criteria	Selection bias	Probably low
	Attrition/exclusion bias	Low
	Selective reporting bias	Low
	Conflict of interest	Low
	Other sources of bias	Low

24. Casas et al. 2017		
Design	Time-series study	
Participants	Human, suicide deaths registered	
Exposure	O_3, PM_{10}	
Comparison		
Outcomes	20533 suicide deaths	
Quality Assessment		
Mustafić's criterion		**Author's judgement**
Exposure	ICD or triad of clinical and laboratory criteria	*
Outcome	Air pollutant measurements frequency and missing data	*
Confounders	Long-term trends, seasonality, temperature and more confounders	*
Risk of Bias Assessment		
Bias Domain		**Author's judgement**
Key criteria	Detection bias, exposure assessment	Probably low
	Detection bias, outcome assessment	Probably low
	Confounding bias	Probably low
Other criteria	Selection bias	Probably low
	Attrition/exclusion bias	Low
	Selective reporting bias	Low
	Conflict of interest	Low
	Other sources of bias	Low

25. Szysykowicy et al. 2010	
Design	Case-crossover study
Participants	Human, emergency department visits with suicide attempt / ideation
Exposure	NO_2, SO_2, O_3, CO, $PM_{10,}$ $PM_{2.5}$
Comparison	
Outcomes	1605 emergency department visits with suicide attempt / ideation
Quality Assessment	

Mustafić's criterion		**Author's judgement**
Exposure	ICD or triad of clinical and laboratory criteria	*
Outcome	Air pollutant measurements frequency and missing data	*
Confounders	Long-term trends, seasonality, temperature and more confounders	
Risk of Bias Assessment		
Bias Domain		**Author's judgement**
Key criteria	Detection bias, exposure assessment	Probably low
	Detection bias, outcome assessment	Probably low
	Confounding bias	Probably high
Other criteria	Selection bias	Probably low
	Attrition/exclusion bias	Low
	Selective reporting bias	Low
	Conflict of interest	Low
	Other sources of bias	Low

26. Rotton, 1993		
Design	Cross-sectional study	
Participants	Human, 584 reports of rape, 674 complaints about obscene phone calls, 288 calls about indecent exposure and 547 more complaints	
Exposure	Ozone and meteorological variables	
Comparison		
Outcomes	Sex crimes reported by police department	
Risk of Bias Assessment		
Bias Domain		**Author's judgement**
Key criteria	Detection bias, exposure assessment	Probably high
	Detection bias, outcome assessment	Probably high
	Confounding bias	Probably low
Other criteria	Selection bias	Probably low
	Attrition/exclusion bias	Low
	Selective reporting bias	Low
	Conflict of interest	Low
	Other sources of bias	Low

27. Chen et al.2017		
Design	Case-crossover study	
Participants	Human, cases of hospital admissions for mental disorder	
Exposure	NO_2, SO_2, CO, O_3, PM_{10}, $PM_{2.5}$	
Comparison		
Outcomes	39143 cases of daily hospital admissions for mental disorder	
Quality Assessment		
Mustafić's criterion		**Author's judgement**
Exposure	ICD or triad of clinical and laboratory criteria	*
Outcome	Air pollutant measurements frequency and missing data	*
Confounders	Long-term trends, seasonality, temperature and more confounders	**
Risk of Bias Assessment		
Bias Domain		**Author's judgement**
Key criteria	Detection bias, exposure assessment	Probably low
	Detection bias, outcome assessment	Low
	Confounding bias	Probably low
Other criteria	Selection bias	Probably low
	Attrition/exclusion bias	Low
	Selective reporting bias	Low
	Conflict of interest	Low
	Other sources of bias	Low

28. Lin et al. 2014		
Design	Cross-sectional study	
Participants	Human, 533 mother-infant pairs	
Exposure	PM_{10}, CO, O_3, SO_2, NO_2	
Comparison		
Outcomes	The 6- and 18-month scales (the Bayley Scales of Infant Development, consists of: gross motor, fine motor, language/communication, social/self-care abilities)	
Risk of Bias Assessment		
Bias Domain		**Author's judgement**
Key criteria	Detection bias, exposure assessment	Probably low
	Detection bias, outcome assessment	Probably low
	Confounding bias	Low
Other criteria	Selection bias	Probably low
	Attrition/exclusion bias	Low
	Selective reporting bias	Low
	Conflict of interest	Low
	Other sources of bias	Low

29. Cho et al. 2015		
Design	Time-series study	
Participants	Human, individuals who visited the emergency department with panic attack	
Exposure	PM_{10}, SO_2, NO_2, O_3, CO	
Comparison		
Outcomes	2320 emergency department visits for panic attacks	
Quality Assessment		
Mustafić's criterion		**Author's judgement**
Exposure	ICD or triad of clinical and laboratory criteria	*
Outcome	Air pollutant measurements frequency and missing data	*
Confounders	Long-term trends, seasonality, temperature and more confounders	*
Risk of Bias Assessment		
Bias Domain		**Author's judgement**
Key criteria	Detection bias, exposure assessment	Probably low
	Detection bias, outcome assessment	Probably low
	Confounding bias	Probably low
Other criteria	Selection bias	Probably low
	Attrition/exclusion bias	Low
	Selective reporting bias	Low
	Conflict of interest	Low
	Other sources of bias	Low

30. Oudin et al. 2018		
Design	Case-crossover study	
Participants	Human, psychiatric emergency visits	
Exposure	PM_{10}, O_3, NO_2	
Comparison		
Outcomes	Number of psychiatric emergency visits was 27 ± 6	
Quality Assessment		
Mustafić's criterion		**Author's judgement**
Exposure	ICD or triad of clinical and laboratory criteria	*
Outcome	Air pollutant measurements frequency and missing data	*
Confounders	Long-term trends, seasonality, temperature and more confounders	
Risk of Bias Assessment		
Bias Domain		**Author's judgement**
Key criteria	Detection bias, exposure assessment	Probably high
	Detection bias, outcome assessment	Low
	Confounding bias	High
Other criteria	Selection bias	Probably low
	Attrition/exclusion bias	Low
	Selective reporting bias	Low
	Conflict of interest	Low
	Other sources of bias	Low

31. Tallon et al. 2017		
Design	Cohort study	
Participants	Human, 412 household-resident older adults aged 57 – 85	
Exposure	$PM_{2.5}$, NO_2, O_3	
Comparison	49073 children followed up from 2000 through 2010	
Outcomes	132 men with erectile dysfunction , 280 men without erectile dysfunction	
Quality Assessment		
Newcastle-Ottawa Quality Assessment Scale-Cohort Study		**Author's judgement**
Selection	Representative of the exposed cohort	*
	Selection of the non-exposed cohort	*
	Ascertainment of exposure	
	Demonstration that outcome of interest was not present at start of study	*
Comparability	Comparability of cohorts on the basis of the design of analysis	**
Outcome	Assessment of outcome	
	Was follow-up long enough for outcome to occur	*
	Adequate of follow up of cohorts	*
Risk of Bias Assessment		
Bias Domain		**Author's judgement**
Key criteria	Detection bias, exposure assessment	Probably low
	Detection bias, outcome assessment	Probably high
	Confounding bias	Probably low
Other criteria	Selection bias	Low
	Attrition/exclusion bias	Low
	Selective reporting bias	Low
	Conflict of interest	Low
	Other sources of bias	Low

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Supplementary D. Criteria for the risk of bias assessment

Criteria for the risk of bias assessment of each study, adapted from the OHAT and Navigation Guide tool

Bias		**Risk of Bias Domains and Ratings**	**Answer**
Key Criteria	**Detection bias, exposure assessment**	Can we be confident in the exposure characterization? List of major considerations: 1) air pollution measurements were performed daily, < 25% missing data 2) more than one monitoring station per a large geographical area 3) models were used for weighting	-LOW risk: There is high confidence that the exposure to ozone is the true average population exposure. -PROBABLY LOW: There is indirect evidence that suggests low risk of bias, or one of the three listed considerations is not applied. -PROBABLY HIGH risk: There is insufficient information to permit a judgment of high risk of bias, but there is indirect evidence that suggests high risk of bias. Additionally, two out of the three listed considerations are not applied. -HIGH risk: There is direct evidence of high risk of misclassification bias, or all three of the listed considerations are not applied.
	Detection bias, outcome assessment	Can we be confident in the outcome assessment?	-LOW risk: Outcome was classified based on diagnosis standard criteria (International Classification System code) and provided by a national or regional database. -PROBABLY LOW: Outcome was assessed based on diagnosis standard criteria and collected by researcher -PROBABLY HIGH risk: Outcome was not assessed based on standard diagnosis criteria AND is accompanied by validation sub-study or sensitivity analysis to suggest that the risk is minimum. -HIGH risk: Outcome was assessed based on self-reports (parents, family) and data collected by the researcher.
	Confounding bias	Did the study design or analysis account for important confounding and modifying variables?	-LOW risk: Study accounted for all important confounders which were measured consistently -PROBABLY LOW: Study accounted for most of confounders AND is not expected to introduce bias -PROBABLY HIGH risk: Study accounted for some but not all of confounders AND is expected to introduce bias -HIGH risk: Study did not account for potential confounders OR were inappropriately measured
Other Criteria	**Selection bias**	Did selection of study participants result in appropriate comparison groups?	-LOW risk: The descriptions of the studied population were sufficiently detailed to support the assertion that risk of selection effects was minimal. -PROBABLY LOW risk: There is insufficient information about population selection to permit a

			judgment of low risk of bias, but there is indirect evidence that suggests low risk of bias. -PROBABLY HIGH risk: There is insufficient information about population selection to permit a judgment of high risk of bias, but there is indirect evidence that suggests high risk of bias. - HIGH risk: There were indications from descriptions of the studied population of high risk of bias.
	Attrition/exclusion bias	Were outcome data complete without attrition or exclusion from analysis?	-LOW risk: There were no missing outcome data or missing data unrelated to true outcome -PROBABLY LOW: There was insufficient information about incomplete data to judge for low risk, but indirect evidence that suggests low risk of bias -PROBABLY HIGH risk: There was insufficient information about incomplete data to judge for high risk, but indirect evidence that suggests high risk -HIGH risk: Missing outcome data is related to true outcome
	Selective reporting bias	Were all measured outcomes reported?	-LOW risk: All of the studies pre-specified outcomes and findings are reported -PROBABLY LOW: There was insufficient information about selective outcome to judge for low risk, but indirect evidence that suggests study was free of selective report -PROBABLY HIGH risk: There was insufficient information about selective reporting to judge for high risk, but indirect evidence suggests that study was not free of selective reporting -HIGH risk: Not all pre-specified outcomes and findings were reported, or one/more of the primary outcomes or analyses were assessed or executed with other methods than the pre-specified one, or one/more of the reported outcomes/findings was/were not pre-specified
	Conflict of interest	Potential source of bias in reporting through source of funding	-LOW risk: The study did not receive funding from an entity with financial interest in the outcome of study -PROBABLY LOW: There is insufficient information to judge for low risk, but indirect evidence suggests study was free of financial interest -PROBABLY HIGH risk: There is insufficient information to judge for high risk, but indirect evidence suggests study was not free of financial interest -HIGH risk: The study received support from an entity with financial interest in the outcome of study
	Other source of bias	Bias due to other problems not covered elsewhere (statistical methods were appropriate and researchers adhere to the study protocol)	-LOW risk: No other sources of bias -PROBABLY LOW: There is insufficient information to judge for low risk, but indirect evidence suggests study was free of other problems -PROBABLY HIGH risk: There is insufficient information to judge for high risk, but indirect evidence suggests study was not free of other problems -HIGH risk: There was at least one important risk of bias

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Curriculum Vitae