ehp ENVIRONMENTAL HEALTH PERSPECTIVES
http://www.ehponline.org

Neurodevelopmental Deceleration by Urban Fine Particles from Different Emission Sources: A Longitudinal Observational Study

Xavier Basagaña, Mikel Esnaola, Ioar Rivas, Fulvio Amato, Mar Alvarez-Pedrerol, Joan Forns, Mònica López-Vicente, Jesús Pujol, Mark Nieuwenhuijsen, Xavier Querol, and Jordi Sunyer

http://dx.doi.org/10.1289/EHP209

Received: 10 March 2015
Revised: 30 September 2015
Accepted: 12 April 2016
Published: 29 April 2016

Environ Health Perspect DOI: 10.1289/EHP209
Advance Publication: Not Copyedited

Neurodevelopmental Deceleration by Urban Fine Particles from Different Emission Sources: A Longitudinal Observational Study

Xavier Basagaña[1,2,3], Mikel Esnaola[1,2,3], Ioar Rivas[1,2,3,4], Fulvio Amato[4], Mar Alvarez-Pedrerol[1,2,3], Joan Forns[1,2,3,5], Mònica López-Vicente[1,2,3], Jesús Pujol[6,7], Mark Nieuwenhuijsen[1,2,3], Xavier Querol[4], and Jordi Sunyer[1,2,3,8]

[1]Centre for Research in Environmental Epidemiology (CREAL), Barcelona, Catalonia, Spain; [2]Universitat Pompeu Fabra (UPF), Barcelona, Catalonia, Spain; [3]CIBER Epidemiología y Salud Pública (CIBERESP), Spain; [4]Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona, Catalonia, Spain; [5]Department of Genes and Environment, Division of Epidemiology, Norwegian Institute of Public Health, Oslo, Norway; [6]MRI Research Unit, CRC Mar, Hospital del Mar, Barcelona, Spain; [7]Centro Investigación Biomédica en Red de Salud Mental, CIBERSAM G21, Barcelona, Spain; [8]IMIM (Hospital del Mar Medical Research Institute), Barcelona, Catalonia, Spain

Address correspondence to X Basagaña, Centre for Research in Environmental Epidemiology (CREAL), c/ Doctor Aiguader 88, 08003 Barcelona, Spain. Telephone: (+34) 932147306. E-mail: xbasagana@creal.cat

Running title: Neurodevelopment and source-specific particles

Acknowledgments: This work was supported by the European Research Council under the ERC [grant number 268479] – the BREATHE project. Fulvio Amato is beneficiary of the Juan de la Cierva postdoctoral Grant (JCI-2012-13473) from the Spanish Ministry of

Economy and Competitiveness. We are acknowledged with all the families participating into the study for their altruism and particularly to the schools Antoni Brusi, Baloo, Betània – Patmos, Centre d'estudis Montseny, Col.legi Shalom, Costa i Llobera, El sagrer, Els Llorers, Escola Pia de Sarrià, Escola Pia Balmes, Escola concertada Ramon Llull, Escola Lourdes, Escola Tècnica Professional del Clot, Ferran i Clua, Francesc Macià, Frederic Mistral, Infant Jesús, Joan Maragall, Jovellanos, La Llacuna del Poblenou, Lloret, Menéndez Pidal, Nuestra Señora del Rosario, Miralletes, Ramon Llull, Rius i Taulet, Pau Vila, Pere Vila, Pi d'en Xandri, Projecte, Prosperitat, Sant Ramon Nonat - Sagrat Cor, Santa Anna, Sant Gregori, Sagrat Cor Diputació, Tres Pins, Tomàs Moro, Torrent d'en Melis, Virolai. We also acknowledge the ESCAPE project for the design and supervision modelling of air pollution. Furthermore, Xavier Mayoral did the technical development of the n-back test; Cecilia Persavento, Judit Gonzalez, Laura Bouso and Pere Figueras contributed to the field work.

Declaration of interests: We have no competing interests.

Abstract

Background: A few studies have reported associations between traffic-related air pollution exposure at schools and cognitive development. The role of PM components or sources other than traffic on cognitive development has been little explored.

Objectives: We aimed to explore the role of PM sources in school air on cognitive development.

Methods: A cohort of 2618 schoolchildren (average age 8.5 years) belonging to 39 schools in Barcelona (Spain) was followed up for a year. Children completed computerized tests assessing working memory, superior working memory and inattentiveness during four visits. Particulate matter < 2.5 μm ($PM_{2.5}$) was measured during two one-week campaigns in each school outdoors and in the classroom. Source apportionment resulted in nine sources: mineral, organic/textile/chalk, traffic, secondary sulphate & organics, secondary nitrate, road dust, metallurgy, sea spray, and heavy oil combustion. Differences in cognitive growth trajectories were assessed with mixed models with age-by-source interaction terms.

Results: An interquartile change increase in indoor traffic-related $PM_{2.5}$ was associated with reductions in cognitive growth equivalent to 22% (95% confidence interval (CI): 2%, 42%) of the annual change in working memory, 30% (95% CI: 6%, 54%) of the annual change in superior working memory, and 11% (95% CI: 0%, 22%) of the annual change in the inattentiveness scale. None of the other $PM_{2.5}$ sources was associated with adverse effects on cognitive development.

Conclusions: Traffic was the only source of fine particles associated with a reduction in cognitive development. Reducing air pollution from traffic at primary schools may result in beneficial effects on cognition.

Introduction

Particulate matter (PM) air pollution is known to produce adverse health effects with important consequences at the population level (Brunekreef and Holgate 2002; Lim et al. 2012; WHO (World Health Organization) 2013). Although the most well-established evidence for a deleterious role of PM concerns cardiovascular and respiratory diseases, emerging evidence suggest that PM exposure can also affect neurodevelopment and cognitive function (Block et al. 2012; WHO (World Health Organization) 2013) . This is supported by animal studies showing neuroinflammation and neuropathological damage in the brain as well as alterations in learning and memory functions in response to air pollution exposure (Block et al. 2012).

Particulate matter is a complex mixture of different components originating from different sources. A better understanding of which components and sources of PM are responsible for the health effects is very important from the regulatory point of view. Using chemical speciation of PM measurements and source apportionment techniques, it is now possible to estimate the concentration attributable to different sources (e.g. traffic, biomass burning, industry or natural sources) (Viana et al. 2008). Recent studies have examined the role of source-specific pollution on health outcomes, mostly cardiovascular and respiratory mortality or hospital admissions. Most of the evidence for harmful effects of air pollution refers to traffic-related air pollution, although the effects of other sources such coal combustion, shipping, road dust or desert dust have also been documented (Cassee et al. 2013; Ostro et al. 2011; WHO (World Health Organization) 2013). The biological mechanisms leading to neurodevelopment effects may be different from those described for cardiovascular and respiratory effects, and the chemical composition of the particles, their

size or their surface area can play a relevant role. For example, suggested mechanisms include disruptions of the nasal and olfactory barrier and the blood-brain barrier allowing direct access of ultrafine particles to the brain. PM was seen in olfactory bulb neurons in children autopsies and PM-associated metals such as nickel (Ni) or vanadium (V) were detected in the brains of dogs (Calderon-Garciduenas et al. 2003; Calderon-Garciduenas et al. 2008).

In a recent longitudinal study of schoolchildren, we reported that cognitive development over 1 year showed a slower increase among children attending schools with high traffic air pollution levels compared to children in less polluted schools (Sunyer et al. 2015). In that study, the air pollution markers used were NO_2, elemental carbon and ultrafine particle number. Interestingly, fine particle ($PM_{2.5}$) mass concentrations at the studied schools were not correlated with traffic pollution, and most of the contribution to $PM_{2.5}$ levels was due to mineral and organic sources (Amato et al. 2014). $PM_{2.5}$ levels are the universal indicator of air quality because of its overwhelming adverse association with many health indicators (WHO (World Health Organization) 2013). Here, we aim to explore the role of all the different sources of $PM_{2.5}$ in school air on cognitive development.

Methods

Design and population

A cohort of schoolchildren in Barcelona (Spain) was followed up for a year (study period: January 2012-March 2013) as part of the BREATHE (BRain dEvelopment and Air polluTion ultrafine particles in scHool childrEn) project. Cluster sampling was performed by first selecting 40 schools and then inviting all students without special needs in grades 2

through 4 (7-10 years of age) to participate. Using a map of nitrogen dioxide (NO_2) levels in the city, pairs of one high-pollution and one low-pollution school matched by socio-economic vulnerability index (census tract-level indicator based on level of education, unemployment, and occupation) and type of school (i.e., public/private) were selected. A total of 39 schools (18 pairs and one trio) accepted to participate and were included in the study (Sunyer et al. 2015). Participating schools were similar to the remaining schools in Barcelona in terms of the socio-economic vulnerability index (Sunyer et al. 2015). Families of 2897 children (59% of those eligible) accepted to participate in the study. All parents or tutors guardians signed the informed consent form approved by the Clinical Research Ethical Committee (No. 2010/41221/I) of the IMIM-Parc de Salut MAR, Barcelona, Spain.

Outcomes: cognitive development

Cognitive development was assessed through long-term change in working memory and attention, as these functions grow steadily during pre-adolescence (Anderson 2002; Rueda et al. 2005). Children were evaluated every three months over four repeated visits using computerized tests. The computerized versions chosen (the n-back task on working memory (Anderson 2002) and the attentional network task, ANT (Rueda et al. 2004)) were validated with brain imaging (Rueda et al. 2004; Thomason et al. 2009) and in the general population (Forns et al. 2014).

Briefly, in the n-back task, subjects are presented a sequence of stimuli in the screen (e.g. a number), one at a time, and they need to respond (i.e. hit a button) only when the current stimulus matches the one presented n steps before. In the present study, we only analyzed 2-back task as a measure of working memory and 3-back task as a measure of superior working memory, and only used the numbers stimuli, although other tests were also

administered. These choices were based on good properties observed for these tests in the same cohort (e.g. clear age-dependent slope and little learning effect) (Sunyer et al. 2015). For each of these two tests, we measured *detectability* (d prime, *d'*), which is the normalized proportion of correctly identified targets minus the normalized proportion of false alarm hits, *d'* = (Z hit rate - Z false alarm rate). A higher *d'* indicates more accurate test performance. In ANT, subjects have to respond whether the central fish in a row is pointing to the left or right by pressing the corresponding button on the mouse. We used the measure *hit reaction time standard error* (HRT-SE), a measure of response speed consistency throughout the test (Sunyer et al. 2015). A high HRT-SE indicates highly variable reactions and is considered a measure of inattentiveness.

Air pollution exposure

Air pollution measurements were taken simultaneously for each pair of schools during two one-week periods separated by 6 months (sampling campaign 1: January to June, 2012; sampling campaign 2: September 2012 to February 2013). Only a pair of schools was measured each week. High-Volume samplers (MCV SA, Spain) for particulate matter < 2.5 μm ($PM_{2.5}$) were installed indoors in a classroom and outdoors in the playground during school hours (09:00 to 17:00 h) from Monday to Thursday. A detailed description of the measurement campaigns and the instruments can be found elsewhere (Amato et al. 2014). Briefly, filters from samplers were divided in different pieces to determine concentrations of major and trace elements via Inductively Coupled Plasma Mass Spectrometry and Atomic Emission Spectrometry (ICP-MS and ICP-AES); concentrations of sulphate, nitrate and chloride ions via Ion Chromatography (IC) and ammonium via a Specific Electrode;

and concentrations of organic carbon (OC) and elemental carbon (EC) via a Thermal-Optical Transmission technique (TOT).

All measurements (including indoor and outdoor measurements) were pooled to conduct the source apportionment analysis, as this was shown to provide the best results in these data (Amato et al. 2014). Source apportionment was performed using a constrained Positive Matrix Factorization (PMF) model based on 33 chemical species. PMF is a weighted least-squares technique that allows accounting for the uncertainty associated with the analytical procedure, and was run by means of the Multilinear Engine program, which allowed the handling of a priori information such as the source profile of local road dust and sea spray (Amato et al. 2014). This technique returned a solution that identified 9 main factors/sources responsible for the variability of $PM_{2.5}$ mass concentrations with an R^2 of 0.95. The nine sources were identified as mineral, organic/textile/chalk, traffic (that included exhaust and non-exhaust contributions), secondary sulphate & organics, secondary nitrate, road dust (re-suspended street dust), metallurgy, sea spray, and heavy oil combustion (mostly from shipping in the study area and period). The elements identifying the sources are summarized in Table 1.

Outdoor and indoor long-term total and source-specific PM levels were obtained by averaging the two one-week measures of each school. To minimize the effect of meteorology and other seasonal effects in the results, we conducted a paired statistical analyses (described below) to restrict comparisons between schools that were measured simultaneously.

Contextual and individual covariates

Socio-demographic factors included questionnaire-based parents' responses on parental education, marital status, environmental tobacco smoke at home, and a neighbourhood socioeconomic status vulnerability index (Sunyer et al. 2015) calculated both at the school and home addresses. Exposure to traffic $PM_{2.5}$ at home was estimated at the geocoded postal address using available maps based on land use regression models (Eeftens et al. 2012; Sunyer et al. 2015). Noise levels in the classroom before children arrived (as a measure of traffic-related noise) were also measured (Sunyer et al. 2015).

Statistical Analysis

Due to the multilevel nature of the data (i.e. visits within children within schools), we used linear mixed effects models with the four repeated cognitive parameters as outcomes and random effects for child and school. Age at each visit (centered at visit 1) was included in the model in order to capture the growth trajectory of the cognitive test. An interaction between age and school concentrations of individual PM sources was included to capture changes in growth trajectory associated with school air pollution exposure. The latter was the effect of interest in this study. Potential confounders were identified using Directed Acyclic Graphs (DAG) as described elsewhere (Sunyer et al. 2015), and they included sex, maternal education (less than/ primary/secondary/university), residential neighbourhood socioeconomic status, and air pollution exposure at home. Indicators of school pair were included in the model to restrict comparisons within pairs of schools measured during the same days, thus removing potential differences in air pollution levels between schools that were due to meteorology or seasonality. The model equation was the following,

$$Y_{psit} = \beta_{0p} + \beta_1 (Age_{psit}-Age_{psi1}) + \beta_2 (PM\_source)_{ps} + \beta_3 (Age_{psit}-Age_{psi1})* (PM\_source)_{ps} + \mathbf{Z}\eta + u_{ps} + v_{psi} + \varepsilon_{psit} ,$$

where Y_{psit} is the cognitive test result for subject *i* in school *s* (belonging to pair *p*) at visit *t*, *t*=(1,2,3,4), β_{0p} are pair-specific intercepts, **Z** is a matrix including all confounders, η is a vector of parameters associated to confounders, u_{ps} are random effects at school level, assumed normally distributed with mean 0 and variance σ_u^2, v_{psi} are random effects associated with subject *i* in school *s*, assumed normally distributed with mean 0 and variance σ_v^2, and ε_{psit} are the model residuals assumed normally distributed with mean 0 and variance σ_e^2. Deviations from linearity were assessed with generalized additive mixed models. Analyses were repeated without the pair indicator and also with further adjustment for total $PM_{2.5}$ levels and the interaction between age and total PM2.5 (Mostofsky et al. 2012). The interactions between age and maternal education (p-value>0.15 for all outcomes) and age and socio-economic status (p-value>0.5 for all outcomes) were unrelated to cognitive development and were not included in the models.

Models included only $PM_{2.5}$ concentrations from a single source at a time, and separate models were fitted for each source. Likewise, separate models were fitted for indoor and outdoor concentrations. Regression coefficients were rescaled to represent the change in the outcome associated with an interquartile change in source-specific $PM_{2.5}$ levels.

We also provided the results using tracers (chemical elements identifying the source) instead of sources for those $PM_{2.5}$ sources showing significant or suggestive adverse effects on cognition. Statistical significance was set at $p<0.05$.

Results

Table 2 summarizes the characteristics of the selected schools with respect to the high vs. low air pollution indicator used at the design stage. Schools with high air pollution showed lower area-level deprivation, less greenness, had a lower percentage of public schools, higher indoor noise levels, were closer to busy roads and their students tended to live closer to the school. Education quality was equivalent in the two groups.

The average age of participants at baseline was 8.5 years. A total of 2,618 (90.3%) children had data on the three outcomes in at least one visit. Children without data on cognitive outcomes belonged more often to public schools (54% vs. 33%) but there were no differences in terms of school vulnerability index (0.45 vs. 0.42). Around half of the children were girls and they attended 2nd, 3rd and 4th grade (37%, 36% and 27%, respectively) in the first visit. Thirty four percent of them attended a public school, while the rest attended a private school (Table 3). More than half of the mothers (58.9%) had university studies, while 12.5% had at most achieved primary education. Thirty one percent of them lived in areas of high deprivation according to the SES vulnerability index. More details of the study population can be found elsewhere (Sunyer et al. 2015).

During the one-year follow-up, working memory increased on average by 13.0%, superior working memory by 16.5% and inattentiveness decreased by 14% (Table 3). At baseline, lower scores were observed for girls, children attending public schools, children from mothers with low education, and children living in more deprived areas. Children from public schools showed a greater change in superior working memory over follow-up than

those from private schools. Change over follow-up was not significantly associated with other characteristics in crude analyses (Table 3).

The median of school-averaged $PM_{2.5}$ mass concentrations were 28μg/m^3 outdoors and 36 μg/m^3 indoors. Mineral (27%) was the source contributing the highest concentration to outdoor $PM_{2.5}$ levels, followed by traffic (17%), organic/textile/chalk (16%), sulphate (14%), nitrate (13%) and smaller contributions of road dust (4%), metallurgy (4%), sea salt (3%) and heavy oil combustion (2%) (Figure 1). Indoor concentrations were in general smaller and followed the same ordering than outdoor sources, with the exception of organic/textile/chalk, with a strong indoor origin and representing the highest contribution to indoor $PM_{2.5}$ (45%). Table 4 describes the variation of concentrations by source across schools. Mineral exhibited the largest variation, with schools below the 25th percentile having mineral concentrations that represented at most 5.5% of the total levels, while in schools above the 75th percentile mineral contributed more than 34% of the total $PM_{2.5}$ levels. The indoor organic/textile/chalk source also showed large variations between schools. The interquartile range for the traffic source was higher for indoor than for outdoor levels, probably reflecting the effect of class orientation on infiltration (Amato et al. 2014). More details on source apportionment results can be found elsewhere (Amato et al. 2014).

Correlations between indoor and outdoor levels of the same sources were generally greater than 0.7 (Supplemental material, Table S1). Exceptions to this pattern were organic/textile/chalk, with indoor-outdoor correlation close to zero, road dust, with a correlation of 0.14, and to a lesser extent, mineral, with a correlation of 0.64. The highest correlations between school levels of outdoor sources were in the 0.5-0.6 range, including the correlations of mineral with organic/textile/chalk, sea salt and road dust, and the pairs

metallurgy-secondary nitrate, heavy oil combustion-secondary sulphate, and road dust-organic/textile/chalk. With regards to indoor sources, the pairs of sources mentioned above for outdoor levels showed similarly high correlations, while secondary nitrate also showed high correlations with secondary sulphate and heavy oil combustion, and traffic showed a negative correlation of -0.52 with road dust (Supplemental material, Table S2).

Figure 2 and Supplemental Material, Table S3 display the change in cognitive outcomes over the follow-up period for an interquartile increase in source-specific $PM_{2.5}$ concentrations. Results in unadjusted analyses were fairly similar to adjusted ones (Supplemental Material, Table S3). Changes from the first to the third quartile in the indoor traffic source were associated with a significant reduction in working memory of -5.6 (95% confidence interval (CI): -10.7, -0.5), equivalent to 22% of the annual change experienced by the participants (Supplemental Material, Table S3); a reduction of superior working memory of -5.1 (95% CI: -9.2, -1.1), equivalent to 30% of the annual change; and an increase of 3.6 (95% CI: 0.0, 7.1) in inattentiveness scale, equivalent to 11% of the annual change. Associations were smaller for outdoor concentrations of traffic $PM_{2.5}$, although results were still significant for superior working memory and inattentiveness. No significant associations were found for $PM_{2.5}$ mass concentrations from other sources, except for a positive association for outdoor concentrations of $PM_{2.5}$ from mineral origin and superior working memory. Outdoor levels of $PM_{2.5}$ from heavy oil combustion showed deleterious effects on inattentiveness and working memory (p-values equal to 0.05 and 0.09, respectively). No important deviations from linearity were detected (data not shown). When analyses were repeated excluding the school pair indicator or adjusting by environmental tobacco smoke exposure at home or traffic-related noise at school results

were almost the same (data not shown). Further adjustment for total PM2.5 levels produced only minimal changes in the results (Supplemental Material, Table S4).

Supplemental material, Figure S1 provides the results when using the tracers for traffic, organic/textile/chalk, secondary nitrate and heavy oil combustion. EC was significantly associated with all outcomes, while copper (Cu) and antimony (Sb) showed significant associations only for inattentiveness. No significant associations were found for tin (Sn), iron (Fe), nitrate (NO_3^-), vanadium (V), nickel (Ni), OC, calcium (Ca) or strontium (Sr).

Discussion

In a longitudinal study assessing cognitive development of schoolchildren over one year, we found that children attending schools with high levels of traffic-related $PM_{2.5}$ showed a slower cognitive development. None of the other $PM_{2.5}$ sources (mineral, organic/textile/chalk, sulphate, nitrate, road dust, metallurgy, and sea spray) showed a deleterious association with cognitive development, although associations for heavy oil combustion were also suggested. Associations with traffic pollution were stronger when considering indoor levels and these associations were detected for working memory, superior working memory and inattentiveness. These results suggest that fine particles from traffic may produce neurotoxic effects, and that exposure to such particles at primary schools can result in a deceleration of cognitive development.

Our previous study was the first to relate primary school levels of air pollution to cognitive development (growth) in a longitudinal setting (Sunyer et al. 2015). A few other studies have related air pollution exposure at schools to neurobehavioral function at a single point in time (van Kempen et al. 2012; Wang et al. 2009), although others found no association

(Clark et al. 2012). Other cross-sectional studies related personal or residential air pollution exposure with cognitive outcomes, and most of them reported positive associations (Chiu et al. 2013; Franco Suglia et al. 2008; Grahame et al. 2014; Guxens et al. 2014; Guxens and Sunyer 2012; Perera et al. 2012). Our study is the first to perform source apportionment of PM and examine the relationship of each individual source with cognitive development in children. In our study, PM from traffic was the only source associated with a slower cognitive development, which agrees with our previously published result on the effects of EC in this same cohort (Sunyer et al. 2015). Although the traffic source includes also non-exhaust particles, the correlation of the source with EC was 0.89. Most of the previous studies used markers of traffic air pollution such as EC or black carbon (BC), NO_2, $PM_{2.5}$ absorbance or PAHs (Chiu et al. 2013; Franco Suglia et al. 2008; Grahame et al. 2014; Guxens et al. 2014; Guxens and Sunyer 2012; Perera et al. 2012).

The role of PM sources other than traffic on cognitive development has been little explored, although some studies exist on industrial pollution. An ecological study in Michigan (USA) found an increased percentage of school failure in schools with higher levels of industrial pollution (Mohai et al. 2011). Other studies in children have found that manganese (Mn) concentrations from mining and industry were associated with impaired verbal intellectual function and motor skills (Lucchini et al. 2012). In our study area, air pollution from industry sources was low, which may be the reason why we did not find associations with this source. Besides, industry emissions depend strongly on industry type and, thus, associations are expected to vary by study setting. Our results in relation to heavy oil combustion were inconclusive. We found an association of outdoor levels of heavy oil combustion with two of the outcomes, but chance could not be excluded due to the p-values

at the limit of significance and the lack of association with indoor levels or with Ni and V, the main elements defining this source. Unexpectedly, we found that exposure to mineral particles was beneficial for superior working memory. This finding was not observed for indoor levels and we do not know of other studies that investigated the link between mineral particles and cognitive development. This result could be a chance finding. Schools with higher mineral concentrations had sandy playgrounds, so an alternative explanation is that they also have more greenness, which can have beneficial effects on cognitive development (Dadvand et al. 2015). However, further adjustment for greenness did not change the mineral results (data not shown).

When examining the effects of chemical elements (tracers of sources) on cognitive development, the most consistent results were found for EC. Cu and Sb were only significantly associated with inattentiveness. There is still debate on which specific components linked to traffic produce health effects, but there seems to be some consensus in that the health effects are not produced by EC alone, but by other co-emissions such as semi-volatile organic compounds (SVOCs) and PAHs which are adsorbed onto the EC core (Grahame et al. 2014). In our study, we also found some suggestions for deleterious effects of particles from heavy oil combustion, which goes along with the hypothesis of particles from combustion being harmful for the brain. Thus, our findings of slower cognitive development associated with exposure to EC may have implications beyond the effects of traffic emissions. For example, biomass burning can also be an important source of EC/BC and PAHs (Grahame et al. 2014), and the high concentrations of indoor pollution from biomass burning in developing countries could have important effects on the cognitive

development of exposed children. In our study area, PM mass concentrations from biomass burning were negligible and this question could not be investigated (Reche et al. 2012).

Toxicological studies support the neurotoxic effects of motor exhaust particles (Grahame et al. 2014). The main biological mechanisms involve proinflammatory and inflammatory effects in the brain following brain deposition of particles or as a result of systemic inflammation produced by deposition of particles in the respiratory tract and alteration of blood-brain barrier function (Block et al. 2012; Calderon-Garciduenas et al. 2008; Grahame et al. 2014). The brain may be especially vulnerable to oxidative stress, and diesel particles (highly enriched in EC) have been shown to activate microglia, which can produce neurotoxicity via oxidative stress (Block et al. 2012; Grahame et al. 2014). Fine particles from other vehicle sources such as breaks could also contribute to the effects beyond motor exhaust, given the association observed for elements generated by breaks abrasion with inattentiveness in our study and their established potential neurotoxicity (Bandmann et al. 2015). Ni and V, which could also lead to oxidative stress, were not associated with cognition in our study.

Our study had several strengths, including its longitudinal design with repeated outcome measurements and the direct measurements of air pollution both indoors and outdoors at schools. The study also had some limitations, such as a relatively small number of schools and the possibility of residual confounding by socioeconomic characteristics. The latter was extensively explored in our previous study and all analyses suggested the observed effects were not due to residual confounding (Sunyer et al. 2015). In our analyses, schools were matched by socioeconomic characteristics and type of school, thus reducing potential differences, and although children from more educated families attended schools with

lower air pollution levels, differences were small. Due to the observational nature of the study, it cannot be ruled out that children attending schools with high levels of pollution shared other unmeasured characteristics (e.g. not captured socioeconomic dimensions, different level of social interaction, etc.) that affected their cognitive development. Differences in cognition were already present at the beginning of the study. This would still be consistent with our hypotheses, as the cognitive functions studied were already developing in the previous years and children were already exposed to school air pollution. Importantly, we observed that these differences widened during the study period, but we could not discard that children of more polluted schools were already in a slower cognitive trajectory because of early life exposures or socioeconomic factors.

Air pollution levels were based on direct measurements at schools on two different seasons. Although this may represent an improvement over previous papers, which used models to estimate air pollution concentrations at schools, our estimations were still imperfect estimates of annual concentrations. This measurement error is unlikely to be related to school characteristics, in which case it would bias the results towards the null. Another limitation was that, in order to increase the statistical power of source apportionment, this was applied to the joint set of all indoor and outdoor measurements, which may generate some artefacts in source identification. Our data could also be affected by other issues in source estimation, e.g. imperfect separation of road dust and the mineral sources. Uncertainty in source estimation, if properly accounted, would widen our confidence intervals (Kioumourtzoglou et al. 2014). We did not have data on other pollutants such as gases or volatile compounds that may be related to cognitive effects, but these are expected to be correlated with the estimated sources. Further studies in different settings are needed

assess the generalizability of these results. Finally, it is worth mentioning that we examined working memory and inattentiveness, but other domains of cognition such as visuospatial ability or language were not evaluated.

Conclusions

This study aimed to investigate if levels of PM at schools were associated with cognitive development separately for each PM source. We found that levels of PM from traffic were associated with important reductions in cognitive growth over a 1-year period in primary school children. Future studies should examine if the effects observed at primary school age are long-lasting and have consequences over the life course.

References

Amato F, Rivas I, Viana M, Moreno T, Bouso L, Reche C, et al. 2014. Sources of indoor and outdoor PM2.5 concentrations in primary schools. Sci Total Environ 490:757-765.

Anderson P. 2002. Assessment and development of executive function (EF) during childhood. Child Neuropsychol 8(2):71-82.

Bandmann O, Weiss KH, Kaler SG. 2015. Wilson's disease and other neurological copper disorders. Lancet Neurol 14(1):103-113.

Block ML, Elder A, Auten RL, Bilbo SD, Chen H, Chen JC, et al. 2012. The outdoor air pollution and brain health workshop. Neurotoxicology 33(5):972-984.

Brunekreef B, Holgate ST. 2002. Air pollution and health. Lancet 360(9341):1233-1242.

Calderon-Garciduenas L, Maronpot RR, Torres-Jardon R, Henriquez-Roldan C, Schoonhoven R, Acuna-Ayala H, et al. 2003. DNA damage in nasal and brain tissues of canines exposed to air pollutants is associated with evidence of chronic brain inflammation and neurodegeneration. Toxicol Pathol 31(5):524-538.

Calderon-Garciduenas L, Solt AC, Henriquez-Roldan C, Torres-Jardon R, Nuse B, Herritt L, et al. 2008. Long-term air pollution exposure is associated with neuroinflammation, an altered innate immune response, disruption of the blood-brain barrier, ultrafine particulate deposition, and accumulation of amyloid beta-42 and alpha-synuclein in children and young adults. Toxicol Pathol 36(2):289-310.

Cassee FR, Heroux ME, Gerlofs-Nijland ME, Kelly FJ. 2013. Particulate matter beyond mass: recent health evidence on the role of fractions, chemical constituents and sources of emission. Inhal Toxicol 25(14):802-812.

Clark C, Crombie R, Head J, van Kamp I, van Kempen E, Stansfeld SA. 2012. Does traffic-related air pollution explain associations of aircraft and road traffic noise exposure on children's health and cognition? A secondary analysis of the United Kingdom sample from the RANCH project. Am J Epidemiol 176(4):327-337.

Chiu YH, Bellinger DC, Coull BA, Anderson S, Barber R, Wright RO, et al. 2013. Associations between traffic-related black carbon exposure and attention in a prospective birth cohort of urban children. Environ Health Perspect 121(7):859-864.

Dadvand P, Nieuwenhuijsen MJ, Esnaola M, Forns J, Basagana X, Alvarez-Pedrerol M, et al. 2015. Green spaces and cognitive development in primary schoolchildren. Proc Natl Acad Sci U S A doi: 10.1073/pnas.1503402112.

Eeftens M, Beelen R, de Hoogh K, Bellander T, Cesaroni G, Cirach M, et al. 2012. Development of Land Use Regression models for PM(2.5), PM(2.5) absorbance, PM(10) and PM(coarse) in 20 European study areas; results of the ESCAPE project. Environ Sci Technol 46(20):11195-11205.

Forns J, Esnaola M, Lopez-Vicente M, Suades-Gonzalez E, Alvarez-Pedrerol M, Julvez J, et al. 2014. The n-back test and the attentional network task as measures of child neuropsychological development in epidemiological studies. Neuropsychology 28(4):519-529.

Franco Suglia S, Gryparis A, Schwartz J, Wright RJ. 2008. Association between traffic-related black carbon exposure and lung function among urban women. Environ Health Perspect 116(10):1333-1337.

Grahame TJ, Klemm R, Schlesinger RB. 2014. Public health and components of particulate matter: the changing assessment of black carbon. J Air Waste Manag Assoc 64(6):620-660.

Guxens M, Garcia-Esteban R, Giorgis-Allemand L, Forns J, Badaloni C, Ballester F, et al. 2014. Air pollution during pregnancy and childhood cognitive and psychomotor development: six European birth cohorts. Epidemiology 25(5):636-647.

Guxens M, Sunyer J. 2012. A review of epidemiological studies on neuropsychological effects of air pollution. Swiss Med Wkly 141:w13322.

Kioumourtzoglou MA, Coull BA, Dominici F, Koutrakis P, Schwartz J, Suh H. 2014. The impact of source contribution uncertainty on the effects of source-specific PM2.5 on hospital admissions: a case study in Boston, MA. J Expo Sci Environ Epidemiol 24(4):365-371.

Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Rohani H, et al. 2012. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380(9859):2224-2260.

Lucchini RG, Guazzetti S, Zoni S, Donna F, Peter S, Zacco A, et al. 2012. Tremor, olfactory and motor changes in Italian adolescents exposed to historical ferro-manganese emission. Neurotoxicology 33(4):687-696.

Mohai P, Kweon BS, Lee S, Ard K. 2011. Air pollution around schools is linked to poorer student health and academic performance. Health Affairs 30(5):852-862.

Mostofsky E, Schwartz J, Coull BA, Koutrakis P, Wellenius GA, Suh HH, et al. 2012. Modeling the association between particle constituents of air pollution and health outcomes. Am J Epidemiol 176(4):317-326.

Ostro B, Tobias A, Querol X, Alastuey A, Amato F, Pey J, et al. 2011. The effects of particulate matter sources on daily mortality: a case-crossover study of Barcelona, Spain. Environ Health Perspect 119(12):1781-1787.

Perera FP, Tang D, Wang S, Vishnevetsky J, Zhang B, Diaz D, et al. 2012. Prenatal polycyclic aromatic hydrocarbon (PAH) exposure and child behavior at age 6-7 years. Environ Health Perspect 120(6):921-926.

Reche C, Viana M, Amato F, Alastuey A, Moreno T, Hillamo R, et al. 2012. Biomass burning contributions to urban aerosols in a coastal Mediterranean city. Sci Total Environ 427-428:175-190.

Rueda MR, Fan J, McCandliss BD, Halparin JD, Gruber DB, Lercari LP, et al. 2004. Development of attentional networks in childhood. Neuropsychologia 42(8):1029-1040.

Rueda MR, Rothbart MK, McCandliss BD, Saccomanno L, Posner MI. 2005. Training, maturation, and genetic influences on the development of executive attention. Proc Natl Acad Sci U S A 102(41):14931-14936.

Sunyer J, Esnaola M, Alvarez-Pedrerol M, Forns J, Rivas I, López-Vicente M, et al. 2015. Association between traffic-related air pollution in schools and cognitive development in primary school children: a prospective cohort study. PLoS Medicine 12(3):e1001792.

Thomason ME, Race E, Burrows B, Whitfield-Gabrieli S, Glover GH, Gabrieli JD. 2009. Development of spatial and verbal working memory capacity in the human brain. J Cogn Neurosci 21(2):316-332.

van Kempen E, Fischer P, Janssen N, Houthuijs D, van Kamp I, Stansfeld S, et al. 2012. Neurobehavioral effects of exposure to traffic-related air pollution and transportation noise in primary schoolchildren. Environ Res 115:18-25.

Viana M, Kuhlbusch TAJ, Querol X, Alastuey A, Harrison RM, Hopke PK, et al. 2008. Source apportionment of particulate matter in Europe: a review of methods and results Journal of Aerosol Science 39(10):827-849.

Wang S, Zhang J, Zeng X, Zeng Y, Wang S, Chen S. 2009. Association of traffic-related air pollution with children's neurobehavioral functions in Quanzhou, China. Environ Health Perspect 117(10):1612-1618.

WHO (World Health Organization). 2013. Review of evidence on health aspects of air pollution – REVIHAAP Project: Final technical report. Bonn: WHO Regional Office for Europe.

Table 1. Main elements identifying the estimated sources.

Source	Identifying species (tracers)
Mineral	Al, Mg, Li, Fe, Ca, Ti, Rb
Traffic	EC, Cu, Sb, Sn, Fe
Organic/Textile/Chalk	OC, Ca, Sr
Secondary sulphate and organics	SO_4^{2-}, NH_4^+
Secondary nitrate	NO_3^-
Road dust	Ca, Fe, Cu, Sb
Metallurgy	Zn, Pb, Cd, Mn, Cu
Sea spray	Na, Cl^-
Heavy oil combustion	V, Ni

Al: aluminum; Ca: calcium; Cd: cadmium; Cl^-: chloride ion; Cu: copper; EC: Elemental Carbon; Fe: iron; Li: lithium; Mg: magnesium; Mn: manganese; Na: sodium; NH_4^+: ammonium cation; Ni: nickel; NO_3^-: nitrate; OC: Organic Carbon; Pb: lead; Rb: rubidium; Sb: antimony; Sn: tin; SO_4^{2-}: sulphate; Sr: strontium; Ti: titanium; V: vanadium; Zn: zinc.

Table 2. Characteristics of selected schools according to the air pollution indicator used at the design stage (city map of NO_2 levels)*.

Characteristic	Low air pollution	High air pollution
Number	20	19
Socioeconomic vulnerability index	0.52 (0.24)	0.41 (0.16)
School greenness (NDVI)	0.31 (0.10)	0.15 (0.03)
Public school	55%	42%
Education quality (PISA 2012)	3.9 (1.3)	3.9 (1.8)
Noise level in classroom (decibels)	37.2 (4.9)	40.1 (5.0)
Distance to busy roads (meters)	369 (357)	118 (178)
Average distance to children home (meters)	2,432 (2338)	1,048 (1613)

Data are number, percent, or mean (standard deviation).

NDVI, Normalized Difference Vegetation Index.

PISA, Programme for International Student Assessment.

* This table is a partial reproduction of published work (Sunyer et al. 2015)

Table 3. Mean (standard deviation) of cognitive outcomes by characteristics of participants.

Characteristics		Working memory (WM) (2-back Numbers, d'×100)		Superior WM (3-back Numbers, d'×100)		Inattentiveness (HRT-SE, ms)	
	n (%) [a]	Baseline	Change	Baseline	Change	Baseline	Change
All	2,618 (100)	224 (126)	30 (156)	118 (100)	20 (130)	272 (90)	-38 (89)
Sex							
Male	1,316 (50.3)	229 (129)	25 (155)	123 (103)	16 (129)	261 (89)*	-35 (89)
Female	1,302 (49.7)	220 (122)	35 (157)	113 (96)	23 (132)	284 (89)	-41 (88)
Type of school							
Public	860 (32.8)	215 (129)*	36 (149)	111 (102)*	32 (126)*	274 (91)	-39 (89)
Private	1,758 (67.2)	229 (124)	27 (159)	121 (99)	14 (132)	271 (89)	-38 (89)
Maternal education							
Primary or less	337 (12.9)	188 (134)*	25 (162)	82 (91)*	25 (130)	308 (88)*	-40 (92)
Secondary	743 (28.4)	213 (124)	32 (160)	118 (102)	14 (132)	274 (90)	-31 (93)
University	1,538 (58.7)	237 (122)	30 (153)	126 (100)	21 (130)	264 (88)	-41 (85)
SES vulnerability at home							
Less deprived	980 (37.4)	233 (124)*	29 (151)	127 (102)*	18 (131)	265 (89)*	-38 (87)
Middle deprived	807 (30.8)	227 (125)	27 (159)	116 (98)	27 (131)	274 (92)	-37 (91)
High deprived	831 (31.7)	212 (128)	34 (159)	110 (100)	15 (130)	281 (88)	-39 (89)
School Pair							
Low polluted	1,328	226 (125)	36 (154)	120 (100)	23 (131)	272 (89)	-42 (86)
High polluted	1,290	222 (126)	24 (158)	116 (100)	16 (130)	273 (90)	-34 (91)

Residential $PM_{2.5}$ from traffic							
1st quartile	635	224 (128)	32 (157)	121 (101)	17 (125)	275 (94)	-38 (83)
2nd quartile	662	229 (124)	22 (159)	117 (103)	21 (139)	272 (90)	-38 (86)
3rd quartile	659	224 (126)	37 (150)	123 (98)	15 (128)	271 (89)	-41 (93)
4th quartile	662	220 (125)	29 (157)	111 (98)	25 (129)	271 (86)	-37 (92)

[a] number of participants with data at baseline

* $p<0.05$ when testing equality between groups.

Table 4. Description of source contributions to PM2.5 in terms of mass and as a percentage of total PM2.5 mass.

Source	Concentration (μg/m^3)				Percentage (%)			
	Percentile			IQR[a]	Percentile			IQR[a]
	25th	50th	75th		25th	50th	75th	
Outdoor $PM_{2.5}$	22.6	28.1	35.8	13.2	-	-	-	-
Mineral	1.2	2.6	12.7	11.5	5.5	11.3	33.7	28.2
Traffic	4.1	5.2	6.8	2.7	12.9	20.5	26.1	13.3
Organic/Textile/Chalk	2.3	4.8	7.1	4.7	10.8	14.7	20.2	9.4
Secondary sulphate and organics	2.6	4.5	5.7	3.1	10.3	13.7	24.7	14.4
Secondary nitrate	1.9	3.2	5.1	3.2	7.1	11.3	15.6	8.5
Road dust	0.6	1.1	1.8	1.2	2.3	4.3	5.5	3.2
Metallurgy	0.9	1.2	1.5	0.6	3.1	3.9	5.4	2.3
Sea spray	0.5	0.7	1.1	0.6	1.8	2.3	3.5	1.7
Heavy oil combustion	0.5	0.6	0.8	0.4	1.6	2.2	2.8	1.1
Indoor $PM_{2.5}$	29.2	35.6	41.5	12.3	-	-	-	-
Mineral	2.0	3.9	7.2	5.2	6.5	11.6	20.6	14.1
Traffic	3.0	4.4	6.8	3.8	9.7	12.7	20.0	10.3
Organic/Textile/Chalk	12.3	15.3	20.1	7.8	37.2	44.8	48.9	11.6
Secondary sulphate and organics	2.3	3.6	5.4	3.1	7.2	10.7	15.0	7.8
Secondary nitrate	0.9	1.1	1.9	1.0	2.6	3.8	5.3	2.8
Road dust	0.5	1.3	2.1	1.6	1.7	3.3	5.2	3.5
Metallurgy	0.7	0.9	1.2	0.5	2.0	2.8	3.3	1.3
Sea spray	0.6	0.7	1.0	0.5	1.7	2.0	2.7	1.1
Heavy oil combustion	0.4	0.6	0.7	0.3	1.1	1.4	2.0	0.9

[a] Interquartile range

Figure Legends

Figure 1. Average source concentrations (panel A) and percent of $PM_{2.5}$ concentrations (panel B) inside (indoor) and outside (outdoor) of schools. Brackets indicate mean + standard deviation.

Figure 2. Change (95% confidence interval) in cognitive growth per interquartile range increase in school source-specific $PM_{2.5}$ mass concentrations. Models were adjusted for age, sex, maternal education, residential neighbourhood socio-economic status, residential $PM_{2.5}$ levels from traffic and school pair; school and subject included as nested random effects. Working memory measured with 2-back Numbers, d'*100. Superior working memory measured with 3-back Numbers, d'*100. Inattentiveness measures with HRT-SE, ms. Black diamonds (♦): indoor concentrations; Empty circles (o): outdoor concentrations.

Environ Health Perspect DOI: 10.1289/EHP209
Advance Publication: Not Copyedited

Figure 1.

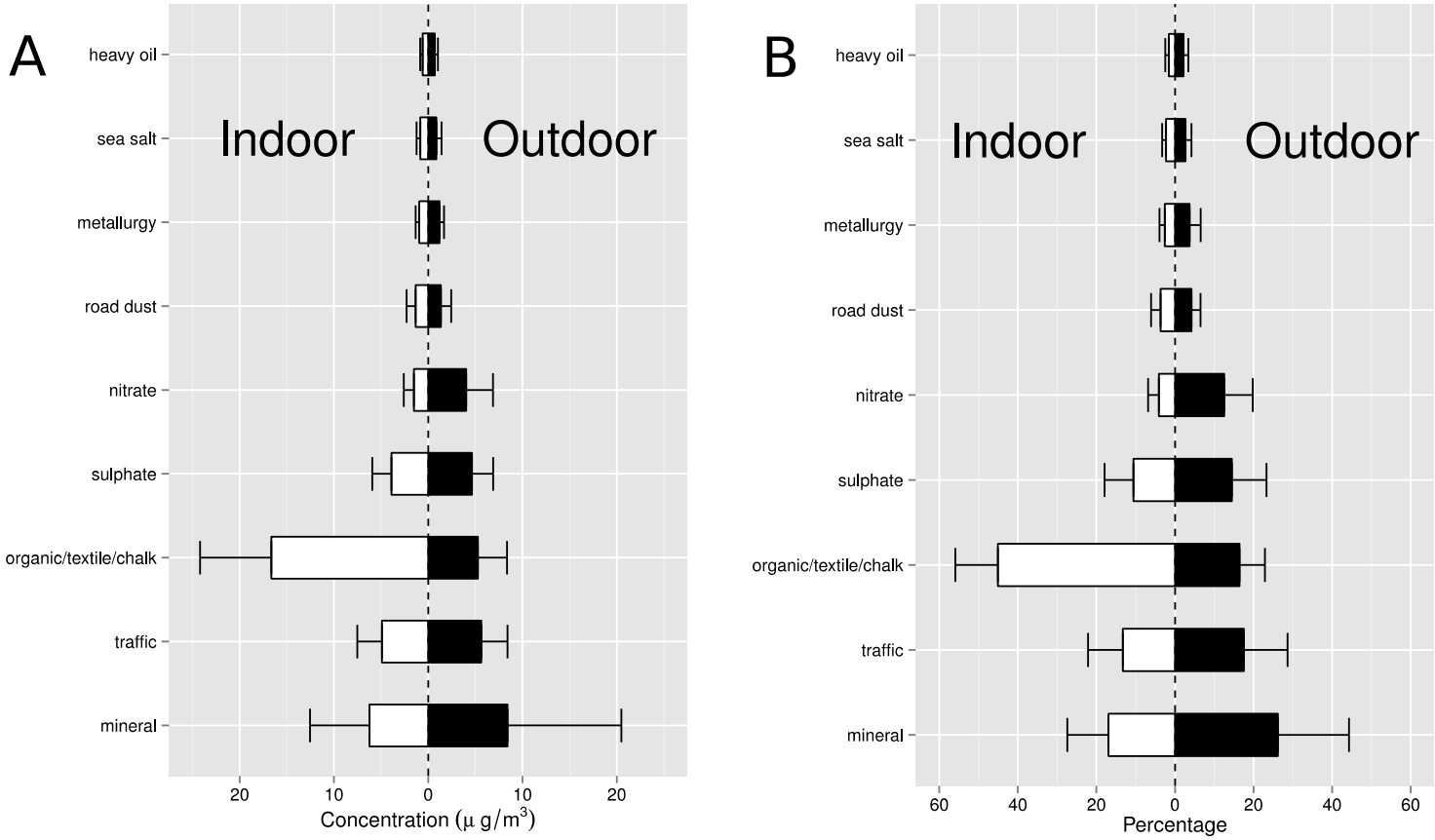
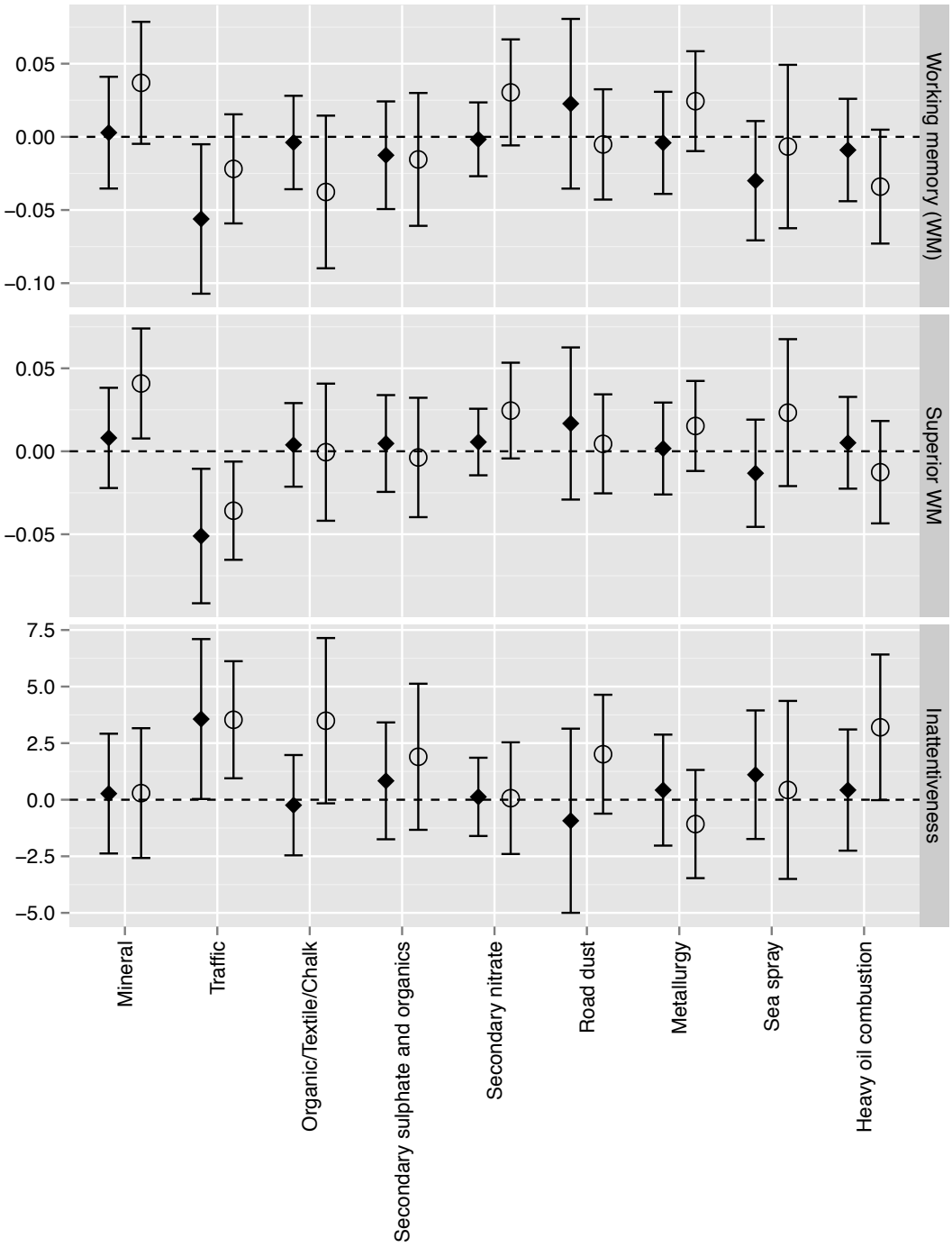


Figure 2.


Working memory (WM)
Superior WM
Inattentiveness
0.05
0.00
−0.05
−0.10
7.5
5.0
2.5
0.0
−2.5
−5.0
Mineral
Traffic
Organic/Textile/Chalk
Secondary sulphate and organics
Secondary nitrate
Road dust
Metallurgy
Sea spray
Heavy oil combustion