

Recent versus chronic exposure to particulate matter air pollution in association with neurobehavioral performance in a panel study of primary schoolchildren.

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1 **Recent *versus* chronic exposure to particulate matter air pollution in association**
2 **with neurobehavioral performance in a panel study of primary schoolchildren**

3

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18

19 **ABSTRACT**

20 Children's neuropsychological abilities are in a developmental stage. Recent air pollution
21 exposure and neurobehavioral performance are scarcely studied. In a panel study, we
22 repeatedly administered to each child the following neurobehavioral tests: Stroop Test
23 (selective attention) and Continuous Performance Test (sustained attention), Digit Span
24 Forward and Backward Tests (short-term memory), and Digit-Symbol and Pattern
25 Comparison Tests (visual information processing speed). At school, recent inside classroom
26 particulate matter ≤ 2.5 or $10 \mu\text{m}$ exposure ($\text{PM}_{2.5}$, PM_{10}) was monitored on each examination
27 day. At the child's residence, recent (same day up to 2 days before) and chronic (365 days
28 before examination) exposures to $\text{PM}_{2.5}$, PM_{10} and black carbon (BC) were modeled.
29 Repeated neurobehavioral test performances ($n=894$) of the children ($n=310$) reflected slower
30 Stroop Test ($p=0.05$) and Digit-Symbol Test ($p=0.01$) performances with increasing recent
31 inside classroom $\text{PM}_{2.5}$ exposure. An interquartile range (IQR) increment in residential
32 outdoor $\text{PM}_{2.5}$ exposure was associated with a total latency of 0.087 seconds ($\text{SE}: \pm 0.034$;
33 $p=0.01$) in the Pattern Comparison Test. Regarding chronic exposure at residence, an IQR
34 increment of $\text{PM}_{2.5}$ exposure was associated with slower performances in the Continuous
35 Performance (9.45 ± 3.47 msec; $p=0.007$) and Stroop Tests (59.9 ± 26.5 msec; $p=0.02$).
36 Similar results were obtained for PM_{10} exposure. In essence, we showed differential
37 neurobehavioral changes robustly and inversely associated with recent or chronic ambient
38 exposure to PM air pollution at residence, i.e., with recent exposure for visual information
39 processing speed (Pattern Comparison Test) and with chronic exposure for sustained and
40 selective attention.

41 **KEYWORDS**

42 particulate matter; air pollution; neurobehavior; children; repeated measures

43 **ABBREVIATIONS**

44 particulate matter with a diameter ≤ 10 or 2.5 μm (PM_{10} , $\text{PM}_{2.5}$); black carbon (BC);

45 interquartile range (IQR); residential proximity to major roads (RPMR); neurobehavioral

46 evaluation system (NES);

47

48 1. INTRODUCTION

49 Polluted air is a complex mixture of water vapor, gases, and solid particles. Evidence is
50 growing that ambient air pollution exposure may be neurotoxic¹. When small particles
51 (particulate matter with a diameter < 10 μm , PM_{10}) deposit in the lungs, they may trigger the
52 release of inflammatory mediators in the systemic circulation^{2,3}. Fine particles ($\text{PM} < 2.5 \mu\text{m}$,
53 $\text{PM}_{2.5}$) can also translocate into the circulation leading to increased systemic inflammation⁴,
54 which may adversely affect the central nervous system (CNS)^{5,6}. Besides the link with
55 systemic inflammation, particles < 0.1 μm might also cause harm to the CNS in a more direct
56 way by crossing the blood-brain-barrier or by retro-axonal translocation via the olfactory
57 nerve^{7,8}. Experimental studies in rodents demonstrated a wide range of biological CNS effects
58 of air pollution exposure including a pro-inflammatory cytokine response, glial activation,
59 oxidative stress, changes in gene expression, and perturbations of levels and turnover of
60 neurotransmitters⁹⁻¹⁴. Epidemiological studies in adults showed that long-term exposure to
61 traffic-related air pollution may contribute to neurodegenerative diseases, such as Parkinson's
62 and Alzheimer's disease^{15,16}. Studies in children suggested that neurotoxic effects of air
63 pollution may translate into observable deterioration of neurobehavioral performance. In
64 children from Boston of approximately 10 years old, average lifetime residential levels of
65 black carbon (BC) were inversely associated with attention, memory, learning, and
66 intelligence^{17,18}. In another prospective cohort study, prenatal air pollution exposure as
67 assessed by personal monitoring of polycyclic aromatic hydrocarbons was inversely
68 associated with neurodevelopmental characteristics (intelligence, behavior) in early
69 childhood¹⁹⁻²¹. Furthermore, cross-sectional studies also reported inverse associations between
70 neurobehavioral performance of children and indicators of chronic air pollution exposure^{22,23}.
71 Recently, it has been shown that children exposed to high traffic-related air pollution have a
72 smaller enhancement in neurobehavioral development after one year in comparison to

73 children exposed to low air pollution²⁴. We found that traffic exposure in adolescents, as
74 reflected by a composite factor combining information about traffic density, time spent in
75 traffic, and urinary concentration of *trans,trans*-muconic acid, was negatively associated with
76 sustained attention²⁵.

77 Despite these studies are suggestive of a neurobehavioral performance deficit associated
78 with fine particle air pollution, there is still insufficient evidence on the consistency of these
79 associations²⁶. Neurobehavioral changes associated with recent air pollution exposure (i.e.,
80 exposure on the day and a few days before the neurobehavioral examination) have been
81 scarcely studied. The aim of this study was to investigate with repeated measures whether
82 neurobehavioral performance was differently associated with recent *versus* chronic air
83 pollution exposure in a panel of primary schoolchildren.

84 **2. MATERIALS & METHODS**

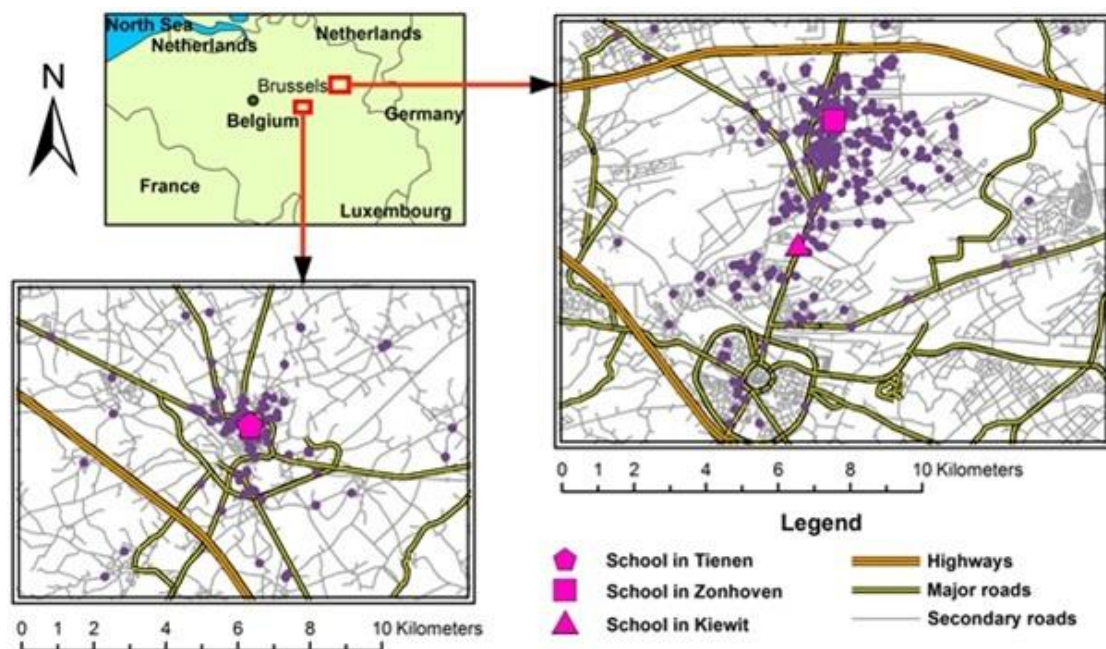
85 ***2.1 Study population***

86 This investigation was part of the COGNAC (COGNition and Air pollution in Children)
87 study. Between 2011 and 2013, we invited children (grades three to six) from three primary
88 schools in Flanders (Belgium) to participate. These schools were located in urban areas with a
89 substantial amount of traffic (Figure 1). Typical particulate matter air pollution (PM_{2.5}) in the
90 recruitment area was mainly characterized by the following components: elemental carbon
91 (3%), organic mass (20%), sea salt (5%), ammonium (12%), nitrate (21%), ammonium sulfate
92 (18%) and mineral dust (3%)²⁷.

93 The parents of the participants filled out a questionnaire to collect information about
94 the current and previous residential addresses, the socioeconomic status of the family, the
95 smoking behavior of the family members, and they provided informed consent for
96 participation. Socioeconomic status was based on the mother's education (up to high school

104 diploma; college or university diploma) and the highest rank of occupation of either parents
105 (unemployed or unqualified worker; qualified worker, white collar assistant, or teaching staff;
106 self-employed, specialist or member of management). The out-of-school sport activities were
107 defined as “none” (no out-of-school sport activities), “low” (≤ 3 hours per week), “middle” ($>$
108 3 to < 6 hours per week) and “high” (≥ 6 hours per week). The study protocol was approved
109 by the medical ethics commissions of Hasselt University and the East-Limburg Hospital.

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115

116 **Figure 1.** Study area with indication of the school locations in the three municipalities and the
117 road system. Dots represent the residential addresses of the schoolchildren.

118
119

120 In total, 334 children agreed to participate in the study, however 24 had to be removed
121 from the database because of missing data on mother’s education and/or occupation of the
122 parents, passive smoking exposure, or residential outdoor exposure. Of the 310 children, 277
123 (89.3%) were examined three times, 30 (9.7%) two times, and 3 (1%) once, amounting to a
124 total number of 894 examinations. The examinations took place between December 2011 and

115 February 2014 on Monday, Tuesday, Thursday, and Friday between 9:00 a.m. and 2:00 p.m..
116 The mean (SD) period of time between two consecutive examinations was 41 (23) days. Each
117 neurobehavioral examination was scheduled for the same time of the day for the same child,
118 but in some cases it was not possible due to school activities. For the same child, the time of
119 the day at which the neurobehavioral examinations took place differed on average (SD) 24
120 (48) min.

121 *2.2 Assessment of PM air pollution exposure*

122 *2.2.1 Air pollution measurements at the schools*

123 At the schools, we used portable devices (AEROCET 531; MetOne Instruments Inc., Grants
124 Pass, OR, USA) to carry out area measurements of particulate matter [PM with a diameter \leq
125 $2.5 \mu\text{m}$ ($\text{PM}_{2.5}$) and $\leq 10 \mu\text{m}$ (PM_{10})] inside the classroom on the examination day (Table 1).
126 Continuous air monitoring was carried out from 9 to 12 a.m. as 2 min interval measurements
127 which were averaged and expressed as $\mu\text{g}/\text{m}^3$.

128 *2.2.2 Modeled outdoor air pollution and traffic indicators at residence*

129 For the child's residence, we used a spatial temporal interpolation method to model the daily
130 residential exposure levels ($\mu\text{g}/\text{m}^3$) of $\text{PM}_{2.5}$, PM_{10} , and BC (Table 1). This method takes into
131 account land-cover data obtained from satellite images (CORINE land-cover data set)²⁸ and
132 pollution data of fixed monitoring stations in combination with a dispersion model^{29,30}. The
133 model calculates the daily interpolated exposure concentrations in a high resolution receptor
134 grid based on information from the Belgian telemetric air quality networks, point sources, and
135 line sources. Overall model performance was evaluated by leave-one-out cross-validation and
136 was based on 34 monitoring points for $\text{PM}_{2.5}$, 58 for PM_{10} , and 14 for BC. Validation statistics
137 of the interpolation tool gave a spatial temporal explained variance of more than 0.80 for
138 $\text{PM}_{2.5}$ ³⁰, 0.70 for PM_{10} ³⁰, and 0.74 for BC³¹. We used this model to estimate the recent

139 exposure at residence up to 48 hours before the neurobehavioral examination as well as the
 140 chronic exposure at residence reflected by the annual mean concentration of the year before
 141 the examination. When a child had more than one residential address at the moment of the
 142 study, we calculated a weighted average using the proportion of time spent at each location.
 143 We calculated also the residential proximity to major roads (RPMR), defined as highways and
 144 other national roads, using geographic information system functions (ArcGIS 9.3).

145 **TABLE 1.** Overview of the PM air pollution exposure indicator measurements or
 146 estimates used for the panel study in schoolchildren.

	PM _{2.5} (µg/m ³)	PM ₁₀ (µg/m ³)	BC (µg/m ³)	RPMR (m)
Recent exposure:				
At schools on day of examination ^a				
Inside the classroom	×	×		
At residential address ^b				
Lag 0 (day of examination)	×	×	×	
Lag 1 (1 day before examination)	×	×	×	
Lag 2 (2 days before examination)	×	×	×	
Chronic exposure:				
At residential address ^b	×	×	×	×

147 PM, particulate matter with aerodynamic diameter < 2.5 µm (PM_{2.5}) or < 10 µm
 148 (PM₁₀); BC, black carbon; RPMR, residential proximity to major road.

149 ^a Actual air pollution measurements by area sampling in the classrooms.

150 ^b Estimates of outdoor air pollution by spatial temporal interpolation modeling.

151 *2.3 Assessment of traffic noise*

152 A GIS-based noise model including the Flemish street and railway networks was used to
 153 estimate traffic noise levels in 5 dB(A)-intervals according to the European Noise Directive
 154 (2002/49/EC)³². The modeling of road noise level included road traffic intensity, vehicle-type-
 155 specific traffic density, type of street surface, small-scale topography of the area, and the
 156 presence or dimensions of buildings and reflecting objects. Railway noise modeling included
 157 the amount of passing trains, type of trains, speed, small-scale topography of the area, and the
 158 presence or dimensions of buildings and reflecting objects. Weighted equivalent noise levels
 159 in dB(A) for traffic over day-time (based on the weighted yearly average noise level between

160 7 a.m. to 7 p.m., and 7 p.m. to 11 p.m.) and at night (yearly average noise level between 11
161 p.m. and 7 a.m) were modeled. Exposure to traffic noise was categorized as ≤ 55 dB, > 55 to
162 ≤ 60 dB, and > 60 dB.

163

164 ***2.4 Neurobehavioral tests***

165 The neurobehavioral examination lasted approximately 20 min. The room where the
166 examinations took place was quiet, appropriately lighted, and ventilated. We administered a
167 computer version of the Stroop Test³³ and the following four tests from the Neurobehavioral
168 Evaluation System 3 (NES3) battery: Continuous Performance, Digit Span, Digit-Symbol,
169 and Pattern Comparison^{34,35}.

170 In the Stroop Test (selective attention domain), four buttons are displayed on the
171 screen (yellow, red, blue, and green). During the test, the name of one of these colors appears
172 on the screen printed in a different color than the name. The task is to touch as fast as possible
173 the button that has the same color as the name, ignoring the color of the printed name. Before
174 the test, eight practice trials take place followed by 48 test trials. The mean reaction time is
175 the average time that passed between the appearance of the name and touching the correct
176 button. This performance indicator was only calculated when the total number of test trails
177 with wrong responses was smaller than or equal to 16.

178 In the Continuous Performance Test (sustained attention domain), silhouettes of
179 animals (e.g., a cat) are displayed on the screen, one at the time and each for approximately
180 200 msec. The task is to immediately respond to the cat's silhouette in this case by pressing
181 the spacebar, but not the silhouette of another animal. A new silhouette is displayed each 1000
182 msec.

183 The Digit Span Test (short-term memory domain) consists of two parts. In the first
184 part, the task is to reproduce a series of digits after an auditory presentation in the order of the
185 presentation. The test starts with a sequence of three digits. In case of a correct answer, a one

186 digit longer sequence is presented. The test continues until two consecutive incorrect answers
187 are given. In the second part of the test, the task is to reproduce the digits in the reverse order
188 of the presentation.

189 In the Digit-Symbol Test (visual information processing speed domain), a row of 9
190 symbols paired with 9 digits is shown at the top of the screen. The same 9 symbols but in a
191 different order are displayed at the bottom of the screen. During the test 27 digits appear
192 consecutively on the screen. When a digit is shown, the task is to indicate as fast as possible
193 the symbol which is paired with this digit in the row of symbols at the bottom of the screen. A
194 new digit appears only after the correct symbol has been indicated.

195 In the Pattern Comparison Test (visual information processing speed domain), three
196 matrices consisting of 10×10 blocks are shown. Two of them are identical. The task is to
197 indicate which pattern is different from the other two patterns. The test includes 25 items.

198 We used as performance parameters, the mean reaction time in the Continuous
199 Performance Test and the Stroop Test, the maximum span forward and backward in the Digit
200 Span Test, and the total latency or the average latency in the Digit-Symbol Test and Pattern
201 Comparison Test respectively.

202 *2.5 Statistical analysis*

203 We performed recent and chronic PM exposure-response analyses using mixed effects models
204 that included random effects for each participant across the neurobehavioral examinations
205 (SAS, version 9.2; SAS Institute Inc., Cary, NC, USA). This method allows each participant
206 to serve as his/her own control over time and eliminates within-subject confounding by
207 personal characteristics that do not change over time. We express the effect estimates for an
208 interquartile range (IQR) increment in recent ($PM_{2.5}$, PM_{10}) and chronic ($PM_{2.5}$, PM_{10} , BC)
209 exposures or living twice as close to major roads (residential proximity to major roads:
210 RPMR). The effect estimates are presented as change in msec for reaction time of the

211 Continuous Performance Test and the Stroop Test, change in number of digits for the Digit
212 Span Forward and Backward Tests, and change in seconds for the latency of the Digit-Symbol
213 Test and Pattern Comparison Test. All analyses were adjusted for *a priori* chosen covariates
214 including sex, age (linear and quadratic term), education of the mother, highest rank of
215 occupation of either parents, passive smoking, out-of-school sport activities, traffic noise
216 (weighted noise during day), hours of computer screen time per week, and day of the week.
217 To capture the non-linear effect of age, we included a quadratic term. Furthermore, a time-
218 varying covariate was included for the measurement occasion (relatedness of examination
219 periods) which is an important predictor of neurobehavioral performance due to the learning
220 effect. In the chronic exposure models, we additionally adjusted for the month of examination
221 to account for seasonality. Since differences are possible for between- and within-subject air
222 pollution effects, we fitted explicit models for recent exposure which included terms for
223 between- and within-subject exposure effects. We reported the within effects. Finally, we
224 tested in a sensitivity analysis the robustness of the findings and replaced residential weighted
225 day-time noise levels by weighted night-time levels.

226 **3. RESULTS**

227 ***3.1 Study population characteristics, neurobehavioral performances, and exposure to PM*** 228 ***air pollution***

229 Characteristics and neurobehavioral test performances of the study group are summarized in
230 Table 2. The number of boys and girls for the three schools combined was approximately
231 equal. The mean (SD) age was 10.2 (1.3) years. The majority (60.9%) of the children's
232 mothers had a college or university diploma and 41 participants were exposed to passive
233 smoking. 41.3% of the children participated up to three hours per week in out-of-school sport
234 activities. For residential traffic noise during the day, 78.4% were exposed to ≤ 55 dB, 13.2%

235 to >55 to ≤ 60 dB, and 8.4% to >60 dB. During the night, 96.8% were exposed to ≤ 55dB and
 236 3.2% to > 55 dB residential traffic noise.

237 Over the examination days, the neurobehavioral test performances averaged ± SD for
 238 sustained attention 593 ± 51.2 msec in the Continuous Performance Test and 1417 ± 377
 239 msec for selective attention in the Stroop Test, for short-term memory 5.26 ± 0.94 and 4.03 ±
 240 0.97 digits in the Digit Span Forward and Backward Tests respectively, and for visual
 241 information processing speed 123 ± 23.5 sec and 4.18 ± 1.01 sec for total latency and average
 242 latency in the Digit-Symbol Test and Pattern Comparison Test respectively.

243

244 **TABLE 2.** Demographic characteristics of the participants.

	N=310
Schools	
Kiewit	69 (22.3%)
Tienen	62 (20.0%)
Zonhoven	179 (57.7%)
Demographic characteristics	
Boys	158 (50.9%)
Age	10.2 ± 1.3
Level of education of the mother	
Up to high school diploma	121 (39.1%)
College or university diploma	189 (60.9%)
Most prestigious category of occupation of either parents,	
Unemployed or not qualified worker	20 (6.4%)
Qualified worker, white-collar assistant, or teaching staff	131 (42.3%)
Self-employed, specialist, or member of management	159 (51.3%)
Passive smoking,	41 (13.2%)
Out-of-school sport activities	
None	36 (11.6%)
≤ 3 hours/week	128 (41.3%)
> 3 to < 6 hours/week	87 (28.1%)
≥ 6 hours/week	59 (19.0%)
Computer screen use, hours per week	4.3 ± 3.8

245 Values represent number (%) or arithmetic mean ± SD.

246 The median (interquartile range; IQR) concentrations of PM air pollution inside the
 247 classrooms at the schools were, for PM_{2.5} 5.14 (8.85) µg/m³, and for PM₁₀ 33.5 (55.2) µg/m³
 248 (Table 3). Table 3 also shows the modeled estimates of recent residential ambient air exposure
 249 to PM_{2.5}, PM₁₀, and BC from Lag 0 to Lag 2. Chronic ambient PM exposure was
 250 characterized by the median residential exposure (IQR) over the year before the examination
 251 [15.7 (1.16) µg/m³ for PM_{2.5}, 21.3 (1.61) µg/m³ for PM₁₀, and 1.54 (0.20) µg/m³ for BC] and
 252 median distance (IQR) from residence to major roads [RPMR, 333 (669) m].

253 **TABLE 3.** Recent and chronic exposure characteristics (N=310).

	Median	25 th percentile	75 th percentile	IQR
Recent (at schools)^a				
PM _{2.5} , µg/m ³	5.14	2.80	11.6	8.85
PM ₁₀ , µg/m ³	33.5	20.9	76.1	55.2
Recent (at residence)^b				
PM _{2.5} , µg/m ³				
Lag 0	16.5	9.10	28.0	18.9
Lag 1	15.2	8.85	27.5	18.7
Lag 2	15.5	8.90	31.8	22.9
PM ₁₀ , µg/m ³				
Lag 0	21.2	13.0	32.2	19.2
Lag 1	19.5	13.0	30.9	17.9
Lag 2	18.9	13.0	36.2	23.2
BC, µg/m ³				
Lag 0	1.55	1.00	2.25	1.25
Lag 1	1.36	1.03	2.03	1.00
Lag 2	1.52	0.93	2.30	1.37
Chronic (at residence)^b				
PM _{2.5} , µg/m ³	15.7	15.2	16.4	1.16
PM ₁₀ , µg/m ³	21.3	20.7	22.3	1.61
BC, µg/m ³	1.54	1.43	1.63	0.20
RPMR, m	333	133	832	699

254 PM, particulate matter with aerodynamic diameter < 2.5 µm (PM_{2.5}) or < 10 µm (PM₁₀); BC, black
 255 carbon; RPMR, residential proximity to major roads.

256 ^a Air pollution at school is obtained by area sampling in the classrooms and averaged over the
 257 examination days.

258 ^b Average ambient air pollution at the residential address over different periods before examination is
 259 obtained by spatial temporal interpolation modeling.

260 *3.2 Associations between recent PM exposure and neurobehavioral performance*

261 For the sustained attention (Continuous Performance Test) and short-term memory (Digit
262 Span Forward and Backward Tests) domains, the repeated neurobehavioral test performances
263 within the same child were not associated with recent inside classroom exposure (PM_{2.5},
264 PM₁₀) at school on the examination day (Table 4). Similarly, no associations were shown for
265 recent exposure at residence (PM_{2.5}, PM₁₀, BC) on the examination day (Lag 0), one day
266 before (Lag 1), and two days (Lag 2) before (Table 5). For selective attention, recent inside
267 classroom PM_{2.5} and PM₁₀ exposures were significantly associated with the Stroop Test
268 showing a 42.7 msec longer mean reaction time [95% confidence interval (CI): -0.40 to 85.8,
269 p=0.05] for an IQR increment in PM_{2.5} exposure (Table 4). The corresponding estimate for
270 recent inside PM₁₀ exposure was 50.2 msec (95% CI: 8.55 to 91.8, p=0.02).

271 For the visual information processing speed domain, significant associations were
272 found between the Digit-Symbol Test performance and recent inside classroom PM_{2.5} or PM₁₀
273 exposure (Table 4). An IQR increment in PM_{2.5} exposure showed a total latency increase of
274 2.05 seconds (95% CI: 0.43 to 3.66; p=0.01). The corresponding result for PM₁₀ was 1.9
275 seconds (p=0.02). The results of the Pattern Comparison Test were adversely associated with
276 recent residential PM_{2.5} and PM₁₀ exposure the day of the examination (Lag 0) (Table 5). For
277 an IQR increment of PM_{2.5} exposure, the average latency increased by 0.087 seconds (95%
278 CI: 0.02 to 0.15; p=0.01), while the corresponding estimate for PM₁₀ was 0.081 seconds
279 (p=0.01). The days before the examination, i.e., at Lag 1 and Lag 2, an IQR increment of
280 PM_{2.5} exposure also showed an increase in average latency of the Pattern Comparison Test
281 with 0.066 seconds (p=0.04) and 0.079 seconds (p=0.03) respectively. For BC, exposure the
282 day before examination (Lag 1) was also associated with the Pattern Comparison Test (0.051
283 seconds in average latency, p = 0.04). For the other recent inside classroom and residential

284 outdoor exposure indicators, no associations were found with the Digit-Symbol or Pattern
 285 Comparison Tests (Table 4 and 5).

286 The associations described above were independent of the covariates sex, age (linear
 287 and quadratic), education of the mother, highest rank of occupation of either parents, passive
 288 smoking, out-of-school sport activities, traffic noise during day, hours per week spent behind
 289 a computer, day of the week, and relatedness of the different examination periods. Additional
 290 adjustment for chronic residential exposure as well as replacement of the traffic noise during
 291 day by traffic noise during night did not change the results.

292 **TABLE 4.** Associations between neurobehavioral test performances and recent inside
 293 classroom PM exposures.

Neurobehavioral Test	Recent indoor PM _{2.5}			Recent indoor PM ₁₀		
	β	95% CI	p-value	β	95% CI	p-value
Attention						
Continuous Performance	-1.29	-5.51 to 2.93	0.55	-2.16	-6.26 to 1.94	0.30
Stroop	42.7	-0.40 to 85.8	0.05	50.2	8.55 to 91.8	0.02
Short-term Memory						
Digit Span Forward	-0.05	-0.16 to 0.05	0.32	-0.002	-0.10 to 0.10	0.97
Digit Span Backward	-0.06	-0.18 to 0.06	0.35	-0.03	-0.15 to 0.08	0.57
Visual Information Processing Speed						
Digit-Symbol	2.05	0.43 to 3.66	0.01	1.9	0.34 to 3.42	0.02
Pattern Comparison	-0.01	-0.11 to 0.09	0.79	-0.03	-0.13 to 0.07	0.54

294 PM, particulate matter with aerodynamic diameter < 2.5 μm (PM_{2.5}) or < 10 μm (PM₁₀); the
 295 metric of PM_{2.5} and PM₁₀ is μg/m³.

296 For an interquartile increment in recent indoor exposure to PM_{2.5} (8.85 μg/m³) or PM₁₀ (55.2
 297 μg/m³), the effect estimates are represented as msec change for the Continuous Performance
 298 Test and the Stroop Test, change in number of digits for the Digit Span Forward and
 299 Backward Tests, and change in sec of latency for the Digit-Symbol and Pattern Comparison
 300 Tests. All analyses were adjusted for sex, age (linear and quadratic), education of the mother,
 301 occupation of the parents, passive smoking, out-of-school physical activity, traffic noise,
 302 hours spent after computers, day of the week, and relatedness of the examination periods.

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304
305

TABLE 5. Associations between neurobehavioral test performances and recent ambient PM exposures at residence.

Attention									
Recent residential exposure	Continuous performance				Stroop				
	β	95% CI		p-value	β	95% CI		p-value	
PM _{2.5} , lag 0	-1.57	-4.45	to 1.32	0.29	-16.7	-46.4	to 12.9	0.27	
PM _{2.5} , lag 1	-0.59	-3.31	to 2.14	0.67	-20.3	-48.3	to 7.66	0.15	
PM _{2.5} , lag 2	-0.11	-3.11	to 2.89	0.94	-26.0	-56.4	to 4.46	0.09	
PM ₁₀ , lag 0	-1.27	-3.99	to 1.45	0.36	-13.8	-41.8	to 14.1	0.33	
PM ₁₀ , lag 1	-0.47	-2.82	to 1.89	0.70	-12.2	-36.4	to 12.1	0.33	
PM ₁₀ , lag 2	-0.10	-2.94	to 2.74	0.94	-22.4	-51.1	to 6.33	0.13	
BC, lag 0	-0.48	-3.17	to 2.20	0.72	-4.48	-32.0	to 23.1	0.75	
BC, lag 1	0.27	-1.83	to 2.37	0.80	-3.96	-25.7	to 17.8	0.72	
BC, lag 2	0.66	-1.84	to 3.16	0.61	-3.80	-29.6	to 22.0	0.77	

Memory									
	Digit Span forward				Digit Span backward				
	β	95% CI		p-value	β	95% CI		p-value	
PM _{2.5} , lag 0	-0.050	-0.124	to 0.024	0.18	0.008	-0.075	to 0.092	0.84	
PM _{2.5} , lag 1	-0.056	-0.126	to 0.015	0.12	-0.028	-0.11	to 0.051	0.49	
PM _{2.5} , lag 2	-0.071	-0.149	to 0.007	0.07	0.006	-0.081	to 0.093	0.89	
PM ₁₀ , lag 0	-0.046	-0.116	to 0.024	0.20	-0.0031	-0.082	to 0.075	0.94	
PM ₁₀ , lag 1	-0.045	-0.106	to 0.016	0.15	-0.034	-0.103	to 0.034	0.33	
PM ₁₀ , lag 2	-0.067	-0.140	to 0.006	0.07	0.000	-0.082	to 0.082	0.99	
BC, lag 0	-0.065	-0.134	to 0.003	0.06	0.019	-0.059	to 0.096	0.64	
BC, lag 1	-0.025	-0.080	to 0.029	0.36	-0.025	-0.086	to 0.036	0.42	
BC, lag 2	-0.051	-0.116	to 0.014	0.12	0.013	-0.060	to 0.086	0.72	

Visual processing speed									
	Digit Symbol				Pattern comparison				
	β	95% CI		p-value	β	95% CI		p-value	
PM _{2.5} , lag 0	-0.77	-1.88	to 0.33	0.17	0.087	0.020	to 0.15	0.01	
PM _{2.5} , lag 1	-0.95	-2.00	to 0.10	0.08	0.066	0.004	to 0.13	0.04	
PM _{2.5} , lag 2	-0.80	-1.95	to 0.35	0.17	0.079	0.010	to 0.15	0.03	
PM ₁₀ , lag 0	-0.50	-1.54	to 0.54	0.35	0.081	0.019	to 0.14	0.01	
PM ₁₀ , lag 1	-0.69	-1.60	to 0.21	0.13	0.046	-0.009	to 0.10	0.10	
PM ₁₀ , lag 2	-0.73	-1.82	to 0.36	0.19	0.057	-0.009	to 0.12	0.09	
BC, lag 0	-0.61	-1.64	to 0.42	0.24	0.041	-0.022	to 0.103	0.20	
BC, lag 1	-0.78	-1.59	to 0.03	0.06	0.051	0.003	to 0.099	0.04	
BC, lag 2	-0.50	-1.47	to 0.47	0.31	0.006	-0.053	to 0.064	0.85	

306 PM, particulate matter with aerodynamic diameter < 2.5 μm (PM_{2.5}) or < 10 μm (PM₁₀); BC,
307 Black carbon; the metric of PM_{2.5}, PM₁₀, and BC is $\mu\text{g}/\text{m}^3$.

308 For an interquartile increment in recent (Lag 0, 1, and 2) indoor exposure to PM_{2.5} (18.9, 18.7,
309 and 22.9 $\mu\text{g}/\text{m}^3$), PM₁₀ (19.2, 17.9, and 23.2 $\mu\text{g}/\text{m}^3$) or BC (1.25, 1.00, and 1.37 $\mu\text{g}/\text{m}^3$), the
310 effect estimates are represented as msec change for the Continuous Performance Test and the
311 Stroop Test, change in number of digits for the Digit Span Forward and Backward Tests, and
312 change in sec of latency for the Digit-Symbol and Pattern Comparison Tests. All analyses
313 were adjusted for sex, age (linear and quadratic), education of the mother, occupation of the
314 parents, passive smoking, out-of-school physical activity, traffic noise, hours of computer
315 screen time per week, day of the week, and relatedness of the examination periods.

316
317
318

319 *3.3 Associations between chronic PM exposure and neurobehavioral performance*

320 In the models studying chronic exposure, we accounted for the following covariates: sex, age
321 (linear and squared term), education of the mother, occupation of the parents, passive
322 smoking, out-of-school sport activities, traffic noise during day , hours per week spent behind
323 a computer, day of the week, month of examination and relatedness of the examination
324 periods. Independent of these covariates, chronic exposure to PM_{2.5} was adversely associated
325 with the attention domain (Continuous Performance and Stroop Tests). For an IQR increment
326 of chronic PM_{2.5} exposure, the reaction time increased by 9.45 msec (95% CI: 2.59 to 16.3;
327 p=0.007) for the Continuous Performance Test and by 59.9 msec (95% CI: 8.1 to 111.6;
328 p=0.02) for the Stroop Test. For PM₁₀, the estimates were in the same direction (Table 6). For
329 BC exposure, we only observed a tendency towards significance for the Continuous
330 Performance Test (5.72 msec; 95% CI: -0.34 to 11.8; p = 0.06) and for residential proximity
331 to major roads (RPMR) no significant associations were found with both attention tests. A
332 sensitivity analysis in which traffic noise during day was replaced by traffic noise during
333 night did not change the main results. For the short-term memory domain, chronic exposures
334 to PM_{2.5}, PM₁₀, BC and residential proximity to major roads did not show significant
335 associations with both short-term memory tests (Table 6). For the visual information
336 processing speed domain, none of the chronic exposure indicators (PM_{2.5}, PM₁₀, BC, and
337 RPMR) was associated with the performances of the Digit-Symbol and Pattern Comparison
338 Tests (Table 6).

339

340 **TABLE 6:** Associations between neurobehavioral test performances and chronic ambient PM
 341 exposures at residence.

Attention									
Chronic exposure	Continuous Performance Test				Stroop Test				
	β	95% CI		p-value	β	95% CI		p-value	
PM _{2.5}	9.45	2.59	to 16.3	0.007	59.9	8.1	to 111.6	0.02	
PM ₁₀	8.66	2.50	to 14.8	0.006	76.5	29.3	to 123.6	0.002	
BC	5.72	-0.34	to 11.8	0.06	6.7	-38.4	to 51.9	0.77	
RPMR	1.92	-1.0	to 4.85	0.20	0.90	-20.6	to 22.4	0.93	
Short-term memory									
	Digit Span Forward Test				Digit Span Backward Test				
	β	95% CI		p-value	β	95% CI		p-value	
PM _{2.5}	-0.025	-0.15	to 0.10	0.70	0.057	-0.071	to 0.18	0.38	
PM ₁₀	-0.063	-0.18	to 0.057	0.30	0.057	-0.064	to 0.18	0.35	
BC	0.025	-0.09	to 0.14	0.66	0.10	-0.011	to 0.20	0.08	
RPMR	-0.047	-0.10	to 0.005	0.08	-0.043	-0.095	to 0.009	0.11	
Visual information processing speed									
	Digit-Symbol Test				Pattern Comparison Test				
	β	95% CI		p-value	β	95% CI		p-value	
PM _{2.5}	2.1	-0.65	to 4.91	0.13	0.050	-0.089	to 0.19	0.48	
PM ₁₀	2.09	-0.39	to 4.57	0.10	0.023	-0.103	to 0.15	0.72	
BC	0.50	-1.99	to 2.99	0.69	0.065	-0.06	to 0.19	0.30	
RPMR	0.39	-0.79	to 1.58	0.51	0.0002	-0.057	to 0.057	1.00	

342 PM, particulate matter with aerodynamic diameter < 2.5 μm (PM_{2.5}) or < 10 μm (PM₁₀); BC,
 343 black carbon; RPMR, residential proximity to major roads. The metric of PM_{2.5}, PM₁₀, and BC
 344 is $\mu\text{g}/\text{m}^3$ and residential proximity to major roads (RPMR) is expressed in m.
 345 For an interquartile increment in chronic exposure to PM_{2.5} (1.16 $\mu\text{g}/\text{m}^3$), PM₁₀ (1.61 $\mu\text{g}/\text{m}^3$),
 346 and BC (0.20 $\mu\text{g}/\text{m}^3$) or for living twice as close to major roads, the effect estimates are
 347 represented as msec change for the Continuous Performance Test and the Stroop Test, change
 348 in number of digits for the Digit Span Forward and Backward Tests, and change in sec of
 349 latency for the Digit-Symbol and Pattern Comparison Tests. All analyses were adjusted for
 350 sex, age (linear and quadratic), education of the mother, occupation of the parents, passive
 351 smoking, out-of-school physical activity, traffic noise, hours of computer screen time per
 352 week, day of the week, and relatedness of the examination periods.
 353

354 **4. DISCUSSION**

355 The CNS of schoolchildren and thus also their neurobehavioral performances are still in a
356 stage of development and may be vulnerable to both recent and chronic PM air pollution. In
357 our panel study of children with repeated measures of neurobehavioral performances, neither
358 recent nor chronic PM exposure did affect short-term memory. However, other findings
359 indicated consistent negative associations of selective attention (Stroop Test) with both recent
360 classroom and chronic ambient residential PM exposure, while decreased sustained attention
361 was associated only with chronic ambient PM exposure at residence. Visual information
362 processing speed seemed to decrease only in conditions of recent PM exposure, either in the
363 classroom (Digit-Symbol Test) or at residence (Pattern Comparison Test). These associations
364 persisted by taking into account the learning effect over the various examination days and by
365 allowing for sex, age, familial socioeconomic position, out-of-school sport activities, and
366 residential traffic noise (day and night). Some of these results highlighted changes in
367 neurobehavioral test performances in association with PM air pollution exposures well below
368 the current EU standards (annual mean PM_{2.5}: 25 µg/m³ and PM₁₀: 40 µg/m³) and just above
369 the current US standard (PM_{2.5}: 12 µg/m³).

370 ***4.1 Recent PM exposure***

371 In contrast to chronic exposure, the neurotoxic potential of recent exposure to PM air
372 pollution in children has not been thoroughly investigated so far. Our panel study of
373 schoolchildren revealed adverse changes in neurobehavioral test performances associated with
374 an IQR increment of recent PM air pollution exposure, i.e., selective attention (Stroop Test)
375 and visual information processing speed (Digit-Symbol and Pattern Comparison Tests).

376 These findings may be interpreted in the context of experimental studies which
377 emphasized the involvement of inflammatory events on neurobehavioral functioning.

378 Administration of bacterial lipopolysaccharide showed in rats a rapid induction of systemic
379 inflammation which strongly impaired memory retrieval in a task thought to require
380 hippocampal pattern separation and context-object discrimination, whereas it did not impair
381 mere memory retrieval in hippocampal dependent tasks³⁶. This finding supports the
382 contention that acute neuro-inflammation may impair context discrimination memory via
383 disruption of pattern separation processes in the hippocampus. Another experimental study on
384 short-term (4 h) air pollution exposures showed rapid modulation of genes in the vaso-
385 regulatory pathway of the brain³⁷. In a randomized cross-over study of humans exposed to
386 diesel motor exhaust (1 h), a significantly lower brain activity (EEG) has been detected in the
387 left frontal cortex as reflected by increased median power frequency within 30 min of
388 exposure, an effect still detectable until 1 h after exposure stopped³⁸. Our observational
389 findings are in line with the hypothesis of PM-induced cerebral inflammation and suggest a
390 prompt neurobehavioral response.

391 ***4.2 Chronic PM exposure***

392 As to chronic PM exposure, the two tests of the attention domain (Continuous Performance
393 Test and Stroop Test) were adversely associated with an IQR increment of chronic ambient
394 PM_{2.5} and PM₁₀ air pollution at residence.

395 Several recent studies reported an inverse association between chronic air pollution
396 exposure and attention-related outcomes. Recently, we observed in adolescents a negative
397 association between sustained attention, assessed using the Continuous Performance Test, and
398 traffic exposure as reflected by a composite indicator comprising traffic density, time spent in
399 traffic, and urinary *trans,trans* muconic-acid²⁵. In a Boston study of 174 children (boys and
400 girls) between 7 and 14 years of age, attention measured by the Continuous Performance Test
401 was negatively associated with residential concentrations of lifetime BC exposure in boys
402 only¹⁸. Though, their findings did not remain comparing the highest BC exposure quartile

403 with the lowest quartile. In our study, we found strong associations between PM air pollution
404 and the Continuous Performance Test, and a negative tendency was observed with chronic BC
405 exposure ($p=0.06$). A possible explanation for the less pronounced effects of BC compared
406 with particulate matter air pollution might be a potential higher exposure misclassification for
407 BC as these models were built using less measuring points (14 for BC compared to 58 for
408 PM_{10}). A Chinese study reported that children whose school was located in an area with low
409 traffic density performed better in the Continuous Performance Test than those from a school
410 with higher traffic-related air pollution²³. In an Indian study, the prevalence of ADHD was
411 higher in urban children than in controls living in an area with low air pollution³⁹. Another
412 study indicated that the average residential elemental carbon concentrations during the first
413 year of life were associated with a higher risk of hyperactivity at 7 years of age as assessed by
414 Parent Rating Scale of the Behavioral Assessment System for Children⁴⁰. A large study in
415 Barcelona including more than 2,700 children showed that those exposed to high traffic-
416 related air pollution levels at school had over a one-year school period a lower development
417 of their working memory²⁴.

418 A plausible explanation of our neurotoxic findings with chronic PM exposure may be
419 linked to alterations in several CNS sub-structures, white matter lesions in cortical areas of the
420 left cerebral hemisphere, and vascular changes^{41,42}. Calderón-Garcidueñas and colleagues⁴³
421 autopsied highly exposed children and young adults who suddenly died in Mexico City
422 (average annual ambient air $PM_{2.5}$: $35.9 \mu\text{g}/\text{m}^3$) and compared their findings with those of
423 “control autopsies” of age-matched persons exposed to lower $PM_{2.5}$ concentrations (< 15
424 $\mu\text{g}/\text{m}^3$). The authors demonstrated in the highly exposed group an up-regulation of cyclo-
425 oxygenase 2 (COX2), interleukin 1β (IL 1β), and CD14 gene expression in the olfactory bulb,
426 frontal cortex, substantia nigra and vagus nerves as well as disruption of the blood-brain-
427 barrier, endothelial activation, and inflammatory cell trafficking. These adverse outcomes

428 may be directly linked to deposition of ultrafine particles, a sub-fraction of PM_{2.5}, in cortical
429 areas or to retro-axonal transport via the olfactory nerve, or alternatively by neuro-
430 inflammation following a systemic inflammatory response to air pollution^{7,8}.

431 ***4.3 Strengths and limitations***

432 Our study has several strengths. First, the risk of bias in the analysis of short-term CNS
433 effects of air pollution was small because of the panel study design and the statistical
434 approach used. More specifically, they allowed to eliminate the risk of reverse causality and
435 confounding of person-related characteristics. Second, recent PM exposure at school was
436 characterized by area sampling in the classroom on the days of the neurobehavioral
437 examinations. Third, recent and chronic ambient exposures to PM_{2.5}, PM₁₀, and BC at
438 residence were estimated for each participant using a high-resolution spatial temporal model.
439 This may explain why significant associations were found with attention tests despite the low
440 spatial contrast in chronic PM_{2.5} exposure (IQR difference 1.16 µg/m³). This observation is in
441 line with other studies such as the Worcester Heart Attack Study which showed a link with
442 acute myocardial infarction for an IQR PM_{2.5} exposure contrast of 0.59 µg/m³⁴⁴. Fourth, we
443 used three estimates for recent ambient exposure at residence to cover the time period of
444 exposure and potential lagged effects up to two days (Lag 0 to 2).

445 We acknowledge some limitations of the study. First, we cannot exclude residual
446 confounding by other neurotoxic substances, such as lead, if these were strongly associated
447 with the air pollution indexes. However, in a recent study we showed in adolescents that the
448 associations between neurobehavioral performance and traffic-related exposure were
449 independent of blood lead⁴⁵. The present study, based on repeated measures, tested an *a priori*
450 hypothesis involving interrelated neurobehavioral outcomes as well as strongly correlated PM
451 exposures (e.g. correlation coefficients for chronic PM₁₀ and PM_{2.5} was r=0.87). Therefore,
452 the neurobehavioral test performances or exposure indicators did not provide a completely

453 independent opportunity for a type I error. For these reasons we did not perform multiple
454 testing. Nevertheless, it seems unlikely that the consistency of negative phenotype-exposure
455 associations would merely occur by chance as reflected by the significantly diminished
456 performances, not only in the two Attention Tests (chronic ambient exposures to PM_{2.5} and
457 PM₁₀ at residence) but also in the Pattern Comparison Test (recent ambient exposures to
458 PM_{2.5}, PM₁₀, or BC on Lag 0-2 at residence). Further study is needed to find out why the
459 inverse associations between the Stroop or Digit-Symbol test results and recent inside
460 classroom PM exposure could not be corroborated for recent ambient PM exposure at
461 residence. It is plausible that recent PM exposure characterized by 3-hour monitoring of
462 classroom air displays different features in comparison to modeled recent PM exposure.
463 Furthermore, the robustness and consistency of prompt neurobehavioral responses to recent
464 exposure might be less outspoken than in the case of chronic ambient PM exposure at
465 residence.

466 **5. CONCLUSIONS**

467 This is the first panel study comparing neurobehavioral changes of recent and chronic PM air
468 pollution exposure. The repeated measurement study design in primary schoolchildren
469 showed differential neurobehavioral changes robustly and inversely associated with recent or
470 chronic ambient exposure to PM air pollution at residence, i.e., with recent exposure for
471 visual information processing speed (Pattern Comparison Test) and with chronic exposure for
472 sustained and selective attention. These neurotoxic findings on behavioral performances in
473 schoolchildren associated with PM air pollution as found in Belgium in the years 2012-2014,
474 strongly support the current tendency to bring the EU limits for ambient air PM-exposure as
475 low as the WHO guidelines.

476

477

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485 *Competing interests*

486 The authors declare they have no conflict of interest.

487

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