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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
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4	Nelly D. Saenen <sup>1</sup> , Eline B. Provost <sup>1,2</sup> , Mineke K Viaene <sup>3</sup> , Charlotte Vanpoucke <sup>4</sup> , Wouter
5	Lefebvre <sup>2</sup> , Karen Vrijens <sup>1</sup> , Harry A Roels <sup>1,5</sup> , Tim S Nawrot <sup>1,6</sup>
6	
7	1. Centre for Environmental Sciences, Hasselt University, Diepenbeek, Belgium.
8	2. Flemish Institute for Technological Research (VITO), Mol, Belgium
9	3. Department of Neurology, Sint Dimphna Hospital, Geel, Belgium.
10	4. Belgian Interregional Environment Agency, Brussels, Belgium.
11	5. Louvain Centre for Toxicology and Applied Pharmacology, Université catholique de
12	Louvain, Brussels, Belgium
13	6. Department of Public Health & Primary Care, Leuven University, Leuven, Belgium.
14	
15	Correspondence to: Tim Nawrot, Hasselt University, Centre for Environmental Sciences,
16	Agoralaan gebouw D, 3590 Diepenbeek, Belgium. Email: tim.nawrot@uhasselt.be. Phone: 32
17	11 268382. Fax: 32 11 26829.

## 19 ABSTRACT

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

## 41 KEYWORDS

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

# 43 ABBREVIATIONS

44 particulate matter with a diameter  $\leq 10$  or 2.5  $\mu$ m (PM<sub>10</sub>, PM<sub>2.5</sub>); black carbon (BC); 45 interquartile range (IQR); residential proximity to major roads (RPMR); neurobehavioral 46 evaluation system (NES);

## 48 1. INTRODUCTION

Polluted air is a complex mixture of water vapor, gases, and solid particles. Evidence is 49 growing that ambient air pollution exposure may be neurotoxic<sup>1</sup>. When small particles 50 (particulate matter with a diameter  $< 10 \mu m$ , PM<sub>10</sub>) deposit in the lungs, they may trigger the 51 release of inflammatory mediators in the systemic circulation<sup>2,3</sup>. Fine particles (PM  $< 2.5 \mu m$ , 52 PM<sub>2.5</sub>) can also translocate into the circulation leading to increased systemic inflammation<sup>4</sup>, 53 which may adversely affect the central nervous system (CNS)<sup>5,6</sup>. Besides the link with 54 systemic inflammation, particles  $< 0.1 \mu m$  might also cause harm to the CNS in a more direct 55 way by crossing the blood-brain-barrier or by retro-axonal translocation via the olfactory 56 nerve<sup>7,8</sup>. Experimental studies in rodents demonstrated a wide range of biological CNS effects 57 58 of air pollution exposure including a pro-inflammatory cytokine response, glial activation, oxidative stress, changes in gene expression, and perturbations of levels and turnover of 59 60 neurotransmitters<sup>9-14</sup>. Epidemiological studies in adults showed that long-term exposure to traffic-related air pollution may contribute to neurodegenerative diseases, such as Parkinson's 61 and Alzheimer's disease<sup>15,16</sup>. Studies in children suggested that neurotoxic effects of air 62 pollution may translate into observable deterioration of neurobehavioral performance. In 63 children from Boston of approximately 10 years old, average lifetime residential levels of 64 black carbon (BC) were inversely associated with attention, memory, learning, and 65 intelligence<sup>17,18</sup>. In another prospective cohort study, prenatal air pollution exposure as 66 assessed by personal monitoring of polycyclic aromatic hydrocarbons was inversely 67 associated with neurodevelopmental characteristics (intelligence, behavior) in early 68 childhood<sup>19-21</sup>. Furthermore, cross-sectional studies also reported inverse associations between 69 neurobehavioral performance of children and indicators of chronic air pollution exposure<sup>22,23</sup>. 70 71 Recently, it has been shown that children exposed to high traffic-related air pollution have a smaller enhancement in neurobehavioral development after one year in comparison to 72

children exposed to low air pollution<sup>24</sup>. We found that traffic exposure in adolescents, as reflected by a composite factor combining information about traffic density, time spent in traffic, and urinary concentration of *trans,trans*-muconic acid, was negatively associated with sustained attention<sup>25</sup>.

Despite these studies are suggestive of a neurobehavioral performance deficit associated with fine particle air pollution, there is still insufficient evidence on the consistency of these associations<sup>26</sup>. Neurobehavioral changes associated with recent air pollution exposure (i.e., exposure on the day and a few days before the neurobehavioral examination) have been scarcely studied. The aim of this study was to investigate with repeated measures whether neurobehavioral performance was differently associated with recent *versus* chronic air pollution exposure in a panel of primary schoolchildren.

## 84 2. MATERIALS & METHODS

#### 85 2.1 Study population

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

The parents of the participants filled out a questionnaire to collect information about the current and previous residential addresses, the socioeconomic status of the family, the smoking behavior of the family members, and they provided informed consent for participation. Socioeconomic status was based on the mother's education (up to high school 97 diploma; college or university diploma) and the highest rank of occupation of either parents 98 (unemployed or unqualified worker; qualified worker, white collar assistant, or teaching staff; 99 self-employed, specialist or member of management). The out-of-school sport activities were 100 defined as "none" (no out-of-school sport activities), "low" ( $\leq$  3 hours per week), "middle' (> 101 3 to < 6 hours per week) and "high" ( $\geq$  6 hours per week). The study protocol was approved 102 by the medical ethics commissions of Hasselt University and the East-Limburg Hospital.

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Figure 1. Study area with indication of the school locations in the three municipalities and the
 road system. Dots represent the residential addresses of the schoolchildren.
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In total, 334 children agreed to participate in the study, however 24 had to be removed from the database because of missing data on mother's education and/or occupation of the parents, passive smoking exposure, or residential outdoor exposure. Of the 310 children, 277 (89.3%) were examined three times, 30 (9.7%) two times, and 3 (1%) once, amounting to a total number of 894 examinations. The examinations took place between December 2011 and February 2014 on Monday, Tuesday, Thursday, and Friday between 9:00 a.m. and 2:00 p.m..
The mean (SD) period of time between two consecutive examinations was 41 (23) days. Each
neurobehavioral examination was scheduled for the same time of the day for the same child,
but in some cases it was not possible due to school activities. For the same child, the time of
the day at which the neurobehavioral examinations took place differed on average (SD) 24
(48) min.

## 121 2.2 Assessment of PM air pollution exposure

## 122 2.2.1 Air pollution measurements at the schools

At the schools, we used portable devices (AEROCET 531; MetOne Instruments Inc., Grants Pass, OR, USA) to carry out area measurements of particulate matter [PM with a diameter  $\leq$ 2.5 µm (PM<sub>2.5</sub>) and  $\leq$  10 µm (PM<sub>10</sub>)] inside the classroom on the examination day (Table 1). Continuous air monitoring was carried out from 9 to 12 a.m. as 2 min interval measurements which were averaged and expressed as µg/m<sup>3</sup>.

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

For the child's residence, we used a spatial temporal interpolation method to model the daily 129 residential exposure levels ( $\mu g/m^3$ ) of PM<sub>2.5</sub>, PM<sub>10</sub>, and BC (Table 1). This method takes into 130 account land-cover data obtained from satellite images (CORINE land-cover data set)<sup>28</sup> and 131 pollution data of fixed monitoring stations in combination with a dispersion model <sup>29,30</sup>. The 132 model calculates the daily interpolated exposure concentrations in a high resolution receptor 133 grid based on information from the Belgian telemetric air quality networks, point sources, and 134 line sources. Overall model performance was evaluated by leave-one-out cross-validation and 135 136 was based on 34 monitoring points for PM<sub>2.5</sub>, 58 for PM<sub>10</sub>, and 14 for BC. Validation statistics of the interpolation tool gave a spatial temporal explained variance of more than 0.80 for 137  $PM_{2.5}\ ^{30}\!\!,$  0.70 for  $PM_{10}\ ^{30}\!\!,$  and 0.74 for BC  $^{31}\!\!.$  We used this model to estimate the recent 138

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).

**TABLE 1**. Overview of the PM air pollution exposure indicator measurements orestimates used for the panel study in schoolchildren.

	<b>PM2.5</b> (μg/m <sup>3</sup> )	<b>PM</b> <sub>10</sub> (μg/m <sup>3</sup> )	BC (μg/m <sup>3</sup> )	<b>RPMR</b> (m)
Recent exposure:				
At schools on day of examination <sup>a</sup>				
Inside the classroom	×	×		
At residential address <sup>b</sup>				
Lag 0 (day of examination)	×	×	×	
Lag 1 (1 day before examination)	×	×	×	
Lag 2 (2 days before examination)	×	×	×	
Chronic exposure:				
At residential address <sup>b</sup>	×	×	×	×

147 PM, particulate matter with aerodynamic diameter  $< 2.5 \ \mu m \ (PM_{2.5}) \ or < 10 \ \mu m$ 

148 (PM<sub>10</sub>); BC, black carbon; RPMR, residential proximity to major road.

<sup>a</sup> Actual air pollution measurements by area sampling in the classrooms.

<sup>b</sup> 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 estimate traffic noise levels in 5 dB(A)-intervals according to the European Noise Directive 153 (2002/49/EC)<sup>32</sup>. The modeling of road noise level included road traffic intensity, vehicle-type-154 specific traffic density, type of street surface, small-scale topography of the area, and the 155 presence or dimensions of buildings and reflecting objects. Railway noise modeling included 156 157 the amount of passing trains, type of trains, speed, small-scale topography of the area, and the presence or dimensions of buildings and reflecting objects. Weighted equivalent noise levels 158 159 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  $\leq 55$  dB, > 55 to 162  $\leq 60$  dB, and > 60 dB.

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## 164 2.4 Neurobehavioral tests

The neurobehavioral examination lasted approximately 20 min. The room where the examinations took place was quiet, appropriately lighted, and ventilated. We administered a computer version of the Stroop Test <sup>33</sup> and the following four tests from the Neurobehavioral Evaluation System 3 (NES3) battery: Continuous Performance, Digit Span, Digit-Symbol, and Pattern Comparison <sup>34,35</sup>.

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

In the Continuous Performance Test (sustained attention domain), silhouettes of animals (e.g., a cat) are displayed on the screen, one at the time and each for approximately 200 msec. The task is to immediately respond to the cat's silhouette in this case by pressing the spacebar, but not the silhouette of another animal. A new silhouette is displayed each 1000 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 digit longer sequence is presented. The test continues until two consecutive incorrect answers
are given. In the second part of the test, the task is to reproduce the digits in the reverse order
of the presentation.

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

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

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

## 202 2.5 Statistical analysis

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

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

## 226 **3. RESULTS**

# 3.1 Study population characteristics, neurobehavioral performances, and exposure to PM air pollution

Characteristics and neurobehavioral test performances of the study group are summarized in Table 2. The number of boys and girls for the three schools combined was approximately equal. The mean (SD) age was 10.2 (1.3) years. The majority (60.9%) of the children's mothers had a college or university diploma and 41 participants were exposed to passive smoking. 41.3% of the children participated up to three hours per week in out-of-school sport activities. For residential traffic noise during the day, 78.4% were exposed to  $\leq$  55 dB, 13.2% to >55 to  $\leq$  60 dB, and 8.4% to >60 dB. During the night, 96.8% were exposed to  $\leq$  55dB and 3.2% to > 55 dB residential traffic noise.

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

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# 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	$10.2 \pm 1.3$
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%)
$\leq$ 3 hours/week	128 (41.3%)
> 3 to < 6 hours/week	87 (28.1%)
$\geq$ 6 hours/week	59 (19.0%)
Computer screen use, hours per week	$4.3\pm3.8$

Values represent number (%) or arithmetic mean  $\pm$  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) $\mu g/m^3,$ and for $PM_{10}$ 33.5 (55.2) $\mu g/m^3$
248	(Table 3). Table 3 also shows the modeled estimates of recent residential ambient air exposure
249	to $PM_{2.5}$ , $PM_{10}$ , 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) $\mu$ g/m <sup>3</sup> for PM <sub>2.5</sub> , 21.3 (1.61) $\mu$ g/m <sup>3</sup> for PM <sub>10</sub> , and 1.54 (0.20) $\mu$ g/m <sup>3</sup> for BC] and
252	median distance (IQR) from residence to major roads [RPMR, 333 (669) m].

	Madian	25 <sup>th</sup>	75 <sup>th</sup>	IOD	
	Median	percentile	percentile	IQK	
Recent (at schools) <sup>a</sup>					
$PM_{2.5}, \mu g/m^3$	5.14	2.80	11.6	8.85	
$PM_{10}, \mu g/m^3$	33.5	20.9	76.1	55.2	
Recent (at residence) <sup>b</sup>					
$PM_{2.5}, \mu g/m^3$					
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	
$PM_{10}, \mu g/m^3$					
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, $\mu g/m^3$					
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) <sup>b</sup>					
$PM_{2.5}, \mu g/m^3$	15.7	15.2	16.4	1.16	
$PM_{10}, \mu g/m^3$	21.3	20.7	22.3	1.61	
BC, $\mu g/m^3$	1.54	1.43	1.63	0.20	
RPMR, m	333	133	832	699	

253	TABLE 3. Recent and	l chronic exposure	characteristics (N=310).
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**254** PM, particulate matter with aerodynamic diameter  $< 2.5 \ \mu m \ (PM_{2.5}) \ or < 10 \ \mu m \ (PM_{10}); BC, black$ 

255 carbon; RPMR, residential proximity to major roads.

<sup>a</sup> Air pollution at school is obtained by area sampling in the classrooms and averaged over the

examination days.

<sup>b</sup> 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

For the sustained attention (Continuous Performance Test) and short-term memory (Digit 261 Span Forward and Backward Tests) domains, the repeated neurobehavioral test performances 262 within the same child were not associated with recent inside classroom exposure (PM<sub>2.5</sub>, 263 PM<sub>10</sub>) at school on the examination day (Table 4). Similarly, no associations were shown for 264 recent exposure at residence (PM<sub>2.5</sub>, PM<sub>10</sub>, BC) on the examination day (Lag 0), one day 265 before (Lag 1), and two days (Lag 2) before (Table 5). For selective attention, recent inside 266 classroom PM<sub>2.5</sub> and PM<sub>10</sub> exposures were significantly associated with the Stroop Test 267 showing a 42.7 msec longer mean reaction time [95% confidence interval (CI): -0.40 to 85.8, 268 p=0.05] for an IQR increment in PM<sub>2.5</sub> exposure (Table 4). The corresponding estimate for 269 recent inside PM<sub>10</sub> exposure was 50.2 msec (95% CI: 8.55 to 91.8, p=0.02). 270

For the visual information processing speed domain, significant associations were 271 272 found between the Digit-Symbol Test performance and recent inside classroom PM2.5 or PM10 exposure (Table 4). An IQR increment in PM2.5 exposure showed a total latency increase of 273 274 2.05 seconds (95% CI: 0.43 to 3.66; p=0.01). The corresponding result for PM<sub>10</sub> was 1.9 seconds (p=0.02). The results of the Pattern Comparison Test were adversely associated with 275 recent residential PM<sub>2.5</sub> and PM<sub>10</sub> exposure the day of the examination (Lag 0) (Table 5). For 276 277 an IQR increment of  $PM_{2.5}$  exposure, the average latency increased by 0.087 seconds (95% CI: 0.02 to 0.15; p=0.01), while the corresponding estimate for  $PM_{10}$  was 0.081 seconds 278 (p=0.01). The days before the examination, i.e., at Lag 1 and Lag 2, an IQR increment of 279 PM<sub>2.5</sub> exposure also showed an increase in average latency of the Pattern Comparison Test 280 with 0.066 seconds (p=0.04) and 0.079 seconds (p=0.03) respectively. For BC, exposure the 281 day before examination (Lag 1) was also associated with the Pattern Comparison Test (0.051 282 seconds in average latency, p = 0.04). For the other recent inside classroom and residential 283

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

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

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

Neuropehavioral Test	R	ecent indoor PN	<b>/I</b> <sub>2.5</sub>	Recent indoor PM <sub>10</sub>				
	β	β 95% CI		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		

PM, particulate matter with aerodynamic diameter  $< 2.5 \ \mu m \ (PM_{2.5}) \ or < 10 \ \mu m \ (PM_{10})$ ; the metric of PM<sub>2.5</sub> and PM<sub>10</sub> is  $\mu g/m^3$ .

For an interquartile increment in recent indoor exposure to  $PM_{2.5}$  (8.85 µg/m<sup>3</sup>) or  $PM_{10}$  (55.2 µg/m<sup>3</sup>), the effect estimates are represented as msec change for the Continuous Performance Test and the Stroop Test, change in number of digits for the Digit Span Forward and Backward Tests, and change in sec of latency for the Digit-Symbol and Pattern Comparison Tests. All analyses were adjusted for sex, age (linear and quadratic), education of the mother, occupation of the parents, passive smoking, out-of-school physical activity, traffic noise, hours spent after computers, day of the week, and relatedness of the examination periods.

Attention									
Recent	Cor	ntinuous perf	forman	ce	Stroop				
residential				p-					p-
exposure	β	95% C	I	value	β	95	% CI	-	value
PM <sub>2.5</sub> , lag 0	-1.57	-4.45 to	1.32	0.29	-16.7	-46.4	to	12.9	0.27
PM <sub>2.5</sub> , lag 1	-0.59	-3.31 to	2.14	0.67	-20.3	-48.3	to	7.66	0.15
PM <sub>2.5</sub> , lag 2	-0.11	-3.11 to	2.89	0.94	-26.0	-56.4	to	4.46	0.09
PM <sub>10</sub> , lag 0	-1.27	-3.99 to	1.45	0.36	-13.8	-41.8	to	14.1	0.33
PM <sub>10</sub> , lag 1	-0.47	-2.82 to	1.89	0.70	-12.2	-36.4	to	12.1	0.33
PM <sub>10</sub> , 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

TABLE 5. Associations between neurobehavioral test performances and recent ambient PM 304 exposures at residence. 305

## Memory

		Digit Spa	an fo	rward		Digit Span backward					
					p-						
	β	95	5% C	I	value	β	959	% CI		p-value	
PM <sub>2.5</sub> , lag 0	-0.050	-0.124	to	0.024	0.18	0.008	-0.075	to	0.092	0.84	
PM <sub>2.5</sub> , lag 1	-0.056	-0.126	to	0.015	0.12	-0.028	-0.11	to	0.051	0.49	
PM <sub>2.5</sub> , lag 2	-0.071	-0.149	to	0.007	0.07	0.006	-0.081	to	0.093	0.89	
PM10, lag 0	-0.046	-0.116	to	0.024	0.20	-0.0031	-0.082	to	0.075	0.94	
PM <sub>10</sub> , lag 1	-0.045	-0.106	to	0.016	0.15	-0.034	-0.103	to	0.034	0.33	
PM <sub>10</sub> , 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

		Dig	it Sy	mbol		Pattern comparison				
	β	9	5% (	CI	p-value	β	959	95% CI		
PM <sub>2.5</sub> , lag 0	-0.77	-1.88	to	0.33	0.17	0.087	0.020	to	0.15	0.01
PM <sub>2.5</sub> , lag 1	-0.95	-2.00	to	0.10	0.08	0.066	0.004	to	0.13	0.04
PM <sub>2.5</sub> , lag 2	-0.80	-1.95	to	0.35	0.17	0.079	0.010	to	0.15	0.03
PM <sub>10</sub> , lag 0	-0.50	-1.54	to	0.54	0.35	0.081	0.019	to	0.14	0.01
PM <sub>10</sub> , lag 1	-0.69	-1.60	to	0.21	0.13	0.046	-0.009	to	0.10	0.10
PM <sub>10</sub> , 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

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

307

For an interquartile increment in recent (Lag 0, 1, and 2) indoor exposure to PM<sub>2.5</sub> (18.9, 18.7, 308 and 22.9  $\mu$ g/m<sup>3</sup>), PM<sub>10</sub> (19.2, 17.9, and 23.2  $\mu$ g/m<sup>3</sup>) or BC (1.25, 1.00, and 1.37  $\mu$ g/m<sup>3</sup>), the 309 effect estimates are represented as msec change for the Continuous Performance Test and the 310 Stroop Test, change in number of digits for the Digit Span Forward and Backward Tests, and 311 change in sec of latency for the Digit-Symbol and Pattern Comparison Tests. All analyses 312 were adjusted for sex, age (linear and quadratic), education of the mother, occupation of the 313 314 parents, passive smoking, out-of-school physical activity, traffic noise, hours of computer screen time per week, day of the week, and relatedness of the examination periods. 315 316

317

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

In the models studying chronic exposure, we accounted for the following covariates: sex, age 320 (linear and squared term), education of the mother, occupation of the parents, passive 321 322 smoking, out-of-school sport activities, traffic noise during day, hours per week spent behind a computer, day of the week, month of examination and relatedness of the examination 323 periods. Independent of these covariates, chronic exposure to PM<sub>2.5</sub> was adversely associated 324 with the attention domain (Continuous Performance and Stroop Tests). For an IQR increment 325 of chronic PM<sub>2.5</sub> exposure, the reaction time increased by 9.45 msec (95% CI: 2.59 to 16.3; 326 p=0.007) for the Continuous Performance Test and by 59.9 msec (95% CI: 8.1 to 111.6; 327 p=0.02) for the Stroop Test. For PM<sub>10</sub>, the estimates were in the same direction (Table 6). For 328 BC exposure, we only observed a tendency towards significance for the Continuous 329 Performance Test (5.72 msec; 95% CI: -0.34 to 11.8; p = 0.06) and for residential proximity 330 331 to major roads (RPMR) no significant associations were found with both attention tests. A sensitivity analysis in which traffic noise during day was replaced by traffic noise during 332 night did not change the main results. For the short-term memory domain, chronic exposures 333 to PM<sub>2.5</sub>, PM<sub>10</sub>, BC and residential proximity to major roads did not show significant 334 associations with both short-term memory tests (Table 6). For the visual information 335 processing speed domain, none of the chronic exposure indicators (PM<sub>2.5</sub>, PM<sub>10</sub>, BC, and 336 RPMR) was associated with the performances of the Digit-Symbol and Pattern Comparison 337 Tests (Table 6). 338

					Att	ention				
Chronic	Continuous Performance				Test	Stroop Test				
exposure	β	95% CI			p-value	β	95% CI			p-value
PM <sub>2.5</sub>	9.45	2.59	to	16.3	0.007	59.9	8.1	to	111.6	0.02
$\mathbf{PM}_{10}$	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-ter	rm memory				
	Digit Span Forward Te				est	Digit Span Backward Test				t
	β	95% CI			p-value	β	95% CI			p-value
PM <sub>2.5</sub>	-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	informatio	on processin	g speed			
	Digit-Symbol Test					Pattern Comparison Test				
	β	95% CI			p-value	β	95% CI			p-value
PM <sub>2.5</sub>	2.1	-0.65	to	4.91	0.13	0.050	-0.08	9 to	o 0.19	0.48
$\mathbf{PM}_{10}$	2.09	-0.39	to	4.57	0.10	0.023	-0.10	3 to	0.15	0.72
BC	0.50	-1.99	to	2.99	0.69	0.065	-0.0	5 to	0.19	0.30
RPMR	0.39	-0.79	to	1.58	0.51	0.0002	-0.05	7 to	0.057	1.00

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

PM, particulate matter with aerodynamic diameter  $< 2.5 \ \mu m \ (PM_{2.5}) \ or <math>< 10 \ \mu m \ (PM_{10}); BC$ , black carbon; RPMR, residential proximity to major roads. The metric of PM<sub>2.5</sub>, PM<sub>10</sub>, and BC is  $\mu g/m^3$  and residential proximity to major roads (RPMR) is expressed in m.

For an interquartile increment in chronic exposure to  $PM_{2.5}$  (1.16 µg/m<sup>3</sup>),  $PM_{10}$  (1.61 µg/m<sup>3</sup>), 345 and BC (0.20 µg/m<sup>3</sup>) or for living twice as close to major roads, the effect estimates are 346 347 represented as msec change for the Continuous Performance Test and the Stroop Test, change in number of digits for the Digit Span Forward and Backward Tests, and change in sec of 348 latency for the Digit-Symbol and Pattern Comparison Tests. All analyses were adjusted for 349 sex, age (linear and quadratic), education of the mother, occupation of the parents, passive 350 smoking, out-of-school physical activity, traffic noise, hours of computer screen time per 351 week, day of the week, and relatedness of the examination periods. 352

## 354 4. DISCUSSION

The CNS of schoolchildren and thus also their neurobehavioral performances are still in a 355 stage of development and may be vulnerable to both recent and chronic PM air pollution. In 356 our panel study of children with repeated measures of neurobehavioral performances, neither 357 recent nor chronic PM exposure did affect short-term memory. However, other findings 358 indicated consistent negative associations of selective attention (Stroop Test) with both recent 359 classroom and chronic ambient residential PM exposure, while decreased sustained attention 360 was associated only with chronic ambient PM exposure at residence. Visual information 361 processing speed seemed to decrease only in conditions of recent PM exposure, either in the 362 363 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 allowing for sex, age, familial socioeconomic position, out-of-school sport activities, and 365 residential traffic noise (day and night). Some of these results highlighted changes in 366 neurobehavioral test performances in association with PM air pollution exposures well below 367 the current EU standards (annual mean PM2.5: 25 µg/m<sup>3</sup> and PM10: 40 µg/m<sup>3</sup>) and just above 368 the current US standard ( $PM_{2.5}$ : 12 µg/m<sup>3</sup>). 369

## 370 4.1 Recent PM exposure

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

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

Administration of bacterial lipopolysaccharide showed in rats a rapid induction of systemic 378 inflammation which strongly impaired memory retrieval in a task thought to require 379 hippocampal pattern separation and context-object discrimination, whereas it did not impair 380 mere memory retrieval in hippocampal dependent tasks<sup>36</sup>. This finding supports the 381 contention that acute neuro-inflammation may impair context discrimination memory via 382 disruption of pattern separation processes in the hippocampus. Another experimental study on 383 short-term (4 h) air pollution exposures showed rapid modulation of genes in the vaso-384 regulatory pathway of the brain<sup>37</sup>. In a randomized cross-over study of humans exposed to 385 diesel motor exhaust (1 h), a significantly lower brain activity (EEG) has been detected in the 386 left frontal cortex as reflected by increased median power frequency within 30 min of 387 exposure, an effect still detectable until 1 h after exposure stopped<sup>38</sup>. Our observational 388 findings are in line with the hypothesis of PM-induced cerebral inflammation and suggest a 389 390 prompt neurobehavioral response.

## 391 *4.2 Chronic PM exposure*

As to chronic PM exposure, the two tests of the attention domain (Continuous Performance
Test and Stroop Test) were adversely associated with an IQR increment of chronic ambient
PM<sub>2.5</sub> and PM<sub>10</sub> air pollution at residence.

Several recent studies reported an inverse association between chronic air pollution 395 396 exposure and attention-related outcomes. Recently, we observed in adolescents a negative association between sustained attention, assessed using the Continuous Performance Test, and 397 traffic exposure as reflected by a composite indicator comprising traffic density, time spent in 398 traffic, and urinary *trans,trans* muconic-acid<sup>25</sup>. In a Boston study of 174 children (boys and 399 girls) between 7 and 14 years of age, attention measured by the Continuous Performance Test 400 was negatively associated with residential concentrations of lifetime BC exposure in boys 401 only<sup>18</sup>. Though, their findings did not remain comparing the highest BC exposure quartile 402

with the lowest quartile. In our study, we found strong associations between PM air pollution 403 and the Continuous Performance Test, and a negative tendency was observed with chronic BC 404 exposure (p=0.06). A possible explanation for the less pronounced effects of BC compared 405 406 with particulate matter air pollution might be a potential higher exposure misclassification for BC as these models were built using less measuring points (14 for BC compared to 58 for 407 PM<sub>10</sub>). A Chinese study reported that children whose school was located in an area with low 408 traffic density performed better in the Continuous Performance Test than those from a school 409 with higher traffic-related air pollution<sup>23</sup>. In an Indian study, the prevalence of ADHD was 410 higher in urban children than in controls living in an area with low air pollution<sup>39</sup>. Another 411 study indicated that the average residential elemental carbon concentrations during the first 412 year of life were associated with a higher risk of hyperactivity at 7 years of age as assessed by 413 Parent Rating Scale of the Behavioral Assessment System for Children<sup>40</sup>. A large study in 414 415 Barcelona including more than 2,700 children showed that those exposed to high trafficrelated air pollution levels at school had over a one-year school period a lower development 416 of their working memory<sup>24</sup>. 417

A plausible explanation of our neurotoxic findings with chronic PM exposure may be 418 linked to alterations in several CNS sub-structures, white matter lesions in cortical areas of the 419 left cerebral hemisphere, and vascular changes <sup>41,42</sup>. Calderón-Garcidueñas and colleagues<sup>43</sup> 420 autopsied highly exposed children and young adults who suddenly died in Mexico City 421 (average annual ambient air PM<sub>2.5</sub>: 35.9  $\mu$ g/m<sup>3</sup>) and compared their findings with those of 422 "control autopsies" of age-matched persons exposed to lower PM<sub>2.5</sub> concentrations (< 15 423  $\mu g/m^3$ ). The authors demonstrated in the highly exposed group an up-regulation of cyclo-424 oxygenase 2 (COX2), interleukin 1 $\beta$  (IL1 $\beta$ ), and CD14 gene expression in the olfactory bulb, 425 frontal cortex, substantia nigra and vagus nerves as well as disruption of the blood-brain-426 barrier, endothelial activation, and inflammatory cell trafficking. These adverse outcomes 427

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<sup>7,8</sup>.

## 431 4.3 Strengths and limitations

Our study has several strengths. First, the risk of bias in the analysis of short-term CNS 432 effects of air pollution was small because of the panel study design and the statistical 433 approach used. More specifically, they allowed to eliminate the risk of reverse causality and 434 435 confounding of person-related characteristics. Second, recent PM exposure at school was characterized by area sampling in the classroom on the days of the neurobehavioral 436 examinations. Third, recent and chronic ambient exposures to PM2.5, PM10, and BC at 437 438 residence were estimated for each participant using a high-resolution spatial temporal model. This may explain why significant associations were found with attention tests despite the low 439 spatial contrast in chronic  $PM_{2.5}$  exposure (IQR difference 1.16  $\mu$ g/m<sup>3</sup>). This observation is in 440 line with other studies such as the Worcester Heart Attack Study which showed a link with 441 acute myocardial infarction for an IQR PM<sub>2.5</sub> exposure contrast of 0.59 µg/m<sup>3</sup> <sup>44</sup>. Fourth, we 442 used three estimates for recent ambient exposure at residence to cover the time period of 443 exposure and potential lagged effects up to two days (Lag 0 to 2). 444

We acknowledge some limitations of the study. First, we cannot exclude residual 445 446 confounding by other neurotoxic substances, such as lead, if these were strongly associated with the air pollution indexes. However, in a recent study we showed in adolescents that the 447 associations between neurobehavioral performance and traffic-related exposure were 448 independent of blood lead<sup>45</sup>. The present study, based on repeated measures, tested an *a priori* 449 hypothesis involving interrelated neurobehavioral outcomes as well as strongly correlated PM 450 exposures (e.g. correlation coefficients for chronic PM<sub>10</sub> and PM<sub>2.5</sub> was r=0.87). Therefore, 451 452 the neurobehavioral test performances or exposure indicators did not provide a completely

independent opportunity for a type I error. For these reasons we did not perform multiple 453 testing. Nevertheless, it seems unlikely that the consistency of negative phenotype-exposure 454 associations would merely occur by chance as reflected by the significantly diminished 455 456 performances, not only in the two Attention Tests (chronic ambient exposures to PM2.5 and PM<sub>10</sub> at residence) but also in the Pattern Comparison Test (recent ambient exposures to 457 PM<sub>2.5</sub>, PM<sub>10</sub>, or BC on Lag 0-2 at residence). Further study is needed to find out why the 458 inverse associations between the Stroop or Digit-Symbol test results and recent inside 459 classroom PM exposure could not be corroborated for recent ambient PM exposure at 460 residence. It is plausible that recent PM exposure characterized by 3-hour monitoring of 461 classroom air displays different features in comparison to modeled recent PM exposure. 462 Furthermore, the robustness and consistency of prompt neurobehavioral responses to recent 463 exposure might be less outspoken than in the case of chronic ambient PM exposure at 464 465 residence.

## 466 **5. CONCLUSIONS**

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

476

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# 485 *Competing interests*

- 486 The authors declare they have no conflict of interest.
- 487

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