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Neurodevelopmental performance among school age children in rural Guatemala is associated with prenatal and postnatal exposure to carbon monoxide, a marker for exposure to woodsmoke

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ABSTRACT

We investigated whether early life chronic exposure to woodsmoke, using personal passive 48-h carbon monoxide (CO) as an indicator, is associated with children's neurodevelopmental and behavioral performance. CO measures were collected every 3 months from 2002 to 2005 among mother–child dyads during the Randomized Exposure Study of Pollution Indoors and Respiratory Effects (RESPIRE) stove intervention trial in San Marcos, Guatemala. From March to June, 2010, study children of age 6–7 years, performed a follow-up non-verbal, culturally adapted neurodevelopmental assessment. We found inverse associations between CO exposure of pregnant mothers during their 3rd trimesters (m = 3.8 ppm ± 3.0 ppm; range: 0.6–12.5 ppm) and child neuropsychological performance. Scores on 4 out of 11 neuropsychological tests were significantly associated with mothers' 3rd trimester CO exposures, including visuo-spatial integration ($p < 0.05$), short-term memory recall ($p < 0.05$), long-term memory recall ($p < 0.05$), and fine motor performance ($p < 0.01$) measured using the Bender Gestalt-II's Copy, Immediate Recall, and an adapted version of a Delayed Recall Figures drawing, and the Reitan-Indiana's Finger Tapping Tests, respectively. These 4 significant finding persisted with adjustment for child sex, age, visual acuity, and household assets (socio-economic status). Summary performance scores were also significantly associated with maternal 3rd trimester CO when adjusted for these covariates. Other variables accounting for variance but were excluded in our final multiple regression models included the following: HOME environment stimulation score, child examiner, WHO height-for-age percentile, and age that the infant stopped breastfeeding. This seems to be the first study on woodsmoke exposure and neurodevelopment, and the first longitudinal birth cohort study on chronic early life CO exposures, determined by high quality measures of mothers' and infants' personal CO exposures, and using well-established, reliable child neuropsychological tests. Further research is needed to replicate our results and inform future interventions and air quality standards for woodsmoke and CO.

1. Background

Wood and other forms of biomass provide readily available and often free cooking and heating fuel for millions of developing world households. Woodsmoke, however, contains thousands of chemicals, in the form of incomplete combustion products, many of which are known to be hazards to health (Naeher et al., 2007). Chronic, elevated biomass smoke exposure during pregnancy has been associated with low birth weight (Boy et al., 2002; Mishra et al., 2004; Pope et al., 2010; Siddiqui et al., 2008; Thompson et al., 2011b). Carbon monoxide (CO), an indicator of woodsmoke exposure and the largest constituent of incomplete combustion, is a neurotoxicant at high exposure levels among adults (Hsiao et al., 2004; Thom et al., 1995) and children (Kim and Coe, 1987; Klees et al., 1985). Initial symptoms of CO exposure among adults at levels equivalent to 5–20% carboxyhemoglobin (COHb) include headache, fatigue, malaise, and difficulty concentrating (EPA, 2010; Hsiao et al., 2004; Thom et al., 1995; Weaver, 1999). The developing nervous system is especially vulnerable to environmental insults (Rice and Barone, 2000).

Abbreviations: Bender Gestalt-II, Bender visual-motor Gestalt test, second edition; CI, confidence interval; CO, carbon monoxide; COHb, carboxyhemoglobin; CRECER, Chronic Respiratory Effects of Early Childhood Exposure to Respirable Particulate Matter; eCO, exhaled breath CO; ETS, environmental tobacco smoke; HOME, Home Observation for Measurement of the Environment; RESPIRE, Randomized Exposure Study of Pollution Indoors and Respiratory Effects; WISC-IV Spanish, Wechsler Intelligence Scale for Children-Fourth Edition in Spanish.

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An oxygen antagonist, CO binds to hemoglobin in red blood cells with an affinity 250 times greater than that of oxygen to form a hypoxia-inducing carboxyhemoglobin (COHb) complex that can also impair the release of oxygen from oxyhemoglobin ($Hb-O_2$) in fetal blood (Cramer, 1982). CO has the ability to cross the blood brain barrier and impair neuronal function, membrane metabolism, and anaerobic energy metabolism (Kondziella et al., 2009), targeting the globus pallidus (a subcortical structure involved in motor and postural control) (Alehan et al., 2007; Hsiao et al., 2004). In utero CO exposure may interrupt sensitive oxygen-dependent neurodevelopmental processes such as myelination (Shprecher and Mehta, 2010), neural packing (Lin et al., 2009), and neuronal migration (Knipp and Bicker, 2009). Following chronic elevated CO exposure, selective up-regulation of GABA-mediated neurotransmission occurs (Benagiano et al., 2007; Storm et al., 1986) and symptoms may manifest differently than those of acute CO exposures.

Among adults, symptoms of chronic CO exposure include impairments in memory (Chen and Schwartz, 2009; Ryan, 1990), executive function, information processing speed, attention and concentration, visuomotor skills, visual spatial planning, visual tracking, and abstract thinking abilities (Raub and Benignus, 2002). Although no previous studies appear to have examined the association between chronic early life CO and neurodevelopment impairments in children, other studies have examined the impact of other pollutants found in woodsmoke. For example, chronic, early life exposures to polycyclic aromatic hydrocarbons (PAHs), nitrogen oxide (NO_2), or black carbon (pollutants also found in woodsmoke), are associated with IQ and learning deficits among urban-dwelling children under 11 years of age (Morales et al., 2009; Perera et al., 2009; Suglia et al., 2008; Wang et al., 2009).

The present study aims to characterize the potential exposure–response relationship between in utero CO exposure from open woodfires and neurodevelopment among children in rural highland Guatemala. Although woodsmoke contains many incomplete combustion products that may have a neurodevelopmental effect, we use CO as an indicator of exposure because it was easier to measure, has the largest mass in incomplete combustion, and itself has properties that support the plausibility of neurotoxic effects.

These children were previously enrolled in a randomized trial birth cohort study called RESPIRE (the Randomized Exposure Study of Pollution Indoors and Respiratory Effects), designed primarily to examine the impact of an improved chimney stove on household air pollution and respiratory health compared to traditional open woodfires (Smith et al., 2011). As part of RESPIRE, personal passive 48-h CO exposures were measured every 3 months among women who had been randomly assigned in 2002 and 2003 to have a chimney woodstove or maintain use of the traditional open woodfire. By 2005, at the end of the trial when infants were 18-months-old, all the households received chimney stoves. At the time of the current study, the participating children were 6–7 years of age.

2. Materials and methods

2.1. Participants and recruitment

This study took place in rural western highland Guatemala (Bruce et al., 2004). Eligible study participants for the current investigation were children born to mothers recruited during pregnancy in the RESPIRE cohort study who were also in the follow-up CRECER study (Chronic Respiratory Effects of Early Childhood Exposure to Respirable Particulate Matter) (CRECER, 2010) for whom infant birth weight and diarrhea information had been collected. From March to June, 2010, we recruited a convenience sample of 39 mother–child dyads from the 117 who participated in RESPIRE/CRECER (Bruce et al., 2004). This convenience sample lived in 5 of the 21 hillside communities in closest proximity to the testing clinic. In addition, we selected those participants who lived in households within a 30-min walk from a road; 100% of those eligible agreed to participate. Study protocols and consents were approved by the Institutional Review Boards from the participating institutions (UC Berkeley and Universidad Del Valle, Guatemala).

2.2. CO exposure

Participants' personal CO exposure was measured in 4 different periods during the study: from (a) mothers during their 2nd and 3rd trimesters of pregnancy; (b) their infants during the first 9 months of life; and (c) from the same children at age 6–7 years at a follow-up neuropsychological assessment. The second trimester of pregnancy personal passive CO exposures were assessed only for a subset of 20 mothers. The mother and infant CO measures were taken using passive 48-h Gastec tubes pinned to the participants' clothing in their breathing zone, and adjusted for simultaneously collected kitchen area CO measured by span-gas calibrated electrochemical CO monitors (McCracken et al., 2009; Smith et al., 2010). For 3 mothers, two measurements taken during the 3rd trimester exposure were averaged. During their first 9 months of life, 8 infants had three and 27 had two measurements taken and averaged; 4 infants only had one measurement taken. Participants took off their CO tubes before going into the chuj (temazcals/sauna houses) and thus measurements do not capture those exposures. Pregnant mothers typically use the chuj once a week.

At the child neuropsychological assessment visit, exhaled breath CO (eCO) was measured using a MicroDirect monitor [803 Webster Street, Lewiston, ME 04240] to detect any acute CO exposures prior to the interview. Children were asked to hold their breaths for 20–30 s and exhale continuously and completely to expel the air in the alveolar lung region (to measure the alveoli-blood CO equilibrium concentration) (Thompson et al., 2011a,b; Lam et al., 2011).

2.3. Neurodevelopmental assessments

Child visits included an assessment of growth (child height and weight), visual acuity (on the (Snellen) "Tumbling E" eye chart) and performance on a battery of 11 brief neuropsychological tests. We selected nonverbal assessment tools that were previously used in other rural Latin American populations (Binder and Roberts, 1980; Chevrier et al., 2009; Grandjean et al., 2006, 1999; Harari et al., 2010). Child neuropsychological scores and administration times were compared to developed countries' reference populations to confirm suitability of the tests (see Table 1 for score comparisons and additional text in Supplemental materials for standard population details). Table 1 lists the tests stratified by neuropsychological domains (the order of administration is shown in Supplemental Table A). We used 3 subtests from the Spanish edition of the Fourth Weschler Intelligence Scale for Children (WISC-IV) including Coding, Symbol Search, and Digit Span forward and backward (Wechsler, 2003). Coding and symbol search standardized (age-adjusted) scores were summed together to generate standardized index scores called the Processing Speed Index (PSI), and Digit Span Total represented forward and backward raw scores summed together. Five Bender Gestalt-II tests included 3 drawing phases (Copy, Immediate Recall, and an adapted Delayed Recall Figures test 30 min later), a motor test (where children draw lines to connect a series of 2 dots), and a perception test (where children match images in a matrix) (Brannigan and Decker, 2003). Fine motor skills were tested using the Reitan Indiana Finger Tap Test (Reitan and Wolfson, 1985) and

Table 1
Guatemalan children's raw and standard (std) scores and comparison to norms (in developed countries).

Cognitive test by domain	Number (all)	Raw scores Mean ± SD	Standard scores Mean ± SD[a]	Compared to std population means (−/+/=)	Children below std average *n* (%)	Children within std norm range *n* (%)
Neuropsychological performance summary score						
% of scores in normal range	39	70.0 ± 22.8	n/a			
Processing speed						
WISC-IV Spanish						
Coding	35	19.5 ± 11.4	5.0 ± 3.0##	−	12 (34)	22 (63)
Symbol search	34	7.8 ± 3.8	5.5 ± 1.9##	−	6 (18)	28 (82)
Processing speed index	34	73.7 ± 10.0	73.7 ± 10.0##	−	11 (32)	23 (68)
Visuo-spatial integration						
Bender Gestalt-II						
Copy figures[b]	39	27.1 ± 5.4	101.2 ± 13.8	+	0 (0)	39 (100)
Perception matrices	35	6.0 ± 1.4	9.0 (M)[c],##	−	34 (97)	1 (3)
Short and long-term memory						
Bender Gestalt-II						
Immediate recall figures	39	9.1 ± 5.3	103.6 ± 16.1*	+	0 (0)	36 (92)
Delayed recall figures[d]	36	8.1 ± 4.6	n/a			
Working memory and attention						
WISC-IV Spanish						
Digit span forward	27	4.3 ± 1.2	7.6 ± 2.3##	−	2 (7)	25 (93)
Digit span total score	27	5.7 ± 2.4	6.0 ± 2.1##	−	9 (33)	18 (67)
Fine motor speed and coordination (dominant hand)						
Reitan-Indiana						
Finger tapping test[e]						
(6 yr olds)	34	24.0 ± 4.3##	n/a	−	7 (21)	26 (76)
(7 yr olds)	3	24.9 ± 1.7#	n/a	−	0 (0)	3 (100)
WRAVMA						
Pegboard[f]	36	27.2 ± 4.9	101.1 ± 15.3	+	1 (3)	34 (94)
(6–6.49 yr olds)	5	25.6 ± 3.6	n/a	=		
(6.5–6.99 yr olds)	28	27.4 ± 5.2	+			
(7.0–7.49 yr olds)	3	28.0 ± 4.6	n/a	=		
Bender Gestalt-II						
Motor connect the dots	38	7.1 ± 2.9##	−	−	19 (50)	19 (50)
Gross motor						
McCarthy leg coordination, abbreviated version						
Walked forward on line (Y), *n* (%)	38 (100)	n/a				
Walked backward on line (Y), *n* (%)	36 (95)					
Balanced on R foot for 10 s, *n* (%)	32 (85)					
Balanced on L foot for 10 s, *n* (%)	35 (92)					

Two-tailed alpha cut-off *p*-values are * ≤0.10, ** ≤0.05, # ≤0.01, ## ≤0.001.
[a] Guatemalan children's mean standardized scores were either better than (+), worse than (−), or equal to (=) standard population scores using two-tailed cut-off *p*-values of * ≤0.10, ** ≤0.05, # ≤0.01, ## ≤0.001.
[b] Standard scores ≤89 are a concern on the Bender Gestalt-II Copy Figures and Immediate Recall Figures Global Scores according to some interpretations (Brannigan, 2004). These scores are considered low/average, falling in the lowest quartile. Using this cut-off score, there were actually 11 with scores low enough to cause concern for the Copy Figures and 6 for the Immediate Recall Figure tests.
[c] Bender Gestalt-II Perception Matrices median was 6 in Guatemala compared to a standard median of 9.
[d] Delayed Recall Figures standard scores are not available.
[e] Finger Tapping Test compared raw scores by age group rather than age-adjusted standardized scores like the rest of the cognitive tests. 6 year olds from developed countries on average completed 27.2 ± 3.3 taps/10 s and 7 year olds had 30.01 ± 3.9 taps/10 s.
[f] Pegboard standard scores had a median of 102 and were compared to a standard score median of 100. Stratified by half year age group, Pegboard's unadjusted standard scores for 6–6.49 year olds were 25.6 ± 4.2, 6.5–6.99 year olds were 26.7 ± 4.9, and 7.0–7.49 year olds were 30.3 ± 5.0 pegs placed in 90 s.

the WRAVMA Grooved Pegboard test (Adams and Sheslow, 1995). Children's gross motor, balance, and coordination were assessed using a subset of the Leg Coordination and Balance games in the McCarthy Scales of Children's Abilities (MCSA) forward and backward line walking and balancing on one foot (McCarthy, 1972). Higher scores on all tests represent more optimal functioning. An additional outcome variable was generated examining the percentage of neuropsychological tests that children completed and scored at the normal range or higher for standardized scores. Higher summary scores represented more consistent performance within normal range across neuropsychological tests and allowed us to examine children who scored consistently above average.

Neurodevelopmental assessments were conducted by two local bilingual Spanish/Mam (an indigenous Mayan language) field workers extensively trained by one of us (LD-C) and tested for reliability. Administration scripts and materials were translated and back-translated from English to Spanish to verify accuracy of translations before they were orally delivered in Mam ($n = 37$) or Spanish ($n = 2$). LD-C and CR reviewed videos taken of the assessments (using back-translations provided orally by a 3rd party Mam- and Spanish-speaking staff member) to ensure administration quality. The Bender Gestalt Copy, Immediate Recall, and Delayed Recall Figures test scores were also verified by a second reviewer.

2.4. Maternal interviews

Mothers were interviewed using a structured questionnaire by bilingual staff in RESPIRE when study children were approximately one-year-old and for the present study when they were 6- to 7-years-old. Questionnaires at year one ascertained demographic and health information including age when infant stopped breastfeeding, home assets, maternal education, and environmental tobacco smoke (ETS) exposure during the first year of life; at year 6–7, questionnaires assessed maternal and child chemical exposure histories (occupational, dietary, and prenatal care and drug use), HOME environment stimulation scores and child education.

2.5. Statistical analyses

Personal 48-h CO exposures levels for study mothers during their 2nd and 3rd trimesters of pregnancy and for children during their first 9 months of life, as well as child exhaled breath CO at the time of their interviews, were examined in relation to child neuropsychological test scores at age 6–7 years in simple (unadjusted) and adjusted least squares regression models for all neurodevelopmental outcomes except for McCarthy Leg coordination abbreviated test, where we used logistic regression. CO exposures (ppm) were $\log_{10}$-transformed to achieve normal distribution (see Fig. A in Supplementary materials). Neuropsychological scores were examined as raw and age-standardized scores. Crude raw scores were analyzed in all cases except PSI, where standard age-adjusted scores were used (given for every 4 months age group representative of US child scores). We used the raw scores adjusted for age in the multivariate regressions because no standardized scores existed for rural Guatemalan children. Coding, Processing Speed Index, and Immediate Recall Figures test scores were $\log_{10}$-transformed in order to meet the assumption of normal distribution for linear regression. Summary scores were regressed against CO exposure measures from all time points. Least square regressions were performed on a subset of the study population for which 2nd trimester natural log-transformed (unadjusted) 48-h CO exposure measures were available.

Several covariates were selected *a priori* based on the literature as related to child neurodevelopment (shown in Table 2). Child sex (categorical) and age (continuous) were included in all final models. Child vision scores were "poor" or "not poor" (poor = "Tumbling E" eye chart scores worse than 20/40). Birth weights were taken within 48-h of birth (Thompson, 2008) and household ownership of a bicycle, television, and radio were summed to generate an 'asset index' (0–3 out of 3; one point for each item). HOME environment stimulation raw scores, assessed at maternal interviews were out of 22, measured using an abbreviated, culturally adapted version of the HOME (Home Observation for Measurement of the Environment) questionnaire used in UNICEF's mid-decade surveys (MICS) for children under 5 and used in previous air pollution and neurodevelopment studies (Perera et al., 2006, 2009). Influential covariates that changed the significance or direction of the association between CO exposure and child's neuropsychological performance were identified in forward univariate and backward stepwise regressions, and retained if they most contributed to the best fit model or were used in previous studies. For all regression analyses, child age and sex interactions were tested using a two-tailed alpha p-value cut-off of <0.10, and we checked that model assumptions were met.

Sensitivity analyses were performed to ensure robustness of regression results (see Fig. 1 and Supplemental Table B for comparison of β-coefficients among significant associations). For example, in subsequent analyses we excluded potentially influential outlier points (scores beyond 2 standard deviations from the mean). In other sensitivity analyses, we excluded participants if their maternal 3rd trimester CO exposure was >10 ppm or if the children's test scores were flagged due to possible alternate explanations for their poor performance (unrelated to CO exposure) such as currently using medications ($n = 2$), prior head trauma resulting in loss of consciousness ($n = 1$), prior convulsion, unwell at the time of assessment ($n = 3$), umbilical cord surgery at birth ($n = 1$), or not in school yet ($n = 1$). We also excluded in sensitivity analyses child scores where there were minor deviation from test administration protocol. Statistical analysis was conducted using STATA/IC 11 (StataCorp. LP, College Station, TX).

Table 2
Sociodemographic characteristics of mothers and children enrolled in study.

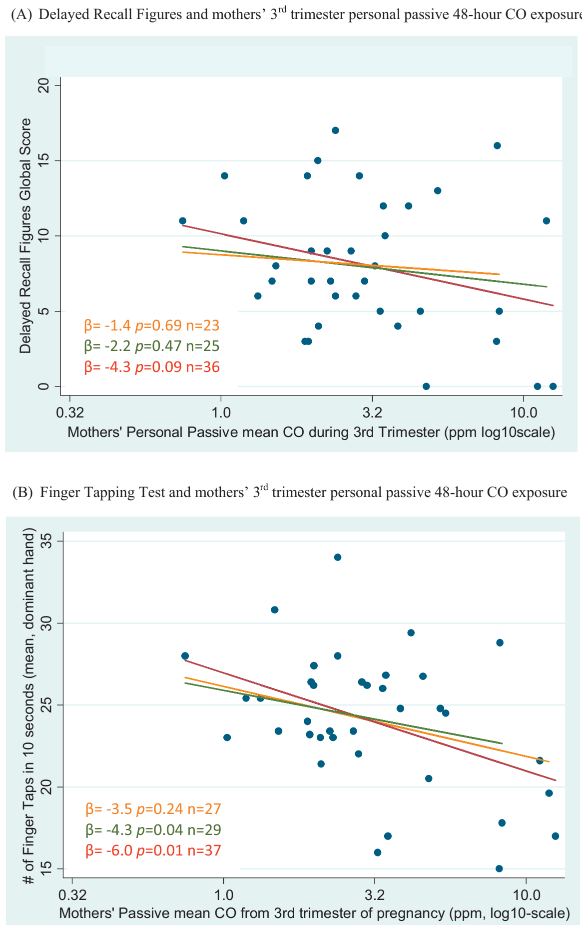
	All children ($n = 39$)
Maternal characteristics	
Age at one year visit, mean ± SD, years	25.7 ± 5.8
Education, n (%)	
Completed primary school	28 (72)
<Primary school completed	11 (28)
HOME environment stimulation abbreviated score, mean ± SD[a]	13.4 ± 2.4
Child characteristics	
Sex, n (%)	
Male	20 (51)
Female	19 (49)
Age at assessment, mean ± SD, years	6.7 ± 0.25
Birth weight, mean ± SD, kg	2.75 ± 0.42
Age stopped breastfeeding, mean ± SD, months	20 ± 4
Household ETS exposure in first year of life, n (%)	
Yes	13 (33)
No	26 (67)
Weight, mean ± SD, kg	17.7 ± 1.6
Height, mean ± SD, cm	104.7 ± 4.2
Body Mass Index (BMI), mean ± SD, kg/m^2	16.1 ± 0.9
WHO standard height-for-age, mean ± SD, percentile[b]	1.2 ± 3.1
Visual acuity, n (%)	
Poor vision (20/60, 20/80, or 20/100)	9 (23)
Good vision (20/40 or 20/20)	30 (77)
Household characteristics	
Altitude, mean ± SD, *m*	2640 ± 156
Assets, n (%)	
Electricity in the house	
Yes	28 (72)
No	11 (28)
Cattle	
Yes	18 (46)
No	21 (54)
Bicycle	
Yes	12 (31)
No	27 (69)
Radio	
Yes	31 (79)
No	8 (21)
TV	
Yes	5 (13)
No	34 (87)
Asset index, n (%)[c]	
0	6 (15)
1	22 (57)
2	7 (18)
3	4 (10)
Chimney stove installed in household before infant was 1.5-years-old, n (%)[d]	
Yes	8 (21)
No	31 (79)

ETS, environmental tobacco smoke from smokers in household.
[a] Mother-reported during maternal interview 2 days prior to child cognitive interview.
[b] World Health Organization age and sex-specific z-score global standards (WHO, 2007).
[c] Asset index: sum of binary indicators for having a bicycle, radio, and television; was published previously with this population (McCracken et al., 2007) and had the best distribution and consistent contribution to models out of possible asset indices combinations from the dataset (scores range from 0 to 3).
[d] Chimney stoves were installed in all RESPIRE study households by the time of the study child reached 1.5-years-old.

3. Results

3.1. Population characteristics

Population characteristics of the 39 mother–child dyads and their households are shown in Table 2. A total of 19 girls and 20 boys aged 6.2–7.4 years participated in this study. No mothers had



Key: Orange = excluding flagged and maternal CO >10ppm; Green= excluding flagged; Red=full sample

Fig. 1. Shown are mothers' CO exposures from the 3rd trimester and child scores on the (A) Bender Gestalt-II's Delayed Recall Figures and (B) the Reitan-Indiana Finger Tapping Test. The 3 fitted lines demonstrate the robustness of the simple linear regression results, using 3 different sample sizes. When influential participants (with alternate explanations for performance or maternal CO during the third trimester >10 ppm) were excluded from linear regression analyses, as indicated by the orange and green lines the inverse direction of association persisted.

completed higher than primary school education, all children and mothers spoke Mam as their first language, and some also spoke Spanish. There were no significant differences in demographic characteristics between those households who were provided a chimney stove before compared to after the study child's birth. A smaller percentage of our study participants had radios (79%) and TVs (13%) in their homes compared to the original RESPIRE cohort of participants (93% and 25% had radios and TVs, respectively; $n = 120$) (McCracken et al., 2007), but were otherwise similar in household assets.

3.2. CO exposures

Table 3 shows personal CO exposure levels. Average maternal 48-h mean passive CO concentrations during the 3rd trimester was 3.8 ± 3.0 ppm (range = 0.6–12.5 ppm; $n = 39$). For the subset of pregnant women ($n = 20$) with 2nd trimester CO measured, the mean personal passive 48-h CO concentration was 3.0 ± 2.5 ppm with a range = 0.8–11.6 ppm (data not shown). During the first 9 months of life, infants' mean personal passive 48-h CO concentration was 2.2 ± 2.0 ppm with a range of 0.3–9.8 ppm ($n = 39$). Log-transformed maternal 3rd trimester and infant's personal passive 48-h CO were associated but not significantly (correlation coefficient = 0.20; $p = 0.12$) (see Supplemental Fig. B). Exhaled CO (eCO) at the time of child testing averaged 2.3 ± 0.9 ppm and all were ≤5 ppm. Seventeen children (44%) reported bathing in the temazcal the day before testing, although these children did not have significantly higher eCO than children who had not used the temazcal the day before (data not shown).

3.3. Neuropsychological scores

Table 1 presents the mean and standard deviation for the raw and standardized scores. Child scores had a wide range with normal distributions for most tests. On average, compared to raw and age-standardized mean scores from developed countries (Adams and Sheslow, 1995; Brannigan and Decker, 2003), a US-representative sample of children according to census sampling methods, Guatemalan children performed non-significantly better on the Copy Figures and Pegboard tests (two-tailed $p \leq 0.10$). On the other hand, they performed significantly worse on the PSI, Perception Matrices, Digit Span Forward and Total, Motor Connect the Dots, Finger Tapping Test and Immediate Recall Figures tests.

Indications of inverse linear relationships (negative β-coefficients) were observed between scores on 9 out of 11 neuropsychological tests, as well as the summary score for overall neuropsychological performance, and maternal 3rd trimester log-CO concentrations, with 4 significant inverse associations: Bender-Gestalt Copy, Immediate Recall, and Delayed Recall Figures, and Finger Tapping Test (see Table 4 and Fig. 2). We found that for every 10-fold increase in maternal CO, Copy Figures scores decreased 4.2 points ($p < 0.10$), Delayed Recall Figures scores decreased 4.3 points ($p = 0.05$), and the average number of finger taps in 10 s decreased by 6 ($p < 0.01$). Log-transformed Immediate Recall scores also decreased 0.3 points, meaning that every 10% increase in maternal CO exposure during the 3rd trimester was significantly associated with a 3% decrease in Immediate Recall scores ($p < 0.05$). After adjustment for child age, sex, visual acuity, and asset index (see Table 4) the significant inverse associations for these 4 tests persisted. The summary scores became significantly inversely associated with maternal 3rd trimester log-CO ($\beta = -0.23$, $p = 0.04$, $n = 39$, $R^2W = 0.30$) when adjusted for these 4 covariates. No significant associations were detected between children's eCO or temazcal use the day before and neuropsychological performance. We used one-tailed cut-off p-values for all of the regression analyses including adjusted models.

Table 3
Mother and infant CO exposures measured by 48-h personal passive diffusion tubes and child exhaled breath CO measures at neuropsychological assessments ($n = 39$).

Exposure	Mean ± SD (ppm)	Median (ppm)	Range (ppm) Minimum–maximum
Mother's CO exposure during the 3rd trimester	3.83 ± 3.03	2.80	0.62–12.52
Infant's CO exposure during 0–9 months of age	2.20 ± 1.98	1.63	0.26–9.77
Child's exhaled breath CO at assessment	2.26 ± 0.86	2.0	1.0–5.0

Table 4
Linear regression results of mother and infant $\log_{10}$-transformed 48-h personal passive CO exposures and child cognitive performance.

Cognitive test	No.	Mother $\log_{10}$CO 3rd trimester β (95% CI)	Adjusted model[a] 3rd trimester β (95% CI)	Infant $\log_{10}$CO 0–9 months β (95% CI)
Neuropsychological performance summary score[b]				
% of scores ≥normal range	39	−0.2 (−0.5, −0.0)	−0.2 (−0.4, −0.0)**	−0.03 (−0.2, 0.2)
Processing speed				
WISC-IV Spanish				
Coding[c]	35	−0.1 (−0.4, 0.1)	NS	−0.02 (−0.2, 0.2)
Symbol search	34	−0.6 (−5.4, 4.2)	NS	−2.6 (−6.0, 0.9)*
Processing speed index [c]	34	−0.01 (−0.1, 0.1)	NS	−0.02 (−0.08, 0.02)
Visuo-spatial integration				
Bender Gestalt-II				
Copy figures	39	−4.2 (−9.8, 1.4)*	−4.4 (−9.5, 0.7)**	−2.2 (−6.8, 2.3)
Perception matrices	34	0.7 (−0.8, 2.2)	NS	0.06 (−1.2, 1.3)
Short and long-term memory				
Bender Gestalt-II				
Immediate recall figures[c]	39	−0.3 (−0.6, 0.02)**	−0.3 (−0.6, .01)**	−0.05 (−0.3, 0.2)
Delayed recall figures	36	−4.3 (−9.4, 0.8)**	−4.8 (−9.8, 0.1)**	0.5 (−3.7, 4.8)
Working memory and attention				
WISC-IV Spanish				
Digit span forward	27	0.03 (−1.6, 1.6)	NS	0.3 (−1.0, 1.5)
Digit span total score [d]	27	−0.1 (−3.0, 2.8)	NS	−0.7 (−2.9, 1.6)
Fine motor speed and coordination (dominant hand)				
Reitan-Indiana				
Finger tapping test	37	−6.0 (−10.3, −1.7)#	−5.7 (−9.7, −1.7)#	0.8 (−3.0, 4.7)
WRAVMA				
Pegboard	36	−0.8 (−6.5, 4.9)	NS	−1.5 (−6.0, 3.1)
Bender Gestalt-II				
Motor connect the dots	38	−1.1 (−4.2, 2.1)	NS	−1.5 (−4.0, 1.0)

One-tailed cut-off p-values are * ≤0.10, ** ≤0.05, # ≤0.01, ## ≤0.00; NS, non-significant.
[a] Multivariate analyses included child age (continuous), child sex (female vs. male), poor vision (yes or no), asset index (0–3; treated as continuous).
[b] When 2 scores with outlier standard residuals were included, maternal CO became significantly associated with summary scores (−0.23, p = 0.05).
[c] Coding, Processing Speed Index, and Immediate Recall Figures tests were $\log_{10}$-transformed.
[d] Only 10 children completed the reverse digit span so it was not analyzed independently (is only shown as a Digit Span Total scores, added to forward digit span scores).

In sensitivity analyses, excluding outliers did not change the inverse direction of the β-coefficients for the 4 tests significantly associated with maternal 3rd trimester CO. Excluding flagged scores (where alternate explanations for their performance existed; $n \leq 13$; as described in Section 2.5), made all 4, except the Finger Tapping Test, become non-significantly inversely associated with maternal 3rd trimester CO ($p < 0.10$, one-tailed) (see Supplemental Table B and Fig. 1). When we also excluded participants with maternal 3rd trimester CO ≥10 ppm, Finger Tapping Test scores remained significantly inversely associated with 3rd trimester CO ($p < 0.05$) (see Fig. 1).

3.4. Maternal second trimester and infant CO exposures

In the subsample of mothers with 2nd trimester CO measurements, 7 out of 11 of the neuropsychological tests had inverse non-significant trends with natural log-transformed maternal CO but only 3 were significant at $p < 0.10$ (one-tailed): coding, PSI, and Immediate Recall Figures (see Supplemental Table C). Symbol search was the only neuropsychological test significantly associated with personal passive 48-h CO among infants aged 0–9 months ($\beta = -2.6$, $p < 0.10$; one-tailed; $n = 34$), with an inverse association persisting after adjustment for child age, sex, and vision ($\beta = -3.5$, $p < 0.05$; one-tailed).

4. Discussion

We found that child scores on 4 out of 11 neuropsychological tests were significantly inversely associated with maternal 3rd trimester CO: visuo-spatial integration, short-term memory recall, long-term memory recall, and fine motor performance. Maternal 3rd trimester CO appeared to influence child neuropsychological performance more than did infant CO in the first 9 months of life, possibly due to the maternal CO's higher mean and greater range of concentrations in the study population.

Our results are consistent with previous epidemiological studies on air pollution and neurodevelopment (Edwards et al., 2010; Perera et al., 2006, 2009). Edwards et al. (2010) found that non-verbal intelligence measured on Raven's Progressive Color Matrices (RPCM) was inversely associated with maternal 3rd trimester personal 48-h PAH exposures among a prospective cohort of 214 children in Krakow, Poland assessed from 2001 to 2006. Rural Guatemalan mothers' mean personal CO levels were higher and their children more malnourished. Unlike the Edwards et al. (2010) and Perera et al. (2006, 2009) studies, we did not assess other environmental exposures, e.g., lead and pesticides which may confound the associations, nor did we measure maternal intelligence. Our study found that maternal log-CO was associated but not significantly with infant log-CO ($p = 0.06$; $\beta = 0.20$; one-tailed). This agrees with previously published RESPIRE study results; CO measures used were representative of chronic CO exposure (McCracken et al., 2009).

This appears to be the first birth cohort study to examine the neurodevelopmental toxicity of woodsmoke. Strengths are its cohort design and multiple longitudinal measures of CO exposures, health status, household assets, and environmental stimulation. As an indicator of woodsmoke exposures, we used validated personal mother and child CO exposures (child eCO and breathing zone passive CO personal exposures taken at multiple time points) which accounted for duration of exposure (48 h). In addition, we used neuropsychological instruments with high reliability, validity and construct clarity among standard US populations that had also been used previously among rural Latin American and Spanish-speaking study populations.

Study limitations include children's performance being below standard averages on a number of neuropsychological tests and

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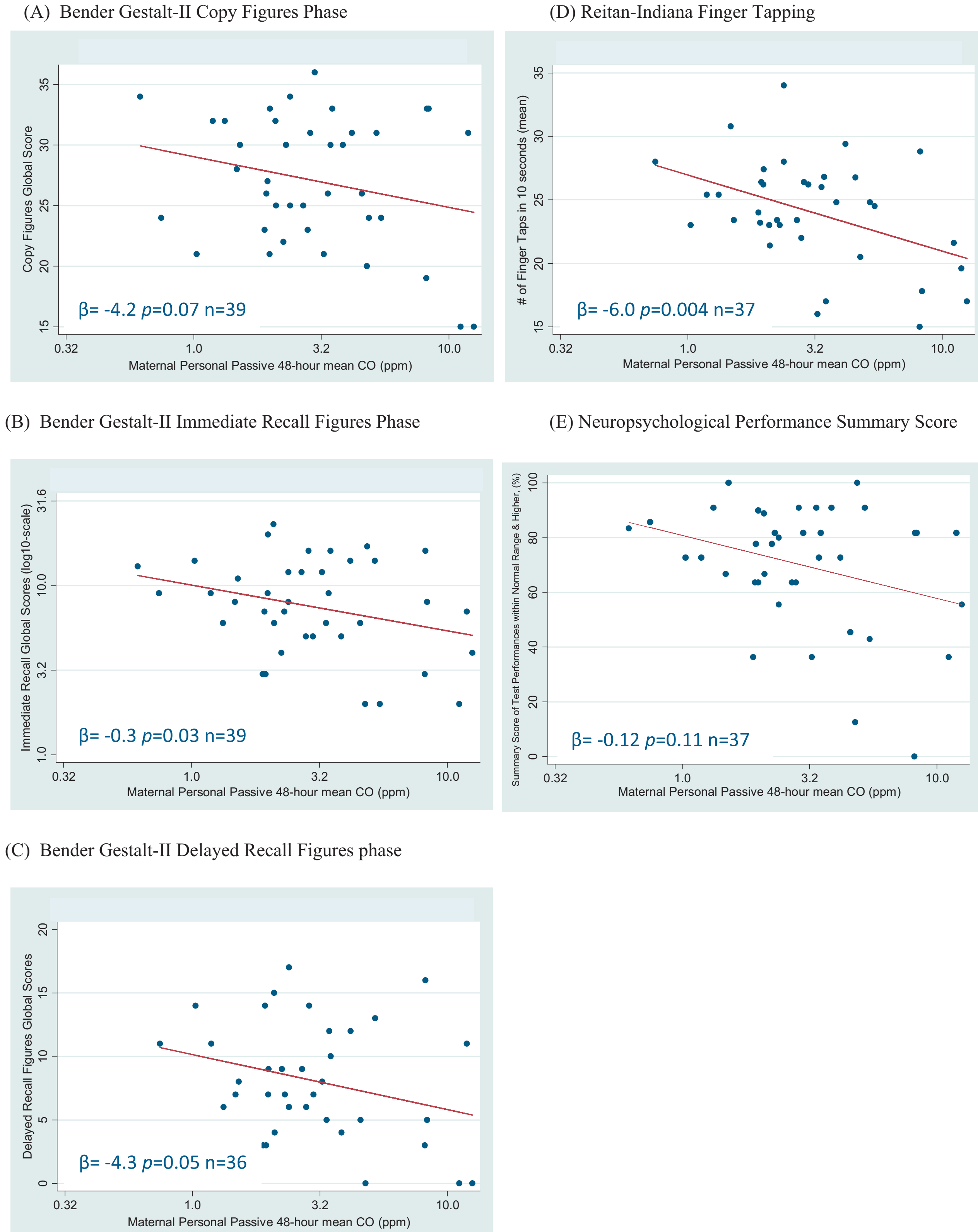

Fig. 2. Results of linear regressions are presented between child neuropsychological performance scores and mothers' 3rd trimester CO exposures. Note that Neuropsychological Performance Summary Scores became significantly inversely associated with maternal 3rd trimester personal passive CO when 2 participants with outlier standard residuals were excluded from simple linear regression analyses.

future studies would benefit from using larger sample sizes to increase results' robustness. Tests were still considered suitable despite low average scores because children in the rural Guatemalan population were able to complete them without difficulty, as indicated by some children who scored in the gifted (above average) range for standard US populations on coding, immediate recall, and pegboard tests. Furthermore, 3 tests were inter-dependent and their scores correlated (the 3 Bender

Gestalt-II Figures drawing tests), so children who did not copy correctly in the first phase subsequently also had poorer recall. Children's performance on the 3 Bender Gestalt Figures drawing tests was probably not due to fine motor ability discrepancies though because these were not related; one child with a low Finger Tapping Test score, scored above average on the Immediate Recall Figures. Short term peak CO concentrations (which of course are much higher during cooking periods than other times e.g., 10-min kitchen CO levels in rural Guatemalan kitchens measured by environmental sampling (not personal) may range up to 150 ppm (Naeher et al., 2007)) were not captured by the passive 48-h monitoring method employed, but this is a common methodological problem for studies measuring and standard setting for chronic exposures. Despite these shortcomings, strength lies in our uniquely highly ethnically and age homogenous birth cohort. Children living in this area of Guatemala may be particularly at risk for neurodevelopmental impairments from woodsmoke because at this elevation (~2600 m) they are already subjected to stress from lower oxygen levels. For example, in RESPIRE mean blood oxygen saturation of healthy infants in the area was found to be 93.2% $\pm$ 3.0% (Bruce et al., 2007). Dietary and drinking water hygiene, maternal mental health, and respiratory disease treatment programs are also priorities in rural Guatemala, with chronic coughs present among half and infant diarrhea (defined as persistent diarrhea for more than 14 days during the first two years of life) experienced among all of the study children. Chronic coughs and respiratory diseases from exposure to woodsmoke may decrease children's school attendance, but coughing at the time of the child interviews did not appear to influence child performance on neuropsychological tests.

5. Conclusions

Although a larger sample size might provide more definitive results, we have identified a wide breadth of specific neuropsychological outcomes that are likely impaired by in utero exposure to woodsmoke at chronic CO levels <13 ppm. In an ethnically and age homogenous birth cohort in a developing country context, child performance on fine motor, visuo-spatial perception, integration, and visual-motor memory tasks, as well a summary performance score were significantly inversely associated with 3rd trimester chronic CO exposure. Chronic personal CO levels measured in Guatemala are comparable to levels measured in some US household kitchens, where smoking and close proximity to high density traffic elevates one's likelihood of exposure. Although not by itself conclusive, this study also suggests that the new WHO 24-h Air Quality Guideline for chronic CO exposure of 7 mg/m^3 (~6 ppm) (WHO, 2010) may not be sufficiently protective for children in utero. With an approximate 50% reduction in personal CO exposures among chimney stove-using mothers compared to open woodfire-using mothers (Smith et al., 2010), future studies may examine whether the chimney stove provides protection from neuropsychological impacts among the children of pregnant women in this population. New, advanced combustion woodstoves that are now becoming available may be capable of even larger reductions. Although there are other pollutants in woodsmoke that may contribute to the neurodevelopmental effects found in this study, our results call for additional studies to explore the effects of chronic woodsmoke and CO exposures among pregnant women on their children's neurodevelopment.

Conflict of interest declaration

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neuro.2011.09.004.

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Neurodevelopmental performance among school age children in rural Guatemala is associated with prenatal and postnatal exposure to carbon monoxide, a marker for exposure to woodsmoke

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ABSTRACT

We investigated whether early life chronic exposure to woodsmoke, using personal passive 48-h carbon monoxide (CO) as an indicator, is associated with children's neurodevelopmental and behavioral performance. CO measures were collected every 3 months from 2002 to 2005 among mother–child dyads during the Randomized Exposure Study of Pollution Indoors and Respiratory Effects (RESPIRE) stove intervention trial in San Marcos, Guatemala. From March to June, 2010, study children of age 6–7 years, performed a follow-up non-verbal, culturally adapted neurodevelopmental assessment. We found inverse associations between CO exposure of pregnant mothers during their 3rd trimesters (m = 3.8 ppm ± 3.0 ppm; range: 0.6–12.5 ppm) and child neuropsychological performance. Scores on 4 out of 11 neuropsychological tests were significantly associated with mothers' 3rd trimester CO exposures, including visuo-spatial integration ($p < 0.05$), short-term memory recall ($p < 0.05$), long-term memory recall ($p < 0.05$), and fine motor performance ($p < 0.01$) measured using the Bender Gestalt-II's Copy, Immediate Recall, and an adapted version of a Delayed Recall Figures drawing, and the Reitan-Indiana's Finger Tapping Tests, respectively. These 4 significant finding persisted with adjustment for child sex, age, visual acuity, and household assets (socio-economic status). Summary performance scores were also significantly associated with maternal 3rd trimester CO when adjusted for these covariates. Other variables accounting for variance but were excluded in our final multiple regression models included the following: HOME environment stimulation score, child examiner, WHO height-for-age percentile, and age that the infant stopped breastfeeding. This seems to be the first study on woodsmoke exposure and neurodevelopment, and the first longitudinal birth cohort study on chronic early life CO exposures, determined by high quality measures of mothers' and infants' personal CO exposures, and using well-established, reliable child neuropsychological tests. Further research is needed to replicate our results and inform future interventions and air quality standards for woodsmoke and CO.

1. Background

Wood and other forms of biomass provide readily available and often free cooking and heating fuel for millions of developing world households. Woodsmoke, however, contains thousands of chemicals, in the form of incomplete combustion products, many of which are known to be hazards to health (Naeher et al., 2007). Chronic, elevated biomass smoke exposure during pregnancy has been associated with low birth weight (Boy et al., 2002; Mishra et al., 2004; Pope et al., 2010; Siddiqui et al., 2008; Thompson et al., 2011b). Carbon monoxide (CO), an indicator of woodsmoke exposure and the largest constituent of incomplete combustion, is a neurotoxicant at high exposure levels among adults (Hsiao et al., 2004; Thom et al., 1995) and children (Kim and Coe, 1987; Klees et al., 1985). Initial symptoms of CO exposure among adults at levels equivalent to 5–20% carboxyhemoglobin (COHb) include headache, fatigue, malaise, and difficulty concentrating (EPA, 2010; Hsiao et al., 2004; Thom et al., 1995; Weaver, 1999). The developing nervous system is especially vulnerable to environmental insults (Rice and Barone, 2000).

Abbreviations: Bender Gestalt-II, Bender visual-motor Gestalt test, second edition; CI, confidence interval; CO, carbon monoxide; COHb, carboxyhemoglobin; CRECER, Chronic Respiratory Effects of Early Childhood Exposure to Respirable Particulate Matter; eCO, exhaled breath CO; ETS, environmental tobacco smoke; HOME, Home Observation for Measurement of the Environment; RESPIRE, Randomized Exposure Study of Pollution Indoors and Respiratory Effects; WISC-IV Spanish, Wechsler Intelligence Scale for Children-Fourth Edition in Spanish.

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An oxygen antagonist, CO binds to hemoglobin in red blood cells with an affinity 250 times greater than that of oxygen to form a hypoxia-inducing carboxyhemoglobin (COHb) complex that can also impair the release of oxygen from oxyhemoglobin (Hb-O_2) in fetal blood (Cramer, 1982). CO has the ability to cross the blood brain barrier and impair neuronal function, membrane metabolism, and anaerobic energy metabolism (Kondziella et al., 2009), targeting the globus pallidus (a subcortical structure involved in motor and postural control) (Alehan et al., 2007; Hsiao et al., 2004). In utero CO exposure may interrupt sensitive oxygen-dependent neurodevelopmental processes such as myelination (Shprecher and Mehta, 2010), neural packing (Lin et al., 2009), and neuronal migration (Knipp and Bicker, 2009). Following chronic elevated CO exposure, selective up-regulation of GABA-mediated neurotransmission occurs (Benagiano et al., 2007; Storm et al., 1986) and symptoms may manifest differently than those of acute CO exposures.

Among adults, symptoms of chronic CO exposure include impairments in memory (Chen and Schwartz, 2009; Ryan, 1990), executive function, information processing speed, attention and concentration, visuomotor skills, visual spatial planning, visual tracking, and abstract thinking abilities (Raub and Benignus, 2002). Although no previous studies appear to have examined the association between chronic early life CO and neurodevelopment impairments in children, other studies have examined the impact of other pollutants found in woodsmoke. For example, chronic, early life exposures to polycyclic aromatic hydrocarbons (PAHs), nitrogen oxide (NO_2), or black carbon (pollutants also found in woodsmoke), are associated with IQ and learning deficits among urban-dwelling children under 11 years of age (Morales et al., 2009; Perera et al., 2009; Suglia et al., 2008; Wang et al., 2009).

The present study aims to characterize the potential exposure–response relationship between in utero CO exposure from open woodfires and neurodevelopment among children in rural highland Guatemala. Although woodsmoke contains many incomplete combustion products that may have a neurodevelopmental effect, we use CO as an indicator of exposure because it was easier to measure, has the largest mass in incomplete combustion, and itself has properties that support the plausibility of neurotoxic effects.

These children were previously enrolled in a randomized trial birth cohort study called RESPIRE (the Randomized Exposure Study of Pollution Indoors and Respiratory Effects), designed primarily to examine the impact of an improved chimney stove on household air pollution and respiratory health compared to traditional open woodfires (Smith et al., 2011). As part of RESPIRE, personal passive 48-h CO exposures were measured every 3 months among women who had been randomly assigned in 2002 and 2003 to have a chimney woodstove or maintain use of the traditional open woodfire. By 2005, at the end of the trial when infants were 18-months-old, all the households received chimney stoves. At the time of the current study, the participating children were 6–7 years of age.

2. Materials and methods

2.1. Participants and recruitment

This study took place in rural western highland Guatemala (Bruce et al., 2004). Eligible study participants for the current investigation were children born to mothers recruited during pregnancy in the RESPIRE cohort study who were also in the follow-up CRECER study (Chronic Respiratory Effects of Early Childhood Exposure to Respirable Particulate Matter) (CRECER, 2010) for whom infant birth weight and diarrhea information had been collected. From March to June, 2010, we recruited a convenience sample of 39 mother–child dyads from the 117 who participated in RESPIRE/CRECER (Bruce et al., 2004). This convenience sample lived in 5 of the 21 hillside communities in closest proximity to the testing clinic. In addition, we selected those participants who lived in households within a 30-min walk from a road; 100% of those eligible agreed to participate. Study protocols and consents were approved by the Institutional Review Boards from the participating institutions (UC Berkeley and Universidad Del Valle, Guatemala).

2.2. CO exposure

Participants' personal CO exposure was measured in 4 different periods during the study: from (a) mothers during their 2nd and 3rd trimesters of pregnancy; (b) their infants during the first 9 months of life; and (c) from the same children at age 6–7 years at a follow-up neuropsychological assessment. The second trimester of pregnancy personal passive CO exposures were assessed only for a subset of 20 mothers. The mother and infant CO measures were taken using passive 48-h Gastec tubes pinned to the participants' clothing in their breathing zone, and adjusted for simultaneously collected kitchen area CO measured by span-gas calibrated electrochemical CO monitors (McCracken et al., 2009; Smith et al., 2010). For 3 mothers, two measurements taken during the 3rd trimester exposure were averaged. During their first 9 months of life, 8 infants had three and 27 had two measurements taken and averaged; 4 infants only had one measurement taken. Participants took off their CO tubes before going into the chuj (temazcals/sauna houses) and thus measurements do not capture those exposures. Pregnant mothers typically use the chuj once a week.

At the child neuropsychological assessment visit, exhaled breath CO (eCO) was measured using a MicroDirect monitor [803 Webster Street, Lewiston, ME 04240] to detect any acute CO exposures prior to the interview. Children were asked to hold their breaths for 20–30 s and exhale continuously and completely to expel the air in the alveolar lung region (to measure the alveoli-blood CO equilibrium concentration) (Thompson et al., 2011a,b; Lam et al., 2011).

2.3. Neurodevelopmental assessments

Child visits included an assessment of growth (child height and weight), visual acuity (on the (Snellen) "Tumbling E" eye chart) and performance on a battery of 11 brief neuropsychological tests. We selected nonverbal assessment tools that were previously used in other rural Latin American populations (Binder and Roberts, 1980; Chevrier et al., 2009; Grandjean et al., 2006, 1999; Harari et al., 2010). Child neuropsychological scores and administration times were compared to developed countries' reference populations to confirm suitability of the tests (see Table 1 for score comparisons and additional text in Supplemental materials for standard population details). Table 1 lists the tests stratified by neuropsychological domains (the order of administration is shown in Supplemental Table A). We used 3 subtests from the Spanish edition of the Fourth Weschler Intelligence Scale for Children (WISC-IV) including Coding, Symbol Search, and Digit Span forward and backward (Wechsler, 2003). Coding and symbol search standardized (age-adjusted) scores were summed together to generate standardized index scores called the Processing Speed Index (PSI), and Digit Span Total represented forward and backward raw scores summed together. Five Bender Gestalt-II tests included 3 drawing phases (Copy, Immediate Recall, and an adapted Delayed Recall Figures test 30 min later), a motor test (where children draw lines to connect a series of 2 dots), and a perception test (where children match images in a matrix) (Brannigan and Decker, 2003). Fine motor skills were tested using the Reitan Indiana Finger Tap Test (Reitan and Wolfson, 1985) and

Table 1
Guatemalan children's raw and standard (std) scores and comparison to norms (in developed countries).

Cognitive test by domain	Number (all)	Raw scores Mean ± SD	Standard scores Mean ± SD[a]	Compared to std population means (−/+/=)	Children below std average *n* (%)	Children within std norm range *n* (%)
Neuropsychological performance summary score						
% of scores in normal range	39	70.0 ± 22.8	n/a			
Processing speed						
WISC-IV Spanish						
Coding	35	19.5 ± 11.4	5.0 ± 3.0##	−	12 (34)	22 (63)
Symbol search	34	7.8 ± 3.8	5.5 ± 1.9##	−	6 (18)	28 (82)
Processing speed index	34	73.7 ± 10.0	73.7 ± 10.0##	−	11 (32)	23 (68)
Visuo-spatial integration						
Bender Gestalt-II						
Copy figures[b]	39	27.1 ± 5.4	101.2 ± 13.8	+	0 (0)	39 (100)
Perception matrices	35	6.0 ± 1.4	9.0 (M)[c,##]	−	34 (97)	1 (3)
Short and long-term memory						
Bender Gestalt-II						
Immediate recall figures	39	9.1 ± 5.3	103.6 ± 16.1*	+	0 (0)	36 (92)
Delayed recall figures[d]	36	8.1 ± 4.6	n/a			
Working memory and attention						
WISC-IV Spanish						
Digit span forward	27	4.3 ± 1.2	7.6 ± 2.3##	−	2 (7)	25 (93)
Digit span total score	27	5.7 ± 2.4	6.0 ± 2.1##	−	9 (33)	18 (67)
Fine motor speed and coordination (dominant hand)						
Reitan-Indiana						
Finger tapping test[e]						
(6 yr olds)	34	24.0 ± 4.3##	n/a	−	7 (21)	26 (76)
(7 yr olds)	3	24.9 ± 1.7#	n/a	−	0 (0)	3 (100)
WRAVMA						
Pegboard[f]	36	27.2 ± 4.9	101.1 ± 15.3	+	1 (3)	34 (94)
(6–6.49 yr olds)	5	25.6 ± 3.6	n/a	=		
(6.5–6.99 yr olds)	28	27.4 ± 5.2	+			
(7.0–7.49 yr olds)	3	28.0 ± 4.6	n/a	=		
Bender Gestalt-II						
Motor connect the dots	38	7.1 ± 2.9##	−	−	19 (50)	19 (50)
Gross motor						
McCarthy leg coordination, abbreviated version						
Walked forward on line (Y), *n* (%)	38 (100)	n/a				
Walked backward on line (Y), *n* (%)	36 (95)					
Balanced on R foot for 10 s, *n* (%)	32 (85)					
Balanced on L foot for 10 s, *n* (%)	35 (92)					

Two-tailed alpha cut-off *p*-values are * ≤0.10, ** ≤0.05, # ≤0.01, ## ≤0.001.
[a] Guatemalan children's mean standardized scores were either better than (+), worse than (−), or equal to (=) standard population scores using two-tailed cut-off *p*-values of * ≤0.10, ** ≤0.05, # ≤0.01, ## ≤0.001.
[b] Standard scores ≤89 are a concern on the Bender Gestalt-II Copy Figures and Immediate Recall Figures Global Scores according to some interpretations (Brannigan, 2004). These scores are considered low/average, falling in the lowest quartile. Using this cut-off score, there were actually 11 with scores low enough to cause concern for the Copy Figures and 6 for the Immediate Recall Figure tests.
[c] Bender Gestalt-II Perception Matrices median was 6 in Guatemala compared to a standard median of 9.
[d] Delayed Recall Figures standard scores are not available.
[e] Finger Tapping Test compared raw scores by age group rather than age-adjusted standardized scores like the rest of the cognitive tests. 6 year olds from developed countries on average completed 27.2 ± 3.3 taps/10 s and 7 year olds had 30.01 ± 3.9 taps/10 s.
[f] Pegboard standard scores had a median of 102 and were compared to a standard score median of 100. Stratified by half year age group, Pegboard's unadjusted standard scores for 6–6.49 year olds were 25.6 ± 4.2, 6.5–6.99 year olds were 26.7 ± 4.9, and 7.0–7.49 year olds were 30.3 ± 5.0 pegs placed in 90 s.

the WRAVMA Grooved Pegboard test (Adams and Sheslow, 1995). Children's gross motor, balance, and coordination were assessed using a subset of the Leg Coordination and Balance games in the McCarthy Scales of Children's Abilities (MCSA) forward and backward line walking and balancing on one foot (McCarthy, 1972). Higher scores on all tests represent more optimal functioning. An additional outcome variable was generated examining the percentage of neuropsychological tests that children completed and scored at the normal range or higher for standardized scores. Higher summary scores represented more consistent performance within normal range across neuropsychological tests and allowed us to examine children who scored consistently above average.

Neurodevelopmental assessments were conducted by two local bilingual Spanish/Mam (an indigenous Mayan language) field workers extensively trained by one of us (LD-C) and tested for reliability. Administration scripts and materials were translated and back-translated from English to Spanish to verify accuracy of translations before they were orally delivered in Mam ($n = 37$) or Spanish ($n = 2$). LD-C and CR reviewed videos taken of the assessments (using back-translations provided orally by a 3rd party Mam- and Spanish-speaking staff member) to ensure administration quality. The Bender Gestalt Copy, Immediate Recall, and Delayed Recall Figures test scores were also verified by a second reviewer.

2.4. Maternal interviews

Mothers were interviewed using a structured questionnaire by bilingual staff in RESPIRE when study children were approximately one-year-old and for the present study when they were 6- to 7-years-old. Questionnaires at year one ascertained demographic and health information including age when infant stopped breastfeeding, home assets, maternal education, and environmental tobacco smoke (ETS) exposure during the first year of life; at year 6–7, questionnaires assessed maternal and child chemical exposure histories (occupational, dietary, and prenatal care and drug use), HOME environment stimulation scores and child education.

2.5. Statistical analyses

Personal 48-h CO exposures levels for study mothers during their 2nd and 3rd trimesters of pregnancy and for children during their first 9 months of life, as well as child exhaled breath CO at the time of their interviews, were examined in relation to child neuropsychological test scores at age 6–7 years in simple (unadjusted) and adjusted least squares regression models for all neurodevelopmental outcomes except for McCarthy Leg coordination abbreviated test, where we used logistic regression. CO exposures (ppm) were $\log_{10}$-transformed to achieve normal distribution (see Fig. A in Supplementary materials). Neuropsychological scores were examined as raw and age-standardized scores. Crude raw scores were analyzed in all cases except PSI, where standard age-adjusted scores were used (given for every 4 months age group representative of US child scores). We used the raw scores adjusted for age in the multivariate regressions because no standardized scores existed for rural Guatemalan children. Coding, Processing Speed Index, and Immediate Recall Figures test scores were $\log_{10}$-transformed in order to meet the assumption of normal distribution for linear regression. Summary scores were regressed against CO exposure measures from all time points. Least square regressions were performed on a subset of the study population for which 2nd trimester natural log-transformed (unadjusted) 48-h CO exposure measures were available.

Several covariates were selected *a priori* based on the literature as related to child neurodevelopment (shown in Table 2). Child sex (categorical) and age (continuous) were included in all final models. Child vision scores were ‘‘poor’’ or ‘‘not poor’’ (poor = ‘‘Tumbling E’’ eye chart scores worse than 20/40). Birth weights were taken within 48-h of birth (Thompson, 2008) and household ownership of a bicycle, television, and radio were summed to generate an ‘asset index’ (0–3 out of 3; one point for each item). HOME environment stimulation raw scores, assessed at maternal interviews were out of 22, measured using an abbreviated, culturally adapted version of the HOME (Home Observation for Measurement of the Environment) questionnaire used in UNICEF’s mid-decade surveys (MICS) for children under 5 and used in previous air pollution and neurodevelopment studies (Perera et al., 2006, 2009). Influential covariates that changed the significance or direction of the association between CO exposure and child’s neuropsychological performance were identified in forward univariate and backward stepwise regressions, and retained if they most contributed to the best fit model or were used in previous studies. For all regression analyses, child age and sex interactions were tested using a two-tailed alpha p-value cut-off of <0.10, and we checked that model assumptions were met.

Sensitivity analyses were performed to ensure robustness of regression results (see Fig. 1 and Supplemental Table B for comparison of β-coefficients among significant associations). For example, in subsequent analyses we excluded potentially influential outlier points (scores beyond 2 standard deviations from the mean). In other sensitivity analyses, we excluded participants if their maternal 3rd trimester CO exposure was >10 ppm or if the children’s test scores were flagged due to possible alternate explanations for their poor performance (unrelated to CO exposure) such as currently using medications ($n = 2$), prior head trauma resulting in loss of consciousness ($n = 1$), prior convulsion, unwell at the time of assessment ($n = 3$), umbilical cord surgery at birth ($n = 1$), or not in school yet ($n = 1$). We also excluded in sensitivity analyses child scores where there were minor deviation from test administration protocol. Statistical analysis was conducted using STATA/IC 11 (StataCorp. LP, College Station, TX).

Table 2
Sociodemographic characteristics of mothers and children enrolled in study.

	All children ($n = 39$)
Maternal characteristics	
Age at one year visit, mean ± SD, years	25.7 ± 5.8
Education, n (%)	
Completed primary school	28 (72)
<Primary school completed	11 (28)
HOME environment stimulation abbreviated score, mean ± SD[a]	13.4 ± 2.4
Child characteristics	
Sex, n (%)	
Male	20 (51)
Female	19 (49)
Age at assessment, mean ± SD, years	6.7 ± 0.25
Birth weight, mean ± SD, kg	2.75 ± 0.42
Age stopped breastfeeding, mean ± SD, months	20 ± 4
Household ETS exposure in first year of life, n (%)	
Yes	13 (33)
No	26 (67)
Weight, mean ± SD, kg	17.7 ± 1.6
Height, mean ± SD, cm	104.7 ± 4.2
Body Mass Index (BMI), mean ± SD, kg/m^2	16.1 ± 0.9
WHO standard height-for-age, mean ± SD, percentile[b]	1.2 ± 3.1
Visual acuity, n (%)	
Poor vision (20/60, 20/80, or 20/100)	9 (23)
Good vision (20/40 or 20/20)	30 (77)
Household characteristics	
Altitude, mean ± SD, *m*	2640 ± 156
Assets, n (%)	
Electricity in the house	
Yes	28 (72)
No	11 (28)
Cattle	
Yes	18 (46)
No	21 (54)
Bicycle	
Yes	12 (31)
No	27 (69)
Radio	
Yes	31 (79)
No	8 (21)
TV	
Yes	5 (13)
No	34 (87)
Asset index, n (%)[c]	
0	6 (15)
1	22 (57)
2	7 (18)
3	4 (10)
Chimney stove installed in household before infant was 1.5-years-old, n (%)[d]	
Yes	8 (21)
No	31 (79)

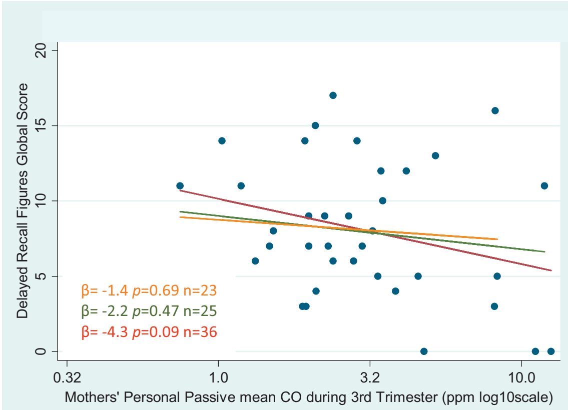
ETS, environmental tobacco smoke from smokers in household.
[a] Mother-reported during maternal interview 2 days prior to child cognitive interview.
[b] World Health Organization age and sex-specific z-score global standards (WHO, 2007).
[c] Asset index: sum of binary indicators for having a bicycle, radio, and television; was published previously with this population (McCracken et al., 2007) and had the best distribution and consistent contribution to models out of possible asset indices combinations from the dataset (scores range from 0 to 3).
[d] Chimney stoves were installed in all RESPIRE study households by the time of the study child reached 1.5-years-old.

3. Results

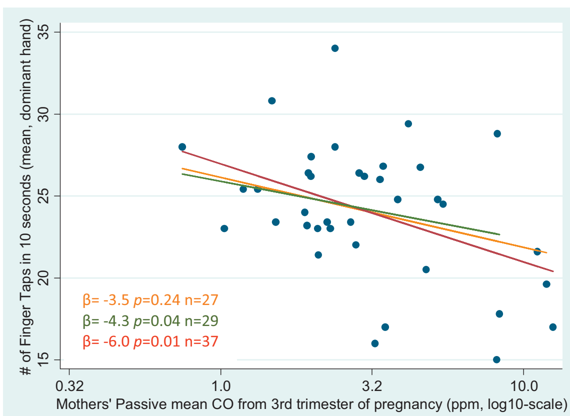
3.1. Population characteristics

Population characteristics of the 39 mother–child dyads and their households are shown in Table 2. A total of 19 girls and 20 boys aged 6.2–7.4 years participated in this study. No mothers had

(A) Delayed Recall Figures and mothers' 3rd trimester personal passive 48-hour CO exposure



(B) Finger Tapping Test and mothers' 3rd trimester personal passive 48-hour CO exposure



Key: Orange = excluding flagged and maternal CO >10ppm; **Green**= excluding flagged; **Red**=full sample

Fig. 1. Shown are mothers' CO exposures from the 3rd trimester and child scores on the (A) Bender Gestalt-II's Delayed Recall Figures and (B) the Reitan-Indiana Finger Tapping Test. The 3 fitted lines demonstrate the robustness of the simple linear regression results, using 3 different sample sizes. When influential participants (with alternate explanations for performance or maternal CO during the third trimester >10 ppm) were excluded from linear regression analyses, as indicated by the orange and green lines the inverse direction of association persisted.

completed higher than primary school education, all children and mothers spoke Mam as their first language, and some also spoke Spanish. There were no significant differences in demographic characteristics between those households who were provided a chimney stove before compared to after the study child's birth. A smaller percentage of our study participants had radios (79%) and TVs (13%) in their homes compared to the original RESPIRE cohort of participants (93% and 25% had radios and TVs, respectively; $n = 120$) (McCracken et al., 2007), but were otherwise similar in household assets.

3.2. CO exposures

Table 3 shows personal CO exposure levels. Average maternal 48-h mean passive CO concentrations during the 3rd trimester was 3.8 ± 3.0 ppm (range = 0.6–12.5 ppm; $n = 39$). For the subset of pregnant women ($n = 20$) with 2nd trimester CO measured, the mean personal passive 48-h CO concentration was 3.0 ± 2.5 ppm with a range = 0.8–11.6 ppm (data not shown). During the first 9 months of life, infants' mean personal passive 48-h CO concentration was 2.2 ± 2.0 ppm with a range of 0.3–9.8 ppm ($n = 39$). Log-transformed maternal 3rd trimester and infant's personal passive 48-h CO were associated but not significantly (correlation coefficient = 0.20; $p = 0.12$) (see Supplemental Fig. B). Exhaled CO (eCO) at the time of child testing averaged 2.3 ± 0.9 ppm and all were $\leq$5 ppm. Seventeen children (44%) reported bathing in the temazcal the day before testing, although these children did not have significantly higher eCO than children who had not used the temazcal the day before (data not shown).

3.3. Neuropsychological scores

Table 1 presents the mean and standard deviation for the raw and standardized scores. Child scores had a wide range with normal distributions for most tests. On average, compared to raw and age-standardized mean scores from developed countries (Adams and Sheslow, 1995; Brannigan and Decker, 2003), a US-representative sample of children according to census sampling methods, Guatemalan children performed non-significantly better on the Copy Figures and Pegboard tests (two-tailed $p \leq 0.10$). On the other hand, they performed significantly worse on the PSI, Perception Matrices, Digit Span Forward and Total, Motor Connect the Dots, Finger Tapping Test and Immediate Recall Figures tests.

Indications of inverse linear relationships (negative β-coefficients) were observed between scores on 9 out of 11 neuropsychological tests, as well as the summary score for overall neuropsychological performance, and maternal 3rd trimester log-CO concentrations, with 4 significant inverse associations: Bender-Gestalt Copy, Immediate Recall, and Delayed Recall Figures, and Finger Tapping Test (see Table 4 and Fig. 2). We found that for every 10-fold increase in maternal CO, Copy Figures scores decreased 4.2 points ($p < 0.10$), Delayed Recall Figures scores decreased 4.3 points ($p = 0.05$), and the average number of finger taps in 10 s decreased by 6 ($p < 0.01$). Log-transformed Immediate Recall scores also decreased 0.3 points, meaning that every 10% increase in maternal CO exposure during the 3rd trimester was significantly associated with a 3% decrease in Immediate Recall scores ($p < 0.05$). After adjustment for child age, sex, visual acuity, and asset index (see Table 4) the significant inverse associations for these 4 tests persisted. The summary scores became significantly inversely associated with maternal 3rd trimester log-CO ($\beta = -0.23$, $p = 0.04$, $n = 39$, $R^2W = 0.30$) when adjusted for these 4 covariates. No significant associations were detected between children's eCO or temazcal use the day before and neuropsychological performance. We used one-tailed cut-off p-values for all of the regression analyses including adjusted models.

Table 3
Mother and infant CO exposures measured by 48-h personal passive diffusion tubes and child exhaled breath CO measures at neuropsychological assessments ($n = 39$).

Exposure	Mean ± SD (ppm)	Median (ppm)	Range (ppm) Minimum–maximum
Mother's CO exposure during the 3rd trimester	3.83 ± 3.03	2.80	0.62–12.52
Infant's CO exposure during 0–9 months of age	2.20 ± 1.98	1.63	0.26–9.77
Child's exhaled breath CO at assessment	2.26 ± 0.86	2.0	1.0–5.0

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Table 4
Linear regression results of mother and infant $\log_{10}$-transformed 48-h personal passive CO exposures and child cognitive performance.

Cognitive test	No.	Mother $\log_{10}$CO 3rd trimester β (95% CI)	Adjusted model[a] 3rd trimester β (95% CI)	Infant $\log_{10}$CO 0–9 months β (95% CI)
Neuropsychological performance summary score[b]				
% of scores ≥normal range	39	−0.2 (−0.5, −0.0)	−0.2 (−0.4, −0.0)**	−0.03 (−0.2, 0.2)
Processing speed				
WISC-IV Spanish				
Coding[c]	35	−0.1 (−0.4, 0.1)	NS	−0.02 (−0.2, 0.2)
Symbol search	34	−0.6 (−5.4, 4.2)	NS	−2.6 (−6.0, 0.9)*
Processing speed index [c]	34	−0.01 (−0.1, 0.1)	NS	−0.02 (−0.08, 0.02)
Visuo-spatial integration				
Bender Gestalt-II				
Copy figures	39	−4.2 (−9.8, 1.4)*	−4.4 (−9.5, 0.7)**	−2.2 (−6.8, 2.3)
Perception matrices	34	0.7 (−0.8, 2.2)	NS	0.06 (−1.2, 1.3)
Short and long-term memory				
Bender Gestalt-II				
Immediate recall figures[c]	39	−0.3 (−0.6, 0.02)**	−0.3 (−0.6, .01)**	−0.05 (−0.3, 0.2)
Delayed recall figures	36	−4.3 (−9.4, 0.8)**	−4.8 (−9.8, 0.1)**	0.5 (−3.7, 4.8)
Working memory and attention				
WISC-IV Spanish				
Digit span forward	27	0.03 (−1.6, 1.6)	NS	0.3 (−1.0, 1.5)
Digit span total score [d]	27	−0.1 (−3.0, 2.8)	NS	−0.7 (−2.9, 1.6)
Fine motor speed and coordination (dominant hand)				
Reitan-Indiana				
Finger tapping test	37	−6.0 (−10.3, −1.7)#	−5.7 (−9.7, −1.7)#	0.8 (−3.0, 4.7)
WRAVMA				
Pegboard	36	−0.8 (−6.5, 4.9)	NS	−1.5 (−6.0, 3.1)
Bender Gestalt-II				
Motor connect the dots	38	−1.1 (−4.2, 2.1)	NS	−1.5 (−4.0, 1.0)

One-tailed cut-off p-values are * ≤0.10, ** ≤0.05, # ≤0.01, ## ≤0.00; NS, non-significant.
[a] Multivariate analyses included child age (continuous), child sex (female vs. male), poor vision (yes or no), asset index (0–3; treated as continuous).
[b] When 2 scores with outlier standard residuals were included, maternal CO became significantly associated with summary scores (−0.23, p = 0.05).
[c] Coding, Processing Speed Index, and Immediate Recall Figures tests were $\log_{10}$-transformed.
[d] Only 10 children completed the reverse digit span so it was not analyzed independently (is only shown as a Digit Span Total scores, added to forward digit span scores).

In sensitivity analyses, excluding outliers did not change the inverse direction of the β-coefficients for the 4 tests significantly associated with maternal 3rd trimester CO. Excluding flagged scores (where alternate explanations for their performance existed; $n \leq 13$; as described in Section 2.5), made all 4, except the Finger Tapping Test, become non-significantly inversely associated with maternal 3rd trimester CO ($p < 0.10$, one-tailed) (see Supplemental Table B and Fig. 1). When we also excluded participants with maternal 3rd trimester CO ≥10 ppm, Finger Tapping Test scores remained significantly inversely associated with 3rd trimester CO ($p < 0.05$) (see Fig. 1).

3.4. Maternal second trimester and infant CO exposures

In the subsample of mothers with 2nd trimester CO measurements, 7 out of 11 of the neuropsychological tests had inverse non-significant trends with natural log-transformed maternal CO but only 3 were significant at $p < 0.10$ (one-tailed): coding, PSI, and Immediate Recall Figures (see Supplemental Table C). Symbol search was the only neuropsychological test significantly associated with personal passive 48-h CO among infants aged 0–9 months ($\beta = -2.6$, $p < 0.10$; one-tailed; $n = 34$), with an inverse association persisting after adjustment for child age, sex, and vision ($\beta = -3.5$, $p < 0.05$; one-tailed).

4. Discussion

We found that child scores on 4 out of 11 neuropsychological tests were significantly inversely associated with maternal 3rd trimester CO: visuo-spatial integration, short-term memory recall, long-term memory recall, and fine motor performance. Maternal 3rd trimester CO appeared to influence child neuropsychological performance more than did infant CO in the first 9 months of life, possibly due to the maternal CO's higher mean and greater range of concentrations in the study population.

Our results are consistent with previous epidemiological studies on air pollution and neurodevelopment (Edwards et al., 2010; Perera et al., 2006, 2009). Edwards et al. (2010) found that non-verbal intelligence measured on Raven's Progressive Color Matrices (RPCM) was inversely associated with maternal 3rd trimester personal 48-h PAH exposures among a prospective cohort of 214 children in Krakow, Poland assessed from 2001 to 2006. Rural Guatemalan mothers' mean personal CO levels were higher and their children more malnourished. Unlike the Edwards et al. (2010) and Perera et al. (2006, 2009) studies, we did not assess other environmental exposures, e.g., lead and pesticides which may confound the associations, nor did we measure maternal intelligence. Our study found that maternal log-CO was associated but not significantly with infant log-CO ($p = 0.06$; $\beta = 0.20$; one-tailed). This agrees with previously published RESPIRE study results; CO measures used were representative of chronic CO exposure (McCracken et al., 2009).

This appears to be the first birth cohort study to examine the neurodevelopmental toxicity of woodsmoke. Strengths are its cohort design and multiple longitudinal measures of CO exposures, health status, household assets, and environmental stimulation. As an indicator of woodsmoke exposures, we used validated personal mother and child CO exposures (child eCO and breathing zone passive CO personal exposures taken at multiple time points) which accounted for duration of exposure (48 h). In addition, we used neuropsychological instruments with high reliability, validity and construct clarity among standard US populations that had also been used previously among rural Latin American and Spanish-speaking study populations.

Study limitations include children's performance being below standard averages on a number of neuropsychological tests and

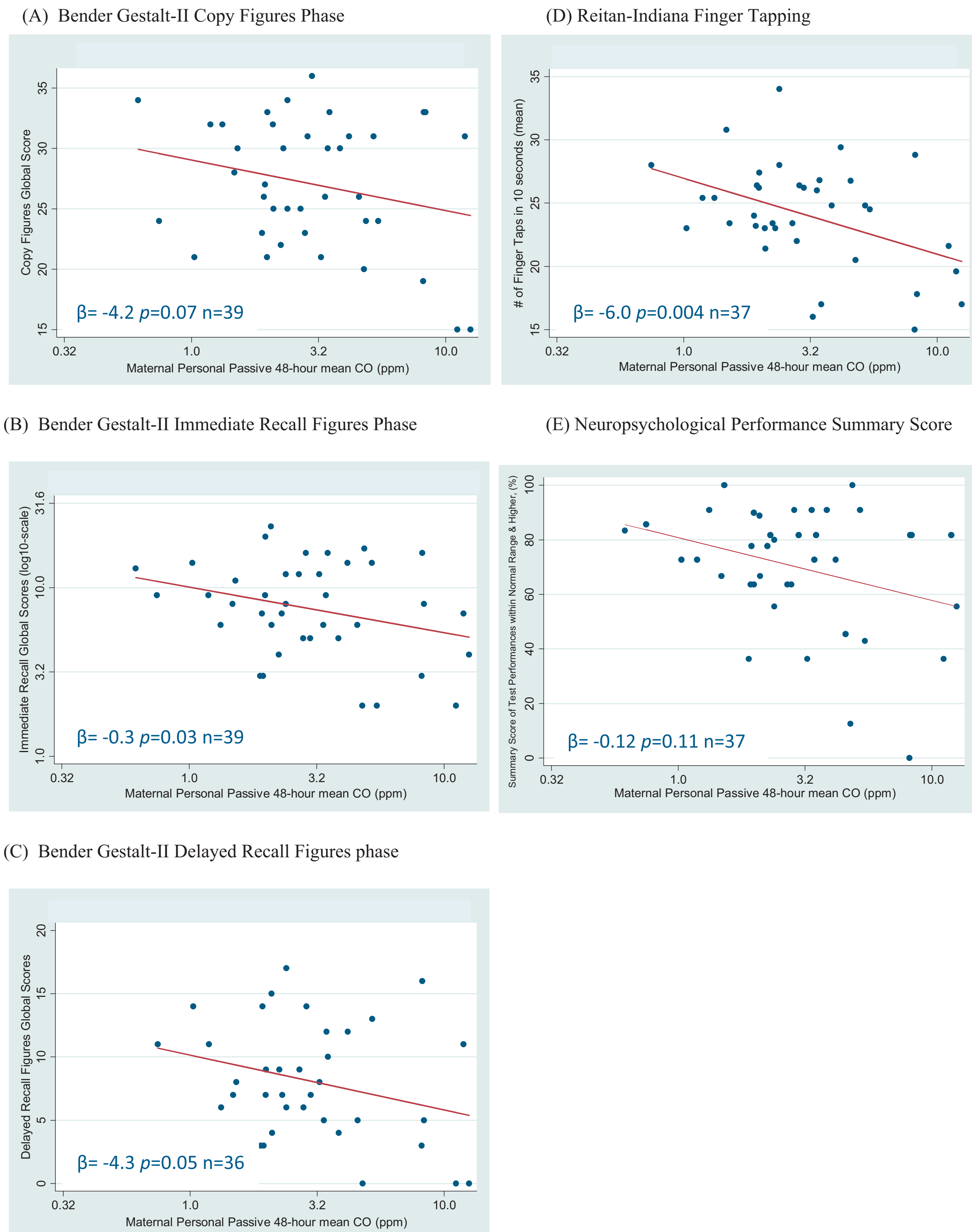

Fig. 2. Results of linear regressions are presented between child neuropsychological performance scores and mothers' 3rd trimester CO exposures. Note that Neuropsychological Performance Summary Scores became significantly inversely associated with maternal 3rd trimester personal passive CO when 2 participants with outlier standard residuals were excluded from simple linear regression analyses.

future studies would benefit from using larger sample sizes to increase results' robustness. Tests were still considered suitable despite low average scores because children in the rural Guatemalan population were able to complete them without difficulty, as indicated by some children who scored in the gifted (above average) range for standard US populations on coding, immediate recall, and pegboard tests. Furthermore, 3 tests were inter-dependent and their scores correlated (the 3 Bender

Gestalt-II Figures drawing tests), so children who did not copy correctly in the first phase subsequently also had poorer recall. Children's performance on the 3 Bender Gestalt Figures drawing tests was probably not due to fine motor ability discrepancies though because these were not related; one child with a low Finger Tapping Test score, scored above average on the Immediate Recall Figures. Short term peak CO concentrations (which of course are much higher during cooking periods than other times e.g., 10-min kitchen CO levels in rural Guatemalan kitchens measured by environmental sampling (not personal) may range up to 150 ppm (Naeher et al., 2007)) were not captured by the passive 48-h monitoring method employed, but this is a common methodological problem for studies measuring and standard setting for chronic exposures. Despite these shortcomings, strength lies in our uniquely highly ethnically and age homogenous birth cohort. Children living in this area of Guatemala may be particularly at risk for neurodevelopmental impairments from woodsmoke because at this elevation (~2600 m) they are already subjected to stress from lower oxygen levels. For example, in RESPIRE mean blood oxygen saturation of healthy infants in the area was found to be 93.2% ± 3.0% (Bruce et al., 2007). Dietary and drinking water hygiene, maternal mental health, and respiratory disease treatment programs are also priorities in rural Guatemala, with chronic coughs present among half and infant diarrhea (defined as persistent diarrhea for more than 14 days during the first two years of life) experienced among all of the study children. Chronic coughs and respiratory diseases from exposure to woodsmoke may decrease children's school attendance, but coughing at the time of the child interviews did not appear to influence child performance on neuropsychological tests.

5. Conclusions

Although a larger sample size might provide more definitive results, we have identified a wide breadth of specific neuropsychological outcomes that are likely impaired by in utero exposure to woodsmoke at chronic CO levels <13 ppm. In an ethnically and age homogenous birth cohort in a developing country context, child performance on fine motor, visuo-spatial perception, integration, and visual-motor memory tasks, as well a summary performance score were significantly inversely associated with 3rd trimester chronic CO exposure. Chronic personal CO levels measured in Guatemala are comparable to levels measured in some US household kitchens, where smoking and close proximity to high density traffic elevates one's likelihood of exposure. Although not by itself conclusive, this study also suggests that the new WHO 24-h Air Quality Guideline for chronic CO exposure of 7 mg/m^3 (~6 ppm) (WHO, 2010) may not be sufficiently protective for children in utero. With an approximate 50% reduction in personal CO exposures among chimney stove-using mothers compared to open woodfire-using mothers (Smith et al., 2010), future studies may examine whether the chimney stove provides protection from neuropsychological impacts among the children of pregnant women in this population. New, advanced combustion woodstoves that are now becoming available may be capable of even larger reductions. Although there are other pollutants in woodsmoke that may contribute to the neurodevelopmental effects found in this study, our results call for additional studies to explore the effects of chronic woodsmoke and CO exposures among pregnant women on their children's neurodevelopment.

Conflict of interest declaration

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neuro.2011.09.004.

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