Implications of combined exposure to household air pollution and HIV on neurodevelopment in Kenyan children

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A thesis

submitted in partial fulfillment of the

requirements for the degree of

Master of Science

University of Washington

2017

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Program Authorized to Offer Degree:

Public Health - Epidemiology

University of Washington

Abstract

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Objectives: Exposure to air pollution is associated with numerous impacts on health, including neurodevelopmental function. The purpose of this study was to estimate the magnitude of air pollution exposure based on environmental carbon monoxide (CO) measures and assessment of 1-hydroxypyrene (1-OHP), a metabolite of polycyclic aromatic hydrocarbons (PAHs), among school-aged HIV-infected and uninfected children in peri urban Kenya and to examine the impacts of these exposures on neurodevelopment.

Methods: We conducted a cross sectional study of 49 HIV uninfected unexposed (HUU) and 45 HIV infected children ages 5-9 and their caregivers in Nairobi, Kenya. We used a battery of neurodevelopmental tests to assess function in 9 domains. Caregiver 24-h personal CO exposure was a proxy for child exposure, and measured child urinary 1-OHP.

Results: Mean 24-h CO exposure was 8.15 ±13.46 ppm and mean urine 1-OHP was 0.81±0.60 µmol/mol creatinine. Overall, 39.4% of children had mean CO exposure >WHO recommended levels. Among HIV-infected children, CO value was associated with lower function in 2 of the 9 neurodevelopmental domains (nonverbal intelligence (β= -0.28, p=0.08) and executive function (β= -0.36, p=0.10)), and better learning function in HUU children (β= 0.29, p=0.09). Among HUU children, a mean CO value exceeding 6.11 ppm was associated with better motor function

(p=0.09). Among HIV-infected children, 1-OHP value was associated with a lower attention score (β=-0.84, p=0.03) and having a 1-OHP value exceeding the median was significantly associated with lower function in the cognitive ability (p=0.01), short term memory (p=0.06), learning ability (p=0.01), delayed memory (p<0.01), and attention domains (p<0.01).

Conclusions: Our results suggest that early life exposure to air pollutants such as PAHs may compromise healthy neurodevelopment, and that HIV-infection may pose added risk. The high prevalence of air pollution exposure in this population highlights the need for effective interventions to reduce exposures.

Introduction:

Worldwide, there are 1.8 million HIV-infected children under 15 years of age.[1] Sub-Saharan Africa (SSA) bears a disproportionate burden of perinatal HIV, with only 12% of the world's population, but 90% of the world's HIV-infected children.[1] In this region, air pollution is also a major public health concern. Outdoor air quality continues to deteriorate with industrialization and population growth.[2,3] Additionally, 82% of households in SSA rely on solid-fuels such as wood, dung, and charcoal for their cooking.[4] Burning of these fuels contributes to household and community air pollution.[4] In SSA, it is estimated that 646 million people are exposed to household air pollution.[5]

Air pollution is a mixture of natural and man-made substances, including particulate matter (PM), carbon monoxide, polycyclic aromatic hydrocarbons (PAHs), and sulfur dioxide.[6] Globally, major sources of air pollution include vehicle exhaust, tobacco smoke, and burning solid fuels such as wood, coal, or dung for heat or cooking.[7,8] Air pollution contributes to numerous acute and chronic illnesses, including pneumonia and cardiovascular events.[2,9]

In addition to cardiopulmonary health impacts, air contaminants may contribute to central nervous system (CNS) developmental toxicity and manifest in neurodevelopmental delays and behavioral problems.[10] Particulate matter in air pollution may activate microglia in the brain, and initiate inflammatory processes of the CNS associated with neurological diseases.[10–12] White matter changes in the brain associated with air pollution may impact brain development and function.[10] A pilot study showed that children in Mexico City were more likely to have white matter lesions than children living in a less-polluted environment.[13]

Carbon monoxide (CO), a product of incomplete combustion of carbon-containing compounds, is one of the main gaseous components of air pollution.[8] Once inhaled, CO binds with hemoglobin, forming carboxyhemoglobin.[14] Since the affinity of hemoglobin is nearly 250 times that of oxygen, this process results in impaired oxygen delivery to tissues.[14] Both animal and human studies have found a relationship between prenatal CO exposure and impairment in neurological function.[15,16] In a study of Guatemalan children aged 6-7 years, 3rd trimester maternal CO exposure, a proxy for wood smoke exposure, was inversely associated with neurocognitive test scores for visuospatial, short and long term memory, and fine motor skills.[17]

There are several proposed mechanisms by which CO in ambient air pollution could result in neurodevelopmental deficiencies. In utero exposure to CO may interrupt sensitive oxygen-dependent neurocognitive processes such as myelination, proliferation, differentiation, and synaptogenesis.[18] Postnatal CO exposure may also adversely impact neurodevelopmental processes. An animal study conducted in 10-day old mice pups found that 3 hours of exposure to low concentrations of CO resulted in impaired memory and learning, and decreased socialization.[19] Carbon monoxide impairs the release of cytochrome c by mitochondria, a vital component in the initiation of apopotosis.[18,19] Thus, CO may interfere with developmental neuroapopotosis, the normal process of cell death that occurs in the late prenatal and early postnatal periods.[18,19]

Polycyclic aromatic hydrocarbons (PAHs) are a group of several hundred chemically related compounds, present in air pollution.[5] Children with higher prenatal PAH exposure had impaired cognitive performance during childhood in recent studies. In a Polish cohort, prenatal airborne PAH exposure was associated with impaired nonverbal reasoning ability at age 5.[20] In a New York City cohort, high prenatal PAH exposure was associated with developmental delay at age 3 and reduced IQ at age 5.[21,22] The exact mechanisms through which PAHs may affect the developing brain are not known and most mechanistic evidence is based on animal studies. Proposed pathways include: endocrine disruption, binding of PAHs to placental growth factors, and oxidative stress.[23–25]

HIV can also cause a broad spectrum of cognitive impairment and neurologic disease.[26–28] These include progressive HIV-encephalopathy (PHE) and behavioral problems such as

ADHD.[28] While the introduction of antiretroviral therapy (ART) has significantly reduced the incidence of severe neurologic impairments such as PHE in HIV-infected children, several studies show that, even with ART, HIV-infected children have lower neurocognitive functioning compared to their uninfected peers.[27,28] Results from a meta-analysis showed that the domains most affected by HIV are working memory and executive function.[26]

Soon after infection, the HIV virus invades the CNS, resulting in the activation of microglia.[29] This inflammatory response initiates a series of inflammatory and neurotoxic events that cause neuronal damage and apoptosis.[29] Damage to white matter resulting from HIV infection may impact cognitive functioning. A study of 75 HIV-infected children in Cape Town, South Africa found infected children have significantly more white matter micro-structural damage, relative to healthy controls.[30] Because HIV and air pollution may impact neurodevelopment through shared pathways, efforts should be made to investigate whether the relationship between air pollution and cognition differs in HIV-infected children. To date, no studies have investigated the impacts of air pollution on neurocognition in an HIV-infected pediatric population.

We measured the magnitude of carbon monoxide and polycyclic aromatic hydrocarbon exposure among school-aged HIV-infected and uninfected children in peri urban Kenya, and examined the relationship between these exposures and neurodevelopment, and whether this relationship differs by child HIV-infection status.

Methods

Participants and recruitment

This study includes early-treated HIV-infected children and HIV unexposed uninfected (HUU) children and their caregivers. Participants were recruited for the Nairobi, Kenya-based Health Impacts of Household Air Pollution on Women's Health and Child Survival and the Impact of HIV on Neurodevelopment in Kenya (INK) studies. HIV-infected children were recruited from 2007-2009 from the ongoing Optimizing HIV-1 Therapy Extension (OPHX) Study, a randomized clinical trial with a two-year pre-randomization period and regular data collection (NCT00428116). As described previously, recruitment criteria included: confirmed HIV-1 DNA positive, no previous ART with the exception of prophylaxis for prevention of mother to child transmission (PMTCT), and age <4.5 months.[31] HIV infected children in this cohort generally initiated ART within 2 weeks of enrollment.

From 2011-2013, HUU children were identified for the INK study, through HIV-negative mothers in Nairobi City Council PMTCT clinics. Eligibility criteria included: age <4.5 months, and biological mother and infant confirmed HIV-1 negative status. Participation required regular follow-up visits, similar to the OPHX visit schedule. Recruitment and cohort information is described previously.[32]

Home visits

To assess household air pollution (HAP), two home visits were conducted by study staff, 24-hours apart, between December 2014 and December 2016. During the first study visit, staff conducted household surveys of cook-stove location and provided and installed HAP monitors. At home visits, study staff collected demographic information and information about typical cooking behaviors and fuel use using standardized questionnaires. Twenty-four hours later, staff returned to collect HAP monitors, and administer questionnaires regarding caregiver adherence to wearing HAP monitors, and behavior related to air pollution exposure over the 24-hour monitoring period. Additionally, study staff collected urine samples from caregivers and children for measurement of biomarkers of HAP exposure.

CO exposure

Caregiver personal CO exposure was measured during a 24-hour monitoring period using Lascar electronic continuous CO monitors. Caregivers were instructed to wear the monitors during waking hours and were asked to perform typical daily household activities. Caregiver CO data was available for 33 HIV-infected children and 38 HUU children. During the same 24-hour monitoring period, household-level CO exposure was measured using a Lascar electronic continuous CO monitors hung in home cooking areas. Household CO data were available for 38 HIV-infected children and 41 HUU children. 24-hour household and caregiver personal exposure to CO was estimated by the mean CO value (in ppm).

Collection, measurement and analysis of urinary 1-OHP

PAH exposure was estimated by determination of a key PAH metabolite, 1-hydroxypyrene (1-OHP). Although PAH exposure is a mixture of compounds, pyrene is typically found in the mixture, making its metabolite, 1-OHP, an appropriate proxy of PAH exposure.[5] Urinary metabolites have been found to be an acceptable biomarker for airborne PAH exposure.[33] Spot urine samples were collected for children and caregivers by study staff immediately following the 24-hour HAP monitoring period and stored at -70 °C on the same day as sample collection. Urine specimens were shipped to the University of Washington on dry ice. Urine samples (10mL) were pH-adjusted with a 5mL buffer containing glucuronidase enzyme and ascorbic acid. Specimens were spiked with deuterated 1-hydroxypyrene (d9-OHP) as an internal standard. Samples were incubated overnight in a 37 °C bath. The deconjugated metabolites were extracted using solid phase extraction (SPE) columns (Supelco, Supelclean 6 mL SPE tubes). PAH metabolites eluted from the SPE columns using 5 mL of an 80:20 methanol: water mixture. Once eluted, the extracts were placed in a TurboVap to be evaporated. The samples were evaporated to approximately 2 mL and then ~4.5 mL of acetonitrile was added to aid in the evaporation process. Once the samples were brought to approximately 1 mL, 20 µL of dimethyl sulfoxide was added and samples were then vortexed for 1 minute. The samples were then brought to near dryness in the TurboVap. Metabolites were reconstituted in 200 µL of 60:40 water: methanol mixture and lastly, the samples were filtered (Fisherbrand, 13mm syringe filter) into high-performance liquid chromatography vials for analysis. Quantification limits were determined by calculating the average of blanks plus 3 standard deviations and by the Lowest Calibration Standard (ng/ml). The limit of detection was set at the higher value between the blanks or the lowest standard.[34] Urinary creatinine analysis was conducted in the same laboratory using the same urine samples. 1-OHP was measured in ng/mL, and values were creatinine-adjusted for dilution and expressed in µmol/mol of creatinine. The following formula was used for adjustment:

$$\frac{1-OHP\ in\ ng/mL}{creatinine\ clearance\ measured\ in\ \mu mol/L} \times \frac{10^6}{218.3 g/mol}$$

1-OHP measured in child urine was available for 32 HIV-infected children and 43 HUU children. 1-OHP measured in caregiver urine was available for 1 HIV-infected child and 17 HUU children.

Neurodevelopmental assessments

A battery of 4 neurodevelopmental assessments were performed by trained study staff. Scripts for each assessment were translated from English to Kiswahili and back-translated to

ensure accuracy. Tests were administered in the preferred language of the child, either Kiswahili or English. The Kaufman Assessment Battery for Children, Second Edition (KABC-II) was used assess the cognitive ability, short term memory, visual-spatial ability, learning, non-verbal intelligence, and delayed memory. [35]The KABC has been used in Senegal[36] and Zaire,[37] and in HIV-infected Ugandan children,[38] and had good construct validity when administered to Ugandan children aged 7-16 years. The Testing of Variables of Attention (TOVA) is a computer-based tool that measures sustained and selective attention based on visual stimuli. We used the TOVA to measure the attention domain. The TOVA was sensitive for long-term neurocognitive deficits in children with a history of cerebral malaria,[39] and has been used in Ugandan HIV-infected children.[38] The Bruininks-Oseretsky Test of Motor Proficiency (BOTMP) is a comprehensive battery of game-like tasks, used to assess fine motor precision, fine motor integration, manual dexterity, bilateral coordination, balance, running speed and agility, upper-limb coordination, and strength. It has been previously used in HIV-infected populations.[38,40] The Behavior Rating Inventory of Executive Function (BRIEF) was used to measure the executive function domain. Unlike the other assessments used for this analysis, it is based on a questionnaire filled out by the child's primary caregiver as a proxy.

The median length of time between the home visit and neurocognitive assessment was 4.75 months (IQR: 1.00, 7.66).

Statistical Analysis:

Descriptive statistics for study population characteristics and neurodevelopmental outcomes(z-score) were calculated, stratified by child HIV infection status. Air pollution variables were examined as both continuous and dichotomous variables. For continuous values of HAP variables, we calculated the arithmetic mean, standard deviation, median, interquartile range, minimum, max, and geometric mean, stratified by child HIV status. We compared geometric means for HIV infected and uninfected children using a 2-sample t-test and compared medians with a non-parametric equality of medians test. The WHO recommends that indoor CO levels do not exceed concentrations of 6.11 ppm (7mg/m^3) over a 24-hour period.[5] Thus we considered participants with caregiver 24-hour or kitchen CO mean levels >6.11ppm to have "high" CO exposure, and those with values ≤6.11 were categorized as "low exposure." Measurements of 1-OHP (µmol/mol creatinine), were dichotomized using the overall median (0.68 µmol/mol creatinine for the cohort. For all dichotomous variables, including child urine 1-OHP, caregiver 1-OHP, caregiver CO, and household-level CO, we calculated the proportion of participants with high versus low levels, stratified by HIV infection status. Chi-square tests were used to determine whether the proportion children with high exposures differed by child HIV infection status.

We used linear regression to evaluate the relationship between potential cofactors for HAP exposure and $\log_{10}$ transformed child urine 1-OHP and $\log_{10}$ transformed caregiver CO. Two-sample t-tests and one-way ANOVA were used to compare geometric mean CO and 1-OHP exposure levels for categorical cofactors with 2 and 3+ levels, respectively. Additionally, we evaluated potential cofactors for high versus low 1-OHP and caregiver CO exposures using two-sample t-tests for continuous cofactors and chi-square tests for categorical cofactors. All analyses were stratified by child HIV-infection status.

We estimated the association between $\log_{10}$ transformed HAP (caregiver CO and child urine 1-OHP) exposure and neurodevelopmental function using univariate and multivariate

linear regression models. Cofactors included in multivariate models were selected *a priori* and included child age and household monthly income. We ran separate models for HIV-infected and HUU children. For dichotomous HAP variables, we calculated mean z-score for neurocognitive assessments by high/low caregiver CO and child urine 1-OHP exposure, then compared the means with a 2-sample t-test.

Finally, using Pearson correlation coefficients, we evaluated the correlation between all natural log-transformed continuous HAP variables including caregiver CO, household CO, child urine 1-OHP, and caregiver urine 1-OHP.

All analyses were performed using Stata 14.0.[41]

Results

Study Population

Study population characteristics for the children and their caregivers are shown in table 1. Our sample included 49 HUU children and 45 HIV infected children with a mean age of 6.6 years at the time of neurodevelopmental testing. Sex distribution varied slightly by HIV infection status (64% HIV infected children were male vs. 45% of HUU children). The majority of primary caregivers were the child's biological mother (93% among HIV-infected, 98% among. Caregivers of children with HIV were more likely to be unemployed, compared to those of HUU children. Household monthly rent was higher among caregivers of HIV infected children (mean 4105 Kenyan Schillings), compared to caregivers of HUU children (2247 Kenyan schillings). The mean age of caregivers was 32.1 years old and caregivers had an average of 9.2 years of education. With the exception of executive function and learning ability, neurodevelopmental function was lower among HIV-infected children in all domains, compared to HUU children.

Household Characteristics

Paraffin (kerosene) was the most common primary type of cooking fuel for both HUU children (76.5%) and HIV-infected children (45.5%), with a significant proportion of the latter households using propane as a primary fuel (34.1%). A larger proportion of households of HUU children reported cooking inside the living area (93.8%), compared to households of HIV-infected children (62.2%). The proportion of households with a smoker was similar between households of HIV-infected children (15.6%) and HUU children (12.8%), as was the proportion reporting garbage being burned by the home (26.7% among HIV-infected, 33.3% among HUU.)

Magnitude of Household Air Pollution

Arithmetic mean, geometric mean, median, IQR, and range for caregiver personal CO, household CO, and child urine 1-OHP can be found in table 2. The arithmetic mean 24-hr caregiver CO levels was 8.15 ppm. Caregiver CO values were higher among HIV-infected children compared to HIV uninfected children (p=0.04), however the geometric mean (p=0.18) and the median (p=0.19) did not differ significantly by HIV infection status. Caregiver 24-h mean CO levels exceed the WHO recommended 6.11ppm value among 39.4% of children and 48.5% and 31.6% of HIV-infected and uninfected children, respectively (p=0.15). The arithmetic mean of 24-hr household CO was 11.37 ppm. Household CO values were higher among HIV-infected children compared to HUU children, but this difference was not statistically significant (p=0.38). 41.3% of all participants and 45.7% and 37.5% of HIV-infected and HUU children, had high household CO levels(p=0.52).

The arithmetic means for 24-hr child urine 1-OHP was 0.81 µmol/mol creatinine and were similar among HIV-infected children (0.89 µmol/mol creatinine) and HUU children (0.75 µmol/mol creatinine) (p=0.56). 49.3% of all children and 43.8% of HIV-infected children had urine 1-OHP values exceeding the median, compared to 53.5% of HUU children (p=0.40).

Correlations between measurements of HAP ranged from weak to strongly correlated (Table 5). Correlation was highest between household and caregiver CO (r=0.70) and lowest for caregiver CO and child 1-OHP (r=0.13).

Cofactors for HAP exposure

HIV Infected Children

Among HIV-infected children, CO exposure differed by employment status, with the highest mean exposure among those in the "other" employment category (p=0.10). The most commonly reported occupation in this category was "business women." Geometric mean child urine 1-OHP values differed by employment status, with the highest means in the "unemployed" and "other" categories (p=0.10). HIV-infected children with high urine 1-OHP had caregivers with fewer years of education (p=0.03) and lower household monthly rent (p=0.05). No other measured cofactors were significantly associated with 1-OHP exposure among HIV-infected children.

HUU Children

Among HUU children, having a smoker in the household was associated with a higher caregiver CO (p=0.04) and high caregiver CO (p=0.11). Among HUU children, no measured cofactors were associated with geometric mean caregiver CO levels, however those with a smoker in the home were more likely to have caregiver CO levels exceeding 6.11 ppm. Geometric mean child urine 1-OHP values were higher among children with smokers in the household (p=0.08), and a greater proportion of HUU children with smokers in the household had 1-OHP values exceeding the median (p=0.10).

Neurodevelopmental Function

HIV-Infected Children

In unadjusted analyses, caregiver 24-h CO value was modestly associated with lower non-verbal and lower non-verbal intelligence (β= -0.27, p=0.07). After adjustment for child age and household monthly rent, higher caregiver 24-h CO value was modestly associated with lower non-verbal intelligence (β= -0.28, p=0.08) and lower executive function (β= -0.36, p=0.10). Having a caregiver CO level exceeding 6.11 ppm was not associated with cognitive function in any of the 9 domains. In unadjusted analyses, child urine 1-OHP value was associated with lower cognitive ability (β= -0.42, p=0.09) and lower function in the attention domain (β= -0.82, p=0.02). However, after adjustment for child age and household monthly rent, only the association with attention remained significant (β=-0.84, p=0.03). Having a high urine 1-OHP value was significantly associated with lower function in the cognitive ability (p=0.01), short term memory (p=0.06), learning ability (p=0.01), delayed memory (p<0.01), and attention domains (p<0.01).

HUU Children

In unadjusted analyses, caregiver 24-h CO value was associated with better motor function (β=0.41, p=0.04). After adjustment for child age and household monthly income, a higher caregiver 24-h CO value was significantly associated with better performance in the learning domain (β= 0.29, p=0.09). Children with high caregiver CO levels had significantly better motor function (p=0.09), compared to children with low caregiver CO levels. In both adjusted and unadjusted analyses, Urine 1-OHP was not associated with cognitive function in any domains.

Correlations Between Measures of HAP

Correlations between measurements of HAP ranged from weak to strongly correlated (Table 5). Correlation was highest between household and caregiver CO (r=0.70) and lowest for caregiver CO and child 1-OHP (r=0.13).

Discussion

We examined the potential adverse neurodevelopmental health consequences of exposure to common air pollutants (CO, PAH) among HIV infected and uninfected children in periurban Kenya. We hypothesized that impacts would be greater among HIV infected children and, consistent with our hypotheses, we observed several statistically significant reduced neurodevelopmental test scores associated with higher 1-OHP concentrations, a marker of PAH exposure, in the HIV infected group. Higher 1-OHP was associated with poorer function in multiple domains (attention, cognitive ability, short term memory, learning ability and delayed memory). We found more modest reductions in two domains (learning, motor function) with increased CO exposure in this group of children. In contrast, among HIV uninfected children, no adverse effects were observed with increased 1-OHP concentrations nor with increased caregiver CO measures.

To our knowledge, these data are the first to assess associations between household air pollution and neurocognitive outcomes in HIV infected children. Our findings of associations between 1-OHP levels and multiple neurocognitive outcomes in HIV infected but not HUU children suggests that the combination of HIV and air pollutants may have synergistic detrimental impact on child neurocognitive outcomes. We and others have shown lower neurodevelopmental functioning among HIV infected than unexposed uninfected children, despite early antiretroviral treatment (ART) (ref 29 and others). Children who have HIV likely have pre-existing neurocognitive compromise that is worsened by exposure to pollutants, while in HUU pollutants appeared to have less discernable impact.

To date, studies of air pollution and neurodevelopment have focused on prenatal exposures to air pollution and subsequent child outcomes. Prenatal exposure to PAH has been associated with impaired cognition in early childhood.[20–22] Dix-Cooper at al,[17] found associations between prenatal exposure to CO and lower cognitive function in the visuo-spatial integration, short term memory recall, long term memory recall or motor domains. Edwards et al,[20] observed an association between prenatal PAH levels and non-verbal intelligence. Our study focused on childhood exposures rather than prenatal exposure and found more impact from PAH than CO exposures in similar but not identical neurocognitive domains as the aforementioned prenatal exposure studies. It is possible that the exposures we measured were similar to earlier prenatal exposures in the same household and that effects may reflect either prenatal or postnatal exposure.

An alarming 39% of children had levels of CO higher than WHO recommended limits for indoor levels. Mean maternal 3rd-trimester personal 48-h CO exposures in a Guatemalan cohort was 3.8 ppm, while the mean 24-hour caregiver CO in our cohort was 8.15 ppm. Similarly, mean 1-OHP levels were also high in our cohort, exceeding levels observed in other cohorts of young children. Mean 1-OHP levels in a cohort of children living near a steel mill in Mariupol, Ukraine was 0.69 µmol/mol creatinine, and 0.34 µmol/mol creatinine in a cohort of children in Kiev, Ukraine[42] The mean level in our cohort was 0.81 µmol/mol creatinine. These high levels underscore need for interventions to decrease indoor air pollution with cleaner fuel cooking and improved home ventilation. Taking a multi-faceted interventional approach– combining biomedical interventions (ART) with interventions to improve indoor air pollution – will be necessary to optimize neurocognitive and respiratory outcomes for children with HIV.

We use two measures of HAP – caregiver CO and urine 1-OHP. The strong correlation between caregiver and household CO measurements (r=0.70) suggests compliance with wearing the monitors, and supports the idea that caregiver CO is an appropriate measurement of CO exposure in the home. The weak correlation between child 1-OHP and household and caregiver CO may reflect the fact that PAH metabolites in the urine are affected by recent dietary intake and are more directly tied to child exposure. Both measures have merit and in this analysis, child 1-OHP was associated with neurodevelopmental outcomes, suggesting that this measure may be more relevant for child outcomes.

To our knowledge, this is the first study to characterize exposures to household air pollution in peri-urban Kenyan children, and the first to examine the relationship between household air pollution and neurodevelopment in this region in the context of pediatric HIV infection. In this setting, kerosene and propane, rather than biomass, are the most common reported cooking fuels. Strengths of this study include detailed neurocognitive assessment data and collection of personal and home air sampling (CO), and collection of biomarkers for PAH exposures. The neurocognitive assessments used in our study have high validity and reliability in Western populations, and that have been used previously among African and HIV infected cohorts. Our study was limited by the timing of collection of air pollution exposure data, performed when children were at school-age. We did not measure exposure during critical developmental windows such as the prenatal period and infancy. While imperfect correlates for early life exposure, ongoing neurodevelopmental processes that continue into school age may also be impacted by exposures experienced into early and later childhood. Additionally, our analysis is limited by a small sample size, which affects our power to detect significant association. We were also unable to control for some potentially important confounders, including nutritional factors, maternal IQ, and exposure to other environmental toxicants. Finally, our analysis only measures exposure to CO and PAH, whereas air pollution is a complex mixture of substances. Other components of air pollution such as non-PAH PM 2.5 constituents and nitrogen dioxide may also impact neurodevelopment.

Conclusions

Despite limitations in timing of exposure assessment and a modest sample size, our results provide further support for emerging evidence that early life exposure to air pollutants such as PAH may compromise healthy neurodevelopment. The enhanced susceptibility among HIV infected children is a novel and important observation. Given the large global population of children co-exposed to higher levels of air pollution and HIV in sub Saharan Africa, continued

emphasis on characterizing and reducing risk factors for poorer neurodevelopmental health in this population is merited.

Furthermore, we observed associations between caregiver CO exposure and impaired child neurodevelopmental function in several neurocognitive domains. The analysis suggests that the WHO 6.11 ppm guideline for CO exposure may not be protective against the adverse impacts on cognition.

Table1: Summary of study population and neurodevelopmental outcomes (z-score), stratified by HIV infection status

	HIV Infected	**HIV Uninfected**
Sociodemographic characteristics	**n(%)**	**n(%)**
Male Sex	29(64.4)	22(44.9)
Caregiver is biological mother	42(93.3)	48(98.0)
Caregiver is married	26(57.8)	33(67.4)
Caregiver employment status		
Employed	11(24.4)	16(34.0)
Unemployed	23(51.1)	16(34.0)
Other	11(24.4)	15(31.9)
	Mean(SD)	**Mean(SD)**
Caregiver age (years)	33.2(6.1)	31.1(5.6)
Child age at neurodevelopmental assessment (years)	6.6(0.8)	6.7(1.4)
Caregiver age (years)	33.2(6.1)	31.1(5.6)
Caregiver education (years)	9.5(2.7)	9.0(2.7)
Household people/room	3.4(2.1)	4.4(1.6)
Household monthly rent (Kenyan Schillings)	4105(4801)	2247(1310)
Household characteristics	**n(%)**	**n(%)**
Smoker in household	7(15.6)	6(12.8)
Cooks in living area	28(62.2)	45(93.8)
Garbage is burned nearby	12(26.7)	16(33.3)
Primary type of cooking fuel		
Wood	2(4.6)	0(0.0)
Propane (LPG)	15(34.1)	8(17.0)
Charcoal	7(15.9)	3(6.4)
Paraffin	20(45.5)	36(76.6)
Neurodevelopmental Function	**Mean(SD)**	**Mean(SD)**
Cognitive ability	-1.90(0.62)	-1.76(0.77)
Short-term memory	-1.48(0.76)	-1.30(0.81)
Visual-spatial	-1.99(0.65)	-1.80(0.95)
Learning	-0.81(0.96)	-0.94(0.85)
Nonverbal	-1.97(0.74)	-1.88(0.89)
Delayed memory	-0.96(0.87)	-0.92(0.75)
Executive function	0.19(0.95)	0.08(0.95)
Attention	-1.41(0.86)	-1.13(1.01)
Motor	-1.93(0.86)	-1.63(1.05)

Table 2: Caregiver CO levels(ppm) and child urine 1-OHP (µmol/mol creatinine), stratified by HIV infection status

	Caregiver 24-h CO, ppm					Caregiver 24-h CO mean >6.11 ppm
	n	Mean (SD)	Median (IQR)	Geometric mean (SD)	Range	n(%)
All	71	8.15(13.46)	4.44(0.53,10.44)	2.31(7.55)	0,82.95	28(39.4)
HIV Infected	33	11.59(18.04)*	6.07(0.82,13.22)	3.22(7.44)	0.03,82.95	16(48.5)
HIV Uninfected	38	5.16(6.50)*	3.71(0.45,7.10)	1.71(7.50)	0,31.63	12(31.6)
	Household 24-h CO, ppm					Household 24-h CO mean >6.11 ppm
	n	Mean (SD)	Median (IQR)	Geometric mean (SD)	Range	n(%)
All	75	11.37(16.52)	4.17(1.05,13.83)	4.07(5.10)	0,95.24	31(41.3)
HIV Infected	35	13.88(19.44)	4.33(1.18,27.02)	4.90(5.46)	0,95.24	16(45.7)
HIV Uninfected	40	9.17(13.33)	3.77(0.95,10.21)	3.47(4.81)	0,54.15	15(37.5)
	Child Urine 1-OHP 1-OHP (µmol/mol creatinine)					High child urine 1-OHP
	n	Mean (SD)	Median (IQR)	Geometric mean (SD)	Range	n(%)
All	75	0.81(0.60)	0.68(0.42,1.00)	0.59(2.43)	0.05,2.74	37(49.3)
HIV Infected	32	0.89(0.73)	0.62(0.44,1.26)	0.60(2.73)	0.05,2.74	14(43.8)
HIV Uninfected	43	0.75(0.48)	0.71(0.42,0.96)	0.59(2.23)	0.07,2.43	23(53.5)

*Indicates that difference by child HIV-infection status. Means were tested with a t-test, geometric means were tested by performing a t-test on the log10 transformed values, and medians were tested with a non-parametric test of equal medians.

Table 3a: Cofactors for high/low caregiver CO exposure and high/low child 1-OHP exposure

	HIV Infected						HIV Uninfected					
	CO≤ 6.11 ppm N=17	CO>6.11 ppm N=16	p	Low urine 1-OHP N=18	High urine 1-OHP N=14	p	CO≤ 6.11 ppm N=26	CO>6.11 ppm N=12	p	Low urine 1-OHP N=20	High urine 1-OHP N=23	p
Household characteristics	**n(%)**	**n(%)**		**n(%)**	**n(%)**		**n(%)**	**n(%)**		**n(%)**	**n(%)**	
Primary type of cooking fuel												
Wood	1(100)	0(--)	0.48	0(0.00)	1(100.0)	0.59	0(0.00)	0(0.00)	0.96	0(0.00)	0(0.00)	0.87
Propane	5(41.7)	7(58.3)		5(71.4)	2(28.6)		5(71.4)	2(28.6)		3(42.9)	4(57.1)	
Charcoal	5(71.4)	2(28.6)		4(57.1)	3(42.9)		2(66.7)	1(33.3)		1(33.3)	2(66.7)	
Paraffin	6(50.0)	6(50.0)		9(56.3)	7(43.8)		17(65.4)	9(34.6)		15(48.4)	16(51.6)	
Smoker in household	2(40.0)	3(60.0)	0.58	4(80.0)	1(20.0)	0.24	1(25.0)	3(75.0)	0.04	1(16.7)	5(83.3)	0.10
Cooks inside living area	8(47.1)	9(52.9)	0.60	15(65.2)	8(34.8)	0.10	23(67.7)	11(32.4)	0.97	19(47.5)	21(52.5)	0.95
Garbage burned nearby with smoke entering kitchen	6(85.7)	1(14.3)	0.04	6(54.6)	5(45.5)	0.89	10(76.9)	3(28.1)	0.37	7(46.7)	8(53.3)	0.93
Socioeconomic indicators												
Caregiver employment status												
Employed	4(50.0)	4(50.0)	0.08	4(66.7)	2(33.3)	0.67	8(66.7)	4(33.3)	0.51	6(50.0)	6(50.0)	0.94
Unemployed	11(68.8)	5(31.3)		11(57.9)	8(43.1)		10(76.9)	3(23.1)		6(42.9)	8(57.1)	
Other	2(22.2)	7(77.8)		3(42.9)	4(57.1)					7(46.7)	8(53.3)	
	Mean(SD)	**Mean(SD)**	**p**	**Mean(SD)**	**Mean(SD)**	**p**	**Mean(SD)**	**Mean(SD)**	**p**	**Mean(SD)**	**Mean(SD)**	**p**
Caregiver education (years)	9.87(3.00)	9.25(2.98)	0.57	9.75(2.11)	7.93(2.27)	0.03	9.71(2.73)	9.17(1.85)	0.54	9.16(2.24)	8.50(3.05)	0.44
Household people/room	3.07(2.35)	3.93(1.88)	0.42	3.32(2.11)	3.98(2.14)	0.48	4.06(1.47)	4.63(1.51)	0.38	3.84(1.21)	4.81(1.87)	0.12
Household monthly rent	4647(4720)	4313(6155)	0.87	3412(2292)	1914(1557)	0.05	2475(1501)	2167(1076)	0.53	2052(952)	2645(1594)	0.16

Table 3a: Cofactors for continuous caregiver CO exposure and continuous child urine 1-OHP

	HIV Infected				HIV Uninfected			
	Caregiver CO(ppm)		Child urine 1-OHP (µmol/mol creatinine)		Caregiver CO(ppm)		Child urine 1-OHP (µmol/mol creatinine)	
Household characteristics	**Geometric Mean(SD)**	**p**	**Geometric Mean(SD)**	**p**	**Geometric Mean(SD)**	**p**	**Geometric Mean(SD)**	**p**
Type of cooking fuel								
Wood	0.82(--)	0.11	2.63(--)	0.30	--	0.78	--	0.15
Propane	3.39(12.82)		0.46(3.11)		3.43(5.42)		0.44(2.75)	
Charcoal	3.74(2.90)		0.62(1.82)		1.97(3.40)		0.74(1.24)	
Paraffin	2.69(7.45)		0.56(2.84)		1.93(6.63)		0.61(2.23)	
Smoker in household								
Yes	3.14(7.75)	0.86	0.65(1.79)	0.86	6.96(1.39)	0.11	0.99(1.55)	0.08
No	3.76(7.16)		0.59(2.92)		1.55(6.29)		0.53(2.28)	
Cooks inside living area								
Yes	3.34(5.91)	0.92	0.52(2.43)	0.17	1.95(5.89)	0.83	0.59(2.25)	0.56
No	3.11(9.83)		0.89(3.41)		2.43(18.4)		0.42(2.73)	
Garbage burned nearby								
Yes	1.94(3.51)	0.46	0.74(2.55)	0.46	1.86(7.62)	0.82	0.59(2.25)	0.76
No	3.70(8.71)		0.54(2.84)		2.05(5.89)		0.60(2.17)	
Socioeconomic Indictors								
Caregiver employment status								
Employed	2.45(13.27)	0.10	0.23(3.07)	0.10	2.62(5.03)	0.51	0.52(2.57)	0.12
Unemployed	2.67(4.05)		0.75(1.96)		1.27(6.65)		0.60(2.66)	
Other	5.75(11.74)		0.74(3.67)		2.04(10.01)		0.62(1.76)	
	log10 caregiver CO		**log 10 Child urine 1-OHP**		**log10 caregiver CO**		**log 10 Child urine 1-OHP**	
	β	**95%CI**	**β**	**95%CI**	**β**	**95%CI**	**β**	**95%CI**
Caregiver education (years)	-0.27	-1.53, 0.99	-1.51	-3.43,0.41	0.18	-0.84,1.23	-0.57	-3.57,1.88
Household people/room	0.63	-0.59,1.86	0.58	-1.06,3.06	0.26	-0.55,1.10	0.70	-0.90,2.37
Household monthly rent	-774	-3062,1514	-1243	-2982,495	-183	-760,394	619	-597,1834

Table 4a: Mean neurodevelopmental test z-scores by caregiver 24-h high/low exposure

	HIV infected					HIV uninfected				
	n	CO≤ 6.11 ppm	n	CO>6.11 ppm	p	n	CO≤ 6.11 ppm	n	CO>6.11 ppm	p
Neurodevelopmental Domain		**mean(SD)**		**mean(SD)**			**mean(SD)**		**mean(SD)**	
Cognitive ability	17	-1.88(0.57)	15	-1.86(0.69)	0.93	23	-1.57(0.85)	12	-1.69(0.56)	0.65
Short-term memory	17	-1.39(0.77)	15	-1.47(0.78)	0.78	23	-1.22(0.61)	12	-1.12(0.23)	0.67
Visuo-spatial	17	-1.96(0.57)	15	-1.97(0.47)	0.95	23	-1.47(1.02)	12	-1.84(0.51)	0.25
Learning	17	-0.75(0.97)	15	-0.72(1.09)	0.93	23	-0.99(0.78)	12	-0.58(0.78)	0.16
Nonverbal	16	-1.76(0.85)	15	-2.08(0.55)	0.24	23	-1.60(0.98)	12	-2.01(0.59)	0.20
Delayed memory	14	-0.92(0.78)	13	-0.84(1.17)	0.83	18	-0.80(0.73)	10	-0.65(0.70)	0.61
Executive function	17	0.44(1.08)	15	-0.04(0.89)	0.18	23	0.18(0.98)	12	-0.34(0.91)	0.14
Attention	15	-1.56(1.12)	12	-1.48(0.53)	0.84	19	-0.98(0.95)	12	-1.05(0.65)	0.83
Motor	17	-1.97(0.85)	15	-1.84(0.77)	0.65	23	-1.82(0.99)	12	-1.22(0.87)	0.09

Table 4b: Mean neurodevelopmental z-scores by child urine PAH high/low exposure

	HIV infected					HIV Uninfected				
	n	Low urine 1-OHP	n	High urine 1-OHP	p	n	Low urine 1-OHP	n	High urine 1-OHP	p
Neurodevelopmental Domain		mean(SD)		mean(SD)			mean(SD)		mean(SD)	
Cognitive ability	18	-1.75(0.52)	14	-2.28(0.60)	0.01	18	-1.80(0.62)	22	-1.71(0.94)	0.74
Short-term memory	18	-1.36(0.66)	14	-1.87(0.80)	0.06	18	-1.43(0.76)	22	-1.14(0.92)	0.29
Visual-spatial	18	-1.77(0.78)	14	-2.19(0.57)	0.11	18	-1.74(0.86)	22	-1.93(1.09)	0.57
Learning	18	-0.66(0.66)	14	-1.45(0.94)	0.01	18	-1.06(0.82)	22	-0.83(0.94)	0.43
Nonverbal	18	-1.89(0.71)	13	-2.20(0.32)	0.15	18	-1.76(0.90)	22	-2.04(0.97)	0.35
Delayed memory	15	0.78(0.36)	13	-1.51(0.71)	<0.01	15	-1.04(0.76)	19	-0.77(0.80)	0.32
Executive function	18	0.16(0.84)	14	0.21(1.09)	0.88	18	-0.05(0.72)	22	-0.10(1.06)	0.62
Attention	15	-0.95(0.76)	12	-1.87(0.87)	<0.01	16	-1.20(1.10)	21	-1.08(1.07)	0.74
Motor	18	-1.73(0.66)	13	-2.09(1.18)	0.29	18	-1.69(1.14)	22	-1.67(1.09)	0.95

Table 5: Spearman correlation coefficient between $\log_{10}$ transformed measurements of household air pollution.

	Child 1-OHP	**Household CO**
Caregiver CO	0.13(n=52)	0.70(n=66)
Household CO	0.24(n=57)	
Caregiver 1-OHP	0.54(n=18)	

Acknowledgements:

First and foremost, I would like to thank Dr. Sarah Benki-Nugent for her kindness, motivation, and helpful comments throughout the entire thesis development and writing process. I am fortunate to have worked with someone so knowledgeable. Thank you also to Drs. Catherine Karr and Grace John-Stewart for their thoughtful feedback on the many iterations of this analysis, and Laurén Gomez for preparing the dataset.

Lastly, I would like to thank my partner, family, and classmates for their love, support, and guidance during the writing of this thesis and my graduate school journey.

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