



Air pollution exposures in early life and brain development in children (ABC): protocol for a pregnancy cohort study

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ABSTRACT

Introduction Air pollution is linked with poor neurodevelopment in high-income countries. Comparable data are scant for low-income countries, where exposures are higher. Longitudinal pregnancy cohort studies are optimal for individual exposure assessment during critical windows of brain development and examination of neurodevelopment. This study aims to determine the association between prenatal ambient air pollutant exposure and neurodevelopment in children aged 12, 24 and 36 months through a collaborative, capacity-enriching research partnership.

Methods and analysis This observational cohort study is based in Nairobi, Kenya. Eligibility criteria are singleton pregnancy, no severe pregnancy complications and maternal age 18 to 40 years. At entry, mothers (n=400) are administered surveys to characterise air pollution exposures reflecting household features and occupational activities and provide blood (for lead analysis) and urine specimens (for polycyclic aromatic hydrocarbon (PAH) metabolites). Mothers attend up to two additional antenatal study visits, with urine collection, and infants are followed through age 36 months for annual neurodevelopment and caregiving behaviour assessment, and child urine and blood collection. Primary outcomes are child motor skills, language and cognition at 12, 24 and 36 months, and executive function at 36 months. The primary exposure is urinary PAH metabolite concentrations. Additional exposure assessment in a subset of the cohort includes residential indoor and outdoor air monitoring for fine particulate matter (PM_{2.5}), carbon monoxide (CO), ultrafine particles (UFP) and black carbon (BC).

Ethics and dissemination This study was approved by the Kenyatta National Hospital - University of Nairobi Ethics and Research Committee, and the University of Washington Human Subjects Division. Results are shared at annual workshops.

INTRODUCTION

Neurodevelopmental disorders (NDDs) are a major global public health concern. Estimates suggest 15.7% of children are significantly delayed in cognitive development, 26.3% in socioemotional development and

WHAT IS ALREADY KNOWN ON THIS TOPIC

- ⇒ Prenatal exposure to air pollutants is associated with worse neurodevelopmental outcomes in high-income countries, where both levels and sources of air pollution are generally lower and less heterogeneous than in lower-income and middle-income countries.
- ⇒ There are few data on exposures indoor and ambient sources of air pollutants in urbanised Africa, and a limited understanding of how pollutants arising from these sources impact child development in this region.

WHAT THIS STUDY HOPES TO ADD

- ⇒ This study will characterise prenatal ambient and household exposures to air pollution in an urban Sub-Saharan Africa (SSA) context.
- ⇒ It will provide a detailed analysis of prenatal air pollution exposures and key neurodevelopmental outcomes in early childhood, including cognitive, language, motor and executive function skills.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- ⇒ This study aims to strengthen the capacity for research on neurotoxicant exposures in early life and child neurodevelopmental outcomes in an urban SSA context and to support links with practitioners and policymakers to inform the translation of research findings to practice and policy.



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36.8% in either or both in lower-income and middle-income countries (LMICs).¹ Modifiable environmental risks, including air pollutants, are an active area of research in high-income countries (HIC). However, research and clinical capacity gaps to characterise NDDs in relation to key contaminants in Sub-Saharan Africa (SSA) stymie development of policy and programmatic actions to reduce exposures for critical populations of pregnant women and children.

In HICs, adverse effects of air pollutants on the developing brain are increasingly defined.^{2–4} Studies focus on regulated ambient pollutants such as fine particulate matter (PM_{2.5}) and nitrogen dioxide, with traffic as a primary source.⁵ Some research examines prenatal exposure to polycyclic aromatic hydrocarbons (PAHs), which are important by-products of incomplete combustion and occur as a component of particulate matter.^{6–9} In HICs, prenatal and first years of life exposure to air pollutants have been associated with autism spectrum disorders,^{10 11} worse psychomotor scores,^{12 13} behaviour problems,^{14 15} lower cognitive functioning^{14 16 17} and poor performance on executive function (EF) tasks.¹⁷

To date, data from SSA have almost exclusively focused on indoor and cookstove emissions in rural settings, where solid fuels predominate.¹⁸ Urban exposures comprise multiple sources, varying by proximity to busy roadways and diesel emissions, unregulated large or small industries, open waste burning, unpaved road dust, household fuel for cooking or heating, and occupational activities.¹⁹ This paper describes a prospective pregnancy cohort study, based in urban SSA, of neurodevelopmental outcomes over 3-year follow-up and with assessment of multiple ambient and indoor air pollutant exposures, including PAHs, PM_{2.5} (ie, particles ≤ 2.5 μm aerodynamic diameter), black carbon (BC), ultrafine particulate matter (UFP) (ie, particles ≤ 0.1 μm) and carbon monoxide (CO).

METHODS AND ANALYSIS

Partner engagement

In September 2019, key stakeholders in child environmental health from research, policy and regulatory spheres in Kenya were identified and invited to participate in a Nairobi-based ‘Kenya Healthy Home Healthy Brain’ Workshop. Priorities for blood lead laboratory procedures, field-based air pollution sampling and neurodevelopmental assessment were emphasised along with interest in research focused on air pollution and neurodevelopment.

Patient and public involvement

Annual workshops throughout the study period are planned to further facilitate engagement with policy-makers and local academic and community partners and seek input on best practices for dissemination of results to participants and the public. Results will be shared accordingly.

Study objectives

The primary goal is to characterise early life exposure to air pollutants and determine the association between prenatal air pollutant exposure and early childhood neurodevelopment. Specific objectives are to characterise early life exposure to air pollutants using urinary biomarkers of PAHs, air monitoring and exposure modelling for PM_{2.5}, CO, UFP and BC. We hypothesise

that air pollution exposure is comparatively high and will correlate with residential proximity to outdoor air pollutant sources, for example, on-road vehicle traffic and industry, and household cooking fuels. This study will test whether high prenatal PAH exposures are associated with worse child neurodevelopmental outcomes at ages 12, 24 and 36 months, including cognitive, language and motor skills, and at 36 months, self-regulation and performance on EF tasks. Finally, this study will explore associations between air pollutant (PM_{2.5}, CO, UFP and BC) exposures and neurodevelopmental outcomes in early childhood.

Study design and setting

The study design is an observational prospective birth cohort study of 400 mother-child pairs followed from pregnancy through child age 36 months. Pregnant individuals are recruited as early as the first trimester and asked to attend up to two additional antenatal study visits (trimester-based), depending on whether they were enrolled in the first second or third trimesters. Mother-child pairs are asked to attend study visits at child age 6 weeks and 6 monthly through child age 36 months (figure 1). In addition to mother’s age (18–40 years), inclusion criteria are residing in Nairobi and planning to reside in the same residence for at least 1 year. Exclusion criteria include the presence of severe pregnancy complications (eg, requiring hospitalisation) and non-singleton pregnancy.

The selection of the recruitment site considered multiple factors, including retention, establishing a ‘neighborhood’ site with minimal travel for study participants and focusing on a catchment population with socio-demographic generalisability to the Nairobi population. The Dandora neighbourhood of Nairobi was of interest, in part owing to the proximity to a large dump site and routine burning of waste for Dandora. In addition, the Dandora 2 Health Centre provides antenatal services and monthly well-child clinic services (immunisation, growth monitoring) during the first year of life, then quarterly or every 6 months to age 5 years.

Screening, enrollment and maternal pregnancy procedures

Beginning in January of 2021, convenience sampling of pregnant women is used to approach women at their routine antenatal visit, and recruit and screen for eligibility to enrol in the study, with a target enrolment of $n=400$. At enrolment, women are interviewed to obtain sociodemographics, and household, occupational and environmental exposures (figure 1), including cooking behaviours, ventilation features and frequency of cooking, lighting, smoking, garbage burning and other combustion-related activities. Medical and obstetric history are obtained, and a brief physical examination is performed. Questions to assess 24-hour exposure to potential contributors to PAH exposure, including cigarette smoking, exposure to secondhand cigarette smoke and consumption of chargrilled or fried foods.²⁰ Urine

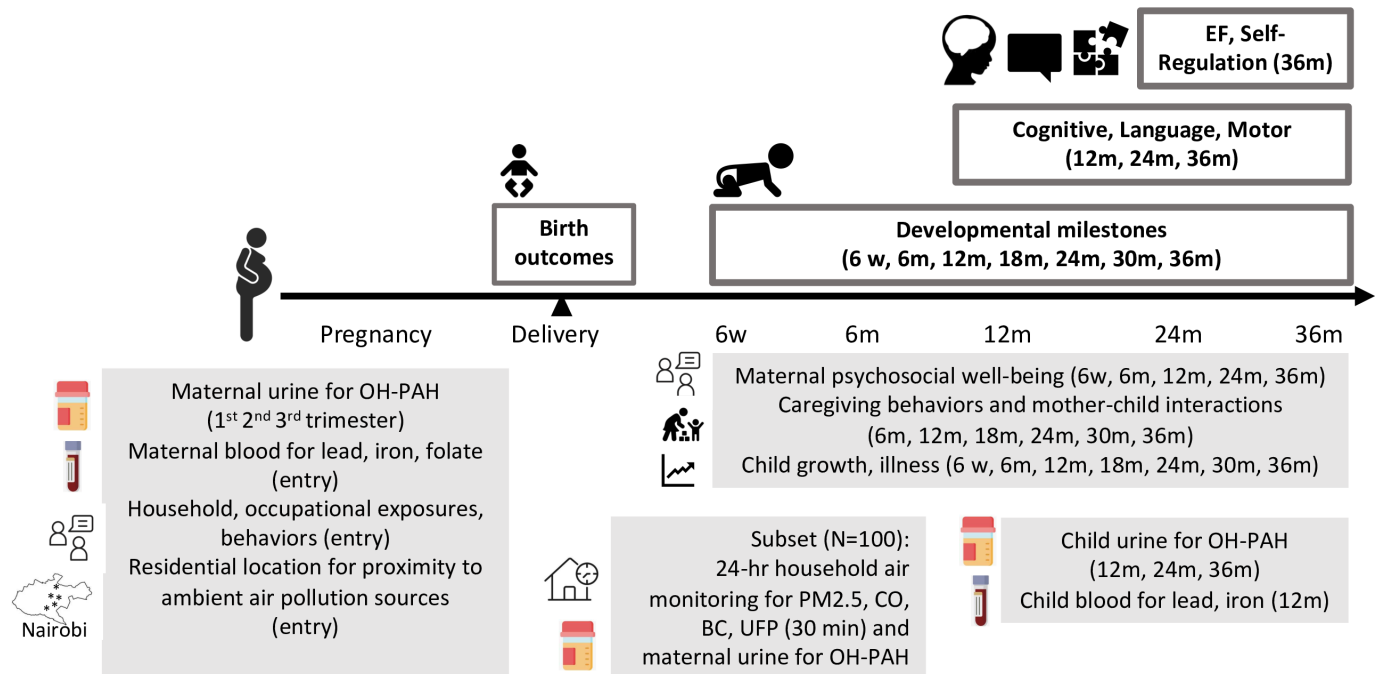


Figure 1 Overview of study environmental exposures, risk/protective factors (grey) and outcomes (white) data collection. BC, black carbon; CO, carbon monoxide; EF, executive function; OH-PAH, hydroxy-polycyclic aromatic hydrocarbon, particulate matter <2.5 µm diameter; UFP, ultra-fine particulate matter.

is collected for ascertainment of urinary metabolites of PAH (hydroxy (uOH)-PAH). A blood sample is collected to determine blood lead level and iron and folate status. Women enrolling during the first and second trimesters are asked to return to the clinic for up to two additional study visits scheduled during the remaining trimesters. A brief physical examination and urine specimen collection are conducted at these visits.

Maternal post-partum procedures

Brief information on delivery (including birth weight, neonatal hospitalisation, birth outcome, infant sex, date of birth, breastfeeding) is collected at the facility for women who give birth at study facility and by telephone for those who give birth elsewhere. In addition, details including any birth complications at delivery, gestational age at birth and Apgar scores are abstracted from medical records (eg, the Kenya Mother & Child Health Handbook) at the next scheduled study visit. Mothers are asked to return to the clinic with their infant at 6 weeks, 6 months and every 6 months thereafter through the first 36 months of life. Psychosocial well-being questionnaires (table 1), including the *Perceived Stress Scale*,²¹ the *Patient Health Questionnaire*^{9,22} and the *Multi-dimensional Scale of Perceived Social Support*²³ are administered at 6 weeks, 6 months and annually thereafter to account for any contribution to neurodevelopment. The *Adverse Childhood Experiences Questionnaire* is administered at 6 months.²⁴ Maternal behaviours related to child cognitive and socioemotional stimulation are assessed using the *UNICEF Multiple Indicator Cluster Survey* (MICS).²⁵ Maternal responsive caregiving and mother-child interactions are

assessed using direct observation, using an adaptation of the *Observed Mother-Child Interactions Assessment* (OMCI).²⁶

Child follow-up procedures

Child data collection includes child medical history, current health status, anthropometrics, including weight, height and head circumference, receipt of vaccines, household hunger²⁷ and specific foods eaten in the past 24 hours using the Minimum Dietary Diversity Food Groups as a guide.²⁸ During follow-up, child urine is collected at annual visits to allow future ascertainment of individual urinary PAH metabolites. At 12 months of age, blood is collected for lead assays and determination of iron status.

Neurodevelopmental assessment of children

When selecting neurodevelopmental outcomes, several aspects were considered, including enhancing the skills of the clinical team (eg, directly administered, observational and caregiver-report measures) and the likelihood the constructs are known or suspected to be sensitive to air pollution. Priority was given to assessments that are used and validated in SSA and that are linked with future neurobehavioural function. At each study visit, developmental milestones corresponding to ages 0 to 24 months and included in the Kenya Mother & Child Health Handbook²⁹ are assessed at age-appropriate windows.

In addition, standardised neurodevelopmental assessments are conducted as described below (table 1). Items are administered in either Kiswahili or English, depending on the mother's preference. All standardised instruments are translated and back-translated to ensure

Table 1 Summary of maternal and child psychosocial and neurodevelopmental assessments

Maternal		
Domain	Measure	Timepoint
Depression, stress, social support	Patient Health Questionnaire-9, Perceived Stress Scale, Multidimensional Scale of Perceived Social Support	6 weeks, 6 months, 12 months, 24 months, 36 months
Early adversity	Adverse Childhood Experiences Questionnaire	12 months
Caregiving behaviours, mother-child interactions	Multi-Indicator Cluster Survey, Early Childhood Development Module, Observed Mother-Child Interactions	6 months, 12 months, 18 months, 24 months, 30 months, 36 months
Child		
Domain	Measure	Timepoint
Developmental milestones	Kenya Ministry of Health Mother Child Handbook, Caregiver-Report Early Development Index	6 weeks, 6 months, 12 months, 18 months, 24 months, 30 months, 36 months
Gross and fine motor, receptive and expressive language, cognition	Bayley Scales of Infant and Toddler Development 4th Ed.	12 months, 24 months, 36 months
Self-regulation	Preschool Self-Regulation Assessment	36 months
Executive function (EF)	EF Touch	36 months
Internalising and externalising symptoms	Achenbach Child Behaviour Checklist	36 months

accuracy. The *Caregiver Reported Early Development Index* (CREDI),³⁰ which assesses motor, language, cognitive, and socioemotional functioning and child mental health, is administered at 6 monthly visits. The CREDI requires minimal training and has a good correlation with the Bayley.³¹ At 12, 24 and 36 months, the *Bayley Scales of Infant and Toddler Development, 4th Edition* (Bayley-4)³² is used to assess cognitive, language and motor outcomes, using a translation and adaptation developed using forward and backward translation and cognitive interviewing.³³ At 36 months, EF is assessed using subtests from the EF Touch tablet-based battery that measure working memory, attention shift and inhibitory control. Tasks have been used extensively, with validation for Kenyan 3 to 6-year-old children.³⁴ During EF Touch administration, a brief standardised questionnaire (*Preschool Self-Regulation Assessment*) is used to assess observed child attention and positive affect.³⁵ Examiner training for the Bayley-4 and EF Touch includes a combination of in-person demonstration and guided practice, video-recorded practice administrations for quality and reliability coding, and ongoing review of video-recorded administrations with participants for quality assurance. Examiners meet weekly to review any administration and scoring concerns. Finally, the *Achenbach Child Behaviour Checklist* (CBCL)³⁶ is used to assess child behaviour problems as self-reported by the mother. Instead of relying on test norms, which may not be representative of participants, we use raw scores and calculated z-scores for neurodevelopmental measures based on the distribution in this sample.

Laboratory and air pollution assessment procedures

Following collection, urine and whole blood specimens are immediately stored on ice (urine) and at room temperature (blood) for same-day transport to the University of Nairobi (UoN), Department of Paediatrics Research Laboratory. A small aliquot of whole blood is used for haemoglobin assays in real-time. The serum is separated from whole blood. Serum, whole blood and urine are then stored at -80°C for future batched assays for folate (serum), lead (whole blood), both done at the UoN, and uOH-PAH assays, done at the University of Washington (UW).

For modelling of air pollutant exposures, all women are scheduled for a brief home visit for the collection of their residential location (ie, latitude and longitude) using handheld global positioning system devices. In a subset ($n=100$), a trained technician conducts a home visit to collect air samples, questionnaires and observational data that will be used to model air pollution exposures in the full cohort. The monitoring includes indoor and outdoor air monitoring for PM_{2.5}, CO (each over 24 hours) and UFP (30 min), and observation of household characteristics. 24-hour PM_{2.5} samples are collected using low-flow pumps and Harvard Impactors fitted with polytetrafluoroethylene filters for gravimetric analysis using standard procedures³⁷ at the UoN. Black carbon is measured on the PM_{2.5} filters using a Multi-Wavelength Black Carbon Instrument (MABI) (Australian Nuclear Science and Technology Organisation, Sydney, NSW, Australia) at the Institute of Nuclear Science and Technology, UoN. Portable CO monitors (EasyLog-USB-CO

Lascar Electronic Ltd., Salisbury, UK) are used to conduct continuous, real-time monitoring of CO. Brief (30 min) sampling of UFP is done indoors and outdoors using a P-Trak 8525 particle counter (TSI Inc., Shoreview, MN, USA). At the end of the 24 hours, a maternal spot urine sample is collected and a brief structured interview is used to collect information on household fuel use and other combustion activities and exposure to outdoor smoke during the previous 24 hours.

Sample size

In prior studies of mother-infant pairs, higher upper quartile and median maternal PAH concentration in air in the third trimester was associated with child cognitive score differences of -4.3 to -5.7 at ages 3 and 5.^{6 7} Assuming 400 mother-child pairs and 10% attrition per year, we anticipate $n=292$ with 36 months of follow-up. For $n=292$, we have 80% power to detect a difference of 0.33 in Bayley-4 z-scores, assuming $\alpha=0.05$.

Statistical analysis

Primary epidemiological analyses will examine the relationship between prenatal exposure to PAHs (third trimester uOH-PAH) and global development (Bayley-4 cognitive, language and motor scales) at 12, 24 and 36 months in the whole cohort, and EF constructs (response inhibition, attentional control, cognitive flexibility) at age 36 months. We hypothesise that prenatal concentrations of these metabolites are associated with poor cognition, language, motor and EF skills. Secondly, we will test the relationship between these exposures and self-regulation. Log-transformed uOH-PAH concentrations will be analysed in regression models including specific gravity as a covariate. Multivariate linear regression with robust standard errors will be used to estimate associations of individual OH-PAHs and the primary continuous neurodevelopmental outcome scores with corresponding 95% CI. Associations will first be tested in a minimal model adjusted for child age, and scatterplots of OH-PAHs by neurodevelopmental scores will be visually inspected to determine whether any associations are driven by outliers. We will examine a model including potential a priori selected confounders (eg, maternal education, socioeconomic status, smoking and secondhand smoke exposure) and potential precision 'risk' variables (eg, low maternal folate, lack of cognitive stimulation, high child blood lead, child undernutrition). Additional stratified models will explore potential effect modifiers (eg, child sex, quality of caregiving, and maternal and child nutritional status). Extended adjusted analyses will explore impact of low birth weight and pre-term birth, which may lie on the causal pathway. Sensitivity analyses will explore bias related to attrition and missingness. Analyses excluding women known to have relocated outside of Nairobi soon after enrolment will be conducted to explore potential misclassification error.

Ethical Considerations

This study is approved by the Kenyatta National Hospital - UoN Ethics and Research Committee (P22/01/2021), the National Commission for Science, Technology and Innovation, and the UW Human Subjects Division (STUDY00012014). Written informed consent for study procedures is obtained from eligible women prior to enrolment. Children observed to have neurodevelopmental or other concerns are referred for care through publicly available services. Mothers are provided with blood lead level results, alongside messaging to identify potential sources of exposure; how to reduce their own and their child's exposures and sources of iron-rich foods to reduce lead absorption.

DISCUSSION

We describe a unique longitudinal pregnancy cohort study of prenatal exposure to air pollution and neurodevelopmental outcomes based in Nairobi, Kenya. To our knowledge, this is the first study to investigate the relationship between neurodevelopment and both ambient and indoor air pollution in an urban African setting. In addition, this study addresses gaps in knowledge on the contribution of specific types of air pollutants, including PAH, fine and ultrafine particulate matter, CO and BC on neurodevelopmental outcomes in SSA and in LMICs in general.

This study uses a non-invasive biomarker of PAH, an important component of air pollution, and is the first to apply this marker in a birth cohort in SSA. Prior studies of the association between neurodevelopment and PAH exposures during the prenatal and early life periods are few and include high-income and middle-income countries, including the USA,^{6 38} China³⁹ and Poland.⁹ In pregnancy and birth cohorts, prenatal PAH exposures have been associated with worse neurodevelopmental scores at ages 2 and 3 years;^{6 39} neurocognitive deficits at age 5 years^{7 9}; symptoms of inattention, anxiety and depression^{8 40}; and white matter differences at older ages.⁴¹ Study of how PAH may impact neurodevelopment in highly urbanised African cities, where exposures could be higher due to rapidly increasing industrialisation as well as cooking fuel emissions, and could occur in combination with additional ubiquitous neurotoxins (eg, lead)⁴² is warranted.

The study design requires collaborative expertise in exposure and atmospheric sciences, maternal child health and early childhood development and assessment, and is a product of collaboration between LMIC and HIC experts in each of these areas, enabling joint mentorship. A focus on enriching capacity for air monitoring and blood lead assays reflects priorities identified by the UoN team. In addition, the study offers unique opportunities for shared training and collaboration on rigorous methods in air monitoring, blood lead measurement and neurodevelopmental assessment that may apply to other contexts.

Air pollution is the fifth leading global cause of mortality and numerous studies have linked this exposure with adverse neurodevelopmental outcomes in high-income and middle-income countries. However, comparable data are lacking for low-income countries. Thus, this study addresses a serious disparity in air pollution research. Importantly, this study informs contemporary exposures to common pollutants of concern and puts into practice targeted activities to support the implementation of rigorous methods and dissemination of study findings to promote research to policy translation.

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