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## A protocol for measuring coal fly ash, heavy metals, and neurobehavioral symptoms in children aged 6-14 residing near coal-fired power plants.

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3 **A protocol for measuring coal fly ash, heavy metals, and neurobehavioral symptoms in**  
4 **children aged 6-14 residing near coal-fired power plants.**  
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9 Kristina M. Zierold<sup>1</sup>, Abby N. Hagemeyer<sup>2</sup>, Clara G. Sears<sup>3</sup>, Guy N. Brock<sup>4</sup>, Barbara J. Polivka<sup>5</sup>,  
10 Charlie H. Zhang<sup>6</sup>, Lonnie Sears<sup>7</sup>.  
11

- 12 1. Department of Environmental Health Sciences, University of Alabama at Birmingham,  
13 Birmingham, Alabama
- 14 2. Department of Epidemiology and Population Health, University of Louisville, Louisville,  
15 Kentucky
- 16 3. Department of Epidemiology, Brown University, Providence, Rhode Island
- 17 4. Department of Biomedical Informatics, The Ohio State University, Columbus, Ohio
- 18 5. School of Nursing, University of Kansas Medical Center, Kansas City, Kansas
- 19 6. Department of Geography & Geosciences, University of Louisville, Louisville, Kentucky
- 20 7. Department of Pediatrics, University of Louisville, Louisville, Kentucky
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30  
31 Corresponding Author:  
32 Kristina M. Zierold, PhD, MS  
33 Associate Professor  
34 Department of Environmental Health  
35 School of Public Health  
36 RPHB 534C  
37 1720 2<sup>nd</sup> Ave S  
38 Birmingham, AL 35294-0022 USA  
39 (502) 934-1091  
40 [kzierold@uab.edu](mailto:kzierold@uab.edu)  
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## ABSTRACT

**Introduction:** Fly ash is a waste product generated from burning coal for electricity. It is comprised of spherical particles ranging in size from 0.1 micrometer to over 100 micrometers in diameter that contain trace levels of heavy metals. Large countries such as China and India generate over 100 million tons per year while smaller countries like Italy and France generate 2-3 million tons per year. The United States generates over 36 million tons of ash, making it one of the largest industrial waste streams in the nation. Fly ash is stored in landfills and surface impoundments exposing communities to fugitive dust and heavy metals that leach into the groundwater. Limited information exists on the health impact of exposure to fly ash. This protocol represents the first research to assess children's exposure to coal fly ash and neurobehavioral outcomes.

**Methods:** We measure exposure to fly ash, heavy metals, and neurobehavioral symptoms in children aged 6-14 years old. Using air pollution samplers and lift tape samples, we collect particulate matter  $\leq 10 \mu\text{m}$  that is analyzed for fly ash and heavy metals. Toenails and fingernails are collected to assess body burden for seventy-two chemical elements. Using the Behavior Assessment and Research System and the Child Behavior Checklist, we collect information on neurobehavioral outcomes. Data collection began in September 2015 and will continue until January 2021.

**Ethics and Dissemination:** This study was approved by the Institutional Review Boards of the University of Louisville (#14.1069) and the University of Alabama at Birmingham (#300003807). We have collected data from 267 children who live within 10 miles of two power plants. Children are at a greater risk for environmental exposure which justifies the rationale for this study. Results of this study will be distributed at conferences, in peer-reviewed journals, and to the participants of the study.

## STRENGTHS AND LIMITATIONS OF THIS STUDY

- A major strength of this innovative study is that it is the first research to measure children's indoor exposure to fly ash, which is an emerging environmental health concern throughout the world.
- This study design includes children aged 6-14 years old, who are more susceptible to environmental exposures, like fly ash and heavy metals.
- Multiple measures of fly ash and heavy metals from environmental (air and lift tape samples) and biological (toenails and fingernails) samples are being collected.
- Neurobehavioral performance and symptoms are measured utilizing two methods: the Behavior Assessment and Research System (BARS) and the Child Behavior Checklist (CBCL).
- Although methods were used to reduce bias in the sample of participants, it is possible that some participants were more concerned about fly ash exposure and hence more likely to participate in the study.

## INTRODUCTION

Coal ash is a waste product that is produced from coal-fired power plants. When coal is burned for energy in pulverized fuel combustion chambers, it generates heat, and produces a molten mineral residue. As heat is extracted by the boiler tubes, flue gas is cooled and the residue hardens and forms an ash. Larger, heavier ash particles fall to the bottom of the combustion chamber. Lighter ash particles remain in the flue gas and are collected in air pollution control devices. These lighter ash particles are termed fly ash and compose 40-80% of coal ash<sup>1-4</sup>.

Coal fly ash is a fine silt of spherical powdery particles with diameters ranging from less than 0.1  $\mu\text{m}$  to over 100  $\mu\text{m}$ . The average size range of the respirable fraction of fly ash is from 1.98  $\mu\text{m}$  to 5.64  $\mu\text{m}$ <sup>5</sup>. Although fly ash is mainly composed of silica, aluminum, iron, calcium, and oxygen, trace elements such as arsenic, chromium, and lead may be found in fly ash<sup>1,5-9</sup>. The composition of fly ash depends on the geochemical properties of the coal, the preparation of the coal, and the burning process, but research has shown that metal concentrations are much greater than those found in the parent coal<sup>10-12</sup>.

In 2018, over 36 million tons of fly ash were generated in the United States (US), making it one of the largest industrial waste streams nationwide<sup>13</sup>. China and India generate more fly ash than the US<sup>14</sup>. Throughout the world, countries vary in the amount of fly ash that is beneficially used in products such as concrete and grout. In the US, approximately 55-65% of fly ash is reutilized<sup>13-14</sup>, however countries like China and India, where coal combustion is increasing, use less than 50% of fly ash<sup>14</sup>. The fly ash that is not reutilized is stored in landfills and surface impoundments with limited regulations, which impose critical environmental and public health concerns.

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3 Landfills and surface impoundments containing fly ash expose nearby communities to  
4 potentially harmful trace elements. Humans can be exposed to fly ash and the metals contained  
5 in the particles by inhaling fugitive dust and ingesting contaminated groundwater. Although the  
6 toxicity and hazard potential of coal ash exposure is high due to potential exposure to trace  
7 elements, there is limited research on the health effects of chronic coal ash exposure among  
8 children. Researchers investigating health among children exposed to fly ash or living in  
9 proximity to power plants have reported greater neurodevelopment conditions, like attention  
10 deficit hyperactivity disorder (ADHD), increased sleep problems, increased respiratory  
11 conditions, and increased gastrointestinal problems<sup>15-17</sup>. These studies were limited in that  
12 residential location or distance from coal-fired power plants was used as a proxy for exposure to  
13 coal ash. None of the studies directly measured in-home exposure to fly ash.  
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28 Children have a higher risk for negative health outcomes related to fly ash exposure.  
29 Compared with adults, children are more likely to breathe through their mouth, breathe more air  
30 relative to their lung size and body weight, are physically closer to the ground-level, are more  
31 likely to engage in hand-to-mouth behaviors and are less likely to stop activity if they experience  
32 respiratory distress. Their brains and lungs are still developing<sup>18-20</sup>.  
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#### 40 **Study Aims**

41  
42 The overall objective of this community-based study is to evaluate fly ash exposure and  
43 the prevalence of neurobehavioral performance and symptoms of 300 children living within 10  
44 miles of two power plants in Jefferson County, Kentucky. Fly ash exposure is measured in  
45 particulate matter  $\leq 10\mu\text{m}$  (PM<sub>10</sub>) samples and lift tape samples. Neurobehavioral outcomes are  
46 assessed by the Behavioral Assessment and Research System (BARS) and the Child Behavior  
47 Checklist (CBCL). The two specific aims from the study that are emphasized in this protocol  
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3 paper are to: (1) Characterize indoor exposure to fly ash and heavy metals in homes of children  
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5 living within close proximity to power plants with coal ash storage facilities and (2) Assess if  
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7 increased fly ash exposure and greater heavy metal body burden is associated with poorer  
8  
9 neurobehavioral health.  
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11  
12 Fly ash is a significant environmental problem with emerging public health impacts. This  
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14 study is novel in that it is the first to measure fly ash in the homes of children. Furthermore, it  
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16 is the first community-based study to utilize these exposure measures to understand the impact  
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18 of exposure on children's neurobehavioral health.  
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## 21 22 23 **METHODS AND ANALYSIS**

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26 This is a cross-sectional study with an exposure assessment. The study takes place in  
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28 Jefferson County and Bullitt County, Kentucky. Jefferson County is home to two power plants  
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30 that are approximately 10 miles apart. Both plants contain large fly ash storage facilities  
31  
32 (landfills and surface impoundments) on site. Data collection began in September 2015 and will  
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34 end on January 31, 2021.  
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### 37 38 **Patient and Public Involvement**

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40 During the design of the grant proposal and this resulting protocol manuscript, no patients  
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42 or the public were involved.  
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### 44 45 **Participant recruitment and sample size**

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47 Our study area represents more than 12 zip codes throughout southwestern Jefferson  
48  
49 County and northern Bullitt County, Kentucky. To ensure participants are representative of the  
50  
51 population throughout the study area, we used Geographical Information Systems (GIS) methods  
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53 to identify and recruit study participants<sup>21</sup>. First, we stratified the study area using a series of  
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3 buffer zones at 2-mile intervals from 0-10 miles from the centroid of the straight line that  
4 connects the two power plants. Additionally, buffer zones were stratified by wedge-shaped  
5 quadrants. This method divided our study area into 20 sampling units (SUs). Prevalence  
6 estimates of neurobehavioral conditions for exposed children were selected to range between  
7 20% and 30%, based on findings from a cross-sectional study that assessed children's health in  
8 four communities residing near a coal-fired power plant<sup>17</sup>. The prevalence of symptoms in the  
9 non-exposed children, were estimated at values of 5% and 10%. These values represent a range  
10 for neurobehavioral conditions in the U.S., such as ADHD (6.8%) and behavioral conduct  
11 problems (3.5%)<sup>22</sup>. Based on a simulated power calculation, we determined that 300 children  
12 needed to be recruited for this study to achieve near 80% power in most scenarios (Table 1).  
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26 Recruitment methods vary and include “shoe-leather” methods, where the research team  
27 goes door-to-door talking with participants about the study and/or leaving flyers at their homes.  
28 Additionally, we mail letters and flyers to potential participants in the zip codes in our study  
29 area, and have used social media, newspaper articles, and television appearances to publicize the  
30 study. Furthermore, we have utilized snowballing methods, asking current participants to ask  
31 their friends and neighbors to recruit additional households that are eligible for this study.  
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#### 40 **Enrollment and consent**

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42 Adult consent and child assent is obtained at the first home visit. Consenting is conducted  
43 in English prior to data collection. Research team members review the consent and assent  
44 documents, answer any questions that the parent/guardian or child has, and confirm the  
45 understanding of the study. Participants and research team members sign two copies of the  
46 consent and assent documents, one for the participants to keep and one for the research team to  
47 keep on file.  
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**TABLE 1:** Power for varying scenarios of sample size per zone, exposures for each zone, and probabilities of symptoms for exposed and unexposed individuals. Grey highlighted cells indicate scenarios where  $\geq 80\%$  power is achieved.

Pr(sym | exposed) and Pr(sym | unexposed)

Sample Size / Zone	Sample Size Total	Exposure / Zone	0.3,0.1	0.3,0.05	0.25,0.1	0.25,0.05
60	300	1,0.8,0.5,0.2,0	0.80	0.97	0.62	0.91
		0.8,0.7,0.5,0.2,0	0.92	1.00	0.75	0.98
		0.7,0.5,0.3,0.1,0	0.91	1.00	0.73	0.97
		0.6,0.5,0.4,0.3,0	0.92	1.00	0.83	0.99
		0.6,0.5,0.4,0.3,0.2	0.99	1.00	0.88	1.00

## Exposure assessment methods

For this study, we conduct air monitoring and collect lift samples to quantify exposure to particulate matter  $< 10 \mu\text{m}$  ( $\text{PM}_{10}$ ) and identify fly ash particles inside children's home environments. In these samples, we also analyze the composition of metals and metalloids in particulate matter and fly ash particles. Additionally, toenails and fingernails are collected from children to assess elemental body burden. A registered nurse (RN) visits the homes and collects the child's vital signs, completes a pediatric health history, and conducts a home exposure assessment. Multiple questionnaires are used to collect additional information. Analytical methods used include Proton-Induced X-Ray Emissions (PIXE), Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray (EDX). Figure 1 details the exposure assessment and analysis methods of the samples, which is provided in detail below.

FIGURE 1 HERE

### Indoor Air measurements

Indoor  $\text{PM}_{10}$  is measured using both continuous particle monitors (EPAM-7500) and a single-stage personal modular impactor (PMI) (SKC Inc.) connected to an AirChek XR5000 pump. The EPAM is a portable particulate monitor that provides real-time measurement and display of  $\text{PM}_{10}$ , particulate matter  $\leq 2.5 \mu\text{m}$  ( $\text{PM}_{2.5}$ ) and particulate matter  $\leq 1.0 \mu\text{m}$ . The EPAM utilizes optical light scattering for real-time measurements. It is placed in participant's homes and configured to measure  $\text{PM}_{10}$  every minute. The EPAM runs for one week.

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3 Inside the cassette of the PMI is a 37 mm polycarbonate filter that collects PM<sub>10</sub>. A 25 mm  
4 pre-oiled disposable impaction disc is inserted onto the top of the filter cassette to decrease  
5 particle bounce and allow for more efficient particle collection. Polycarbonate membrane filters  
6 were selected because of their properties that allow for analysis by optical microscopy  
7 techniques. To determine the total mass of PM<sub>10</sub> that is collected, gravimetric analysis is  
8 conducted. Prior to being inserted into the cassette of the PMI, each polycarbonate filter is  
9 weighed three times using a BM-20 analytical microbalance. The average of these measurements  
10 is known as the pre-weight. Once the PMI is removed from the field, the filter is weighed three  
11 times. The average of these measurements is known as the post-weight. Subtracting the pre-  
12 weight from the post-weight provides the total mass of PM<sub>10</sub> that is collected from the home.  
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26 The PMI is connected to an AirChek XR5000 air sampling pump via ¼ inch diameter tygon  
27 tubing. These small, lightweight pumps are specifically designed to provide accurate ( $\pm 5\%$  of  
28 set-point) airflows between 1-5 liters per minute (L/min) by using an isothermal closed loop flow  
29 sensor. The isothermal closed loop flow sensor directly measures and constantly maintains the  
30 set flow rate. To compensate for fluctuations in temperature after the pump has been calibrated,  
31 the AirChek XR5000 has a built-in sensor. In the case of excessive backpressure, for example if  
32 the filter becomes overloaded, the AirChek XR5000 is designed to stop after >15 seconds. The  
33 pump will display a flow fault icon on the screen and attempt to restart up to five times every 15  
34 seconds. Before placing the pumps into the homes, they are calibrated using a MesaLabs DryCal  
35 Defender 510 in the laboratory. After calibration, three flow rate readings are taken one minute  
36 apart and recorded. All readings are within  $\pm 5\%$  of 3 L/min, which is the recommended flow  
37 rate for optimal PMI performance. The initial flow rate is calculated by averaging these three  
38 readings.  
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3 Using tripod stands, the PMI is placed roughly 1-1.5 meters above the ground to simulate the  
4 breathing zone of an average child. Additionally, strategic placement of the PMI and air pump  
5 avoids windows, doors to the outside, air vents, fireplaces, stoves, and electronic devices to  
6 avoid resuspension of particles. Once in place, the PMI and air pump are turned on and continue  
7 to run in the participant's home for approximately one week. At the end of the air sampling  
8 period, three to four flow rate measurements are taken with the DryCal and recorded. The  
9 average of these measurements is known as the final flow rate. The overall flowrate is  
10 determined by taking the average of the sum of the initial flowrate and final flowrate. Using the  
11 overall flowrate and the total mass of PM<sub>10</sub>, as determined by gravimetric analysis, the  
12 concentration of PM<sub>10</sub> is determined. Calculating the mass concentration on the filters is a vital  
13 step in determining the elemental distribution in subsequent laboratory methods. To determine  
14 the elemental composition of PM<sub>10</sub>, PIXE is used. To determine the presence of fly ash and the  
15 composition of fly ash, SEM/EDX is used.  
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### 35 Analytical methods used

#### 36 *Proton Induced X-ray Emission Spectroscopy (PIXE)*

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38 PIXE is useful if the elemental concentrations are low or if the elements are present at  
39 unknown concentrations. PIXE is an analytical method in which energetic protons transfer  
40 kinetic energy to the inner shell electrons of the target atom, forcing the electrons from the atom  
41 resulting in X-ray production<sup>23</sup>. The X-ray spectrum and energies are unique to the element from  
42 which they were emitted and the amount of X-rays emitted corresponds to the mass of the  
43 particular element being assessed in the sample<sup>23</sup>. There are several advantages to PIXE analysis.  
44 First, because it is a non-destructive analysis method, errors from sample digestion and  
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3 preparation are alleviated. Secondly, PIXE is capable of simultaneously analyzing 72 inorganic  
4 elements from sodium to uranium in liquid, solid, and aerosol filter samples.  
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### 8 9 *Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (SEM/EDX)*

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11 Fly ash particles are distinguished from other particles by their morphological and  
12 chemical properties. Fly ash particles are smoothly spherical, which are very distinct from other  
13 metallurgical emissions. Therefore, fly ash particles can be identified through microscopic  
14 methods. In addition to morphological differences, fly ash is chemically different than other  
15 particulate matter. For example, metallurgical emissions are characterized by the elements, FE  
16 (iron), MN (manganese), and SI (silicon). Particles from the steel industry are characterized by  
17 FE, MN, SI, and aluminum (AL). Fly ash particles are characterized by SI, sulfur (S), potassium  
18 (K), calcium (CA), and FE. This metal “fingerprint” is used to identify the presence of fly ash in  
19 our samples.  
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32 SEM/EDX is a quick, non-destructive surface analytical technique that creates high  
33 resolution images of surface topography. Primary electrons, produced from the scanning electron  
34 beam, bombard the sample’s surface and thus generate secondary electrons. The secondary  
35 electron’s low energy intensity is greatly affected by the surface topography of the sample. The  
36 surface image is generated by measuring the intensity of the secondary electron as a function of  
37 the scanning electron beam’s position. Because of the primary electron beam’s ability to focus  
38 on an area <10 nm in size, high resolution images are possible. Primary electron bombardment  
39 from the scanning beam also creates backscattered electrons that indicate the elements in the  
40 sample<sup>24</sup>. Identification of an element is possible because the backscatter electron intensity is  
41 associated with the atomic number of a specific element.  
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55 In addition to secondary and backscattered electrons, the scanning electron beam creates  
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3 X-rays. As previously discussed in the PIXE section, X-rays are unique to the corresponding  
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5 element. Therefore, analysis of the X-ray can provide semi-quantitative information on the  
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7 elements in the sample<sup>24</sup>.

### 10 Lift Tape Samples

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12 During the first home visit, lift tape sampling is conducted. Lift sampling is a simple  
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14 method for removing particles from a surface to determine their number and size distribution.  
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16 We use Stick-To-It Lift Tape (SKC, Inc) to identify the presence of fly ash on multiple surfaces  
17  
18 in children's bedrooms. Stick-to-it Lift Tape is a flexible plastic microscope slide with an  
19  
20 adhesive area that can be used for sampling inorganic dust contamination on surfaces. These lift  
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22 tapes are non-destructible and have a consistent sample area. In each child's bedroom, three  
23  
24 standard locations, a bedframe, window, and dresser, are sampled. The lift tape samples undergo  
25  
26 optical microscopy to determine the presence or absence of fly ash in the dust samples and  
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28 provide the percent of fly ash on the samples, as well as the elemental concentration of fly ash  
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30 particles.  
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### 36 Activity Assessment

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38 In addition to air sampling and lift sampling, an activity diary is filled out by each  
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40 participant. The types of activities recorded include: cooking, use of secondary heating sources,  
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42 use of indoor fans, burning candles or incense, cleaning activities and use of chemicals,  
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44 construction, presence of pets, open/closed windows, and smoking. This information will  
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46 provide insight into differences in fly ash and metal concentrations that occur among the  
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48 samples.  
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### 54 Registered Nurse Visit



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3 After air sampling and lift sampling is completed, an RN schedules an appointment with  
4 the parents/guardians to visit the home. The nurse's visit takes approximately one hour to  
5 complete. While at the home, the RN measures the child's height, weight, and blood pressure,  
6 and completes the Pediatric Health History Interview and Environmental Home Assessment.  
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### 13 *Pediatric Health History Interview.*

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16 The Pediatric Health History Interview form includes demographic information about the  
17 participant and parents, current and past health conditions, past hospitalizations, current  
18 medications use, parents' perception of health and behavior, immunizations history, details of  
19 pregnancy complications and use of substances during pregnancy and delivery, breastfeeding,  
20 early childhood development, the child's current participation in school activities and behavior at  
21 school and at home, and a brief health history of the immediate family living in the home. The  
22 interview form was developed by investigators of the study, after evaluating several standard  
23 pediatric health assessment forms.  
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### 35 *Environmental Home Assessment.*

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38 A visual assessment of the home is conducted using the publicly-available Pediatric  
39 Environmental Home Assessment (PEHA) tool developed by the National Center for Healthy  
40 Housing. The PEHA includes a subjective determination of general home characteristics and  
41 indoor pollutants and observation of the general home environment, the sleep environment, and  
42 home safety<sup>25</sup>. Information such as type of house, age of home, type of foundation, number of  
43 floors, sources of heating and cooling, the presence of indoor pollutants (presence of molds,  
44 lead-based paints, asbestos, radon, environmental smoke), the RN's assessment of the cleanliness  
45 of the home environment, details of the participants sleep environment (number of beds in room,  
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3 allergens, pillows, bedding, flooring, etc.), and home safety (renovations, lighting, poison  
4 control, fire hazards, appropriate storage of chemicals and hot liquids, window guards, etc.) is  
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6 collected.  
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### 10 11 12 13 Toenails and Fingernails 14

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16 Heavy metal body burden is assessed by collecting toenails and fingernails from the child  
17 participants. Toenails and fingernails are a useful measure of metals because they represent long-  
18 term exposure given the slow growth rate, are less likely to be contaminated, are non-invasive,  
19 and are easy to collect and store. Toenails and fingernails reflect exposure integrated over the  
20 preceding 3-12 months and concentrations of elements may vary due to age, gender, behaviors,  
21 and diet<sup>26, 27</sup>.  
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30 Parents are asked to begin collecting their child's toenails and fingernails during the  
31 initial phone conversation, prior to the initial visit. During the initial visit, any nails the child had  
32 already cut are collected, in addition to any nails the child cuts during the visit. For each  
33 participant, approximately 150 mg of nails are collected over.  
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40 Once the total amount of nails is collected, they are cleaned using one acetone wash and  
41 two deionized water washes. The nails are then dried and weighed a final time before being  
42 placed in a container to transport to the laboratory for analysis. Children's nails are cryogenically  
43 frozen, ground, and bound into a 3/8-inch pellet, with the natural binding agent Somar-Mix  
44 Power #210, a mixture of boric acid and water. The pellet is then analyzed by PIXE to determine  
45 the amount and type of elements in the sample.  
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## Study Questionnaires

Parents or guardians of the participating children complete the Environmental Health History Questionnaire (EHH) and Home Cleaning Questionnaire (HC). The EHH consists of 108 questions and is based on existing pediatric environmental exposure history guides such as the Pediatric Environmental History<sup>28</sup>, the pediatric exposure history questions to be included in a well-child visit<sup>29</sup>, and the American Academy of Pediatrics guidance on taking an environmental history<sup>30</sup> as well as The Agency for Toxic Substances and Disease Registry's "Taking an Exposure History,<sup>31</sup>" and the rapid questionnaire of environmental exposures to pregnant women<sup>32</sup>. The HC has 9 questions related to cleaning behaviors. The questionnaires are left with the parents or guardians for approximately one week and returned upon completion.

## **Study outcomes being measured**

To assess neurobehavioral performance and symptoms, we utilize the Behavior Assessment and Research System (BARS) and CBCL. Both were completed at the participant's home.

### **Child Behavior Checklist (CBCL)**

Although there are several instruments available that assess problem behaviors in children, the CBCL is among the most respected and widely used; it has been translated into over 90 languages<sup>33, 34</sup>. The CBCL is a psychometrically-sound, research tool for evaluating children's emotional, behavioral, and social functioning. Although there are CBCL forms available for different age groups, this study focuses on the CBCL for ages 6-18 years of age.

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2  
3 Additionally, there are parent, teacher, and child report forms. For this study, we are using the  
4 parent-report form. The CBCL's questions are associated with problems on a syndrome scale in  
5  
6 parent-report form. The CBCL's questions are associated with problems on a syndrome scale in  
7  
8 eight different categories: anxious/depressed, withdrawn/depressed, somatic complaints, social  
9  
10 problems, thought problems, attention problems, rule-breaking behavior, and aggressive  
11  
12 behavior.  
13

14  
15 Anxious/depressed, withdrawn/depressed, and somatic complaints are broadly  
16  
17 categorized as internalizing behaviors. Rule-breaking behaviors and aggressive behaviors are  
18  
19 broadly categorized as externalizing behaviors. Overall, the CBCL yields scores for internalizing  
20  
21 and externalizing behaviors, total problems, and six Diagnostic and Statistical Manual of Mental  
22  
23 Disorders (DSM)- oriented subscales. The six DSM-oriented subscales include attention  
24  
25 deficit/hyperactivity problems, anxiety problems, oppositional defiant problems, affective  
26  
27 problems, conduct problems, and somatic problems<sup>35</sup>. Based on age and sex, these scores are  
28  
29 compared to clinical cut off points for the particular comparison group.  
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32  
33 The CBCL is left with the parents/guardians for one week and returned upon completion.  
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35 Based on parents'/guardians' responses to the 124-item questionnaire, t-scores are calculated  
36  
37 using standardized norms for age and gender. If a participant scores in the clinical or borderline  
38  
39 range on any of the CBCL subscales, the child psychologist follows-up with the  
40  
41 parents/guardians of the child by conducting a Structured Clinical Interview for Diagnosis of  
42  
43 DSM Disorders.  
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#### 46 47 48 49 Behavior Assessment and Research System (BARS)

50  
51 Neurobehavioral performance is assessed in all children using the Behavioral Assessment  
52  
53 and Research System (BARS)<sup>36</sup>. BARS, which administers a series of neurobehavioral tests,  
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3 includes a 9-button device that sits on top of a  
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5 standard laptop. Child participants hit a button  
6  
7 from 1 to 9 corresponding to their answer.  
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10 BARS was developed by the Oregon Health  
11  
12 and Science University to provide a series of  
13  
14 neurobehavioral tests that are optimized to  
15  
16 detect neurotoxicity<sup>36</sup>. It has been adapted for  
17  
18 use with children as young as preschool age<sup>37-</sup>  
19  
20 <sup>39</sup>. BARS has been used for children exposed to  
21  
22 neurotoxic chemicals (pesticides) but has not previously been utilized for children exposed to fly  
23  
24 ash in their community<sup>37, 38</sup>.  
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29  
30 The six BARS tests that are used to assess neurobehavioral performance are displayed in  
31  
32 Table 2. Comparisons in formal studies have shown that BARS tests have comparable test-retest  
33  
34 reliabilities with the tests given in their original testing formats<sup>36</sup>. In addition to the BARS tests,  
35  
36 three additional tests are used: the Recall of Objects Immediate and Recall of Objects Delayed,  
37  
38 Purdue Pegboard, and Beery-Buktenica Developmental Test of Visual-Motor Integration. These  
39  
40 nine tests cover a range of neurobehavioral performance  
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Table 2. Neurobehavioral Tests

Test	Measured Functions
<b>BARS Tests</b>	
Symbol-Digit	Speed, Attention/Integration
Finger Tapping	Response Speed and Coordination
Digit Span	Memory and Attention
Continuous Performance	Attention
Matching-to-Sample	Visual Memory
Selective Attention	Attention

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5 A child psychologist administers the  
6  
7 nine tests in the evening hours during the  
8  
9 weekdays or on a Sunday afternoon. The

<b>Additional Tests</b>	
Recall of Objects Immediate and Recall of Objects Delayed	Recall and Recognition Memory
Purdue Pegboard	Dexterity
Visual Motor Integration	Hand-eye Coordination

12 BARS tests are administered continuously as the child sits comfortably at a table. While the  
13  
14 study team members are present throughout the entire test and answer questions as they arise,  
15  
16 there is minimal interaction with the children during the BARS tests. The children interact with  
17  
18 the computer. These tests are given continuously and in the same order for each child. When the  
19  
20 BARS is completed, the psychologist administers the other three tests. It takes approximately 40  
21  
22 minutes to complete the testing.  
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### 28 **Planned statistical analysis**

30  
31 Characterization of the metal concentrations on filters and in nail samples will be  
32  
33 stratified by sampling zone and evaluated using exploratory data analysis methods including  
34  
35 boxplots, histograms, and kernel density estimates. Sampling units will be grouped into exposure  
36  
37 zones on the basis of the minimal distance from either of the two plants. Differences between  
38  
39 these exposure zones will be evaluated using one-way analysis of variance (ANOVA) or the  
40  
41 Kruskal-Wallis test, depending on whether the data are normally distributed. The use of  
42  
43 transformations (e.g. log, Box-Cox power transformation) will be explored. Additionally,  
44  
45 associations between metal concentrations and individual distance from the nearest plant for each  
46  
47 household will be explored using Pearson/Spearman correlations and linear regression models.  
48  
49  
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51 For toenails and fingernail samples, if the majority (e.g.,  $\geq 75\%$ ) of children have levels  
52  
53 below the Limit of Detection (LOD), concentrations will be dichotomized as present / absent and  
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3 analyzed for differences between zones using logistic regression. If the majority of  
4  
5 concentrations are detectable, then differences between children within each exposure zone will  
6  
7 be evaluated using either one-way ANOVA or the non-parametric Kruskal-Wallis test. If there  
8  
9 is a mixture of detectable and below the LOD concentrations, a total metal score will be  
10  
11 calculated, similar to the method of Cave et al, 2010. Briefly, since metal concentrations are on  
12  
13 different scales, each metal concentration will be ranked and then aggregated and grouped into  
14  
15 quartiles of overall metal concentration<sup>40</sup>.  
16  
17

18  
19 Percentage of fly ash found in the filter samples will be analyzed in a similar fashion to  
20  
21 the metal concentrations. Presence of fly ash will also be dichotomized into present/absent, and  
22  
23 evaluated for differences between exposure zones. Adjustment for other environmental factors  
24  
25 and activities potentially influencing metal concentrations (e.g. smoking,) will be accounted for  
26  
27 using multivariable regression models.  
28  
29

30  
31 Association between the BARS tests / CBCL t-scores and exposure zone / distance from  
32  
33 the plant will be evaluated using a linear regression model, with possible transformations (Box-  
34  
35 Cox) when responses are non-normally distributed. A similar model will be used to investigate  
36  
37 potential associations between BARS and CBCL scores and fly ash exposure / heavy metal body  
38  
39 burden. In addition to investigating associations with continuous CBCL scores, CBCL scores  
40  
41 will be dichotomized at a level indicative of a disorder and analyzed for association with fly ash  
42  
43 exposure using either logistic regression or the Cochran-Mantel-Haenszel test (with exposure  
44  
45 zone or sampling unit as the strata). Initially, each exposure variable (fly ash, heavy metal  
46  
47 concentration) will be analyzed individually to determine significant marginal associations with  
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49 BARS / CBCL t-scores, with p-values adjusted for multiple comparisons to control the false-  
50  
51 discovery rate using the Benjamini-Hochberg approach<sup>41</sup>. After any significant marginal  
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3 associations have been identified, potential confounding variables (demographics, exposure and  
4 activity history) will be adjusted for using multivariable regression models. Since missing values  
5 can have a compounding effect in multivariable regression models, percentage of missing values  
6 will be evaluated for each variable and checked for association with zone and other important  
7 covariates. If found to depend on these variables, multiple imputation strategies will be used to  
8 impute missing values and fit multivariable regression models.  
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## 19 **ETHICS AND DISSEMINATION**

### 21 **Ethics**

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24 Written informed consent is obtained from the parents/guardians and written informed  
25 assent is obtained from all participating children. Trained study personnel explain the informed  
26 consent documents to the parents/guardians and the assent document to the children. After the  
27 explanation, parents/guardians and children sign the documents. Two copies of the  
28 consent/assent documents are signed; one copy is kept by the parents/guardians and one copy is  
29 kept by the researchers. These consenting procedures were approved by the Institutional Review  
30 Board of the University of Louisville (IRB # 14.1069) and the University of Alabama at  
31 Birmingham (IRB#:300003807), where the principal investigator (PI) of the grant is currently  
32 employed.  
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### 49 **Dissemination**

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51 All findings from this study will be disseminated through publications in peer-reviewed journals  
52 and presentations at national and international conferences. In addition, results will be provided  
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3 to the participants of the study. Within three months, the child psychologist makes multiple  
4 attempts to contact and discuss the neurobehavioral outcomes with parents/guardians if the child  
5 has scored poorly on the CBCL. The environmental results will be returned after the study  
6 concludes with the final enrollment. At this time, the researchers will create summary statistics  
7 based on the community that can be compared.  
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## 17 **CONCLUSION, STRENGTHS, AND LIMITATIONS**

19 This protocol paper describes our research that represents the first study to assess  
20 children's exposure to in-home fly ash and prevalence neurobehavioral outcomes. The health  
21 impacts of fly ash are unknown, but the potential risks are immense. Currently most countries of  
22 the world do not consider fly ash as a hazardous waste, so the regulations regarding its storage  
23 and disposal are limited. Disposal methods permit fugitive dust to escape leading to increases in  
24 ambient air pollution. Numerous epidemiological studies have associated particulate matter with  
25 cancer, heart disease, asthma, and/or increased mortality. The potential impact of this innovative  
26 study is great as it will provide evidence to describe the environmental health impacts of fly ash  
27 exposure. Better understanding the exposure that communities living near fly ash storage  
28 facilities may help to provide impetus for better regulations for its storage.  
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### 47 **Strengths**

49 This study has several strengths. Regarding exposure, we are able to quantify indoor  
50 PM<sub>10</sub> concentrations and determine if fly ash is found in the home. Children spend the majority  
51 of time indoors<sup>42</sup> and the EPA reports that concentrations of pollutants can be 2-5 times higher  
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3 indoors than outdoors<sup>43</sup>. For measurement of fly ash, we are using both air sampling and lift tape  
4 sampling which provides us a characterization of in-home exposure. For the assessment of the  
5 outcomes, we are utilizing several measures of neurobehavioral assessment including BARS and  
6 the CBCL. BARS is administered at the homes of the participating children by a child  
7 psychologist. The same psychologist conducts all the testing, assuring consistency in the  
8 protocol. Community members were involved in recruitment of participants for this study.  
9  
10 Research has shown that studies that involve community members have lowered attrition,  
11 increased compliance, improved accuracy and greater applicability and usability in the settings  
12 where community-based research occurs<sup>44-48</sup>.  
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#### 23 Limitations

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26 Although there are many strengths of this innovative study, there are some limitations.  
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28 First, we assume that a week-long in-home air sample is representative of children's chronic  
29 exposure. While children spend hours in their homes, they also spend times in other indoor  
30 locations. In Jefferson County, Kentucky, children do not necessarily attend their neighborhood  
31 schools, so exposure may be increased or decreased depending on location of their school.  
32  
33 Second, we are not measuring weather patterns. Although we are trying to recruit equal numbers  
34 of children by season, sampling occurs on different days and during different years. We hope to  
35 consider these differences by performing additional analyses that will account for wind speed,  
36 wind direction, moisture, and temperature. Third, participants who are more concerned about fly  
37 ash pollution or whose children have pre-existing health problems may be more likely to enroll  
38 in this study. To address this potential bias, recruitment materials do not have references to the  
39 health outcome we are assessing.  
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For peer review only

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6 **Author's Contributions:** KMZ is the principal investigator of the study, conceptualized the  
7 initial project design, and led the writing of this manuscript. ANH and CGS made substantial  
8 contributions to the writing of the manuscript and editing. LS, GNB, CZ, and PJB are co-  
9 investigators of the study and contributed to the conceptualization of the project and assisted in  
10 the writing and editing of the manuscript. All authors read and approved the final manuscript.  
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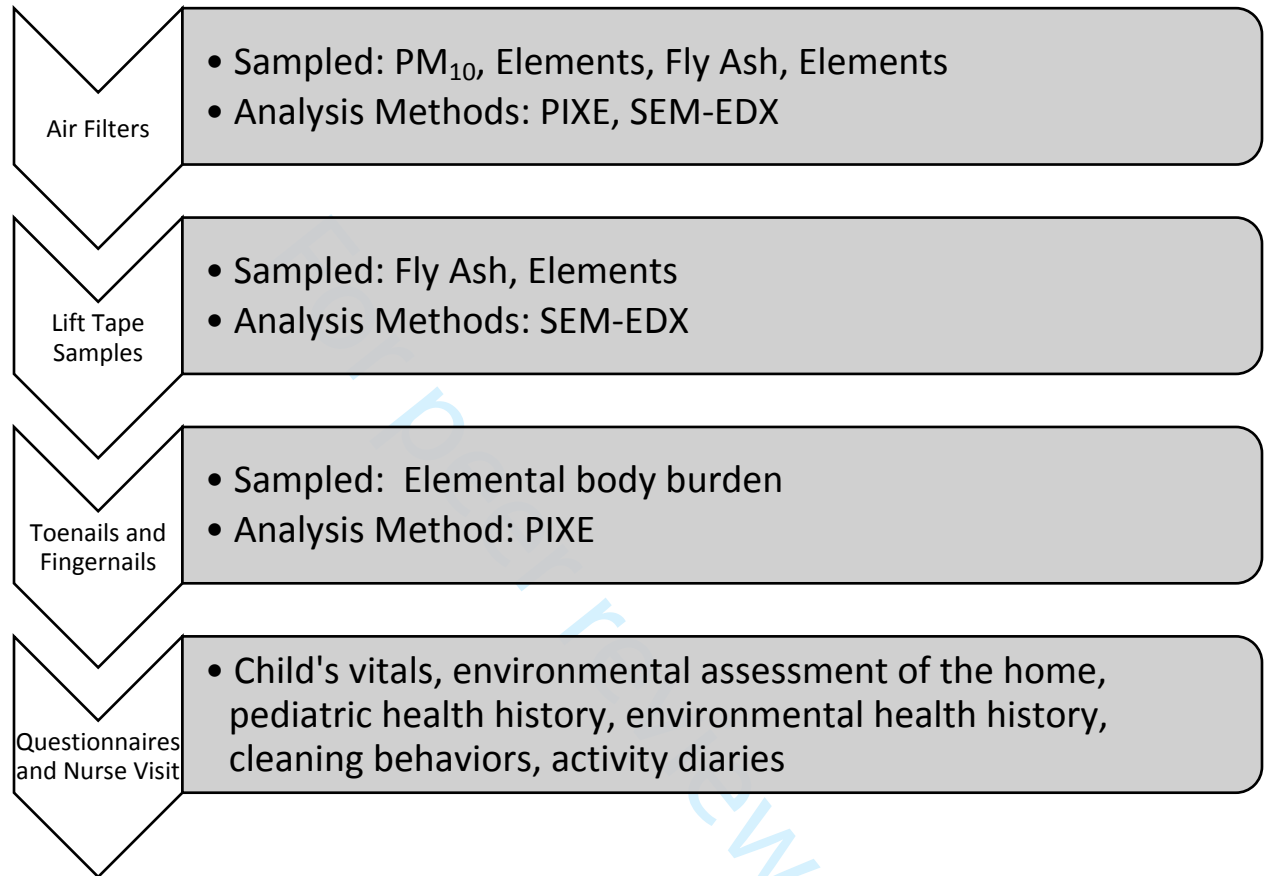


Figure 1. Exposure assessment, analytical methods used, and outcomes from the assessment.

# BMJ Open

## A protocol for measuring indoor exposure to coal fly ash and heavy metals, and neurobehavioral symptoms in children aged 6-14 residing near coal-fired power plants.

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3 **A protocol for measuring indoor exposure to coal fly ash and heavy metals, and**  
4 **neurobehavioral symptoms in children aged 6-14 residing near coal-fired power plants.**  
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9 Kristina M. Zierold<sup>1</sup>, Abby N. Hagemeyer<sup>2</sup>, Clara G. Sears<sup>3</sup>, Guy N. Brock<sup>4</sup>, Barbara J. Polivka<sup>5</sup>,  
10 Charlie H. Zhang<sup>6</sup>, Lonnie Sears<sup>7</sup>.  
11

- 12 1. Department of Environmental Health Sciences, University of Alabama at Birmingham,  
13 Birmingham, Alabama
- 14 2. Department of Epidemiology and Population Health, University of Louisville, Louisville,  
15 Kentucky
- 16 3. Department of Epidemiology, Brown University, Providence, Rhode Island
- 17 4. Department of Biomedical Informatics, The Ohio State University, Columbus, Ohio
- 18 5. School of Nursing, University of Kansas Medical Center, Kansas City, Kansas
- 19 6. Department of Geography & Geosciences, University of Louisville, Louisville, Kentucky
- 20 7. Department of Pediatrics, University of Louisville, Louisville, Kentucky
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30  
31 Corresponding Author:  
32 Kristina M. Zierold, PhD, MS  
33 Associate Professor  
34 Department of Environmental Health  
35 School of Public Health  
36 RPHB 534C  
37 1720 2<sup>nd</sup> Ave S  
38 Birmingham, AL 35294-0022 USA  
39 (502) 934-1091  
40 [kzierold@uab.edu](mailto:kzierold@uab.edu)  
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## ABSTRACT

**Introduction:** Fly ash is a waste product generated from burning coal for electricity. It is comprised of spherical particles ranging in size from 0.1 micrometer to over 100 micrometers in diameter that contain trace levels of heavy metals. Large countries such as China and India generate over 100 million tons per year while smaller countries like Italy and France generate 2-3 million tons per year. The United States generates over 36 million tons of ash, making it one of the largest industrial waste streams in the nation. Fly ash is stored in landfills and surface impoundments exposing communities to fugitive dust and heavy metals that leach into the groundwater. Limited information exists on the health impact of exposure to fly ash. This protocol represents the first research to assess children's exposure to coal fly ash and neurobehavioral outcomes.

**Methods:** We measure indoor exposure to fly ash and heavy metals, and neurobehavioral symptoms in children aged 6-14 years old. Using air pollution samplers and lift tape samples, we collect particulate matter  $\leq 10 \mu\text{m}$  that is analyzed for fly ash and heavy metals. Toenails and fingernails are collected to assess body burden for seventy-two chemical elements. Using the Behavior Assessment and Research System and the Child Behavior Checklist, we collect information on neurobehavioral outcomes. Data collection began in September 2015 and will continue until February 2021.

**Ethics and Dissemination:** This study was approved by the Institutional Review Boards of the University of Louisville (#14.1069) and the University of Alabama at Birmingham (#300003807). We have collected data from 267 children who live within 10 miles of two power plants. Children are at a greater risk for environmental exposure which justifies the rationale for this study. Results of this study will be distributed at conferences, in peer-reviewed journals, and to the participants of the study.

## STRENGTHS AND LIMITATIONS OF THIS STUDY

- A major strength of this innovative study is that it is the first research to measure children's indoor exposure to fly ash, which is an emerging environmental health concern throughout the world.
- This study design includes children aged 6-14 years old, who are more susceptible to environmental exposures, like fly ash and heavy metals.
- Multiple measures of fly ash and heavy metals from environmental (air and lift tape samples) and biological (toenails and fingernails) samples are being collected.
- Neurobehavioral performance and symptoms are measured utilizing two methods: the Behavior Assessment and Research System (BARS) and the Child Behavior Checklist (CBCL).
- Although methods were used to reduce bias in the sample of participants, it is possible that some participants were more concerned about fly ash exposure and hence more likely to participate in the study.



## INTRODUCTION

Coal ash is a waste product that is produced from coal-fired power plants. When coal is burned for energy in pulverized fuel combustion chambers, it generates heat, and produces a molten mineral residue. As heat is extracted by the boiler tubes, flue gas is cooled and the residue hardens and forms an ash. Larger, heavier ash particles fall to the bottom of the combustion chamber. Lighter ash particles remain in the flue gas and are collected in air pollution control devices. These lighter ash particles are termed fly ash and compose 40-80% of coal ash<sup>1-4</sup>.

Coal fly ash is a fine silt of spherical powdery particles with diameters ranging from less than 0.1  $\mu\text{m}$  to over 100  $\mu\text{m}$ . The average size range of the respirable fraction of fly ash is from 1.98  $\mu\text{m}$  to 5.64  $\mu\text{m}$ <sup>5</sup>. Although fly ash is mainly composed of silicon, aluminum, iron, calcium, and oxygen, trace elements such as arsenic, chromium, and lead may be found in fly ash<sup>1,5-9</sup>. The composition of fly ash depends on the geochemical properties of the coal, the preparation of the coal, and the burning process, but research has shown that metal concentrations are much greater than those found in the parent coal<sup>10-12</sup>.

In 2018, over 36 million tons of fly ash were generated in the United States (US), making it one of the largest industrial waste streams nationwide<sup>13</sup>. China and India generate more fly ash than the US<sup>14</sup>. Throughout the world, countries vary in the amount of fly ash that is beneficially used in products such as concrete and grout. In the US, approximately 55-65% of fly ash is reutilized<sup>13-14</sup>, however countries like China and India, where coal combustion is increasing, use less than 50% of fly ash<sup>14</sup>. The fly ash that is not reutilized is stored in landfills and surface impoundments with limited regulations, which impose critical environmental and public health concerns.

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3 Landfills and surface impoundments containing fly ash expose nearby communities to  
4 potentially harmful trace elements. Humans can be exposed to fly ash and the metals contained  
5 in the particles by inhaling fugitive dust and ingesting contaminated groundwater. Children have  
6 a higher risk for negative health outcomes related to fly ash exposure. Compared with adults,  
7 children are more likely to breathe through their mouth, breathe more air relative to their lung  
8 size and body weight, are physically closer to the ground-level, are more likely to engage in  
9 hand-to-mouth behaviors and are less likely to stop activity if they experience respiratory  
10 distress. Their brains and lungs are still developing<sup>15-17</sup>.

11  
12 Although the toxicity and hazard potential of coal ash exposure is high due to potential  
13 exposure to trace elements, there is limited research on the health effects of chronic coal ash  
14 exposure among children. Researchers investigating health among children exposed to fly ash or  
15 living in proximity to power plants have reported greater neurodevelopment conditions, like  
16 attention deficit hyperactivity disorder (ADHD), increased sleep problems, increased respiratory  
17 conditions, and increased gastrointestinal problems<sup>18-20</sup>. These studies were limited in that  
18 residential location or distance from coal-fired power plants was used as a proxy for exposure to  
19 coal ash. None of the studies directly measured in-home exposure to fly ash.

20  
21 Research has shown that Americans spend approximately 90% of their time indoors<sup>21</sup>,  
22 where the concentrations of some pollutants can be 2 to 5 times higher than outdoor  
23 concentrations<sup>22</sup>. Furthermore, fly ash can enter the home through windows, doors, or ventilation  
24 systems. So, indoor exposure is a potential public health concern, especially for children.  
25 However, little research has investigated whether children who reside in the vicinity of coal-fired  
26 power plants with coal ash storage facilities are at greater risk of neurobehavioral problems using  
27 data on exposure collected in participants' homes.

## Study Aims

The overall objective of this community-based study is to evaluate indoor fly ash exposure and the prevalence of neurobehavioral performance and symptoms of 300 children living within 10 miles of two power plants in Jefferson County, Kentucky. Fly ash exposure is measured in particulate matter  $\leq 10\mu\text{m}$  ( $\text{PM}_{10}$ ) samples and lift tape samples. Neurobehavioral outcomes are assessed by the Behavioral Assessment and Research System (BARS) and the Child Behavior Checklist (CBCL). The two specific aims from the study that are emphasized in this protocol paper are to: (1) Characterize indoor exposure to fly ash and heavy metals in homes of children living within close proximity to power plants with coal ash storage facilities and (2) Assess if increased fly ash exposure and greater heavy metal body burden is associated with poorer neurobehavioral health.

Fly ash is a significant environmental problem with emerging public health impacts. This study is novel in that it is the first to measure fly ash in the homes of children. Furthermore, it is the first community-based study to utilize these exposure measures to understand the impact of exposure on children's neurobehavioral health.

## METHODS AND ANALYSIS

This is a cross-sectional study with an exposure assessment. Data collection began in September 2015 and will end on January 31, 2021. The study takes place in Jefferson County and Bullitt County, Kentucky.

### Power Plants in Jefferson County Kentucky, USA

Jefferson County is home to two power plants that are approximately 10 miles apart. The Cane Run Generating Station was built in the 1950s and began operation in November 1954. It is

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3 located approximately 8 miles from downtown Louisville, KY and occupies over 500 acres along  
4 the Ohio river<sup>23</sup>. This plant has five ponds, two of which stored coal ash. The main coal ash  
5 pond, which was opened in 1972 and sits approximately 1,200 feet east of the Ohio River, has a  
6 surface area of approximately 50 acres, with a capacity of 2 million cubic yards<sup>24, 25</sup>. This pond  
7 stored fly ash, bottom ash, and other materials<sup>24,25</sup>. It received a high hazard rating by the United  
8 States Environmental Protection Agency (EPA) indicating that collapse of the pond could lead to  
9 loss of life or major damage to dwellings, buildings, or important utilities<sup>26</sup>. In 2015 the plant  
10 was refitted for natural gas. In 2017, the main ash pond was closed and capped. In addition to the  
11 capped pond, Cane Run has a large on-site ash landfill that opened in the early 1980s<sup>27</sup> and it is  
12 now capped<sup>28</sup>. It was last estimated to be 110 acres and over 130 feet high<sup>29</sup>.

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The Mill Creek Generating Station is located downstream from the Cane Run Plant. It  
began operating in the early 1970s, occupies over 500 acres, and is the largest coal-fired power  
plant owned by Louisville Gas and Electric<sup>30</sup>. The plant's main coal ash pond, which opened at  
the same time as the plant<sup>31</sup>, is in proximity to residential homes. The coal ash pond sits on over  
40 acres and stores an estimated 6.4 million cubic yards of material<sup>31,32</sup>. It has been given a high  
hazard rating by the EPA. Mill Creek's coal ash landfill opened in the 1980s, has a maximum  
elevation of 598 feet, and contains approximately 13.5 million cubic yards of coal ash<sup>33</sup>.

### **Patient and Public Involvement**

During the design of the grant proposal and this resulting protocol manuscript, no patients  
or the public were involved.

### Participant recruitment and sample size

Our study area represents more than 12 zip codes throughout southwestern Jefferson County and northern Bullitt County, Kentucky. To ensure participants are representative of the population throughout the study area, we used Geographical Information Systems (GIS) methods to identify and recruit study participants<sup>34</sup>. First, we stratified the study area using a series of buffer zones at 2-mile intervals from 0-10 miles from the centroid of the straight line that connects the two power plants. Additionally, buffer zones were stratified by wedge-shaped quadrants. This method divided our study area into 20 sampling units (SUs). Prevalence estimates of neurobehavioral conditions for exposed children were selected to range between 20% and 30%, based on findings from a cross-sectional study that assessed children's health in four communities residing near a coal-fired power plant<sup>20</sup>. The prevalence of symptoms in the non-exposed children, were estimated at values of 5% and 10%. These values represent a range for neurobehavioral conditions in the U.S., such as ADHD (6.8%) and behavioral conduct problems (3.5%)<sup>35</sup>. Based on a simulated power calculation, we determined that 300 children needed to be recruited for this study to achieve near 80% power in most scenarios (Table 1).

**TABLE 1:** Power for varying scenarios of sample size per zone, exposures for each zone, and probabilities of symptoms for exposed and unexposed individuals. Grey highlighted cells indicate scenarios where  $\geq 80\%$  power is achieved.

Pr(sym | exposed) and Pr(sym | unexposed)

Sample Size / Zone	Sample Size Total	Exposure / Zone	0.3,0.1	0.3,0.05	0.25,0.1	0.25,0.05
60	300	1,0.8,0.5,0.2,0	0.80	0.97	0.62	0.91
		0.8,0.7,0.5,0.2,0	0.92	1.00	0.75	0.98
		0.7,0.5,0.3,0.1,0	0.91	1.00	0.73	0.97
		0.6,0.5,0.4,0.3,0	0.92	1.00	0.83	0.99
		0.6,0.5,0.4,0.3,0.2	0.99	1.00	0.88	1.00

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5 Recruitment methods vary and include “shoe-leather” methods, where the research team  
6 goes door-to-door talking with participants about the study and/or leaving flyers at their homes.  
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8 Additionally, we mail letters and flyers to potential participants in the zip codes in our study  
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10 area, and have used social media, newspaper articles, and television appearances to publicize the  
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12 study. Furthermore, we have utilized snowballing methods, asking current participants to ask  
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14 their friends and neighbors to recruit additional households that are eligible for this study.  
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### 19 **Inclusion and Exclusion Criteria of Study Participants**

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21 For this study, both children and their parents/guardians are being recruited. To be  
22 included in the study, the family must have lived at their address or within the sampling units for  
23 at least two years. Most of the families in our study are non-transient and remain within the study  
24 area. In order for parents/guardians to participate, they have to consent for their child to take part  
25 in the study, complete three questionnaires, help their child collect fingernails and toenails, allow  
26 a registered nurse into their home to take the vitals of the child and complete a pediatric health  
27 history and home inspection, and permit the research team to conduct the in-home exposure  
28 assessment. Additionally, if parents/guardians are smokers, they must agree to smoke outside  
29 during the week that the air pollution samplers are running inside the home.  
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42 In order for the child to take part in this study, he/she must assent to participate, allow  
43 researchers to take dust samples in his/her room, agree to assist his/her parents with toenail and  
44 fingernail collection, take a battery of computer tests and manual tests that measure  
45 neurobehavioral performance. Children are excluded from this study if they have a genetic  
46 disorder that is known to cause neurobehavioral problems, such as Down syndrome.  
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3 For this study, we assent all children. If their parent/guardian wants to participate, but the  
4 child does not assent, we do not enroll the child or parent/guardian into the study.  
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### 10 **Exposure assessment methods**

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12 For this study, we conduct air monitoring and collect lift samples to quantify exposure to  
13 particulate matter < 10  $\mu\text{m}$  (PM<sub>10</sub>) and identify fly ash particles inside children's home  
14 environments. In these samples, we also analyze the composition of metals and metalloids in  
15 particulate matter and fly ash particles. Additionally, toenails and fingernails are collected from  
16 children to assess elemental body burden. A registered nurse (RN) visits the homes and collects  
17 the child's vital signs, completes a pediatric health history, and conducts a home exposure  
18 assessment. Multiple questionnaires are used to collect additional information. Analytical  
19 methods used include Proton-Induced X-Ray Emissions (PIXE), Scanning Electron Microscopy  
20 (SEM) and Energy Dispersive X-ray (EDX). Figure 1 details the exposure assessment and  
21 analysis methods of the samples, which is provided in detail below.  
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FIGURE 1 HERE

### 42 Indoor Air measurements

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45 Indoor PM<sub>10</sub> is measured using both continuous particle monitors (EPAM-7500) and a  
46 single-stage personal modular impactor (PMI) (SKC Inc.) connected to an AirChek XR5000  
47 pump. The EPAM is a portable particulate monitor that provides real-time measurement and  
48 display of PM<sub>10</sub>, particulate matter  $\leq 2.5 \mu\text{m}$  (PM<sub>2.5</sub>) and particulate matter  $\leq 1.0 \mu\text{m}$ . The EPAM  
49 utilizes optical light scattering for real-time measurements. It is placed in participant's homes  
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3 and configured to measure PM<sub>10</sub> every minute. The EPAM runs for one week.  
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5 Inside the cassette of the PMI is a 37 mm polycarbonate filter that collects PM<sub>10</sub>. A 25 mm  
6 pre-oiled disposable impaction disc is inserted onto the top of the filter cassette to decrease  
7 particle bounce and allow for more efficient particle collection. Polycarbonate membrane filters  
8 were selected because of their properties that allow for analysis by optical microscopy  
9 techniques. To determine the total mass of PM<sub>10</sub> that is collected, gravimetric analysis is  
10 conducted. Prior to being inserted into the cassette of the PMI, each polycarbonate filter is  
11 weighed three times using a BM-20 analytical microbalance. The average of these measurements  
12 is known as the pre-weight. Once the PMI is removed from the field, the filter is weighed three  
13 times. The average of these measurements is known as the post-weight. Subtracting the pre-  
14 weight from the post-weight provides the total mass of PM<sub>10</sub> that is collected from the home.  
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28 The PMI is connected to an AirChek XR5000 air sampling pump via ¼ inch diameter tygon  
29 tubing. These small, lightweight pumps are specifically designed to provide accurate ( $\pm 5\%$  of  
30 set-point) airflows between 1-5 liters per minute (L/min) by using an isothermal closed loop flow  
31 sensor. The isothermal closed loop flow sensor directly measures and constantly maintains the  
32 set flow rate. To compensate for fluctuations in temperature after the pump has been calibrated,  
33 the AirChek XR5000 has a built-in sensor. In the case of excessive backpressure, for example if  
34 the filter becomes overloaded, the AirChek XR5000 is designed to stop after >15 seconds. The  
35 pump will display a flow fault icon on the screen and attempt to restart up to five times every 15  
36 seconds. Before placing the pumps into the homes, they are calibrated using a MesaLabs DryCal  
37 Defender 510 in the laboratory. After calibration, three flow rate readings are taken one minute  
38 apart and recorded. All readings are within  $\pm 5\%$  of 3 L/min, which is the recommended flow  
39 rate for optimal PMI performance. The initial flow rate is calculated by averaging these three  
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5 Using tripod stands, the PMI is placed roughly 1-1.5 meters above the ground to simulate the  
6 breathing zone of an average child. Additionally, strategic placement of the PMI and air pump  
7 avoids windows, doors to the outside, air vents, fireplaces, stoves, and electronic devices to  
8 avoid resuspension of particles. Once in place, the PMI and air pump are turned on and continue  
9 to run in the participant's home for approximately one week. At the end of the air sampling  
10 period, three to four flow rate measurements are taken with the DryCal and recorded. The  
11 average of these measurements is known as the final flow rate. The overall flowrate is  
12 determined by taking the average of the sum of the initial flowrate and final flowrate. Using the  
13 overall flowrate and the total mass of PM<sub>10</sub>, as determined by gravimetric analysis, the  
14 concentration of PM<sub>10</sub> is determined. Calculating the mass concentration on the filters is a vital  
15 step in determining the elemental distribution in subsequent laboratory methods. To determine  
16 the elemental composition of PM<sub>10</sub>, PIXE is used. To determine the presence of fly ash and the  
17 composition of fly ash, SEM/EDX is used.  
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### 38 Analytical methods used

#### 39 *Proton Induced X-ray Emission Spectroscopy (PIXE)*

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42 PIXE is useful if the elemental concentrations are low or if the elements are present at  
43 unknown concentrations. PIXE is an analytical method in which energetic protons transfer  
44 kinetic energy to the inner shell electrons of the target atom, forcing the electrons from the atom  
45 resulting in X-ray production<sup>36</sup>. The X-ray spectrum and energies are unique to the element from  
46 which they were emitted and the amount of X-rays emitted corresponds to the mass of the  
47 particular element being assessed in the sample<sup>36</sup>. There are several advantages to PIXE analysis.  
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3 First, because it is a non-destructive analysis method, errors from sample digestion and  
4 preparation are alleviated. Secondly, PIXE is capable of simultaneously analyzing 72 inorganic  
5 elements from sodium to uranium in liquid, solid, and aerosol filter samples.  
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### 10 11 *Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (SEM/EDX)* 12

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14 Fly ash particles are distinguished from other particles by their morphological and  
15 chemical properties. Fly ash particles are smoothly spherical, which are very distinct from other  
16 metallurgical emissions. Therefore, fly ash particles can be identified through microscopic  
17 methods. In addition to morphological differences, fly ash is chemically different than other  
18 particulate matter. For example, metallurgical emissions are characterized by the elements, Fe  
19 (iron), Mn (manganese), and Si (silicon). Particles from the steel industry are characterized by  
20 Fe, Mn, Si, and aluminum (Al). Fly ash particles are characterized by Si, sulfur (S), potassium  
21 (K), calcium (Ca), and Fe. This metal “fingerprint” is used to identify the presence of fly ash in  
22 our samples.  
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34 SEM/EDX is a quick, non-destructive surface analytical technique that creates high  
35 resolution images of surface topography. Primary electrons, produced from the scanning electron  
36 beam, bombard the sample’s surface and thus generate secondary electrons. The secondary  
37 electron’s low energy intensity is greatly affected by the surface topography of the sample. The  
38 surface image is generated by measuring the intensity of the secondary electron as a function of  
39 the scanning electron beam’s position. Because of the primary electron beam’s ability to focus  
40 on an area <10 nm in size, high resolution images are possible. Primary electron bombardment  
41 from the scanning beam also creates backscattered electrons that indicate the elements in the  
42 sample<sup>37</sup>. Identification of an element is possible because the backscatter electron intensity is  
43 associated with the atomic number of a specific element.  
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3 In addition to secondary and backscattered electrons, the scanning electron beam creates  
4 X-rays. As previously discussed in the PIXE section, X-rays are unique to the corresponding  
5 element. Therefore, analysis of the X-ray can provide semi-quantitative information on the  
6 elements in the sample<sup>37</sup>.  
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### 11 Lift Tape Samples

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13 During the first home visit, lift tape sampling is conducted. Lift sampling is a simple  
14 method for removing particles from a surface to determine their number and size distribution.  
15 We use Stick-To-It Lift Tape (SKC, Inc) to identify the presence of fly ash on multiple surfaces  
16 in children's bedrooms. Stick-to-it Lift Tape is a flexible plastic microscope slide with an  
17 adhesive area that can be used for sampling inorganic dust contamination on surfaces. These lift  
18 tapes are non-destructible and have a consistent sample area. In each child's bedroom, three  
19 standard locations, a bedframe, window, and dresser, are sampled. The lift tape samples undergo  
20 optical microscopy to determine the presence or absence of fly ash in the dust samples and  
21 provide the percent of fly ash on the samples, as well as the elemental concentration of fly ash  
22 particles.  
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### 38 Activity Assessment

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40 In addition to air sampling and lift sampling, an activity diary is filled out by each  
41 participant. The types of activities recorded include: cooking, use of secondary heating sources,  
42 use of indoor fans, burning candles or incense, cleaning activities and use of chemicals,  
43 construction, presence of pets, open/closed windows, and smoking. This information will  
44 provide insight into differences in fly ash and metal concentrations that occur among the  
45 samples.  
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### Registered Nurse Visit

After air sampling and lift sampling is completed, an RN schedules an appointment with the parents/guardians to visit the home. The nurse's visit takes approximately one hour to complete. While at the home, the RN measures the child's height, weight, and blood pressure, and completes the Pediatric Health History Interview and Environmental Home Assessment.

#### *Pediatric Health History Interview.*

The Pediatric Health History Interview form includes demographic information about the participant and parents, current and past health conditions, past hospitalizations, current medications use, parents' perception of health and behavior, immunizations history, details of pregnancy complications and use of substances during pregnancy and delivery, breastfeeding, early childhood development, the child's current participation in school activities and behavior at school and at home, and a brief health history of the immediate family living in the home. The interview form was developed by investigators of the study, after evaluating several standard pediatric health assessment forms.

#### *Environmental Home Assessment.*

A visual assessment of the home is conducted using the publicly-available Pediatric Environmental Home Assessment (PEHA) tool developed by the National Center for Healthy Housing. The PEHA includes a subjective determination of general home characteristics and indoor pollutants and observation of the general home environment, the sleep environment, and home safety<sup>38</sup>. Information such as type of house, age of home, type of foundation, number of floors, sources of heating and cooling, the presence of indoor pollutants (presence of molds, lead-based paints, asbestos, radon, environmental smoke), the RN's assessment of the cleanliness

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3 of the home environment, details of the participants sleep environment (number of beds in room,  
4 allergens, pillows, bedding, flooring, etc.), and home safety (renovations, lighting, poison  
5 control, fire hazards, appropriate storage of chemicals and hot liquids, window guards, etc.) is  
6 collected.  
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### 15 Toenails and Fingernails

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18 Heavy metal body burden is assessed by collecting toenails and fingernails from the child  
19 participants. Toenails and fingernails are a useful measure of metals because they represent long-  
20 term exposure given the slow growth rate, are less likely to be contaminated, are non-invasive,  
21 and are easy to collect and store. Toenails and fingernails reflect exposure integrated over the  
22 preceding 3-12 months and concentrations of elements may vary due to age, gender, behaviors,  
23 and diet<sup>39,40</sup>.  
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32 Parents/guardians are asked to begin collecting their child's toenails and fingernails  
33 during the initial phone conversation, prior to the initial visit. During the initial visit, any nails  
34 the child had already cut are collected, in addition to any nails the child cuts during the visit. For  
35 each participant, approximately 150 mg of nails are collected over.  
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42 Once the total amount of nails is collected, they are cleaned using one acetone wash and  
43 two deionized water washes. The nails are then dried and weighed a final time before being  
44 placed in a container to transport to the laboratory for analysis. Children's nails are cryogenically  
45 frozen, ground, and bound into a 3/8-inch pellet, with the natural binding agent Somar-Mix  
46 Power #210, a mixture of boric acid and water. The pellet is then analyzed by PIXE to determine  
47 the amount and type of elements in the sample.  
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## Study Questionnaires

Parents or guardians of the participating children complete the Environmental Health History Questionnaire (EHH) and Home Cleaning Questionnaire (HC). The EHH consists of 108 questions and is based on five existing pediatric environmental exposure history guides including the Pediatric Environmental History<sup>41</sup>, the pediatric exposure history questions to be included in a well-child visit<sup>42</sup>, and the American Academy of Pediatrics guidance on taking an environmental history<sup>43</sup> as well as The Agency for Toxic Substances and Disease Registry's "Taking an Exposure History,<sup>44</sup>" and the rapid questionnaire of environmental exposures to pregnant women<sup>45</sup>. The HC has 9 questions related to cleaning behaviors. The questionnaires are left with the parents or guardians for approximately one week and returned upon completion.

## **Study outcomes being measured**

To assess neurobehavioral performance and symptoms, we utilize the Behavior Assessment and Research System (BARS) and the Child Behavior Checklist. Both were completed at the participant's home.

## Child Behavior Checklist (CBCL)

Although there are several instruments available that assess problem behaviors in children, the CBCL is among the most respected and widely used; it has been translated into over 90 languages<sup>46,47</sup>. The CBCL is a psychometrically-sound, research tool for evaluating children's emotional, behavioral, and social functioning. Although there are CBCL forms available for

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3 different age groups, this study focuses on the CBCL for ages 6-18 years of age. There are  
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5 parent, teacher, and child report forms. For this study, we are using the parent-report form. The  
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7 CBCL's questions are associated with problems on a syndrome scale in eight different categories:  
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10 anxious/depressed, withdrawn/depressed, somatic complaints, social problems, thought  
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12 problems, attention problems, rule-breaking behavior, and aggressive behavior.  
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15 Anxious/depressed, withdrawn/depressed, and somatic complaints are broadly  
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17 categorized as internalizing behaviors. Rule-breaking behaviors and aggressive behaviors are  
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19 broadly categorized as externalizing behaviors. Overall, the CBCL yields scores for internalizing  
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21 and externalizing behaviors, total problems, and six Diagnostic and Statistical Manual of Mental  
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23 Disorders (DSM)-oriented subscales. The six DSM-oriented subscales include attention  
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25 deficit/hyperactivity problems, anxiety problems, oppositional defiant problems, affective  
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27 problems, conduct problems, and somatic problems<sup>48</sup>. Based on age and sex, these scores are  
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29 compared to clinical cut off points for the particular comparison group.  
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33 The CBCL is left with the parents/guardians for one week and returned upon completion.  
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35 Based on parents'/guardians' responses to the 124-item questionnaire, t-scores are calculated  
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37 using standardized norms for age and gender. If a participant scores in the clinical or borderline  
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39 range on any of the CBCL subscales, the child psychologist follows-up with the  
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41 parents/guardians of the child by conducting a Structured Clinical Interview for Diagnosis of  
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43 DSM Disorders.  
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#### 49 Behavior Assessment and Research System (BARS)

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51 Neurobehavioral performance is assessed in all children using the Behavioral Assessment  
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53 and Research System (BARS)<sup>49</sup>. BARS, which administers a series of neurobehavioral tests,  
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includes a 9-button device that sits on top of a standard laptop. Child participants hit a button from 1 to 9 corresponding to their answer.

BARS was developed by the Oregon Health and Science University to provide a series of neurobehavioral tests that are optimized to detect neurotoxicity<sup>49</sup>. It has been adapted for use with children as young as preschool age<sup>50-52</sup>. BARS has been used for children exposed to neurotoxic chemicals (pesticides) but has not previously been utilized for children exposed to fly ash in their community<sup>50,51</sup>.

The six BARS tests that are used to assess neurobehavioral performance are displayed in Table 2. Comparisons in formal studies have shown that BARS tests have comparable test-retest reliabilities with the tests given in their original testing formats<sup>49</sup>. In addition to the BARS tests, three additional tests are used: the Recall of Objects Immediate and Recall of Objects Delayed, Purdue Pegboard, and Beery-Buktenica Developmental Test of Visual-Motor Integration. These nine tests cover a range of neurobehavioral performance.

Table 2. Neurobehavioral Tests

Test	Measured Functions
<b>BARS Tests</b>	
Symbol-Digit	Speed, Attention/Integration
Finger Tapping	Response Speed and Coordination
Digit Span	Memory and Attention
Continuous Performance	Attention
Matching-to-Sample	Visual Memory
Selective Attention	Attention
<b>Additional Tests</b>	
Recall of Objects Immediate and Recall	Recall and Recognition



A child psychologist administers the nine tests in the evening hours during the weekdays or on a Sunday afternoon. The

of Objects Delayed	Memory
Purdue Pegboard	Dexterity
Visual Motor Integration	Hand-eye Coordination

BARS tests are administered continuously as the child sits comfortably at a table. While the study team members are present throughout the entire test and answer questions as they arise, there is minimal interaction with the children during the BARS tests. The children interact with the computer. These tests are given continuously and in the same order for each child. When the BARS is completed, the psychologist administers the other three tests. It takes approximately 40 minutes to complete the testing.

### Planned statistical analysis

Characterization of the metal concentrations on filters and in nail samples will be stratified by sampling zone and evaluated using exploratory data analysis methods including boxplots, histograms, and kernel density estimates. Sampling units will be grouped into exposure zones on the basis of the minimal distance from either of the two plants. Differences between these exposure zones will be evaluated using one-way analysis of variance (ANOVA) or the Kruskal-Wallis test, depending on whether the data are normally distributed. The use of transformations (e.g. log, Box-Cox power transformation) will be explored. Additionally, associations between metal concentrations and individual distance from the nearest plant for each household will be explored using Pearson/Spearman correlations and linear regression models.

For toenails and fingernail samples, if the majority (e.g.,  $\geq 75\%$ ) of children have levels below the Limit of Detection (LOD), concentrations will be dichotomized as present / absent and analyzed for differences between zones using logistic regression. If the majority of

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3 concentrations are detectable, then differences between children within each exposure zone will  
4 be evaluated using either one-way ANOVA or the non-parametric Kruskal-Wallis test. If there  
5 is a mixture of detectable and below the LOD concentrations, a total metal score will be  
6 calculated, similar to the method of Cave et al, 2010. Briefly, since metal concentrations are on  
7 different scales, each metal concentration will be ranked and then aggregated and grouped into  
8 quartiles of overall metal concentration<sup>53</sup>.  
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12 Presence of fly ash found in the filter samples will be analyzed in a similar fashion to the  
13 metal concentrations. Presence of fly ash will also be dichotomized into present/absent, and  
14 evaluated for differences between exposure zones. Adjustment for other environmental factors  
15 and activities potentially influencing metal concentrations (e.g. smoking,) will be accounted for  
16 using multivariable regression models.  
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20 Association between the BARS tests / CBCL t-scores and exposure zone / distance from  
21 the plant will be evaluated using a linear regression model, with possible transformations (Box-  
22 Cox) when responses are non-normally distributed. A similar model will be used to investigate  
23 potential associations between BARS and CBCL scores and fly ash exposure / heavy metal body  
24 burden. In addition to investigating associations with continuous CBCL scores, CBCL scores  
25 will be dichotomized at a level indicative of a disorder and analyzed for association with fly ash  
26 exposure using either logistic regression or the Cochran-Mantel-Haenszel test (with exposure  
27 zone or sampling unit as the strata). Initially, each exposure variable (fly ash, heavy metal  
28 concentration) will be analyzed individually to determine significant marginal associations with  
29 BARS / CBCL t-scores, with p-values adjusted for multiple comparisons to control the false-  
30 discovery rate using the Benjamini-Hochberg approach<sup>54</sup>. After any significant marginal  
31 associations have been identified, potential confounding variables (demographics, exposure and  
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3 activity history) will be adjusted for using multivariable regression models. Since missing values  
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5 can have a compounding effect in multivariable regression models, percentage of missing values  
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7 will be evaluated for each variable and checked for association with zone and other important  
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9 covariates. If found to depend on these variables, multiple imputation strategies will be used to  
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11 impute missing values and fit multivariable regression models.  
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## 16 17 **Geographical Information Systems and Spatial Analysis**

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19 Geographic information systems (GIS) and spatial analysis methods are utilized in  
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21 several stages of this project. First, similar to Allpress et al., (2008), GIS is used to create  
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23 quadrats and distance buffers that are overlaid with the census data for the spatial sampling  
24  
25 procedures<sup>34</sup>. Second, GIS is used to geocode recruited participants on maps and measure their  
26  
27 distance to the two coal-fired power plants. Third, more advanced spatial statistical analysis  
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29 techniques such as Hotspot Analysis and bivariate local Moran's I are used to investigate the  
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31 clustering patterns of high-level coal ash and heavy metals surrounding the two power plants and  
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33 their storage facilities and explore their associations with the distribution of children's  
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35 neurobehavioral problems across the study area. In general, GIS and spatial analysis methods  
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37 are useful for us to examine distance decay effects of exposure to air toxicants from the power  
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39 plants and identify evidence of exposure induced adverse health outcomes in children.  
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## 46 47 **ETHICS AND DISSEMINATION**

### 48 49 **Ethics**

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51 Written informed consent is obtained from the parents/guardians and written informed  
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53 assent is obtained from all participating children. Trained study personnel explain the informed  
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3 consent documents to the parents/guardians and the assent document to the children. After the  
4 explanation, parents/guardians and children sign the documents. Two copies of the  
5 consent/assent documents are signed; one copy is kept by the parents/guardians and one copy is  
6 kept by the researchers. These consenting procedures were approved by the Institutional Review  
7 Board of the University of Louisville (IRB # 14.1069) and the University of Alabama at  
8 Birmingham (IRB#:300003807), where the principal investigator (PI) of the grant is currently  
9 employed.  
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## 21 Dissemination

22 All findings from this study will be disseminated through publications in peer-reviewed journals  
23 and presentations at national and international conferences. In addition, results will be provided  
24 to the participants of the study. Within three months, the child psychologist makes multiple  
25 attempts to contact and discuss the neurobehavioral outcomes with parents/guardians if the child  
26 has scored poorly on the CBCL. The environmental results will be returned after the study  
27 concludes with the final enrollment. At this time, the researchers will create summary statistics  
28 based on the community that can be compared.  
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## 44 **STRENGTHS, AND LIMITATIONS**

45 This protocol paper describes our research that represents the first study to assess  
46 children's exposure to in-home fly ash and prevalence neurobehavioral outcomes. The health  
47 impacts of fly ash are unknown, but the potential risks are immense. Currently most countries of  
48 the world do not consider fly ash as a hazardous waste, so the regulations regarding its storage  
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3 and disposal are limited. Disposal methods permit fugitive dust to escape leading to increases in  
4 ambient air pollution. Numerous epidemiological studies have associated particulate matter with  
5 cancer, heart disease, asthma, and/or increased mortality. The potential impact of this innovative  
6 study is great as it will provide evidence to describe the environmental health impacts of fly ash  
7 exposure. Better understanding the exposure that communities living near fly ash storage  
8 facilities may help to provide impetus for better regulations for its storage.  
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### 19 Strengths

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21 This study has several strengths. Regarding exposure, we are able to quantify indoor  
22 PM<sub>10</sub> concentrations and determine if fly ash is found in the home. Children spend the majority  
23 of time indoors<sup>21</sup> and the EPA reports that concentrations of pollutants can be 2-5 times higher  
24 indoors than outdoors<sup>22</sup>. For measurement of fly ash, we are using both air sampling and lift tape  
25 sampling which provides us a characterization of in-home exposure. For the assessment of the  
26 outcomes, we are utilizing several measures of neurobehavioral assessment including BARS and  
27 the CBCL. BARS is administered at the homes of the participating children by a child  
28 psychologist. The same psychologist conducts all the testing, assuring consistency in the  
29 protocol. Community members were involved in recruitment of participants for this study.  
30 Research has shown that studies that involve community members have lowered attrition,  
31 increased compliance, improved accuracy and greater applicability and usability in the settings  
32 where community-based research occurs<sup>55-59</sup>.  
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### 51 Limitations

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3 Although there are many strengths of this innovative study, there are some limitations.  
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5 First, we assume that a week-long in-home air sample is representative of children's chronic  
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7 exposure. While children spend hours in their homes, they also spend times in other indoor  
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9 locations, such as schools. In Jefferson County, Kentucky, children do not necessarily attend  
10  
11 their neighborhood schools, so exposure may be increased or decreased depending on location of  
12  
13 their school. Second, during the week-long sampling period, participants may have interfered  
14  
15 with the sampling equipment. Although the pumps require a series of steps to be physically shut  
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17 down and they were contained in soundproof cases which make turning on and off the pumps  
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19 difficult, participants could have turned the pump off by the electrical switch that was connected  
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21 to the outlet where the pump was plugged in. Additionally, children could have put their hands  
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23 over the impactor, which would have changed the flowrates and hence the amount of PM<sub>10</sub>  
24  
25 collected. When we installed the samplers in the homes of the participants, several things were  
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27 done to prevent participant interference. We ensured that the sampling equipment was placed in  
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29 a location that was not in the way of the family's general movement, such as in a corner of the  
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31 room with the impactor facing the main area. Furthermore, we checked the flowrate of the  
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33 pumps in the middle of the week and again at the end of the sampling period . This ensured that  
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35 they were running at the 3 liters/minute required for the sampler and that they were still running.  
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37 In a few instances, we believed that participants did interfere with the sampler, because (1) the  
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39 pump shut off early in the sampling week, or (2) the filter became overloaded and the pump shut  
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41 off. In these instances, the participant was either removed from the study, or agreed to allow us  
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43 to conduct the sampling again.  
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51 Third, we are not directly measuring temperature, humidity, and air velocity in the home.  
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53 These conditions could have an effect on PM<sub>10</sub> measurement. We do ask participants to keep an  
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3 activity diary of events around the home, including the opening and closing of windows. Fourth,  
4 we are not measuring exposure to other pollutants in the home. We are only focusing on fly ash,  
5 PM<sub>10</sub>, and metals. Other potential pollutants such as volatile organic compounds could explain  
6 some neurobehavioral symptoms in children. Fifth, participants who are more concerned about  
7 fly ash pollution or whose children have pre-existing health problems may be more likely to  
8 enroll in this study. To address this potential bias, recruitment materials do not have references to  
9 the health outcome we are assessing.

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19 The final potential limitation of this study is that we have only included the parent form  
20 of the CBCL. The validity and reliability of the CBCL is high for assessing childhood behavior  
21 and emotional problems and has been addressed in many studies<sup>60-63</sup>. Chronbach's alpha's of  
22 the CBCL range from a low of 0.72 for anxiety problems to a high of 0.97 for total problems.  
23 However, we did not utilize the teacher report of behavior which is commonly used to ascertain  
24 behavioral problems such as ADHD<sup>64</sup>. Problems such as attentiveness are often most apparent in  
25 school and teacher input may have improved identification of children with behavioral problems.  
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31  
32 initial project design, and led the writing of this manuscript. ANH and CGS made substantial  
33  
34 contributions to the writing of the manuscript and editing. LS, GNB, CZ, and PJB are co-  
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36 investigators of the study and contributed to the conceptualization of the project and assisted in  
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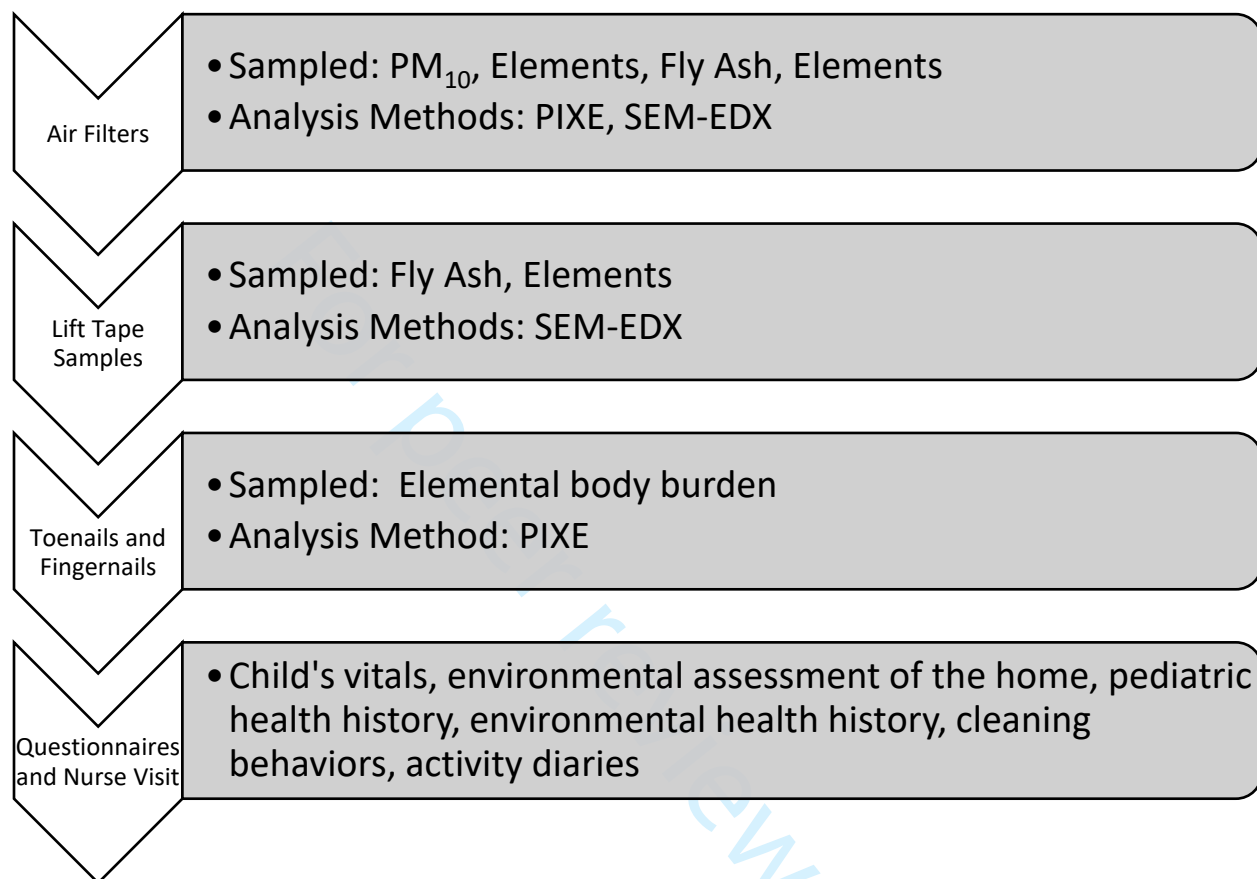


Figure 1. Exposure assessment, analytical methods used, and outcomes from the assessment.

Abbreviations: PM<sub>10</sub> = particulate matter  $\leq$  10 micrometers, PIXE = Proton Induced X-Ray Emissions, SEM = Scanning Electron Microscopy, EDX = Energy Dispersive X-ray

# BMJ Open

## A protocol for measuring indoor exposure to coal fly ash and heavy metals, and neurobehavioral symptoms in children aged 6-14 years old.

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4 **neurobehavioral symptoms in children aged 6-14 years old.**  
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9 Kristina M. Zierold<sup>1</sup>, Clara G. Sears<sup>2</sup>, Abby N. Hagemeyer<sup>3</sup>, Guy N. Brock<sup>4</sup>, Barbara J. Polivka<sup>5</sup>,  
10 Charlie H. Zhang<sup>6</sup>, Lonnie Sears<sup>7</sup>.  
11  
12  
13

- 14 1. Department of Environmental Health Sciences, University of Alabama at Birmingham,  
15 Alabama
- 16 2. Department of Epidemiology, Brown University, Providence, Rhode Island
- 17 3. Department of Epidemiology and Population Health, University of Louisville, Louisville,  
18 Kentucky
- 19 4. Department of Biomedical Informatics and Center for Biostatistics, The Ohio State  
20 University, Columbus, Ohio
- 21 5. School of Nursing, University of Kansas Medical Center, Kansas City, Kansas
- 22 6. Department of Geography & Geosciences, University of Louisville, Louisville, Kentucky
- 23 7. Department of Pediatrics, University of Louisville, Louisville, Kentucky
- 24
- 25
- 26
- 27
- 28
- 29

30  
31 Corresponding Author:  
32 Kristina M. Zierold, PhD, MS  
33 Associate Professor  
34 Department of Environmental Health  
35 School of Public Health  
36 RPHB 534C  
37 1720 2<sup>nd</sup> Ave S  
38 Birmingham, AL 35294-0022 USA  
39 (502) 934-1091  
40 [kzierold@uab.edu](mailto:kzierold@uab.edu)  
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## ABSTRACT

**Introduction:** Fly ash is a waste product generated from burning coal for electricity. It is comprised of spherical particles ranging in size from 0.1 micrometer to over 100 micrometers in diameter that contain trace levels of heavy metals. Large countries such as China and India generate over 100 million tons per year while smaller countries like Italy and France generate 2-3 million tons per year. The United States generates over 36 million tons of ash, making it one of the largest industrial waste streams in the nation. Fly ash is stored in landfills and surface impoundments exposing communities to fugitive dust and heavy metals that leach into the groundwater. Limited information exists on the health impact of exposure to fly ash. This protocol represents the first research to assess children's exposure to coal fly ash and neurobehavioral outcomes.

**Methods:** We measure indoor exposure to fly ash and heavy metals, and neurobehavioral symptoms in children aged 6-14 years old. Using air pollution samplers and lift tape samples, we collect particulate matter  $\leq 10 \mu\text{m}$  that is analyzed for fly ash and heavy metals. Toenails and fingernails are collected to assess body burden for seventy-two chemical elements. Using the Behavior Assessment and Research System and the Child Behavior Checklist, we collect information on neurobehavioral outcomes. Data collection began in September 2015 and will continue until February 2021.

**Ethics and Dissemination:** This study was approved by the Institutional Review Boards of the University of Louisville (#14.1069) and the University of Alabama at Birmingham (#300003807). We have collected data from 267 children who live within 10 miles of two power plants. Children are at a greater risk for environmental exposure which justifies the rationale for this study. Results of this study will be distributed at conferences, in peer-reviewed journals, and to the participants of the study.

## STRENGTHS AND LIMITATIONS OF THIS STUDY

- A major strength of this innovative study is that it is the first research to measure children's indoor exposure to fly ash, which is an emerging environmental health concern throughout the world.
- This study design includes children aged 6-14 years old, who are more susceptible to environmental exposures, like fly ash and heavy metals.
- Multiple measures of fly ash and heavy metals from environmental (air and lift tape samples) and biological (toenails and fingernails) samples are being collected.
- Neurobehavioral performance and symptoms are measured utilizing two methods: the Behavior Assessment and Research System (BARS) and the Child Behavior Checklist (CBCL).
- Although methods were used to reduce bias in the sample of participants, it is possible that some participants were more concerned about fly ash exposure and hence more likely to participate in the study.

## INTRODUCTION

Coal ash is a waste product that is produced from coal-fired power plants. When coal is burned for energy in pulverized fuel combustion chambers, it generates heat, and produces a molten mineral residue. As heat is extracted by the boiler tubes, flue gas is cooled and the residue hardens and forms an ash. Larger, heavier ash particles fall to the bottom of the combustion chamber. Lighter ash particles remain in the flue gas and are collected in air pollution control devices. These lighter ash particles are termed fly ash and compose 40-80% of coal ash<sup>1-5</sup>

Coal fly ash is a fine silt of spherical powdery particles with diameters ranging from less than 0.1  $\mu\text{m}$  to over 100  $\mu\text{m}$ <sup>5-7</sup>. The average size range of the respirable fraction of fly ash is from 1.98  $\mu\text{m}$  to 5.64  $\mu\text{m}$ <sup>8</sup>. Although fly ash is mainly composed of silicon, aluminum, iron, calcium, and oxygen, trace elements such as arsenic, chromium, and lead may be found in fly ash<sup>1,5, 8-13</sup>. The composition of fly ash depends on the geochemical properties of the coal, the preparation of the coal, and the burning process, but research has shown that metal concentrations are much greater than those found in the parent coal<sup>14-16</sup>.

In 2018, over 36 million tons of fly ash were generated in the United States (US), making it one of the largest industrial waste streams nationwide<sup>17</sup>. China and India generate more fly ash than the US<sup>18</sup>. Throughout the world, countries vary in the amount of fly ash that is beneficially used in products such as concrete and grout. In the US, approximately 55-65% of fly ash is reutilized<sup>17-18</sup>, however countries like China and India, where coal combustion is increasing, use less than 50% of fly ash<sup>18</sup>. The fly ash that is not reutilized is stored in landfills and surface impoundments with limited regulations, which impose critical environmental and public health concerns.



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3 Landfills and surface impoundments containing fly ash expose nearby communities to  
4 potentially harmful trace elements. Humans can be exposed to fly ash and the metals contained  
5 in the particles by inhaling fugitive dust and ingesting contaminated groundwater. Children have  
6 a higher risk for negative health outcomes related to fly ash exposure. Compared with adults,  
7 children are more likely to breathe through their mouth, breathe more air relative to their lung  
8 size and body weight, are physically closer to the ground-level, are more likely to engage in  
9 hand-to-mouth behaviors and are less likely to stop activity if they experience respiratory  
10 distress. Their brains and lungs are still developing<sup>19-21</sup>.

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12 Although the toxicity and hazard potential of coal ash exposure is high due to potential  
13 exposure to trace elements, there is limited research on the health effects of chronic coal ash  
14 exposure among children. Researchers investigating health among children exposed to fly ash or  
15 living in proximity to power plants have reported greater neurodevelopment conditions, like  
16 attention deficit hyperactivity disorder (ADHD), increased sleep problems, increased respiratory  
17 conditions, and increased gastrointestinal problems<sup>22-24</sup>. These studies were limited in that  
18 residential location or distance from coal-fired power plants was used as a proxy for exposure to  
19 coal ash. None of the studies directly measured in-home exposure to fly ash.

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21 Research has shown that Americans spend approximately 90% of their time indoors<sup>25</sup>,  
22 where the concentrations of some pollutants can be 2 to 5 times higher than outdoor  
23 concentrations<sup>26</sup>. Furthermore, fly ash can enter the home through windows, doors, or ventilation  
24 systems. So, indoor exposure is a potential public health concern, especially for children.  
25 However, little research has investigated whether children who reside in the vicinity of coal-fired  
26 power plants with coal ash storage facilities are at greater risk of neurobehavioral problems using  
27 data on exposure collected in participants' homes.

## Study Aims

The overall objective of this community-based study is to evaluate indoor fly ash exposure and the prevalence of neurobehavioral performance and symptoms of 300 children living within 10 miles of two power plants in Jefferson County, Kentucky. Fly ash exposure is measured in particulate matter  $\leq 10\mu\text{m}$  (PM<sub>10</sub>) samples and lift tape samples. Neurobehavioral outcomes are assessed by the Behavioral Assessment and Research System (BARS) and the Child Behavior Checklist (CBCL). The two specific aims from the study that are emphasized in this protocol paper are to: (1) Characterize indoor exposure to fly ash and heavy metals in homes of children living within close proximity to power plants with coal ash storage facilities and (2) Assess if increased fly ash exposure and greater heavy metal body burden is associated with poorer neurobehavioral health.

Fly ash is a significant environmental problem with emerging public health impacts. This study is novel in that it is the first to measure fly ash in the homes of children. Furthermore, it is the first community-based study to utilize these exposure measures to understand the impact of exposure on children's neurobehavioral health.

## METHODS AND ANALYSIS

This is a cross-sectional study with an exposure assessment. Data collection began in September 2015 and will end on February, 2021. The study takes place in Jefferson County and Bullitt County, Kentucky.

### Power Plants in Jefferson County Kentucky, USA

Jefferson County is home to two power plants that are approximately 10 miles apart and owned by the same parent company. The Cane Run Generating Station was built in the 1950s

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3 and began operation in November 1954. It is located approximately 8 miles from downtown  
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5 Louisville, KY and occupies over 500 acres along the Ohio river<sup>27</sup>. This plant has five ponds,  
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7 two of which stored coal ash. The main coal ash pond, which was opened in 1972 and sits  
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9 approximately 1,200 feet east of the Ohio River, has a surface area of approximately 50 acres,  
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11 with a capacity of 2 million cubic yards<sup>28, 29</sup>. This pond stored fly ash, bottom ash, and other  
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13 materials<sup>28,29</sup>. It received a high hazard rating by the United States Environmental Protection  
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15 Agency (EPA) indicating that collapse of the pond could lead to loss of life or major damage to  
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17 dwellings, buildings, or important utilities<sup>30</sup>. In 2015 the plant was refitted for natural gas. In  
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19 2017, the main ash pond was closed and capped. In addition to the capped pond, Cane Run has a  
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21 large on-site ash landfill that opened in the early 1980s<sup>31</sup> and it is now capped<sup>32</sup>. It was last  
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23 estimated to be 110 acres and over 130 feet high<sup>33</sup>.

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30 The Mill Creek Generating Station is located downstream from the Cane Run Plant. It  
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32 began operating in the early 1970s, occupies over 500 acres, and is the largest coal-fired power  
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34 plant owned by Louisville Gas and Electric<sup>34</sup>. The plant's main coal ash pond, which opened at  
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36 the same time as the plant<sup>35</sup>, is in proximity to residential homes. The coal ash pond sits on over  
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38 40 acres and stores an estimated 6.4 million cubic yards of material<sup>35,36</sup>. It has been given a high  
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40 hazard rating by the EPA. Mill Creek's coal ash landfill opened in the 1980s, has a maximum  
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42 elevation of 598 feet, and contains approximately 13.5 million cubic yards of coal ash<sup>37</sup>.

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47 Both the Cane Run and Mill Creek plants are pulverized coal, subcritical fired steam  
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49 generators<sup>38</sup> that receive coal from the Illinois Basin of Western Kentucky and Indiana by rail or  
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51 barge<sup>39</sup>. The coal from this area is mid-range sulfur, low moisture content, moderate ash content,  
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53 and high BTU (British Thermal Unit), bituminous thermal coal. Affolter and Hatch (2011) stated  
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3 that the main coals in the Western Kentucky region consist of Danville-Baker, Herrin, and  
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5 Springfield Coals<sup>40</sup>. Table 1 reports the characteristics of these coals.  
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9 Table 1. Characteristics of coal from the Illinois Basin of Western Kentucky  
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Coal Type	Mean Ash Yield, % (Range, %)	Mean Sulfur Content, % (Range, %)	Mean Calorific Value, BTU/lb Range (BTU/lb)
Danville -Baker	11.9 (4.2-44.2)	2.9 (0.3-9.7)	10,920 (5,800–12,990)
Herrin	10.9 (2.4-43.6)	3.0 (0.3-14.5)	11,170 (5,770–13,420)
Springfield	11.2 (2.8-49.7)	3.5 (0.5-19.5)	11,280 (4,810–13,910)

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29 Before coal is burned for energy, it is washed to remove or decrease impurities. In  
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31 Western Kentucky coal, sulfur and ash are the two predominate impurities that are removed  
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33 during the coal washing process. Washing the coal reduces sulfur content by 0.5% to 2.5% and  
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35 reduces ash content by 9-13%<sup>41</sup>. As previously noted, elements that may be harmful to human  
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37 health can become concentrated in coal ash<sup>5, 14-16</sup>. Affolter and Hatch (2011) reported mean  
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39 elemental concentrations of thirteen different potentially harmful elements found in coals  
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41 throughout the Illinois Basin<sup>40</sup>. Table 2 presents the ranges of these elements.  
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Table 2. Range of potentially toxic elements found in coals throughout the Illinois Basin.

Element	Range (ppm)
Antimony	0.7 – 2.3
Arsenic	5.8 - 34
Beryllium	1.6 – 3.7
Cadmium	0.14 – 1.3
Chromium	15 - 20
Cobalt	3.6 – 9.2
Lead	7.7 - 24
Manganese	17 - 62
Mercury	0.08 – 0.14
Nickel	12 - 36
Selenium	1.3 – 3.7
Thorium	1.7 – 2.5
Uranium	1.3 – 3.3

### Patient and Public Involvement

During the design of the grant proposal and this resulting protocol manuscript, no patients or the public were involved.

### Participant recruitment and sample size

Our study area represents more than 12 zip codes throughout southwestern Jefferson County and northern Bullitt County, Kentucky. To ensure participants are representative of the population throughout the study area, we used Geographical Information Systems (GIS) methods to identify and recruit study participants<sup>42</sup>. First, we stratified the study area using a series of buffer zones at 2-mile intervals from 0-10 miles from the centroid of the straight line that connects the two power plants. Additionally, buffer zones were stratified by wedge-shaped quadrants. This method divided our study area into 20 sampling units (SUs). Prevalence estimates of neurobehavioral conditions for exposed children were selected to range between 20% and 30%, based on findings from a cross-sectional study that assessed children's health in four communities residing near a coal-fired power plant<sup>24</sup>. The prevalence of symptoms in the non-exposed children, were estimated at values of 5% and 10%. These values represent a range for neurobehavioral conditions in the U.S., such as ADHD (6.8%) and behavioral conduct problems (3.5%)<sup>43</sup>. Based on a simulated power calculation, we determined that 300 children needed to be recruited for this study to achieve near 80% power in most scenarios (Table 3).

**TABLE 3:** Power for varying scenarios of sample size per zone, exposures for each zone, and probabilities of symptoms for exposed and unexposed individuals. Grey highlighted cells indicate scenarios where  $\geq 80\%$  power is achieved.

Pr(sym | exposed) and Pr(sym | unexposed)

Sample Size / Zone	Sample Size Total	Exposure / Zone	0.3,0.1	0.3,0.05	0.25,0.1	0.25,0.05
60	300	1,0.8,0.5,0.2,0	0.80	0.97	0.62	0.91
		0.8,0.7,0.5,0.2,0	0.92	1.00	0.75	0.98
		0.7,0.5,0.3,0.1,0	0.91	1.00	0.73	0.97
		0.6,0.5,0.4,0.3,0	0.92	1.00	0.83	0.99
		0.6,0.5,0.4,0.3,0.2	0.99	1.00	0.88	1.00

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5 Recruitment methods vary and include “shoe-leather” methods, where the research team  
6 goes door-to-door talking with participants about the study and/or leaving flyers at their homes.  
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8 Additionally, we mail letters and flyers to potential participants in the zip codes in our study  
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10 area, and have used social media, newspaper articles, and television appearances to publicize the  
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12 study. Furthermore, we have utilized snowballing methods, asking current participants to ask  
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14 their friends and neighbors to recruit additional households that are eligible for this study.  
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### 21 **Inclusion and Exclusion Criteria of Study Participants**

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23 For this study, both children and their parents/guardians are being recruited. To be  
24 included in the study, the family must have lived at their address or within the sampling units for  
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26 at least two years. Most of the families in our study are non-transient and remain within the study  
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28 area. In order for parents/guardians to participate, they have to consent for their child to take part  
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30 in the study, complete three questionnaires, help their child collect fingernails and toenails, allow  
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32 a registered nurse into their home to take the vitals of the child and complete a pediatric health  
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34 history and home inspection, and permit the research team to conduct the in-home exposure  
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36 assessment. Additionally, if parents/guardians are smokers, they must agree to smoke outside  
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38 during the week that the air pollution samplers are running inside the home.  
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45 In order for the child to take part in this study, he/she must assent to participate, allow  
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47 researchers to take dust samples in his/her room, agree to assist his/her parents with toenail and  
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49 fingernail collection, take a battery of computer tests and manual tests that measure  
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51 neurobehavioral performance. Children are excluded from this study if they have a genetic  
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53 disorder that is known to cause neurobehavioral problems, such as Down syndrome.  
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3 For this study, we assent all children. If their parent/guardian wants to participate, but the  
4 child does not assent, we do not enroll the child or parent/guardian into the study.  
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### 10 **Exposure assessment methods**

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12 For this study, we conduct air monitoring and collect lift samples to quantify exposure to  
13 particulate matter < 10  $\mu\text{m}$  ( $\text{PM}_{10}$ ) and identify fly ash particles inside children's home  
14 environments. In these samples, we also analyze the composition of metals and metalloids in  
15 particulate matter and fly ash particles. Additionally, toenails and fingernails are collected from  
16 children to assess elemental body burden. A registered nurse (RN) visits the homes and collects  
17 the child's vital signs, completes a pediatric health history, and conducts a home exposure  
18 assessment. Multiple questionnaires are used to collect additional information. Analytical  
19 methods used include Proton-Induced X-Ray Emissions (PIXE), Scanning Electron Microscopy  
20 (SEM) and Energy Dispersive X-ray (EDX). Figure 1 details the exposure assessment and  
21 analysis methods of the samples, which is provided in detail below.  
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FIGURE 1 HERE

### 42 Indoor Air measurements

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45 Indoor  $\text{PM}_{10}$  is measured using both continuous particle monitors (EPAM-7500) and a  
46 single-stage personal modular impactor (PMI) (SKC Inc.) connected to an AirChek XR5000  
47 pump. The EPAM is a portable particulate monitor that provides real-time measurement and  
48 display of  $\text{PM}_{10}$ , particulate matter  $\leq 2.5 \mu\text{m}$  ( $\text{PM}_{2.5}$ ) and particulate matter  $\leq 1.0 \mu\text{m}$ . The EPAM  
49 utilizes optical light scattering for real-time measurements. It is placed in participant's homes  
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3 and configured to measure PM<sub>10</sub> every minute. The EPAM runs for one week.  
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5 Inside the cassette of the PMI is a 37 mm polycarbonate filter that collects PM<sub>10</sub>. A 25 mm  
6 pre-oiled disposable impaction disc is inserted onto the top of the filter cassette to decrease  
7 particle bounce and allow for more efficient particle collection. Polycarbonate membrane filters  
8 were selected because of their properties that allow for analysis by optical microscopy  
9 techniques. To determine the total mass of PM<sub>10</sub> that is collected, gravimetric analysis is  
10 conducted. Prior to being inserted into the cassette of the PMI, each polycarbonate filter is  
11 weighed three times using a BM-20 analytical microbalance. The average of these measurements  
12 is known as the pre-weight. Once the PMI is removed from the field, the filter is weighed three  
13 times. The average of these measurements is known as the post-weight. Subtracting the pre-  
14 weight from the post-weight provides the total mass of PM<sub>10</sub> that is collected from the home.  
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28 The PMI is connected to an AirChek XR5000 air sampling pump via ¼ inch diameter tygon  
29 tubing. These small, lightweight pumps are specifically designed to provide accurate (± 5% of  
30 set-point) airflows between 1-5 liters per minute (L/min) by using an isothermal closed loop flow  
31 sensor. The isothermal closed loop flow sensor directly measures and constantly maintains the  
32 set flow rate. To compensate for fluctuations in temperature after the pump has been calibrated,  
33 the AirChek XR5000 has a built-in sensor. In the case of excessive backpressure, for example if  
34 the filter becomes overloaded, the AirChek XR5000 is designed to stop after >15 seconds. The  
35 pump will display a flow fault icon on the screen and attempt to restart up to five times every 15  
36 seconds. Before placing the pumps into the homes, they are calibrated using a MesaLabs DryCal  
37 Defender 510 in the laboratory. After calibration, three flow rate readings are taken one minute  
38 apart and recorded. All readings are within ±5 % of 3 L/min, which is the recommended flow  
39 rate for optimal PMI performance. The initial flow rate is calculated by averaging these three  
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3 readings.

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5 Using tripod stands, the PMI is placed roughly 1-1.5 meters above the ground to simulate the  
6 breathing zone of an average child. Additionally, strategic placement of the PMI and air pump  
7 avoids windows, doors to the outside, air vents, fireplaces, stoves, and electronic devices to  
8 avoid resuspension of particles. Once in place, the PMI and air pump are turned on and continue  
9 to run in the participant's home for approximately one week. At the end of the air sampling  
10 period, three to four flow rate measurements are taken with the DryCal and recorded. The  
11 average of these measurements is known as the final flow rate. The overall flowrate is  
12 determined by taking the average of the sum of the initial flowrate and final flowrate. Using the  
13 overall flowrate and the total mass of PM<sub>10</sub>, as determined by gravimetric analysis, the  
14 concentration of PM<sub>10</sub> is determined. Calculating the mass concentration on the filters is a vital  
15 step in determining the elemental distribution in subsequent laboratory methods. To determine  
16 the elemental composition of PM<sub>10</sub>, PIXE is used. To determine the presence of fly ash and the  
17 composition of fly ash, SEM/EDX is used.  
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### 38 Analytical methods used

#### 39 *Proton Induced X-ray Emission Spectroscopy (PIXE)*

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42 PIXE is useful if the elemental concentrations are low or if the elements are present at  
43 unknown concentrations. PIXE is an analytical method in which energetic protons transfer  
44 kinetic energy to the inner shell electrons of the target atom, forcing the electrons from the atom  
45 resulting in X-ray production<sup>44</sup>. The X-ray spectrum and energies are unique to the element from  
46 which they were emitted and the amount of X-rays emitted corresponds to the mass of the  
47 particular element being assessed in the sample<sup>44</sup>. There are several advantages to PIXE analysis.  
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3 First, because it is a non-destructive analysis method, errors from sample digestion and  
4 preparation are alleviated. Secondly, PIXE is capable of simultaneously analyzing 72 inorganic  
5 elements from sodium to uranium in liquid, solid, and aerosol filter samples.  
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### 10 *Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (SEM/EDX)*

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13 Fly ash particles are distinguished from other particles by their morphological and  
14 chemical properties. Fly ash particles are smoothly spherical, which are very distinct from other  
15 metallurgical emissions. Therefore, fly ash particles can be identified through microscopic  
16 methods. In addition to morphological differences, fly ash is chemically different than other  
17 particulate matter. For example, metallurgical emissions are characterized by the elements, Fe  
18 (iron), Mn (manganese), and Si (silicon). Particles from the steel industry are characterized by  
19 Fe, Mn, Si, and aluminum (Al). Fly ash particles are characterized by Si, sulfur (S), potassium  
20 (K), calcium (Ca), and Fe. This metal “fingerprint” is used to identify the presence of fly ash in  
21 our samples.  
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34 SEM/EDX is a quick, non-destructive surface analytical technique that creates high  
35 resolution images of surface topography. Primary electrons, produced from the scanning electron  
36 beam, bombard the sample’s surface and thus generate secondary electrons. The secondary  
37 electron’s low energy intensity is greatly affected by the surface topography of the sample. The  
38 surface image is generated by measuring the intensity of the secondary electron as a function of  
39 the scanning electron beam’s position. Because of the primary electron beam’s ability to focus  
40 on an area <10 nm in size, high resolution images are possible. Primary electron bombardment  
41 from the scanning beam also creates backscattered electrons that indicate the elements in the  
42 sample<sup>45</sup>. Identification of an element is possible because the backscatter electron intensity is  
43 associated with the atomic number of a specific element.  
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3 In addition to secondary and backscattered electrons, the scanning electron beam creates  
4 X-rays. As previously discussed in the PIXE section, X-rays are unique to the corresponding  
5 element. Therefore, analysis of the X-ray can provide semi-quantitative information on the  
6 elements in the sample<sup>45</sup>.  
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### 11 Lift Tape Samples

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14 During the first home visit, lift tape sampling is conducted. Lift sampling is a simple  
15 method for removing particles from a surface to determine their number and size distribution.  
16 We use Stick-To-It Lift Tape (SKC, Inc) to identify the presence of fly ash on multiple surfaces  
17 in children's bedrooms. Stick-to-it Lift Tape is a flexible plastic microscope slide with an  
18 adhesive area that can be used for sampling inorganic dust contamination on surfaces. These lift  
19 tapes are non-destructible and have a consistent sample area. In each child's bedroom, three  
20 standard locations, a bedframe, window, and dresser, are sampled. The lift tape samples undergo  
21 optical microscopy to determine the presence or absence of fly ash in the dust samples and  
22 provide the percent of fly ash on the samples, as well as the elemental concentration of fly ash  
23 particles.  
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### 38 Activity Assessment

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40 In addition to air sampling and lift sampling, an activity diary is filled out by each  
41 participant. The types of activities recorded include: cooking, use of secondary heating sources,  
42 use of indoor fans, burning candles or incense, cleaning activities and use of chemicals,  
43 construction, presence of pets, open/closed windows, and smoking. This information will  
44 provide insight into differences in fly ash and metal concentrations that occur among the  
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### Registered Nurse Visit

After air sampling and lift sampling is completed, an RN schedules an appointment with the parents/guardians to visit the home. The nurse's visit takes approximately one hour to complete. While at the home, the RN measures the child's height, weight, and blood pressure, and completes the Pediatric Health History Interview and Environmental Home Assessment.

#### *Pediatric Health History Interview.*

The Pediatric Health History Interview form includes demographic information about the participant and parents, current and past health conditions, past hospitalizations, current medications use, parents' perception of health and behavior, immunizations history, details of pregnancy complications and use of substances during pregnancy and delivery, breastfeeding, early childhood development, the child's current participation in school activities and behavior at school and at home, and a brief health history of the immediate family living in the home. The interview form was developed by investigators of the study, after evaluating several standard pediatric health assessment forms.

#### *Environmental Home Assessment.*

A visual assessment of the home is conducted using the publicly-available Pediatric Environmental Home Assessment (PEHA) tool developed by the National Center for Healthy Housing. The PEHA includes a subjective determination of general home characteristics and indoor pollutants and observation of the general home environment, the sleep environment, and home safety<sup>46</sup>. Information such as type of house, age of home, type of foundation, number of floors, sources of heating and cooling, the presence of indoor pollutants (presence of molds, lead-based paints, asbestos, radon, environmental smoke), the RN's assessment of the cleanliness

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3 of the home environment, details of the participants sleep environment (number of beds in room,  
4 allergens, pillows, bedding, flooring, etc.), and home safety (renovations, lighting, poison  
5 control, fire hazards, appropriate storage of chemicals and hot liquids, window guards, etc.) is  
6 collected.  
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### 15 Toenails and Fingernails

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18 Heavy metal body burden is assessed by collecting toenails and fingernails from the child  
19 participants. Toenails and fingernails are a useful measure of metals because they represent long-  
20 term exposure given the slow growth rate, are less likely to be contaminated, are non-invasive,  
21 and are easy to collect and store. Toenails and fingernails reflect exposure integrated over the  
22 preceding 3-12 months and concentrations of elements may vary due to age, gender, behaviors,  
23 and diet<sup>47,48</sup>.  
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32 Parents/guardians are asked to begin collecting their child's toenails and fingernails  
33 during the initial phone conversation, prior to the initial visit. During the initial visit, any nails  
34 the child had already cut are collected, in addition to any nails the child cuts during the visit. For  
35 each participant, approximately 150 mg of nails are collected over.  
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42 Once the total amount of nails is collected, they are cleaned using one acetone wash and  
43 two deionized water washes. The nails are then dried and weighed a final time before being  
44 placed in a container to transport to the laboratory for analysis. Children's nails are cryogenically  
45 frozen, ground, and bound into a 3/8-inch pellet, with the natural binding agent Somar-Mix  
46 Power #210, a mixture of boric acid and water. The pellet is then analyzed by PIXE to determine  
47 the amount and type of elements in the sample.  
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## Study Questionnaires

Parents or guardians of the participating children complete the Environmental Health History Questionnaire (EHH) and Home Cleaning Questionnaire (HC). The EHH consists of 108 questions and is based on five existing pediatric environmental exposure history guides including the Pediatric Environmental History<sup>49</sup>, the pediatric exposure history questions to be included in a well-child visit<sup>50</sup>, and the American Academy of Pediatrics guidance on taking an environmental history<sup>51</sup> as well as The Agency for Toxic Substances and Disease Registry's "Taking an Exposure History,<sup>52</sup>" and the rapid questionnaire of environmental exposures to pregnant women<sup>53</sup>. The HC has 9 questions related to cleaning behaviors. The questionnaires are left with the parents or guardians for approximately one week and returned upon completion.

## **Study outcomes being measured**

To assess neurobehavioral performance and symptoms, we utilize the Behavior Assessment and Research System (BARS) and the Child Behavior Checklist. Both were completed at the participant's home.

### Child Behavior Checklist (CBCL)

Although there are several instruments available that assess problem behaviors in children, the CBCL is among the most respected and widely used; it has been translated into over 90 languages<sup>54, 55</sup>. The CBCL is a psychometrically-sound, research tool for evaluating children's emotional, behavioral, and social functioning. Although there are CBCL forms

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3 available for different age groups, this study focuses on the CBCL for ages 6-18 years of age.  
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5 There are parent, teacher, and child report forms. For this study, we are using the parent-report  
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7 form. The CBCL's questions are associated with problems on a syndrome scale in eight different  
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9 categories: anxious/depressed, withdrawn/depressed, somatic complaints, social problems,  
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11 thought problems, attention problems, rule-breaking behavior, and aggressive behavior.  
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15 Anxious/depressed, withdrawn/depressed, and somatic complaints are broadly  
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17 categorized as internalizing behaviors. Rule-breaking behaviors and aggressive behaviors are  
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19 broadly categorized as externalizing behaviors. Overall, the CBCL yields scores for internalizing  
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21 and externalizing behaviors, total problems, and six Diagnostic and Statistical Manual of Mental  
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23 Disorders (DSM)-oriented subscales. The six DSM-oriented subscales include attention  
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25 deficit/hyperactivity problems, anxiety problems, oppositional defiant problems, affective  
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27 problems, conduct problems, and somatic problems<sup>56</sup>. Based on age and sex, these scores are  
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29 compared to clinical cut off points for the particular comparison group.  
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34 The CBCL is left with the parents/guardians for one week and returned upon completion.  
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36 Based on parents'/guardians' responses to the 124-item questionnaire, t-scores are calculated  
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38 using standardized norms for age and gender. If a participant scores in the clinical or borderline  
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40 range on any of the CBCL subscales, the child psychologist follows-up with the  
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42 parents/guardians of the child by conducting a Structured Clinical Interview for Diagnosis of  
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44 DSM Disorders.  
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#### 49 Behavior Assessment and Research System (BARS)

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52 Neurobehavioral performance is assessed in all children using the Behavioral Assessment  
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54 and Research System (BARS)<sup>57</sup>. BARS, which administers a series of neurobehavioral tests,  
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3 includes a 9-button device that sits on top of a standard laptop. Child participants hit a button  
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5 from 1 to 9 corresponding to their answer. BARS was developed by the Oregon Health and  
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7 Science University to provide a series of neurobehavioral tests that are optimized to detect  
8  
9 neurotoxicity<sup>57</sup>. It has been adapted for use with children as young as preschool age<sup>58-60</sup>. BARS  
10  
11 has been used for children exposed to neurotoxic chemicals (pesticides) but has not previously  
12  
13 been utilized for children exposed to fly ash in their community<sup>58,59</sup>.  
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18 The six BARS tests that are used to assess neurobehavioral performance are displayed in  
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20 Table 4. Comparisons in formal studies have shown that BARS tests have comparable test-retest  
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22 reliabilities with the tests given in their original testing formats<sup>57</sup>. In addition to the BARS tests,  
23  
24 three additional tests are used: the Recall of Objects Immediate and Recall of Objects Delayed,  
25  
26 Purdue Pegboard, and Beery-Buktenica Developmental Test of Visual-Motor Integration. These  
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28 nine tests cover a range of neurobehavioral performance.  
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32 A child psychologist administers the nine tests in the evening hours during the weekdays  
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34 or on a Sunday afternoon. The BARS tests are administered continuously as the child sits  
35  
36 comfortably at a table. While the study team members are present throughout the entire test and  
37  
38 answer questions as they arise, there is minimal interaction with the children during the BARS  
39  
40 tests. The children interact with the computer. These tests are given continuously and in the same  
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42 order for each child. When the BARS is completed, the psychologist administers the other three  
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44 tests. It takes approximately 40 minutes to complete the testing.  
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Table 4. Neurobehavioral tests

Test	Measured Functions
<b>BARS Tests</b>	
Symbol-Digit	Speed, Attention/Integration
Finger Tapping	Response Speed and Coordination
Digit Span	Memory and Attention
Continuous Performance	Attention
Matching-to-Sample	Visual Memory
Selective Attention	Attention
<b>Additional Tests</b>	
Recall of Objects Immediate and Recall of Objects Delayed	Recall and Recognition Memory
Purdue Pegboard	Dexterity
Visual Motor Integration	Hand-eye Coordination

### Planned statistical analysis

Characterization of the metal concentrations on filters and in nail samples will be stratified by sampling zone and evaluated using exploratory data analysis methods including boxplots, histograms, and kernel density estimates. Sampling units will be grouped into exposure zones on the basis of the minimal distance from either of the two plants. Differences between these exposure zones will be evaluated using one-way analysis of variance (ANOVA) or the Kruskal-Wallis test, depending on whether the data are normally distributed. The use of transformations (e.g. log, Box-Cox power transformation) will be explored. Additionally,

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3 associations between metal concentrations and individual distance from the nearest plant for each  
4 household will be explored using Pearson/Spearman correlations and linear regression models.  
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8 For toenails and fingernail samples, if the majority (e.g.,  $\geq 75\%$ ) of children have levels  
9 below the Limit of Detection (LOD), concentrations will be dichotomized as present / absent and  
10 analyzed for differences between zones using logistic regression. If the majority of  
11 concentrations are detectable, then differences between children within each exposure zone will  
12 be evaluated using either one-way ANOVA or the non-parametric Kruskal-Wallis test. If there  
13 is a mixture of detectable and below the LOD concentrations, a total metal score will be  
14 calculated, similar to the method of Cave et al, 2010. Briefly, since metal concentrations are on  
15 different scales, each metal concentration will be ranked and then aggregated and grouped into  
16 quartiles of overall metal concentration<sup>61</sup>.  
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28 Presence of fly ash found in the filter samples will be analyzed in a similar fashion to the  
29 metal concentrations. Presence of fly ash will also be dichotomized into present/absent, and  
30 evaluated for differences between exposure zones. Adjustment for other environmental factors  
31 and activities potentially influencing metal concentrations (e.g. smoking,) will be accounted for  
32 using multivariable regression models.  
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40 Association between the BARS tests / CBCL t-scores and exposure zone / distance from  
41 the plant will be evaluated using a linear regression model, with possible transformations (Box-  
42 Cox) when responses are non-normally distributed. A similar model will be used to investigate  
43 potential associations between BARS and CBCL scores and fly ash exposure / heavy metal body  
44 burden. In addition to investigating associations with continuous CBCL scores, CBCL scores  
45 will be dichotomized at a level indicative of a disorder and analyzed for association with fly ash  
46 exposure using either logistic regression or the Cochran-Mantel-Haenszel test (with exposure  
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3 zone or sampling unit as the strata). Initially, each exposure variable (fly ash, heavy metal  
4 concentration) will be analyzed individually to determine significant marginal associations with  
5 BARS / CBCL t-scores, with p-values adjusted for multiple comparisons to control the false-  
6 discovery rate using the Benjamini-Hochberg approach<sup>62</sup>. After any significant marginal  
7 associations have been identified, potential confounding variables (demographics, exposure and  
8 activity history) will be adjusted for using multivariable regression models. Since missing values  
9 can have a compounding effect in multivariable regression models, percentage of missing values  
10 will be evaluated for each variable and checked for association with zone and other important  
11 covariates. If found to depend on these variables, multiple imputation strategies will be used to  
12 impute missing values and fit multivariable regression models.  
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## 28 **Geographical Information Systems (GIS) and GeoSpatial Methods**

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31 In addition to facilitating the spatial sampling procedure described above, GIS and  
32 advanced geospatial statistical methods will be utilized in the analysis stage of this project. GIS  
33 will be used to geocode participants' residential addresses and measure distance from  
34 participant's residence to the two power plants, as well as spatially interpolate and integrate the  
35 exposure observations (i.e. fly ash, PM<sub>10</sub>, and heavy metals) and health outcome data.  
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42 Geospatial statistical techniques such as Hotspot Analysis and bivariate local Moran's I  
43 will be used to investigate the clustering patterns of fly ash and heavy metals and explore the  
44 associations between these patterns and children's neurobehavioral problems across the study  
45 area. These analyses will help characterize the geospatial patterns in neurobehavioral problems  
46 related with indoor fly ash exposure in the vicinity of the power plants and coal ash storage  
47 facilities.  
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3 Furthermore, exposure modeling will be used to investigate the spatial dispersion of  
4 pollutants in the study area while considering local meteorological factors (e.g. temperature,  
5 wind speed, wind direction, etc.). To estimate the spatial dispersion of air pollution from the two  
6 plants, we will utilize fate and transport modeling via map algebra<sup>63</sup> and the AERSCREEN  
7 model, which is based on the EPA's AERMOD<sup>64</sup>. AERSCREEN produces estimates of "worse-  
8 case" concentrations of pollutants from a single source, for many times intervals, ranging from 1-  
9 hour, 3-hour, 8-hour, 24-hour up to annual. We anticipate that AERSCREEN will be particularly  
10 useful for estimating overlapping exposures from both power plants and storage facilities. In  
11 general, these geospatial analysis methods will allow us to examine distance decay effects on  
12 exposure to air toxicants and identify areas that may have the highest levels of exposure to  
13 pollutants from the power plants.  
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## 30 **ETHICS AND DISSEMINATION**

### 31 Ethics

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35 Written informed consent is obtained from the parents/guardians and written informed  
36 assent is obtained from all participating children. Trained study personnel explain the informed  
37 consent documents to the parents/guardians and the assent document to the children. After the  
38 explanation, parents/guardians and children sign the documents. Two copies of the  
39 consent/assent documents are signed; one copy is kept by the parents/guardians and one copy is  
40 kept by the researchers. These consenting procedures were approved by the Institutional Review  
41 Board of the University of Louisville (IRB # 14.1069) and the University of Alabama at  
42 Birmingham (IRB#:300003807), where the principal investigator (PI) of the grant is currently  
43 employed.  
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## Dissemination

All findings from this study will be disseminated through publications in peer-reviewed journals and presentations at national and international conferences. In addition, results will be provided to the participants of the study. Within three months, the child psychologist makes multiple attempts to contact and discuss the neurobehavioral outcomes with parents/guardians if the child has scored poorly on the CBCL. The environmental results will be returned after the study concludes with the final enrollment. At this time, the researchers will create summary statistics based on the community that can be compared.

## STRENGTHS, AND LIMITATIONS

This protocol paper describes our research that represents the first study to assess children's exposure to in-home fly ash and prevalence neurobehavioral outcomes. The health impacts of fly ash are unknown, but the potential risks are immense. Currently most countries of the world do not consider fly ash as a hazardous waste, so the regulations regarding its storage and disposal are limited. Disposal methods permit fugitive dust to escape leading to increases in ambient air pollution. Numerous epidemiological studies have associated particulate matter with cancer, heart disease, asthma, and/or increased mortality. The potential impact of this innovative study is great as it will provide evidence to describe the environmental health impacts of fly ash exposure. Better understanding the exposure that communities living near fly ash storage facilities may help to provide impetus for better regulations for its storage.

## Strengths

This study has several strengths. Regarding exposure, we are able to quantify indoor PM<sub>10</sub> concentrations and determine if fly ash is found in the home. Children spend the majority of time indoors<sup>25</sup> and the EPA reports that concentrations of pollutants can be 2-5 times higher indoors than outdoors<sup>26</sup>. For measurement of fly ash, we are using both air sampling and lift tape sampling which provides us a characterization of in-home exposure. For the assessment of the outcomes, we are utilizing several measures of neurobehavioral assessment including BARS and the CBCL. BARS is administered at the homes of the participating children by a child psychologist. The same psychologist conducts all the testing, assuring consistency in the protocol. Community members were involved in recruitment of participants for this study. Research has shown that studies that involve community members have lowered attrition, increased compliance, improved accuracy and greater applicability and usability in the settings where community-based research occurs<sup>65-69</sup>.

## Limitations

Although there are many strengths of this innovative study, there are some limitations. First, we assume that a week-long in-home air sample is representative of children's chronic exposure. While children spend hours in their homes, they also spend times in other indoor locations, such as schools. In Jefferson County, Kentucky, children do not necessarily attend their neighborhood schools, so exposure may be increased or decreased depending on location of their school. Second, during the week-long sampling period, participants may have interfered with the sampling equipment. Although the pumps require a series of steps to be physically shut down and they were contained in soundproof cases which make turning on and off the pumps

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3 difficult, participants could have turned the pump off by the electrical switch that was connected  
4 to the outlet where the pump was plugged in. Additionally, children could have put their hands  
5 over the impactor, which would have changed the flowrates and hence the amount of PM<sub>10</sub>  
6 collected. When we installed the samplers in the homes of the participants, several things were  
7 done to prevent participant interference. We ensured that the sampling equipment was placed in  
8 a location that was not in the way of the family's general movement, such as in a corner of the  
9 room with the impactor facing the main area. Furthermore, we checked the flowrate of the  
10 pumps in the middle of the week and again at the end of the sampling period. This ensured that  
11 they were running at the 3 liters/minute required for the sampler and that they were still running.  
12 In a few instances, we believed that participants did interfere with the sampler, because (1) the  
13 pump shut off early in the sampling week, or (2) the filter became overloaded and the pump shut  
14 off. In these instances, the participant was either removed from the study, or agreed to allow us  
15 to conduct the sampling again.  
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33 Third, we are not directly measuring temperature, humidity, and air velocity in the home.  
34 These conditions could have an effect on PM<sub>10</sub> measurement. We do ask participants to keep an  
35 activity diary of events around the home, including the opening and closing of windows. Fourth,  
36 we are not measuring exposure to other pollutants in the home. We are only focusing on fly ash,  
37 PM<sub>10</sub>, and metals. Other potential pollutants such as volatile organic compounds could explain  
38 some neurobehavioral symptoms in children. Fifth, participants who are more concerned about  
39 fly ash pollution or whose children have pre-existing health problems may be more likely to  
40 enroll in this study. To address this potential bias, recruitment materials do not have references to  
41 the health outcome we are assessing.  
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3 The final potential limitation of this study is that we have only included the parent form  
4 of the CBCL. The validity and reliability of the CBCL is high for assessing childhood behavior  
5 and emotional problems and has been addressed in many studies<sup>70-73</sup>. Chronbach's alpha's of  
6 the CBCL range from a low of 0.72 for anxiety problems to a high of 0.97 for total problems.  
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8 However, we did not utilize the teacher report of behavior which is commonly used to ascertain  
9 behavioral problems such as ADHD<sup>74</sup>. Problems such as attentiveness are often most apparent in  
10 school and teacher input may have improved identification of children with behavioral problems.  
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23 Figure 1. Exposure assessment, analytical methods used, and outcomes from the assessment.  
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26 Abbreviations: PM10 = particulate matter  $\leq 10$  micrometers, PIXE = Proton Induced X-Ray  
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29 Emissions, SEM = Scanning Electron Microscopy, EDX = Energy Dispersive X-ray  
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6 **Author's Contributions:** KMZ is the principal investigator of the study, conceptualized the  
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8 contributions to the writing of the manuscript and editing. LS, GNB, CZ, and PJB are co-  
9 investigators of the study and contributed to the conceptualization of the project and assisted in  
10 the writing and editing of the manuscript. All authors read and approved the final manuscript.  
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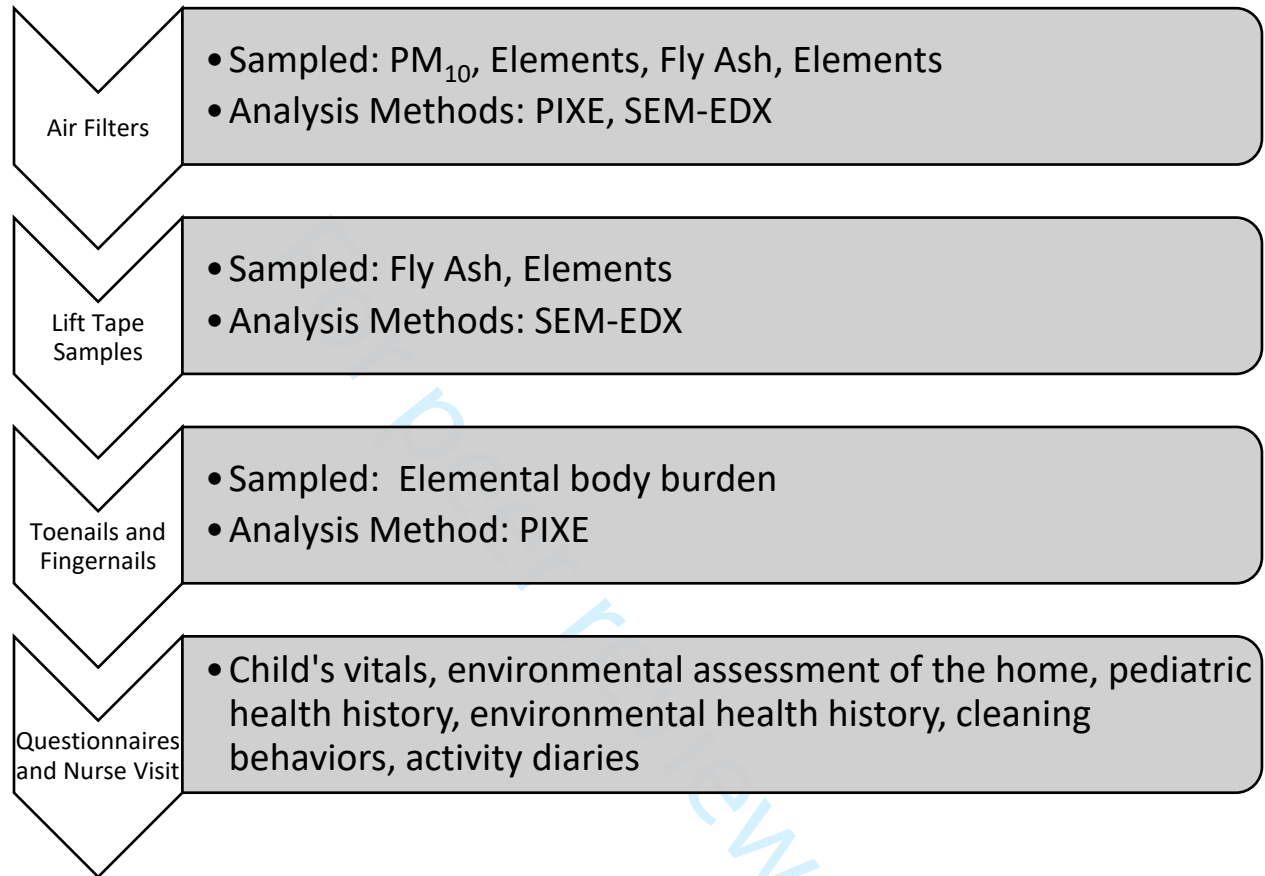


Figure 1. Exposure assessment, analytical methods used, and outcomes from the assessment.

Abbreviations: PM<sub>10</sub> = particulate matter  $\leq$  10 micrometers, PIXE = Proton Induced X-Ray Emissions, SEM = Scanning Electron Microscopy, EDX = Energy Dispersive X-ray