PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	A protocol for measuring indoor exposure to coal fly ash and heavy metals, and neurobehavioral symptoms in children aged 6-14 years old.
AUTHORS	Zierold, Kristina; Sears, Clara; Hagemeyer, Abby; Brock, Guy; Polivka, Barbara; Zhang, Charlie; Sears, Lonnie

VERSION 1 – REVIEW

REVIEWER	Alex G Stewart University of Exeter, UK
REVIEW RETURNED	13-Jun-2020

GENERAL COMMENTS	This protocol examines associations between neurobehavioural attributes of children and exposure to fly ash arising from waste stores at nearby coal-fired power stations. The protocol is clear and well explained. However, I would like more consideration of possible biases (1) from potential interference from families hosting the air sampling equipment for one week, and (2) in parental reporting in the Child Behavior Check List. Also, the note about possible bias from weather should be included, with details of potential analyses to examine this, in the methods section and not slipped into the last paragraph. While acknowledging the study is well under way and that methods will not be changed, I have the following questions: 1) Have the authors considered movement of families into the study area, reducing the length of time the children would be exposed? 2) Is there any record of schools attended and any way of examining this as a possible source of exposure? 3) Is there any information on the metal content of the coal burnt in the power stations? 4) Is there any information on outdoor fly ash concentrations moving away from the sources? 5) How will the authors consider the overlapping exposure of two fly ash sources, which may be different in terms of metal content?

REVIEWER	Francesca Gorini National Research Council
REVIEW RETURNED	04-Jul-2020

GENERAL COMMENTS	In this study, the authors aimed to evaluate the neurobehavioral performance and symptoms in children aged 6-14 years associated with exposure fly ash and heavy metals emitted by two power plants

	within 10 miles from the study area. The protocol involve quantifying PM10 exposure and identifying fly ash particles in children's home environments. Neurobehavioral performance is assessed by Behavior Assessment and Research System and Child Behavior Checklist. Furthermore, individual body burdern of heavy metals is evaluated by collecting toenails and fingernails from the child participants. Overall, the paper is interesting and well written, and it deals with an unexplored topic, i.e., health impact of exposure to fly ash. Stenghts and limits of the study are clearly stated. Minor comments. Figure 1. Please provide a list of abbreviations in the legend. Page 13. Please use the proper abbreviations of metals mentioned (i.e., Fe, Mn, etc.). Page 17. It is unclear how many environmental exposure history guides the Environmental Health History Questionnaire is based on. Page 17, line 37. Please provide the full name of CBCL.

REVIEWER	Manorama Patri Ravenshaw University, Cuttack, Odisha, India
REVIEW RETURNED	02-Aug-2020

GENERAL COMMENTS	Comments for the author The manuscript entitled 'A protocol for measuring coal fly ash, heavy metals, and neurobehavioral symptoms in children aged 6-14 residing near coal-fired power plants' is a good attempt. The investigation seems interesting and has practical significance in the developing countries. Major comments (1) The title shows the author only wants to investigate the impact on children aged (6-14) residing near coal-fired power plants. But, how the exposure (outdoor) assessment not included in this study? What is the possibilities of the indoor exposures containing fly ash? Does it mean that the children are not susceptible for other indoor pollutants exposure (lift tape samples)? The age of participants mentioned also vital for respiratory diseases (early post natal period). (2) The protocol consists two specific aims; so the question is how many children in the control group? How coal ash storage facilities was maintained with duration? Please do describe them. (3) There are no inclusion or exclusion criteria in the Epidemiological Studies, so how to conduct the study and then come out the conclusion that the indoor environmental pollution is the major cause? We all know that the occurrence of behavioral phenotype is complex and many factors may affect this process. (4) The duration and particular timing of collection and measurement of pollutants like flyash of this study did not show us their scientific hypothesis. The relationship between exposure and neurobehavior seems reasonable but it is so weak. (5) The level of environment pollutants like fly ash emitted from power plants of both Jefferson County and Bullitt County (detectable LOD concentration) whether having same/similar? Please mention in detail. Did the authors use the criteria of international standard or choose another place as control for seasonal variation of PM as well as heavy metal?

VERSION 1 – AUTHOR RESPONSE

Reviewer #1 Comments	Author's Responses
This protocol examines associations between neurobehavioural attributes of children and exposure to fly ash arising from waste stores at nearby coal-fired power stations. The protocol is clear and well explained.	Thank you for your comment.
However, I would like more consideration of possible biases (1) from potential interference from families hosting the air sampling equipment for one week, and (2) in parental reporting in the Child Behavior Check List.	1) Thank you for recognizing this potential limitation. We have added some discussion about the potential interference from families to the limitations. We have included the following text: 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 difficult, participants could have turned the pump off by the electrical switch that was connected to the outlet where the pump was plugged in. Additionally, children could have put their hands over the impactor, which would have changed the flowrates and hence the amount of PM_{10} collected. When we installed the samplers in the homes of the participants, several things were done to prevent participant interference. We ensured that the sampling equipment was placed in a location that was not in the way of the family's general movement, such as in a corner of the room with the impactor facing the main area. Furthermore, we checked the flowrate of the pumps in the middle of the week and again at the end of the sampling period . This ensured that they were running at the 3 liters/minute required for the sampler and that they were still running. In a few instances, we believed that participants did interfere with the sampler, because (1) the pump shut off early in the sampling week, or (2) the filter became overloaded and the pump shut off. In these instances, the participant was either removed from the study, or agreed to allow us to conduct the sampling again. 2) We acknowledge that there is a limitation in only including the parent report of the Child Behavior Checklist, as the teacher report is not included in this study. However, the reliability and validity of the Child Behavior Checklist has been proven in many research studies. We have added the following to the limitations:

	The final potential limitation of this study is that we have only included the parent form of the CBCL. The validity and reliability of the CBCL is high for assessing childhood behavior and emotional problems and has been addressed in many studies[60-63] . Chrombach's alpha's of the CBCL range from a low of 0.72 for anxiety problems to a high of 0.97 for total problems. However, we did not utilize the teacher report of behavior which is commonly used to ascertain behavioral problems such as ADHD[64]. Problems such as attentiveness are often most apparent in school and teacher input may have improved identification of children with behavioral problems.
Also, the note about possible bias from weather should be included, with details of potential analyses to examine this, in the methods section and not slipped into the last paragraph.	Thank you for bringing this point to our attention. This protocol and the corresponding grant are focused on in-home fly ash exposure. While we have planned in the future to investigate the impact of weather overall, to prevent confusion, about the outdoor and indoor air pollution measurements for the aims of this grant, we changed the limitation to read: Third, we are not directly measuring temperature, humidity, and air velocity in the home. These conditions could have an effect on PM_{10} measurement. We do ask participants to keep an activity diary of events around the home, including the opening and closing of windows. We also added a section on GIS and Spatial Analysis in the Methods and Analysis section of the manuscript. We have added: **Geographical Information Systems and Spatial Analysis** Geographic information systems (GIS) and spatial analysis methods are utilized in several stages of this project. First, similar to Allpress et al., (2008), GIS is used to create quadrats and distance buffers that are overlaid with the census data for the spatial sampling procedures[34]. Second, GIS is used to geocode recruited participants on maps and measure their distance to the two coal-fired power plants. Third, more advanced spatial statistical analysis techniques such as Hotspot Analysis and bivariate local Moran's I are used to investigate the clustering patterns of high-level coal ash and heavy metals surrounding the two power plants and their storage facilities and explore their associations with the distribution of children's neurobehavioral problems across the study area. In general, GIS and spatial analysis methods are useful for us to examine distance

	decay effects of exposure to air toxicants from the power plants and identify evidence of exposure induced adverse health outcomes in children. However, when the grant aims are completed, we will use exposure modeling to investigate outdoor pollutant concentrations. Kind of like a side interest. We propose to use fate and transport modeling via map algebra (Pistocchi, 2008) and EPA's AERSCREEN model to estimate the spatial dispersion of air pollution from the two power plants in the study area. We anticipate that AERSCREEN will be particularly useful for examining concentrations of coal ash from both sites (one at a time) and spatially overlaying results.
Have the authors considered movement of families into the study area, reducing the length of time the children would be exposed?	Yes. One of the inclusion criteria of the study was that children had to live within the study area for at least 2 years. However, this population is predominately non-transient and if they move, they do stay in the same area of Jefferson County/Bullitt County. We did take an address history. To clarify this concern, we added the inclusion criteria and exclusion criteria of the study into the Methods and Analysis section. We have added the following: **Inclusion and Exclusion Criteria of Study Participants** For this study, both children and their parents/guardians are being recruited. To be included in the study, the family must have lived at their address or within the sampling units for at least two years. Most of the families in our study are non-transient and remain within the study area. In order for parents/guardians to participate, they have to consent for their child to take part in the study, complete three questionnaires, help their child collect fingernails and toenail, allow a registered nurse into their home to take the vitals of the child and complete a pediatric health history and home inspection, and permit the research team to conduct the in-home exposure assessment. Additionally, if parents/guardians are smokers, they must agree to smoke outside during the week that the air pollution samplers are running inside the home. In order for the child to take part in this study, he/she must assent to participate, allow researchers to take dust samples in his/her room, agree to assist his/her parents with toenail and fingernail collection, take a battery of computer tests and manual tests that measure neurobehavioral performance.

	Children are excluded from this study if they have a genetic disorder that is known to cause neurobehavioral problems, such as Down syndrome. For this study, we assent all children. If their parent/guardian wants to participate, but the child does not assent, we do not enroll the child or parent/guardian into the study.
Is there any record of schools attended and any way of examining this as a possible source of exposure?	Thank you for this insightful comment. We do have a record of the school that the child is currently attending. We did consider school environments initially in our study design. However, one of the major concerns regarding sampling in schools is that in this area in the state of Kentucky, children do not attend their neighborhood schools. Some children might attend school quite far from our study area. For example, a child living in the Southwest region of Jefferson County, where our study area is located might be school bussed to a school 20 miles away in the Eastern part of Jefferson County. Although this is a great suggestion, we cannot introduce this into the study now, because it would require us to place monitors into the schools and have the funding from the granting agency to do so. For this study, which is really the first to address health in children potentially exposed to fly ash, we were really focused on the home environment.
Is there any information on the metal content of the coal burnt in the power stations?	Thank you for this additional insightful comment. We do not have information on the metal content of the coal burnt in the power stations. Coal for both plants came from the same source. We assume that there is difference between the metal content in the parent coal and that in the fly ash. Metal content in the fly ash could be affected by the burning technique, time in the combustion chamber, the cleaning process of the coal, etc., Previous research has found that the concentrations of metals in coal ash are greater than the concentrations in parent coal. We wanted a sample of coal ash from the landfills and surface impoundments, but were not permitted to have one.
Is there any information on outdoor fly ash concentrations moving away from the sources?	There is no information on outdoor fly ash concentrations moving away from the sources for **this** research location and very limited studies in general.

	In 2011, the power company in Louisville completed some lift sampling on three houses directly across from one of the power plants. The power company did report finding significant number of fly ash particles on the houses. There have been a few studies that evaluated "power plant emissions" and health. But the emissions were not directly measured as fly ash. Here is a link to one study that found emissions 20-30 miles away from the power plant, and the impact on low birthweight. https://pubmed.ncbi.nlm.nih.gov/28653819/ All studies have used location or proximity to power plants as proxies for exposure. Power plant emissions can include other pollutants besides fly ash. This is the first study to directly measure fly ash and human health.
How will the authors consider the overlapping exposure of two fly ash sources, which may be different in terms of metal content?	First, we identify fly ash based on morphology, so differences in the neurotoxic metal composition due to plant source won't impact the overall identification of fly ash presence in homes. It is used as a dichotomized variable (Yes presence/No presence). However, we are also analyzing the metal content of the fly ash particles by EDX, so in the future, we can see if the metal composition of fly ash particles varies geographically. Statistically we have written in the protocol: To account for potential exposure from both plants, SU 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 ANOVA or the Kruskal-Wallis test, depending on whether the data are normally distributed or not.
Reviewer #2 Comments	**Author's Responses**
In this study, the authors aimed to evaluate the neurobehavioral performance and symptoms in children aged 6-14 years associated with exposure fly ash and heavy metals emitted by two power plants within 10 miles from the study area. The protocol involve quantifying PM10 exposure and identifying fly ash particles in children's home environments. Neurobehavioral performance is assessed by Behavior Assessment and Research System and Child Behavior Checklist. Furthermore, individual body burdern of heavy metals is evaluated by collecting toenails and fingernails from the child participants.	Thank you for your comment regarding our study.

Overall, the paper is interesting and well written, and it deals with an unexplored topic, i.e., health impact of exposure to fly ash. Stenghts and limits of the study are clearly stated.	
Figure 1. Please provide a list of abbreviations in the legend.	Corrected as requested. We added the list of abbreviations below the figure.
Page 13. Please use the proper abbreviations of metals mentioned (i.e., Fe, Mn, etc.).	Corrected as requested.
Page 17. It is unclear how many environmental exposure history guides the Environmental Health History Questionnaire is based on.	Thank you. We have clarified that the EHH was based on 5 environmental exposure history guides. We have added the following to the text: The EHH consists of 108 questions and is based on five existing pediatric environmental exposure history guides including the Pediatric Environmental History[41] , the pediatric exposure history questions to be included in a well-child visit[42], and the American Academy of Pediatrics guidance on taking an environmental history[43] as well as The Agency for Toxic Substances and Disease Registry's "Taking an Exposure History,[44]"and the rapid questionnaire of environmental exposures to pregnant women[45].
Page 17, line 37. Please provide the full name of CBCL.	Corrected as requested.
Reviewer #3 Comments	**Author's Responses**
The manuscript entitled 'A protocol for measuring coal fly ash, heavy metals, and neurobehavioral symptoms in children aged 6-14 residing near coal-fired power plants' is a good attempt. The investigation seems interesting and has practical significance in the developing countries.	Thank you. This study has implications for developing countries since many developing countries are increasing the use of coal, and hence generating much more coal ash. Storage is a problem.
(1) The title shows the author only wants to investigate the impact on children aged (6-14) residing near coal-fired power plants. But, how the exposure (outdoor) assessment not included in this study?	Yes, the purpose of this grant is to investigate **indoor** fly ash exposure on children aged 6-14 years old. We did not include outdoor assessment. This study focuses on indoor exposure. American children spend 90% of their time indoors, so we proposed in the grant to investigate indoor air pollution. Indoor air pollution is a major problem and the concentration of pollutants can be 2-5 times greater in the home. We added the following to the text in the Introduction: Research has shown that Americans spend approximately 90% of their time indoors[21], where the concentrations of some pollutants can be 2 to 5 times higher than outdoor concentrations[22]. Furthermore, fly ash can enter the home through windows, doors, or ventilation systems.

	So, indoor exposure is a potential public health concern, especially for children. However, little research has investigated whether children who reside in the vicinity of coal-fired power plants with coal ash storage facilities are at greater risk of neurobehavioral problems using data on exposure collected in participants' homes. We have changed the title to show that this study focuses on indoor exposure. The new title is: **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.**
What is the possibilities of the indoor exposures containing fly ash?	The possibility of homes containing fly ash is great, as it is comprised of small particles which easily make their way into homes. As other particulate matter exists in homes, so does fly ash, which ranges in size from <0.1 micrometers to greater than 100 micrometers. In preliminary analysis of our study data to date, we have found fly ash in many homes (>50%). Particulate matter (fly ash) can enter the home through windows, doors, ventilation systems, etc.
Does it mean that the children are not susceptible for other indoor pollutants exposure (lift tape samples)?	Yes, children are susceptible to other pollutants, but the important aspect of this study and focus of this grant is to assess fly ash and metals in homes. No other research has addressed if children are exposed to fly ash in home. We have added the following text to the limitations: Fourth, we are not measuring exposure to other pollutants in the home. We are only focusing on fly ash, PM_{10}, and metals. Other potential pollutants such as volatile organic compounds could explain some neurobehavioral symptoms in children We are confused about the (list tape samples?) comment. Lift tape samples are being used to

The age of participants mentioned also vital for respiratory diseases (early post natal period).	pick up dust. They collect particles and we analyze them to see if fly ash is on the lift tape. This grant is measuring exposure to fly ash and the elements in fly ash. We are not looking for fungal or biological sources of exposure. We are specifically investigating fly ash. Yes, this is true. But for the NIH grant, we proposed to investigate neurobehavioral outcomes, because metals, which are found in fly ash are associated with neurobehavioral and neurodevelopmental problems. In the Pediatric Health History Questionnaire, we take a full history of the child's health. This will tell us if they have a respiratory disease. But the grant that was funded was specifically to investigate neurobehavioral outcomes.
(2) The protocol consists two specific aims; so the question is how many children in the control group?	This is a cross-sectional study with 300 children. This is not a case-control study or cohort study. This is a type of epidemiological study called a cross-sectional study. A cross-sectional study investigates potential exposure and health outcomes among all the participants in the study. Then outcomes are evaluated and prevalence odds ratios can be determined. Furthermore, there are also many other statistical methods to investigate those children exposed to coal ash and those children not exposed to coal ash. Case-control or cohort studies have "controls" or control-groups.

How coal ash storage facilities was maintained with duration? Please do describe them.	We have provided detail on the coal ash storage sites. We have added the following: **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 located approximately 8 miles from downtown Louisville, KY and occupies over 500 acres along the Ohio river[23]. This plant has five ponds, two of which stored coal ash. The main coal ash pond, which was opened in 1972 and sits approximately 1,200 feet east of the Ohio River, has a surface area of approximately 50 acres, with a capacity of 2 million cubic years[24, 25]. This pond stored fly ash, bottom ash, and other materials[24,25]. It received a high hazard rating by the United States Environmental Protection Agency (EPA) indicating that collapse of the pond could lead to loss of life or major damage to dwellings, buildings, or important utilities[26]. In 2015 the plant was refitted for natural gas. In 2017, the main ash pond was closed and capped. In addition to the capped pond, Cane Run has a large on-site ash landfill that opened in the early 1980s[27] and it is now capped[28]. It was last estimated to be 110 acres and over 130 feet high[29]. 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[30]. The plant's main coal ash pond, which opened at the same time as the plant[31], 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[31,32]. 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 s of coal ash[33].
(3) There are no inclusion or exclusion criteria in the Epidemiological Studies, so how to conduct the study and then come out the conclusion that the indoor environmental pollution is the major cause? We all know that the occurrence of behavioral phenotype is complex and many factors may affect this process.	There are inclusion and exclusion criteria. We have added them into the protocol paper. In the Methods and Analysis section, we have added the following text: **Inclusion and Exclusion Criteria of Study Participants** For this study, both children and their parents/guardians are being recruited. To be

	included in the study, the family must have lived at their address or within the sampling units for at least two years. Most of the families in our study are non-transient and remain within the study area. In order for parents/guardians to participate, they have to consent for their child to take part in the study, complete three questionnaires, help their child collect fingernails and toenails, allow a registered nurse into their home to take the vitals of the child and complete a pediatric health history and home inspection, and permit the research team to conduct the in-home exposure assessment. Additionally, if parents/guardians are smokers, they must agree to smoke outside during the week that the air pollution samplers are running inside the home. In order for the child to take part in this study, he/she must assent to participate, allow researchers to take dust samples in his/her room, agree to assist his/her parents with toenail and fingernail collection, take a battery of computer tests and manual tests that measure neurobehavioral performance. Children are excluded from this study if they have a genetic disorder that is known to cause neurobehavioral problems, such as Down syndrome. For this study, we assent all children. If their parent/guardian wants to participate, but the child does not assent, we do not enroll the child or parent/guardian into the study. Reviewer 3 brings up good points about other sources of exposure and that behavior is complicated. However, American children spend most of their time inside, so we decided to investigate the impact of indoor fly ash exposure on health. We also note that although there are multiple factors in the child's behavior understanding the effect of environmental factors could lead to prevention efforts for child behavior disorders. We added the following to the limitations: Fourth, we are not measuring exposure to other pollutants in the home. We are only focusing on fly ash, PM_{10}, and metals. Other potential pollutants such as volatile organic compounds could explain some neurobehavioral symptoms in children
(4) The duration and particular timing of collection and measurement of pollutants like flyash of this study did not show us their scientific hypothesis. The relationship between exposure and neurobehavior seems reasonable	Thank you for the comment, however, the relationship between fly ash exposure and neurobehavioral performance and symptoms is not weak. The hypothesis may seem “weak” because there is no research that has directly

but it is so weak.	measured the impact of fly ash exposure on neurobehavioral performance and symptoms. This is the first. Our scientific hypothesis is that exposure to neurotoxic metals during childhood development in particular (compared to prenatally or in adulthood) impacts behavior. This hypothesis is similar to the significant evidence from childhood vs prenatal lead exposure and the effects on neurodevelopment. So we measured fly ash during childhood because we hypothesize that exposure during the childhood period is relevant to neurodevelopment, this is supported by literature on other metals and on the fact that the brain develops until adulthood. We do know from previous studies, that fly ash contains many neurotoxic metals as cited in our introduction. We also know that metal concentration increases as particle size decreases, so the concentration of metals in coal ash is much greater than the concentrations found in the parent coal. From our introduction, with multiple references regarding the neurotoxic metals in fly ash: *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[1,5-9]. 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[10-12].* Furthermore, limited studies that have assessed exposure to coal ash or emissions from power plants have determined neurobehavioral differences in children exposed to the pollutants. From our protocol: *Researchers investigating health among children exposed to fly ash or living in proximity to power plants have reported greater neurodevelopment conditions, like attention deficit hyperactivity disorder (ADHD), increased sleep problems, increased respiratory conditions, and increased gastrointestinal problems[15-17]. These studies were limited in that residential location or distance from coal-fired power plants was used as a proxy for exposure to coal ash. None of the studies directly measured in-home exposure to fly ash.* In addition, our environmental sampling methods provide an estimate of long-term exposure. We sample for one week in the

	home and we collect dust samples that have been in the home from areas that are generally not cleaned.
(5) The level of environment pollutants like fly ash emitted from power plants of both Jefferson County and Bullitt County (detectable LOD concentration) whether having same/similar? Please mention in detail.	I apologize, but we do not understand this comment. We have tried to respond to what we think you are requesting. We first identify fly ash based on morphology, so differences in neurotoxic metal composition due to plant source won't impact the overall identification of fly ash presence in homes. We have a dichotomous variable (fly ash in home/no fly ash in home). There is no LOD in a microscopy techniques (SEM, PLM). When fly ash is found by microscopy, we use EDX to determine the metals in the fly ash. EDX is roughly sensitive to about 0.1% by weight for individual elements in an homogeneous material. The actual sensitivity depends on the specific element under consideration (for example, EDX does not detect hydrogen or helium and is very insensitive to boron and nitrogen) and the conditions of the sample. If elements of interest are present as discrete domains within a matrix material (such as iron oxide particles in a silica powder sample), it is often possible to identify the presence of trace levels of these elements. While the accuracy of EDX data is best described as semi-quantitative (approximate), the precision of the data is quite good. That is, analyses of similar samples will result in data that reflect even small differences in concentration (perhaps a couple of tenths of a percent) despite the inability to actually fully quantify that data. Since we are also analyzing the metal content of the fly ash particles by EDX, we can see if the metal composition of fly ash particles varies geographically.
Did the authors use the criteria of international standard or choose another place as control for seasonal variation of PM as well as heavy metal?	Since this study is taking place in the United States, we utilized the seasons as defined in the United States.

VERSION 2 – REVIEW

REVIEWER	Alex G Stewart University of Exeter, UK
REVIEW RETURNED	26-Aug-2020

GENERAL COMMENTS	The authors of this protocol for an investigation into the effect of fly ash from coal fired power stations into neuro-behavioural issues in children have responded clearly to most of the reviewers' comments. However, I would like to make the following points, which refer to my (Reviewer #1) comments on the metal content of the coal, outdoor fly ash concentrations and overlapping exposure of fly ash from the two sources, which is probably point 5 of Reviewer #3 as well: The study will measure fly ash content as found in the homes of the participating children. Indoor fly ash will reflect source(s) of the local outdoor fly ash. In order to further understand the interactions between the source (coal fired power station) and to increase the generalisability of this study, further consideration of the source coal and resulting fly ash is important. The source-pathway-receptor approach is common in environmental investigations; this study justifiably concentrates on the end of the pathway and the receptors. However, some further consideration of the geochemistry of the source and early pathway will be necessary as the study findings develop and the investigators think about what their findings are telling them. The authors themselves state that "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". It is thus important to have some information on the sources and early pathways (some sort of characterisation of the different dusts, information on the nature of the processes and materials generating them). The authors state that the two stations use the same coal (this should be referenced in some way), but given that it is likely that there are different preparation practices and burning conditions of the coal, four different areas of distribution of fly ash are likely: unpolluted, polluted by source 1, by source 2, by both sources. This will be in addition to the changing concentrations with distance (and possibly weather patterns). All this information is important for the interpretation of the results, as well as for the non-health readers of the work and the wider relevance of the research outcomes of this useful and interesting study. I wish you well with the work. The new references are in a different format to the previous references. Additionally, there are about seven references (out of >60) since 2017. Perhaps there should be a few more?

REVIEWER	Manorama Patri Ravenshaw University
REVIEW RETURNED	07-Sep-2020

GENERAL COMMENTS	Accept.

VERSION 2 – AUTHOR RESPONSE

Reviewer Comments	Author Responses
The study will measure fly ash content as found in the homes of the participating children. Indoor fly ash will reflect source(s) of the local outdoor fly ash. In order to further understand the interactions between the source (coal fired power station) and to increase the generalisability of this study, further consideration of the source coal and resulting fly ash is important.	Thank you for the suggestion. We added information to highlight that the two plants studied are owned and operated by the same company. The facilities are 10 miles apart and largely used the same source of coal; bituminous thermal coal from the Illinois Basin of Western Kentucky. Furthermore, the plants are both pulverized coal, sub-critical fired steam generators. We have added this information to a section on the power plants and the coal in: Power Plants in Jefferson County Kentucky, USA. Both the Cane Run and Mill Creek plants are pulverized coal, subcritical fired steam generators[38] that receive coal from the Illinois Basin of Western Kentucky and Indiana by rail or barge[39]. The coal from this area is mid-range sulfur, low moisture content, moderate ash content, and high BTU (British Thermal Unit), bituminous thermal coal. Affolter and Hatch (2011) stated that the main coals in the Western Kentucky region consist of Danville-Baker, Herrin, and Springfield Coals[40]. Table 1 reports the characteristics of these coals. Table 1. Before coal is burned for energy, it is washed to remove or decrease impurities. In Western Kentucky coal, sulfur and ash are the two predominate impurities that are removed during the coal washing process. Washing the coal reduces sulfur content by 0.5% to 2.5% and reduces ash content by 9-13%[41].As previously noted, elements that may be harmful to human health can become concentrated in coal ash[5, 14-16.] Affolter and Hatch (2011) reported mean elemental concentrations of thirteen different potentially harmful elements found in coals throughout the Illinois Basin[40]. Table 2 presents the ranges of these elements Table 2.
The source-pathway-receptor approach is common in environmental investigations; this study justifiably concentrates on the end of the pathway and the receptors. However, some further consideration of the geochemistry of the source and early pathway will be necessary as the study findings develop and the investigators think about what their findings are telling them. The authors themselves state that “The composition of fly ash depends on the	Yes, the source-pathway-receptor approach is common in environmental investigations, and some of the authors have expertise with the modeling methods commonly used to investigate source-receptor relationships. We agree that focusing on the end of the pathway and receptors is justifiable and critical for this health-focused epidemiologic study.

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". It is thus important to have some information on the sources and early pathways (some sort of characterisation of the different dusts, information on the nature of the processes and materials generating them). The authors state that the two stations use the same coal (this should be referenced in some way), but given that it is likely that there are different preparation practices and burning conditions of the coal, four different areas of distribution of fly ash are likely: unpolluted, polluted by source 1, by source 2, by both sources. This will be in addition to the changing concentrations with distance (and possibly weather patterns).	We added information to highlight the geochemistry of the parent coal and the similar ownership and operational procedures of these facilities (i.e. early pathway information). See above. Due to these similarities in the ownership, facility types, operations, and source coal it is unlikely that we will be able to attribute fly ash to a specific source based on what our grant was funded to investigate. Other air sampling methods and analytical methods would be needed for a source-receptor investigation. However, the reviewer makes a good point that including more information about the source and early pathway is important because it will help the reader assess the generalizability of our study design and results to areas surrounding other power plants using coal from different regions and different plant designs. We have added detail regarding dispersion analysis that was planned to estimate concentrations at residential addresses, which will consider the impact of meteorological factors and distance from both facilities. We have updated the text in the Geographical Information Systems and Geospatial Methods: In addition to facilitating the spatial sampling procedure described above, GIS and advanced geospatial statistical methods will be utilized in the analysis stage of this project. GIS will be used to geocode participants' residential addresses and measure distance from participant's residence to the two power plants, as well as spatially interpolate and integrate the exposure observations (i.e. fly ash, PM_{10}, and heavy metals) and health outcome data. Geospatial statistical techniques such as Hotspot Analysis and bivariate local Moran's I will be used to investigate the clustering patterns of fly ash and heavy metals and explore the associations between these patterns and children's neurobehavioral problems across the study area. These analyses will help characterize the geospatial patterns in neurobehavioral problems related with indoor fly ash exposure in the vicinity of the power plants and coal ash storage facilities. Furthermore, exposure modeling will be used to investigate the spatial dispersion of pollutants in the study area while considering local meteorological factors (e.g. temperature, wind speed, wind direction, etc.). To estimate

	the spatial dispersion of air pollution from the two plants, we will utilize fate and transport modeling via map algebra[63] and the AERSCREEN model, which is based on the EPA's AERMOD[64]. AERSCREEN produces estimates of "worse-case" concentrations of pollutants from a single source, for many times intervals, ranging from 1-hour, 3-hour, 8-hour, 24-hour up to annual. We anticipate that AERSCREEN will be particularly useful for estimating overlapping exposures from both power plants and storage facilities. In general, these geospatial analysis methods will allow us to examine distance decay effects on exposure to air toxicants and identify areas that may have the highest levels of exposure to pollutants from the power plants.
All this information is important for the interpretation of the results, as well as for the non-health readers of the work and the wider relevance of the research outcomes of this useful and interesting study.	We appreciate the reviewer's interest in wanting to understand the information regarding the sources and outdoor pollution. This study was funded to investigate the indoor air and health impact of fly ash on children, hence why the protocol is focused on characterizing indoor exposure and not developing air pollution models. This study protocol was proposed and funded as an epidemiological study. We have added some information regarding the source coal and the plants that generate the ash.
The new references are in a different format to the previous references.	Corrected
Additionally, there are about seven references (out of >60) since 2017. Perhaps there should be a few more?	We have added some updated references, however it is important to recognize that there is very limited research on the human health impacts of coal ash. The lack of "newer" references reflects this. Furthermore, many of the recent literature focuses on leachability or beneficial use of coal ash. There is limited information on exposure to coal ash as an air pollutant.