Title: Cognitive Deve**L**opment in the **U**rban **E**nvironment (The **CLUE** study)

1.Importance: This proposal aims to investigate the emerging hypothesis that children growing up in urban environments display impaired cognitive development due to long-term exposures to traffic-related air pollutants[1] and the noise associated with residential proximity to busy roads.[2] Cognitive deficits have potential life-limiting impacts at both the individual and societal level, with significant economic importance to the nation. While there has been a focus on early years, very few studies have considered the impact of urban environments on development in adolescence. Brain development in adolescence exhibits sensitive periods related to higher-order cognitive skills that are important in educational achievement.[3,4] CLUE will examine whether the effects of air pollution and noise on cognitive development are independent, additive or act in synergy through the Study of Cognition, Adolescents and Mobile Phones (SCAMP), an ongoing prospective cohort study of over 6,000 secondary school children across Greater London.[5] The CLUE study will encompass a number of innovations targeting significant scientific uncertainties to comprehensively advance this research area, through: (**1**) simultaneous consideration of multiple environmental stressors (air pollutants and noise); (**2**) validation, augmentation and enhancement of state-of-the-art source-specific air pollution[6] and noise models[7] developed as part of the NERC-MRC funded Traffic project[8] and validated through the MRC-funded COPE project (ref: MR/L019744/1), using personal monitoring data including individual time-activity patterns and monitoring of indoor environments (school and home), to produce source and chemical specific, location-based, dynamic exposures for the full cohort; **(3)** a longitudinal epidemiological analysis of the associations between air pollution and noise and cognitive and behavioural development during adolescence, employing for the first time, dynamic exposure assessment for cohort individuals; **(4)** an analysis of the effects of early life exposures to air pollution and noise, assessed retrospectively using residential postcodes at birth, and cognitive development during adolescence; and **(5)** an investigation of the potential underlying causal mechanisms, focusing on: (a) biomarkers of exposure (urinary metals) and response (oxidative stress[9] and neuronal injury[10]), their relationship with each other and with short (daily) to longer-term modelled exposures based on the enhanced dynamic modelling developed within this application; (b) the relationship between exposure and response biomarkers with baseline cognition and cognitive development over the study period. With increasing population growth and continuing urbanization, air quality and urban noise have emerged as important determinants of the global and European burden of disease[11] as well as public health within cities.[12,13] Despite predicted reductions in emissions from all sources, air pollution concentrations and noise levels on and around London's busiest roads continue to exceed UK Air Quality Strategy Objective (AQS) and WHO recommended Limit/Guideline Values.[14]

Associations between air pollution and cognitive development: Neurological effects of traffic-related air pollutants have already been highlighted in epidemiological studies.[15,16] Due to the brain growth spurt during the first two years of life[17], earlier studies focused on the neurotoxic effects of prenatal and early postnatal exposures to air pollutants.[18,19] However, the brain continues to mature until early adulthood and thus remains susceptible to developmental injury. Indeed there is an emerging literature demonstrates measurable cognitive deficits and neuropsychological delays in executive function (attention and working memory), motor skills, language development, decreased intelligence quotient (IQ), poorer academic performance in children exposed to elevated air pollutant concentrations (particularly with exposures to polycyclic aromatic hydrocarbons, nitrogen oxides, $PM_{2.5}$ and particle associated metals).[1,10,20] Associations have been seen with indicators of high traffic exposure, such as proximity to busy roads, or the proportion of diesel traffic.[21,22] A recent prospective study of 7 to 10-year-old children (n=2715) in Barcelona revealed significant delays in cognitive development for those attending schools in highly polluted areas compared to those from less polluted areas.[23] A biological basis for these observations has been proposed, based on the translocation of inhaled ultra-fine particles into the brain, with the associated induction of a low level chronic neuroinflammation and oxidative stress resulting in glial activation, and white matter injury.[24] There is evidence from animal studies that inhaled ultrafine particles can penetrate into the olfactory bulb, the frontal cortical and subcortical areas of the brain[25] and that inhalation of ambient air pollution[26] and diesel exhaust can elicit inflammatory changes[27] and altered electro-encephalographic patterns within the brain consistent with cortical stress.[28] Oxidative stress is well established in the pathophysiology of many central nervous system diseases associated with impaired cognition, as well as with the age-related functional decline.[29] For air pollution, the imposition of oxidative stress is thought to arise either through capacity of inhaled pollutants to elicit oxidative injury to the lung, related to their intrinsic capacity to

oxidise biomolecules (nitrogen dioxide), or in the case of particulate matter their content of organic (polyaromatic hydrocarbons and quinones) and inorganic redox (transition metals) catalysts.[30] Consistent with this role, concentrations of 8-isoprostane have been shown to be elevated in exhaled breath condensate and the urine of subjects after exposure to traffic derived air pollution.[31,32]

Associations between noise and cognitive development: There is evidence indicating that chronic noise exposure is associated with impaired cognitive outcomes (particularly those involving central processing and language) and academic attainment among children, however much of this evidence comes from cross-sectional studies that focussed on aircraft noise.[33] Results from the only two longitudinal studies investigating the detrimental effects of aircraft noise on cognitive performance in children, the Munich Airport study[34] and the UK RANCH study[35], are inconsistent, although both studies suggest a link between aircraft noise and impaired reading comprehension in children. Cross-sectional studies specifically analysing the relationship between road-traffic noise exposure and cognitive development/academic performance in children are inconsistent [36-38] and to date, no longitudinal studies have explored the effects of road traffic or rail noise exposure on cognition or academic performance in children. Many of these previous studies had limited ability to explore the potentially additive, independent or synergistic associations with air pollution exposure. Proposed mechanisms for noise effects on cognition include reduction of speech intelligibility,[39] impaired attention,[40,41] learned helplessness,[42] indiscriminate filtering out of noise,[43] and elevated stress responses due to arousal,[44] annoyance/irritation, frustration,[45] and repeated stimulation of the endocrine and autonomic nervous systems.[33] In adults, studies have shown that chronic exposure to environmental noise is associated with elevated cortisol[46], which, in turn, is related to impaired cognitive function.[47] Thus, the stress response to noise has been proposed as a potential mechanistic pathway for any effects of noise exposure on cognition in children.[33] However, very few studies have directly examined the stress response in children, using urinary/salivary cortisol or self-perceived stress, in association with noise exposure, and available studies have produced inconsistent results.[48,49]

Current exposure assessment for air pollution and noise:

Accurate exposure estimates are an essential part of the chain linking environmental stress, such as noise and air pollution, to health outcomes, such as cognitive development. Historically, air pollution exposure assessments including proximity models, land-use regression (LUR) and atmospheric dispersion have been used in epidemiological studies with their various limitations.[50] Recent developments in exposure assessment have seen the incorporation of space-time–activity data and personal measurements, resulting in "Hybrid" or dynamic exposure models.[51] By considering the study population as a living, moving entity, rather than using a static estimation based on residential address, these dynamic models come closer to a "best" estimate of human exposure. Importantly, dynamic exposure models are able to quantify and contrast micro-environments of exposure – travelling, at work/home etc. – highlighting which activities contribute most significantly to overall short and long-term exposure patterns. Furthermore, exposure patterns can be estimated for a range of pollutants and pollution sources, such as size fractionated particulate matter (PM), tyre and brake wear, tail-pipe emissions etc. However, these models require bespoke validation datasets to correctly assign mobility and behavioural patterns, as well as micro-environmental exposures. This information can be gathered by providing personal monitoring equipment to a cohort subset. Consequently, there are few examples of dynamic exposure modelling being used for large scale population health studies. One example of the combined use of personal monitoring and dynamic exposure modelling is "COPE: Characterisation of COPD exacerbations using environmental exposure modelling" study which utilises the London Human Exposure Model (LHEM), a dynamic model developed as part of the TRAFFIC study.[4] The CLUE study will enhance the LHEM beyond the TRAFFIC and COPE studies by providing, on a larger scale, both child-specific mobility and exposure data and paired indoor/outdoor pollutant measurements in the home and school environments. Outdoor noise levels will be estimated using the TRANEX (TRAffic Noise Exposure) model.[7,52,53] TRANEX is based on the UK Calculation of Road Traffic Noise methodology[54] and can estimate road traffic noise levels, for annual A-weighted noise metrics (e.g. daytime, night-time) at multiple facades of dwellings with spatial accuracy of <1m. TRANEX was specifically developed for London as part of the TRAFFIC study; it includes >63,000 road links with information on traffic composition and speed, and uses detailed information on building geometry/heights and ground cover to assess noise propagation routes and noise diffraction between road sources and receivers (i.e. address point locations). Little is known about indoor residential noise exposures and how different characteristics of the built environment (e.g.

window glazing and openings) combine to modify these exposures.[55] In this study we aim to extend current noise exposure modelling to translate outdoor noise levels to estimates of indoor exposures in homes and schools.

This cross-disciplinary research will generate a rich and unique dataset to address current gaps in evidence, build on and enhance exposure modelling in complex urban environments, and advance our understanding of biological pathways of exposures and responses. CLUE will extend the evidence base with which to inform policy across a range of sectors.

2. Scientific Potential- People and Track Record: The research team all have a track record of working together on projects including SCAMP, the TRAFFIC project and a number of NIHR-funded air pollution grants. **Dr Mireille Toledano** is Reader in Epidemiology at Imperial College London (ICL) with over 15 years' specialized expertise in environmental epidemiology and the design, set up and follow-up of prospective cohort studies for environment and health. She is PI of the SCAMP cohort study, co-PI of the COSMOS cohort study. **Prof Michael Thomas** is Professor in Cognitive Neuroscience at Birkbeck, University of London, and the director of the Centre for Educational Neuroscience (CEN). He has substantial expertise on cognitive and brain development during adolescence, both in terms of developmental processes in children and in the final cognitive structures they produce in the adult. . **Dr Ian Mudway** is a lecturer in Respiratory Toxicology at KCL. He has 20 years' experience in air pollution research and assessing the toxicity of ambient pollutants. He played a leading role in the delivery of the NIHR-BRC funded EXHALE project examining the respiratory health of children in London. **Prof Frank Kelly** holds the chair in Environmental Health at KCL, where he is Director of the Environmental Research Group. He is PI of the above mentioned TRAFFIC study and is Chairman of the Department of Health's Expert Committee on the Medical Effects of Air Pollution (COMEAP). **Dr Ben Barratt** is a Lecturer in Air Quality Science at KCL. He specialises in air quality exposure assessments for health cohort studies. He is PI on the MRC-funded study COPE and the US HEI study "HKD3D – a 3D dynamic exposure model for Hong Kong". **Dr Sean Beevers** is a Senior Lecturer in Air Quality Modelling at KCL. He has 20 years' experience in air quality modelling for policy development and public health research, in particular developing advanced air quality exposure modelling systems including the LHEM. **Dr John Gulliver** is Senior Lecturer in Environmental Science at ICL and has ~20 years of expertise in using geographical information systems (GIS) to model environmental exposures, developing both UK[5] and EU-scale[56] noise models. In addition, SCAMP has other co-investigators to draw knowledge from to support this proposal such as Dr Iroise Dumontheil who is a Reader in Cognitive Neuroscience at Birkbeck, University of London and Prof Andy Tolmie who is Professor of Psychology and Human Development at the UCL-Institute of Education.

Environment: The proposed research will be led by ICL. MT, FK, JG, IM, SB and BB are all members of the MRC-PHE Centre for Environment and Health, an interdisciplinary research Centre with world-leading expertise in the fields of environment and health. As a cross-centre collaboration, the CLUE project will be supported by the Centre's infrastructure and benefit from this excellent collaborative environment. CLUE will also benefit from large in kind contributions as a nested study within the existing infrastructure of the SCAMP study – a 4.5 year prospective cohort study commissioned by the Department of Health. SCAMP is exploring cognitive and behavioural development in relation to the use of mobile phones and other wireless devices. It is the largest cohort of its type worldwide, following over 6,000 secondary school pupils at Year 7 (age 11-12 years), focusing specifically on executive functions as these are mainly supported by the frontal lobes which continue to develop during adolescence.[57] In comparison to other UK and international cohort studies (including those that have contributed to, for example, the ENRIECO collaboration), the SCAMP cohort is unique in terms of its specific combination of cognitive outcomes, age at cognitive assessments and biosample collection, and assessment of both air pollution and noise exposure. Crucially, the SCAMP study location makes it the only cohort able to take advantage of the pre-existing TRANEX and LHEM), and thus incorporate dynamic exposure modelling.

Research plans- Aims and hypotheses: This is a longitudinal study of air pollution, noise, biomarkers and cognition. The core hypothesis to be tested is that exposure to individual air pollutants (source specific fractions of PM, NO_2 and O_3), pollutant mixtures, and noise, either separately or in synergy, during adolescence are associated with cognitive impairments in adolescents living within Greater London. The proposal will also test the hypothesis that early life exposure to air pollutants and noise are associated with cognitive development later on in adolescence. Further, that cognitive deficits are associated with the induction of systemic oxidative stress, and in the case of air pollution that these are related to pro-oxidant components of the urban airshed (transition metals associated with ambient particles and oxidative gases), resulting in

neuronal injury and altered neurochemical homeostasis in the brain. **Specific objectives:** Objectives will be carried out in 5 work packages (WP) as shown in Figure 1: **(1)** To enhance existing noise and air pollution models by using already collected detailed measurements (including fixed-site measurements of noise levels, $PM_{2.5}$, PM_{10}, NO_2, O_3 and particle number concentrations at home and school, simultaneously indoors and outdoors, and personal time activity/mobility) to validate, calibrate, and extend the existing London Human Exposure Model (LHEM) air pollution and TRANEX noise exposure models (WP1). **(2)** To link residential postcodes at birth to modelled outdoor air quality and noise estimates to create early life exposures for each child from conception through pregnancy to the end of the first year of their life (WP2). **(3)** To conduct epidemiological analysis (WP3) of (i) changes in cognitive and behavioural outcomes between SCAMP baseline and follow-up assessments in relation to estimates of noise and air pollution from the enhanced validated exposure models in the 6,000 SCAMP cohort; and (ii) the relationship between early life exposures to air pollution and noise and cognitive and behavioural development during adolescence. **(4)** To investigate possible causal mechanisms of the impact of noise and air pollution on cognition using biomarkers of exposure and biomarkers of response: a) by performing biological analysis on the urine and saliva samples (WP4); and b) relating these markers to i) modelled air pollution and noise exposures and ii) cognitive performance and behavioural problems measured at baseline and longitudinally over 2 years (WP5).

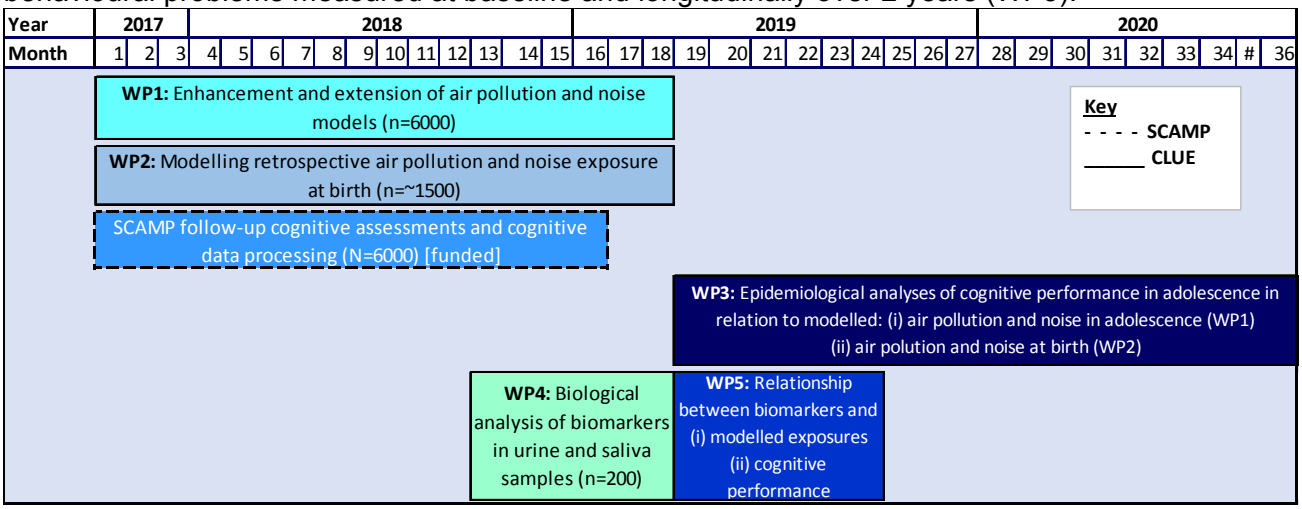

Figure 1. CLUE Timeline of Work Packages

SCAMP data contributions to CLUE

The proposed study utilises the following resources from the SCAMP study:

(a) Assessment of cognitive development, behavioural problems and other covariates: Computer-based cognitive assessments comprising 9 cognitive tasks undertaken together with other questionnaires under examination conditions for a period of 1 hour. Baseline assessment (T1) of 6000 children is complete and follow-up (T2) assessments began in November 2016. The cognitive battery provides three principal types of measure: non-verbal fluid intelligence (Cattell Culture Fair test); language processing, and particularly its resilience in the face of environmental noise (the Speech-in-Noise task); and executive function (EF) skills, capturing respectively cognitive flexibility (Trail-making task), working memory (Backwards Digit Span, Corsi Span), inhibition ('Answer phones' task), and sustained attention (Continuous Performance task). This combined set of executive function skills provides for a robust composite measure of this facet of cognition, using the main dimension of variance in a principal components analysis. Non-verbal intelligence, language processing, and EF are aspects of cognitive function that continue to develop across adolescence, exhibiting reliable changes over a 2-year follow-up.[4,58,59] Behavioural problems are assessed at T1 and T2 via the Strength and Difficulties Questionnaire (SDQ).[60] The scores of 4 SDQ subscales (emotional symptoms, conduct problems, hyperactivity/inattention, peer relationship problems) measure adverse behavioural outcomes and will be combined to form a total difficulties score (TDS). Fluid intelligence, language processing, EF, and TDS will be primary outcome measures in longitudinal analyses (WP3 & 5). A rich dataset on potential confounders such as socio-economic status (SES) (e.g. parental education/occupation, household size), ethnicity, residential history, medical history, physical activity, diet, mode of commuting to school, home environment including ETS and window glazing etc, and anthropometric measures is also available from SCAMP.

(b) Birth data (n=~1500): To model early life exposure to air pollutants and noise retrospectively, residential address of children at birth is required. Residential address at birth will be obtained from national birth registration records. To date, ~1500 parents of SCAMP children have consented to routine data linkage and, as consent is ongoing, it is expected to increase over time.

(c) Measurements and biosampling of SCAMP subset (n=200), the 'investigative cohort': Urine and saliva samples have been/are being collected at three time points (baseline, personal monitoring and follow-up phases) in SCAMP. As part of the personal monitoring study, 48-72 hour personal time activity history (using GPS and a smartphone-based mobility app); and concurrent first morning void spot urine and saliva samples are being collected.At the same time, micro-environmental measurements of air pollutants and noise levels have been/are being gathered, funded by the MRC Centre for Environment and Health and the NIHR GSTFT/KCL Biomedical Research Centre. Paired indoor/outdoor continuous measurements of gases (carbon monoxide, nitrogen dioxide, nitric oxide, ozone) and particulate matter ($PM_{2.5}$ and PM_{10} derived from particle number counts) are taken by AQMesh (Geotech Ltd, UK) and Tzoa (RD02, Tzoa Wearables, California, USA) devices respectively over a period of 48-72 hours at each child's home (n=200) and a subset of SCAMP schools (40%). Paired indoor/outdoor noise measurements using Class 1 Optimus CR:171B sound level meters are taken in the home over a period of one hour at the end of the personal monitoring period and in a sub set of schools (25%). The indoor noise measurements were taken in a room close to the bedroom on a tripod. Noise measurements are summarised as hourly LAeq (i.e. A-weighted noise) and hourly maximum noise levels (Lmax). Noise measurements will be analysed for quiet periods mainly night-time to determine the contribution of environmental noise to indoor noise levels. To date, personal monitoring has been conducted on 90 of 200 expected participants – with monitoring on the remaining participants scheduled for completion by July 2017. All instruments were operated to defined QA/QC standards, with regular calibration against reference monitors. Air quality and noise measurements are being collected over weekdays and weekends and also across different seasons to account for potential variations. All data from SCAMP that will be available as inputs to modelling in this proposal (WP1) are summarised in Table 1. Children in the investigative cohort come from all SCAMP participating schools (both state and private) have similar characteristics i.e. age, sex, ethnicity and SES of the entire SCAMP cohort.

Enhanced air pollutant and noise modelling (WP1): Using individually modelled time-location-activity patterns LHEM is capable of estimating an individuals' pollutant exposure as they move around the UK, including in-vehicle, train, cycling, indoors at home and at work/school. It achieves this by combining an hourly outdoor air pollution model (CMAQ-urban)[61], which predicts air pollution every 20m with indoor-outdoor ratios for pollutant infiltration into buildings[62], and micro-environmental mass balance models, estimating exposure to $PM_{10/2.5}$, PM associated chemical constituents and gaseous pollutants. Currently, the exposure of ~45,000 Londoners is predicted, based upon the London Travel Demand Survey (LTDS)[63] and representative of 6.8 million people. In this proposal, the personal and micro-environmental pollutant monitoring described above, will be used to evaluate and calibrate the existing LHEM model, improving its exposure estimates for important indoor environments at home and at school and to validate the LHEM's modelling of time activity patterns (mode and duration of travel) specifically for children aged 11-15 years.. In combination with long-term personal exposure measurements in different indoor and outdoor transport microenvironments, made available from the MRC COPE study, as well as recent and comprehensive measurements in the London Underground, the LHEM model will be subject to unprecedented development to improve its accuracy. While the paired indoor-outdoor home and school measurements will provide minute by minute information on exposure sources and patterns for 200 children over a relatively short period, the LHEM model will be required to extend the exposure estimates to represent average exposure for different seasons, weekend vs weekday predicted by the CMAQ-urban model. LHEM exposure predictions will be improved through a model 'calibration' exercise. Where the model does not agree with observations, adjustments will be made to indoor/outdoor ratios and deposition rates by hour of the day to reflect average indoor exposure and to associate these with specific building characteristics/types. Likewise the average exposure of the participants whilst travelling will be calculated by LHEM using GPS location and mobility data collected during the SCAMP personal monitoring study, to give location details and mode of transport from the activity history. In transport environments the LHEM will have benefitted from a 'calibration' against COPE personal measurements and will benefit from the use of the new London Underground measurements incorporated into LHEM as part of the project. Combining the

newly evaluated LHEM model with information available for the extended cohort of 6000 children including: home and school location, smoking, building type, journey details and mode of transport, individual exposures will be calculated across the SCAMP study period, 2015-2017 for $PM_{10/2.5}$, source specific PM-associated chemical constituents, NO_X, NO_2, O_3 for each of the 6000 children for longitudinal epidemiological analysis and analysis against biomarkers in the investigative cohort. Importantly, as CMAQ urban is built on a chemical transport model it is ultimately capable of providing exposure estimates at an equivalent spatial and temporal resolution across the UK, as well as the European domain. Therefore the enhanced model has the potential to be applied to other cohorts, to cross validate findings at a later date.

Monitoring		**Duration**	**Procedure/Device**	**Data inputs to…**
Fixed-site	**Noise** home + school	48-72hr	Paired indoor/outdoor monitoring. Class 1 OptimusCR:171B sound level meters.	**TRANEX**: validate/extend with **indoor noise data.**
	Air pollution home + school	48-72hr	Paired indoor/outdoor monitoring. $PM_{2.5}$, PM_{10}, NO, NO_2, O_3, CO via AQMesh - multipollutant electrochemical sensor; and particle count via TZOA.	**LHEM**: evaluate the model **indoors**
Personal	**Mobility**	48-72hr	Smartphone app (GPS data)	**LHEM**: provide **child mobility data for use in the model**
			Time activity diary smartphone app (Travel mode)	

Table1. Application of different monitoring components in LHEM and TRANEX models

For the noise exposure modelling, we will use modelled outdoor noise levels as the basis for predicting indoor noise levels in exposure assessment. Outdoor noise from different transport sources will be modelled for each address and school location. Road traffic noise will be modelled for each building façade using TRANEX noise,[5] whereas rail and aircraft noise will be modelled for a single address point location. Annual average airport noise data for Heathrow and London City airport are already held by ICL for some relevant years and data for further years will be obtained from the Civil Aviation Authority. Moreover, rail noise modelled at all ~3.1 million addresses in London is already held at ICL. Noise monitoring data collected for the investigative cohort will be used as follows: Outdoor noise measurements will be compared with modelled outdoor noise levels to further evaluate the TRANEX model, then linked to the indoor noise measurements via multiple regression analysis, accounting for information characteristics of each location (e.g. number and type of windows, building volume, height above ground), to model noise levels inside dwellings. Cross-validation techniques will be used to evaluate the model. Novel outputs from this study will be a first attempt at developing an empirical regression model to predict indoor, daytime (school), evening (bedroom) and night-time (bedroom) noise levels. The validated, calibrated and enhanced TRANEX model will then be used to estimate noise exposures for all 6000 SCAMP participants.

Air pollution and noise modelling at birth (WP2): To obtain retrospective air pollution levels at time of birth (2003/04), pre-existing modelled air pollution estimates for London will be linked to the residential postcodes of children at birth to provide cumulative exposures from conception through pregnancy and over the first year of life for each child. Similarly, the TRANEX model will be used to provide noise exposure estimates using traffic data for 2003/2004 on an annual basis (supplied by KCL from the TRAFFIC project) linked to each child's residential postcode at birth.

Epidemiological analysis (WP3): We will evaluate the association between changes in cognitive and behavioural outcomes from baseline to follow-up assessments and exposure to noise and air pollution. Our epidemiological analysis will include all SCAMP participants who have completed both baseline and follow-up cognitive assessments. Primary outcome measures will be as follows: for cognition - fluid intelligence, language processing, and the composite EF measure at baseline (T1) and follow-up (T2); and for behavioural problems - total difficulties score (TDS) from the SDQ at T1 and T2. Specific EF component skills (cognitive flexibility, working memory, inhibition, sustained attention) and SDQ subscale scores would be secondary outcome measures, assessing possible differential effects of environmental influences. Due to the multilevel nature of the data collected i.e. repeated assessments nested within each child and children nested within schools, we will use linear mixed effects models with the outcome measures at both T1 and T2 included as dependent variables, and random effects for child and school. The models will evaluate differences

in cognitive performance/behavioural problems, at baseline and 2-year change, according to air pollution and noise exposures. Primary exposure measures will be modelled $PM_{2.5}$, PM_{10}, NO_X, NO_2, O_3, and indoor daytime (school), evening (bedroom) and night-time (bedroom) noise levels at baseline. For comparison with previous research, modelled outdoor noise levels (annual A-weighted noise metrics for day and night time) from TRANEX will also be analysed. Air pollution and noise exposure measures will be included as independent variables in single-pollutant, and joint noise-air pollutant models. Possible secondary analyses of multi-air pollutant models will depend upon pollutant correlations. Exposures will be included as categorical variables (dichotomous or quartiles), unless crude models demonstrate linear relationships between modelled air pollution and noise exposures and EF scores. Models evaluating change in cognitive performance will be adjusted for individual baseline to follow-up age difference (to account for the influence of age on cognitive test response). Models will be adjusted for covariates such as sex, SES (at individual and small-area level), ethnicity, BMI, smoking in the home, mode of travel to school, season. Sensitivity analyses would exclude participants to elevated daytime (≥50dB; 13.5%) and night-time (≥50dB; 5.1%) noise levels due to aircraft and/or rail noise, those who changed residential address in the 6 months prior to SCAMP baseline to account for potential exposure misclassification. We will also stratify analysis by sex, type of school, SES, and ethnicity to investigate potential effect modification. We will use similar analytical strategies to evaluate the relationship between exposure to air pollution and noise in early-life (at birth) and cognitive and behavioural development in adolescence. This will be undertaken in the subsample of SCAMP children on whom we have consent for linkage to birth records (~1500).

Biomarker analysis (WP4&5): Biomarker analysis will take place using 3 samples from each of the 200 subjects within the investigative cohort, reflecting samples collected during baseline, personal monitoring and follow-up assessments of SCAMP. Exposure biomarkers will include urinary metals, assessed by inductively coupled plasma mass spectrometry following sample digestion in nitric acid. Focus will be on metal and metalloid elements to provide exposure biomarkers indicative of varying traffic sources (brake wear (Cu, Sb, Ba), tyre wear (Zn), resuspension of road dust (Al, Ca), mechanical abrasion from the engine (Fe, Mo, Mn) and tail pipe markers indicative of oil/fuel combustion (Cr, Ni, V, As)[31]. We will examine the association between these exposure biomarkers and the LHEM modelled pollutant estimates; examining short-term (24 hour and monthly) and long-term (annual) exposures to NO_2, O_3, PM_{10} and $PM_{2.5}$; as well as source informative PM-chemical components derived from the CMAQ urban model, including elemental carbon, primary and secondary organic carbon and diesel indicative intermediate-volatility organic compounds after controlling for ETS exposure, sex and ethnicity. We will also examine the relationship of these exposure biomarkers with baseline cognitive function and change in cognitive performance (using composite EF factor) over the two year follow up period. As both noise[65] and air pollutant exposures[9] have been associated with the induction of systemic oxidative stress we will also examine whether modelled pollutant exposures and measures of cognitive function are associated with lipid and DNA oxidation markers (biomarkers of response) in the children's urine; 8-isoprostane (8-isoP) and 8-Oxo-2'-deoxyguanosine (8-oxo-dG), respectively. Furthermore, we will examine the relationship between modelled exposure estimates, cognitive performance, and biomarkers of response indicative of neuronal injury. These will be neuron specific enolase (NSE) and calcium-binding protein S100B in saliva, both of which have been shown to be elevated in acute brain injury, neuropsychiatric conditions and disorders associated with neuroinflammation.[65-67] Urinary 8-oxo-dG and 8-isoP will be quantified using commercially available competitive enzyme-linked immunosorbent assays according to the manufacturer's instructions (Oxis Health Products, Portland OR, USA and Cayman Chemical Company, Ann Arbor, MI, USA, respectively), with data corrected for urinary creatinine (Cayman Chemical Company, Ann Arbor, MI, USA). Neuron specific enolase and S100B will be determined using commercially available ELISAs from R&D Systems and Abnova, respectively. In addition, salivary cortisol concentrations will be measured using diagnostic kits (Salimetrics, UK) as indicators of hypothalamus-pituitary-adrenal (HPA) axis activity. Determination of urinary cotinine concentrations, as a control for potential environmental tobacco smoke (ETS) exposures will be performed using a commercial microplate enzyme immunoassay (Cozart Forensic Microplate EIA for cotinine, product no. M155B1) from Concateno (Abingdon, UK), with evidence of exposure to ETS assigned at a creatinine corrected cotinine value of more than 30 ng/mg.[68]

Study power and data analysis: Assuming baseline test scores (at 11-12 years old) have a mean of 23.65 and standard deviation of 9.22 (e.g., the CANTAB spatial working memory task[69]), and estimating standard deviation of change in cognitive scores over 2 years from estimated test-retest

correlation, the epidemiological analysis of 6,000 children (WP3) will have 80% power at 5% significance to detect small differences (ranging from 0.63–1.01 in absolute units) in the change of cognitive scores between high and low NO_x exposure groups. In the investigative cohort (n=200) analyses (WP5) we will have 80% power at 5% significance to detect correlations of 0.25 or more between air pollution/noise exposure estimates and urinary oxidative stress biomarkers, as have been observed in previous research.[70]

3. Ethics and research governance: Parents and children are provided with study information for informed consent. Study participation is voluntary, and participants have the right to withdraw at any time. Procedures for consent and confidentiality/security of data will be based on those already in place for SCAMP, which have been approved by NRES Committee North West-Haydock and the HSCIC Information Governance Toolkit Team. Access to personal identifying information is restricted and all members of the academic research team at ICL sign non-disclosure agreements. Before commencing CLUE, we will obtain additional Sponsorship and Indemnity cover from ICL's Joint Research Compliance Office and ethical approval.

4. Exploitation and dissemination: Study results will be published in high-ranking peer-reviewed scientific journals and high-profile conferences. Dissemination to wider stakeholders will be via the MRC-PHE Centre for Environment & Health's Community Advisory Board, comprising NGOs, politicians, and representatives of press and industry, and through the activity of the Outreach Committee, which will facilitate public and school engagement with research. Study findings will be shared with participants, parents, and schools via newsletters and the study website.

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