# Joint Research Management Office (JRMO) Research Protocol for Research Studies

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|---|---|
| **Full Title** | Respiratory impacts of tailpipe and non-tailpipe particulates on adults with asthma: A feasibility study |
| **Short Title** | **(IONA) Assessing the impact of non-tailpipe emissions from traffic on the asthmatic airway** |
| **Sponsor** | Queen Mary University of London (Queen Mary)<br><br>Contact person:<br><br>Dr Mays Jawad<br>Research & Development Governance Operations Manager<br>Joint Research Management Office<br>Empire House<br>67-75 New Road<br>Whitechapel, E1 1HH Phone: 020 7882 7275/6574<br>Email: research.governance@qmul.ac.uk |
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# 1. Contents

## 2. Glossary

AUKCAR - Asthma and Lung UK Centre for Applied Research
BMI – Body Mass Index
CO-I - Co-Investigator
FEF – Forced Expiratory Flow
FeNO - Fractional Exhaled Nitric Oxide
FEV1 – Forced Expiration in One Second
FVC – Forced Vital Capacity
GCP – Good Clinical Practice
HGV – Heavy Goods Vehicles
ICL – Imperial College London
IONA – Assessing the impact of non-tailpipe emission from traffic on the asthmatic airway
IgE - Immunoglobulin E
ISC – Independent Scientific Committee
Kph – Kilometres Per Hour
LAQN – London Air Quality Network
LGV Light Goods Vehicles
Mph – Miles Per Hour
NTE – Non-tailpipe emissions
NO2 – Nitrogen Dioxide
O3 - ozone
PI – Principal Investigator
PM – Particulate Matter
PMG – Project Management Group
PPI – Public and patient Involvement
QMUL – Queen Mary, University of London
SOP – Standard Operating Procedures.
TLC – Tidal Lung Capacity
UK – United Kingdom

## 3. Signature page

**CI Agreement**

The study, as detailed within this Research Protocol, will be conducted in accordance with the principles of Good Clinical Practice (GCP), the UK Policy Framework for Health and Social Care Research, and the Declaration of Helsinki and any other applicable regulations. I agree to take responsibility for the statistical analysis and oversight of this study.

**CI Name:** **Dr James Scales**

**Signature:** 

**Date:** **23/06/2022**

## 4. Summary and synopsis

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| Short title | (IONA) Assessing the impact of non-tailpipe emissions from traffic on the asthmatic airway |
| **Methodology** | Randomised experimental three condition crossover panel study |
| **Objectives / aims** | **Objective 1**: Assess and compare the acute health effects: changes in lung function and airway inflammation, in mild/moderate asthmatics exposed at three selected microenvironments with contrasting traffic profiles to increase contrast in particulates from brake wear, tyre wear, and road abrasion.<br><br>**Objective 2:** Provision of an air pollutant database ($PM_{2.5}$ and $PM_{10}$ mass and chemical composition, PNC and $NO_2$) and a time series of source apportioned $PM_{2.5}$ and $PM_{10}$ covering all exposure days at the three |

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| | selected sites.<br><br>**Objective 3**: Examine the relationship between variations in daily non-tailpipe source fractions derived from tire, brake and road wear, with the deposited dose determined in the nasal airways and the physiologic and immunologic responses observed.<br><br>**Objective 4**: Provision of a biobank of plasma samples for future work examining molecular signature of exposure and response. |
| **Number of participants** | 48 |
| **Inclusion and exclusion criteria** | **Inclusion criteria:**<br>• Doctor diagnosed asthma starting on or before age 16 years<br>• Prescribed regular inhaled corticosteroid medication<br>• Able to use a static exercise bicycle for the study duration<br><br>**Exclusion criteria:**<br>• Tobacco smoking, or living with smoker<br>• Body mass index (BMI) > 30<br>• Asthma hospitalisation within 12 months<br>• Three or more asthma episodes requiring oral corticosteroid medication within 12 months<br>• Other major lung disease<br>• Chest surgery within 6 months<br>• Unable to give informed consent<br>• Occupational exposure to particulate matter (PM) or high levels of air pollution |
| **Statistical methodology and analysis (if applicable)** | Mixed effects models, taking into account repeated measurements within participants. |
| **Study duration** | 01/08/2022- 1/12/2024 |

# 5. Introduction

## 5.1. Background

The global burden of disease survey estimated air pollution contributed to one in eight deaths in 2019[1]. Urban areas such as London are a heterogeneous patchwork of different air pollutants, which all have different impacts on human health. Air pollution disproportionally impacts people living with asthma, increasing hospital admissions[2] and the likelihood of death[3]. Reports suggest that 65% of asthma deaths could be avoided by suitable primary care support[4]. Understanding how different air quality environments affects people living with asthma will improve asthma management in primary care by increasing the specificity guidance provided to people with asthma.

In an effort to reduce air pollution a number of government and industrial policies have been developed to increase sales of electric and hybrid cars. Electric and hybrid vehicles produce less air pollution from exhausts but due to their increased weight, they increase air pollution from tyre and road wear with an uncertain contribution from brake wear. Particles arising from tire and brake wear, as well as the resuspension of road dust represent a greater proportion of roadside PM by mass than direct tailpipe emissions. As tailpipe emissions will decrease as nations strive to meet their NetZero commitments, greater attention on non-tailpipe sources is urgently required to evaluate their relative hazard compared with other pollutant sources. Our previous *in vitro* studies on dendritic cells and airway macrophages have indicated enhanced immune responses with the coarse particulate fraction ($PM_{2.5-10}$) from non-tailpipe constituents[5]. To date no research has explored the health impacts of non-tailpipe air pollution on human health in a real-world setting.

## 5.2. Rationale

In an effort to reduce air pollution a number of government and industrial policies have been developed to increase sales of electric and hybrid cars. Electric and hybrid vehicles produce less air pollution from exhausts but due to their increased weight, they increase air pollution from tyre and road wear with an uncertain contribution from brake wear. Air pollution particles arising from tire and brake wear, as well as resuspension of road dust, now represent a greater proportion of roadside PM by mass than direct tailpipe emissions. These Non-Tailpipe Emissions (NTE) remain unregulated, and their health impacts under explored, particularly in vulnerable groups.

Previous work by our team [6-12] demonstrated the feasibility of a quasi-experimental human real-world exposures using a crossover design for disentangling relative contributions of pollutant metrics on acute cardiopulmonary endpoints in healthy adults. Whilst this work showed the key role played by primary gaseous and particulate emissions, the study focused on responses in healthy adults and did not

examine biological pathways likely to lead to symptom exacerbation in vulnerable asthmatic populations. The study did examine a range of metals such as Fe, Cu, Ni and V, reflecting their capacity to cause oxidative stress, but did not explicitly relate these to NTEs, nor employ them for detailed source appointment.

Our previous work has consistently shown that coarse mode PM is more effective at driving dendritic cell maturation and T-cell activation, than $PM_{2.5}$ samples from urban background and roadside sites, or freshly collected diesel tailpipe PM. Given that such changes were observed in vitro this provides suitable rationale that health changes may be observed in asthmatic participants as people living with asthma are uniquely sensitive to the impacts of air pollution.

To date, no research has explored whether real-world NTE are causally related to worsening of asthma. Therefore, we aim to explore the hypothesis that clinically important adverse acute asthmatic responses are driven by non-tailpipe components within coarse mode PM.

# 6. Study objectives

## 6.1. Primary objective

**Objective 1:** Assess and compare the acute health effects: changes in lung function and airway inflammation, in mild/moderate asthmatics exposed at three selected microenvironments with contrasting traffic profiles to increase contrast in particulates from brake wear, tyre wear, and road abrasion.

## 6.2. Secondary objective

**Objective 2**: Provision of an air pollutant database ($PM_{2.5}$ and $PM_{10}$ mass and chemical composition, PNC and $NO_2$) and a time series of source apportioned $PM_{2.5}$ and $PM_{10}$ covering all exposure days at the three selected sites.

**Objective 3**: Examine the relationship between variations in daily non-tailpipe source fractions derived from tire, brake and road wear, with the deposited dose determined in the nasal airways and the physiologic and immunologic responses observed.

**Objective 4**: Provision of a biobank of plasma and nasal mucus samples for future work examining molecular signature of exposure and response.

## 6.3. Primary endpoint

- Lung function as measured by Forced Expiratory Volume in one second (FEV1)

**Secondary endpoint**

- Spirometry (FVC, FVC/FEV1 ratio, z Scores)
- Fractional Expired Nitric Oxide (FeNO)
- Peak expiratory flow
- Oscillometry
- Asthma symptoms
- Asthma control Test
- Quality of Life: AQLQ
- Immune responses: damage-associated molecular patterns (DAMPs) and alarmins, such as high mobility group box 1 (HMGB1) S100 proteins, myeloperoxidase-DNA complexes (MPO-DNA), IL-33, Th2 and Th17 cytokines and metal deposition in the nasal airways

**Control variables**

- Estimated VO2max
- Heart rate
- Air quality measured as: PM10 and PM2.5, particle number concentration (PNC) and gaseous pollutants ozone (O3), nitrogen dioxide (NO2), black carbon.
- Pollen count
- Temperature and humidity
- Physical activity (accelerometry)
- Personal air quality (Plume labs)

**Biobanking**

- Venous blood
- Nasal Mucus
- Urine

**Banking**

- PM filters

## 7. Study population

Asthma is a complex disease now recognized to include multiple phenotypes. Therefore, to ensure maximum generalisability of our findings, we will recruit participants with the most common phenotype: allergic asthma that started in childhood/adolescence. We will exclude those with a BMI >30 to exclude obese, steroid-resistant phenotypes. Participants (male and female) recruited to the study will be over 18 years old with mild to moderate stable asthma. We will exclude those

hospitalized with an asthma attack in the last 12 months, and those with more than three episodes requiring oral corticosteroids in the last 12 months. To reduce the impact of confounders participants must neither smoke, nor live with current smokers, must live within greater London, and have no occupational exposure to PM or oxidant gases.

With input and guidance from our active patient and public involvement group at the Asthma UK Centre for Applied Research (AUKCAR), participants will be recruited from primary care.

## 7.1. Recruitment

***Primary Care settings***

Guided by experts within the Primary Care CRN teams and by NOCLOR, we will use a PIC site approach. In the first instance participants will be recruited via general practice. We will approach general practices in East London with the help of Dr Victoria Tzortziou-Brown, research lead for North East London Integrated Care Board, inviting them to act as PIC sites and other areas of central London with the help of NOCLOR.

With guidance from the study team, a member of the practice administrative team, GP or practice nurse will run searches of their patient electronic health records using READ / SNOMED codes to identify eligible patients according to the study inclusion / exclusion criteria.

Eligible participants identified by searches will be approached via secure electronic mail out using AccuRx, with an invitation to join the study. The invitation will provide a very brief summary of the study with a link to the Patient Information Sheet and further information on the IONA study website, through which they can express an interest in participation, and provide contact details. A draft text can be found at *SMS text exampleV0.2 28.02.23*

Once potential participants have expressed an interest in the study, Dr James Scales will contact them by phone or a Teams/Zoom call to discuss the study, clarifying any points and answering questions. Potential participants will have at least 24hours to consider participation. To confirm their consent, they will have the option of emailing copies of signed consent forms to Dr Scales, or signing consent forms at the first study visit.

As an NIHR portfolio study, we will seek Service Support Costs and CRN support for recruitment.

***Non primary care settings***

If recruitment in primary care proves ineffective, our secondary recruitment approach will target people with asthma who study and work in Queen Mary, University of London and Imperial College London. With input and guidance from our active patient and public involvement group at the Asthma UK Centre for Applied Research (AUKCAR), participants will be recruited from research lists, lecture visits, posters and social media mail outs across the university campuses and general population. Participants may be university students and staff. However if participants are members of staff or students they will not be recruited by members of staff in direct supervisory roles.

***Example recruitment documents:***

Lecture slide (LINK)

Poster (LINK)

SMS template (LINK)

Email template (LINK)

Website Copytext. (LINK)

### 7.2. Inclusion criteria

**Inclusion criteria:**

- Doctor diagnosed asthma starting on or before age 16 years
- Prescribed regular inhaled corticosteroid medication
- Able to use a static exercise bicycle for the study duration

### 7.3. Exclusion criteria

- Tobacco smoking, or living with smoker
- BMI > 30
- Asthma hospitalisation within 12 months
- Three or more asthma episodes requiring oral corticosteroid medication within 12 months
- Other major lung disease
- Chest surgery within 6 months
- Unable to give informed consent
- Occupational exposure to PM or high levels of air pollution
- Under the age of 18
- Women at any stage of pregnancy
- Any breast feeding women

Occupational exposure is defined as people who work as Taxi drivers, couriers, waste removal drivers and utility services drivers. If other occupations with high exposure to pollution need to be considered the study team will discuss potential eligibility with the PMG group, comprising of experts in air pollution and asthma management.

**8. Study design**

Randomized cross-over semi-experimental panel study

# 9. Study procedures

## 9.1. Pilot Study

A pilot study will be completed to future explore the efficacy of the study design. The pilot will be completed following the same protocol outlined in this document in sections 9.2 to 19.1 with the following alterations:

***Recruitment:***

20 participants will be recruited in a convenience sample using the following inclusion and exclusion criteria:

***Inclusion:***

- Healthy participants with no known respiratory health issues.
- Able to exercise intermittently for two hours
- No surgery within 12months

***Exclusion:***

- Unable to give informed consent
- Occupational exposure to PM or high levels of air pollution
- Under the age of 18
- Women at any stage of pregnancy
- Any breast feeding women

***Screening:***

As the exclusion criteria prohibits participants with respiratory health issues pilot participants will not provide a screening blood sample (4 x 10ml in single draw) for eosinophil levels, and specific common aero-allergens immunoglobulin E (IgE) levels.

***Expenses:***

Only expenses pertaining to travel will be paid to participants of the pilot study.

***Biobank:***
Due to the novelty of the research we will biobank samples from the pilot study in accordance to the procedures outlined in section 14.

***Dissemination:***

- Findings will be disseminated to the study team and PPI group to inform any decision making prior to the commencement of data collection for IONA.
- There is no intention at this time to compare health to asthmatic data, due to likely differences in air quality. However findings will still be highly novel and we will still seek to publish in peer reviewed journals.

### 9.2. Overview

48 participants with mild and moderate asthma will be recruited from QMUL and Imperial College London universities and research lists into a single experimental group. Participants will visit the field-testing sites located in three different air quality environments in a randomised cross over design. Sites targeted will be:

(1) A busy road junction characterized by **stop-go traffic** to enhance emissions from brake wear
(2) **High speed continuous traffic,** to enhance tire wear emissions
(3) An **urban background** site away from clear traffic sources

During the exposure protocol participants will ride on a static bike for two hours at a standardised intermittent intensity. Participants will perform identical respiratory health assessment following the exercise task. In parallel to exposures at sites we will perform real-time measurements of PM10 and PM2.5, particle number concentration (PNC) and gaseous pollutants ozone (O3), nitrogen dioxide (NO2), black carbon.

### 9.3. Participant pathway summary

Participants will be invited to take part by either Dr James Scales or by a primary care practitioner in their General Practice clinics. Participants will provide informed consent after they have read the study information sheets and have time consider their involvement (minimum 24 hours) and ask any questions.

Upon providing informed consent the participants will visit QMUL laboratories for a screening assessment. During this one hour visit they will complete a submaximal exercise test on a static bike (to provide estimates of maximal exercise capacity to standardise exercise exposure protocol) and perform pre and post bronchodilator

spirometry and exhaled nitric oxide testing and oscillometry. At this point participants will be sent an online link to the symptom surveys. Surveys will be explained to the participants and they will be asked to complete the surveys they days before the testing visits. Participants will be provided with personal air quality monitor, heart rate monitor and physical activity monitor to wear three days before the exposure visit.

Participants will be invited to visit one of three field based study sites in a random order. Study sites will be located in walled gazebos next to air quality monitoring sites (managed by our collaborators at ICL) positioned near roads to provide the required air quality exposure. The protocol for the three visits will be identical and each visit will be separated by at least two weeks (washout). Participants will initially provide a urine sample at home and will bring the sample with them to the testing site. To control for participant exposures before testing, a taxi will be provided for the participant. Participants will travel with car windows closed, and air recirculation turned on. Participants will arrive that the study site at 10:00am, and will be joined by up to three other participants. In the first hour the participants will perform the respiratory health assessments and will provide a blood and a nasal mucus sample. After performing all the pre exercise tests participants will wear a heart rate monitor and will commence the exercise protocol (six exercise bouts of 15 minutes separated by fifteen-minute rest periods on a static exercise bike). Half-way through the protocol participants will perform spirometry, impulse oscillometry and FeNO testing. Upon completing the two-hour exercise protocol participants will complete the same battery of health assessments and will provide nasal mucus samples. Participants will leave the assessment area at about 14:00pm. Air pollution measurements will be taken for the duration of the time that the participant visits the exercise location.

24 hours after the exercise protocol participants will visit the QMUL laboratories to perform the health assessment tests again and to provide a mucus sample via nasal lavage, blood sample via venepuncture and provide a urine sample. After repeating this process three times (one visit to each field site) they will receive an expenses payment of £200 and leave the study. Participants will be paid £60 per exposure visit and £20 pre-screening visit. Participants will be paid for each visit they attend and will be paid when they complete participation or withdraw from the study.

### 9.4. Exposure site selection:

The two air quality supersites in London, located in a suburban park in South London (Honor Oak Park) and close to a major trunk road in Central London (Marylebone Road), will be used as a basis for this study. These are owned and managed by ICL and provide the required facilities and the highly time resolved chemical composition measurements required to quantify the non- tailpipe component of PM. An additional measurement location will be established at ICL's White City campus for the duration of the study using ICL's mobile measurement facility,[13] equipped with the same highly time resolved chemical composition measurement capability as the supersites. This location provides the contrast in non-tailpipe contributions being next to the high

speed A40 flyover and the required facilities to support the trial which are not available at other LAQN measurement locations.

*Figure 1: Air quality supersites: Marylebone Road (left), Honor Oak Park (centre), mobile measurement station (right)*

The Marylebone Road station is located 2m from kerbside, this major trunk road carries 51,000 vehicles per day - 4% Heavy goods vehicles (HGV), 3% Buses, 16% light goods vehicles (LGV);[14] average speed is 34 kph (21 mph). The stop-go traffic on this congested road increases brake wear relative to tire wear and resuspension. This is confirmed by the LAEI, which estimates 51% of NTE as brake wear compared to 39% as resuspension and 10% as tyre wear. There is a large area of sidewalk next to the cabin that will be segregated from the public for the exercise compound and public toilets are less than 100m away, equipment will be stored in the station between exposures.

The proposed location on ICL's White City campus is 5 m from the kerbside of the busy A40 flyover, carrying 93,000 vehicles per day - 6% HGVs, 17% LGVs;[14] average speed was 33-59 kph (21-37mph). This road is a major route into and out of Central London, the high number of HGV and associated vehicle weight will lead to increased resuspension and tire wear.[15,16] Due to the higher speeds and more free flowing traffic, the LAEI estimates this location to have only 85% of the brake wear emissions of the Marylebone Road measurement location thereby increasing the contrast between the two traffic locations. There is ample room on the campus to segregate as an exercise compound and the adjacent Michael Uren Engineering Hub will provide equipment storage and toilet facilities.

### 9.5. Air Pollution Measurements and Characterization of Non-Tailpipe Emissions:

The air quality monitoring stations will provide, measurement data according to the data quality assurance procedures described below, they will also enable a robust determination of the sources of tailpipe, non-tailpipe and other urban and regional sources of $PM_{10}$ and $PM_{2.5}$.

*Table 1: Air quantity station measurement configuration*

| **Measurement** | **Stop-go traffic** | **High speed continuous traffic** | **Urban background** |
|---|---|---|---|
| | **Marylebone Road** | **White City** | **Honor Oak Park** |
| $PM_{10}$ and $PM_{2.5}$ | Optical Particle Counter, Fidas 200E, Palas, DE | | |
| $NO_2$ | T200 Chemiluminescence NO/ NOx, Teledyne, USA | | T500U Cavity Attenuated Phase Shift (CAPS), Teledyne, USA |
| NO, $NO_X$ or $NO_Y$ | | | T200U Chemiluminescence NO/ $NO_Y$, Teledyne, USA |
| $O_3$ | T400, UV Absorption O3 Analyzer, Teledyne, USA | | |
| 37 elements (Al, Si, P, S, Cl, K, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Ge, As, Se, Br, Rb, Sr, Y, Zr, Nb, Cd, In, Sn, Sb, I, Ba, Hg, Tl, Pb, Bi) | Xact Multi-Metals Monitoring System, Cooper Environmental Services, USA | | |
| Organic Mass, $NO_3$, $SO_4$, $NH_4$ | Aerosol Chemical Speciation Monitor (ACSM), Aerodyne Research Inc, USA | | |
| Black Carbon | Aethalometer AE33, Magee Scientific, USA | | |
| | | | |

The measurement stations are all part of the London Air Quality Network (www.londonair.org) and or the UK government's Automatic Urban and Rural Network (https://uk-air.defra.gov.uk/networks/network-info?view=aurn)

- PM10 and PM2.5 is measured using the Fidas 200E which is certified for use on UK air quality networks and have demonstrated equivalence to the reference

method through extensive laboratory and field tests in the UK and EU (https://uk-air.defra.gov.uk/networks/monitoring-methods?view=mcerts-scheme).

- Oxides of nitrogen (NO, NOX, NO2) is measured using ozone chemiluminescent; this is the standard approach for monitoring networks in the UK and internationally.

- The urban background station is more focused on atmospheric research and is therefore equipped with more highly specified measurement equipment; it measures NO2 using Cavity Attenuated Phase Shift (CAPS) and NOY rather than NOX to minimize sample loss in this lower concentration environment.

- O3 is measured at all stations using UV Adsorption.

The air quality supersites stations at Honor Oak Park and Marylebone Road are equipped with high time resolution chemical composition measurement techniques and particle counters as part of the UK's Government's Particle Numbers and Concentrations Network or through funding from UK Research and Innovation funding and are managed and operated by Imperial College London

The Xact Multi-Metals Monitoring System (Cooper Environmental, USA) measures 37 elements between Al and Bi at an hourly time resolution using ED-XRF (Tremper et al., 2018). To maximise size and chemical composition information, PM2.5 and PM10 are collected onto the Teflon filter tape on alternate hours using a switching valve and intermediate hours are interpolated as described by (Manousakas et al., 2022).

The ACSM (Aerodyne Research Inc, USA) is a widely research instrument (Ng et al., 2011) and is extensively being used in advanced measurement networks around the world to measure the non-refractory composition of aerosols.(Bressi et al., 2021) All ACSMs used in this study utilize a quadrupole mass spectrometer, the Honor Oak Park instrument uses a PM2.5 lens(Joo et al., 2021) while the roadside instruments use a PM1 lens to minimize lens transmission losses of freshly emitted aerosols close to vehicles(Xu et al., 2017).

The AE33 Aethalometer measures the attenuation of light transmitted through the sample collected on a filter, which is then converted to a black carbon measurement.

The road surface conditions will be collected from surface sensors at Marylebone Road (DRS511, Vaisala, Finland) and using a camera at White City Flyover (DSC111, Vaisala, Finland), this will be used to differentiate wet and dry road surface conditions a per Hicks et al 2021(Hicks et al., 2021).

### 9.5.2 Source Apportionment

The equipment discussed will identify sources of NTE by quantifying and source apportioning the high time resolution chemical composition measurements described above to produce time series and factor profiles of source emissions in organic aerosols and elemental compositions.

Using high time resolution data in this way addresses many of the challenges faced when quantifying the different sources in the urban environment as the higher time resolution yields a greater variability from changing emission characteristics, boundary layer dynamics, and reflects short lived events which are obscured by the long sampling time of filter-based approaches(Wang et al., 2018). This variability is fundamental to improving the performance of multivariate statistical approaches which are used to estimate the source contributions and fingerprints to solve a mass balance. Positive Matrix Factorization (PMF) (Paatero and Tapper, 1994) is the most commonly used of these and the solutions describe the complex, time-dependent aerosol composition as a linear combination of factor profiles and their contributions. It has been used extensively for the identification of heavy metals, water soluble and carbonaceous sources (Hopke et al., 2020) and for high-time resolution elemental data from the Xact (Font et al., 2022b, Hasheminassab et al., 2020, Liu et al., 2019, Rai et al., 2020, Rai et al., 2021, Wang et al., 2018, Yu et al., 2019, Manousakas et al., 2022).

PMF will be applied to the PM10 and PM2.5 Xact data and ACSM data from all 3 stations using the Source Finder software (SoFi Pro, https://datalystica.com/), which has been used by the ICL team in numerous studies (Font et al., 2022b, Fröhlich et al., 2015, Manousakas et al., 2022, Reyes-Villegas et al., 2016, Visser et al., 2015b) to provide hourly source factor time series and factor profiles for the duration of the exposures. For the ACSM data, the Sofi software has been used in many recent advances to reduce the uncertainty associated with rotational ambiguity by introducing a priori information about the source profiles (Canonaco et al., 2013, Canonaco et al., 2021) and is now widely used in that community.(Crippa et al., 2014) Recent work on data from Zurich (Manousakas et al., 2022) has shown that source apportionment of the XACT PM10 and PM2.5 data can be used to quantify the emissions from a range of sources in urban background locations.

Separating the tire and road wear sources is not straight forward as they are internally mixed and generated by the same frictional forces. To help understand some of these processes in greater depth, additional size fractionated measurements of polymers are planned at Honor Oak Park and Marylebone Road during 2022/23 under a separate project (NERC, ‘Understanding UK airborne microplastic pollution: sources, pathways and fate’).

### 9.6. Pre-assessment screening

Following provision of written informed consent, study volunteers will undergo pre-assessment screening at QMUL. They will complete baseline FeNO and spirometry, pre- and post-bronchodilator, to confirm asthma status (based on $FEV_1$ reversibility and patient interview). A blood sample (10ml in single draw) will be collected to assess a full blood count including eosinophil levels, and specific immunoglobulin E (IgE) levels across a panel of common aero-allergens (to be included as a study confounding variable). Participants will then perform a submaximal cycle ergometer exercise test (Latin Protocol) to establish the exercise watt levels to be used at the site visits. If they wish participants can warm up by walking on a treadmill prior to the exercise test. Participants will be given a personal air quality monitor to wear for three days before the exposure visit.

## 9.7. Randomization

The study will use a 6X3 Williams crossover inequality test of paired differences.[17] as such the participants will be randomised to a sequence for their exposures to the three sites. Six sequences will be used (ABC/ BCA/ CBA/ ACB/ BAC/ CAB).

## 9.8. Health effects assessment on exposure visit days:

Participant flow through an exposure session is summarised in figure 2, while participant flow through the study is summarised in figure 3. On the morning of the site visits participants will first provide a urine sample while at home (9:00 am) Participants will be provided with a taxi to the study site. The participants will be transported taxi with closed windows and air circulation enabled, to the exposure sites.

Once at the exposure sites (10:00am-11:00am) baseline spirometry and determination of FeNO and sample collection (4x10 ml blood collection and baseline nasal lavage) will be performed at the field laboratory. At this time a brief health questionnaire will be administered to assess recent asthma control and ensure the volunteer is free of respiratory infection and has not experienced a recent exacerbation of their asthma.

During the two and a half hour exposure and exercise protocol (11:00am-13:30 pm), participants' heart rate will be monitored throughout the exposure period; at the half way point, after sufficient recovery (12:15am) participants will perform spirometry FeNO and Oscillometry. Immediately post-exposure the participants will perform spirometry, oscillometry FeNO and mucus sample collection at the field lab. The following day, volunteers will return for 28-hour post measurements; biosamples, spirometry and FeNO.

| | IONA: Schedule of assessment and exposure | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Pre-Study | Screening | 3 day pre | Pre | Exposure | Post | 24 hour post | 3 days post |
| | | Phys lab | Self assess | Field lab | Field lab | Field lab | Phys lab | Self assess |
| Consent | x | | | | | | | |
| Exercise test | | x | | | | | | |
| FeNO | | x | | x | x | x | x | |
| Oscillometry | | x | | x | x | x | x | |
| Spirometry | | x | | x | x | x | x | |
| Asthma control test | | x | | | | | | |
| QOL: AQLQ | | x | | | | | | |
| Baseline questionare | | x | | | | | | |
| Daily Symptom Q | | x | x | x | | x | x | x |
| PEF Test | | | x | | | | x | |
| Personal air quality | | | x | | | | | |
| Physical activity | | | x | | | | | |
| Venepuncture | | | | x | | | x | |
| Nasal lavage | | | | x | | x | x | |
| Urine sample | | | | x | | | x | |
| Heart rate | | | | | x | | | |

*Figure 2. Flow of participants through IONA study days*

Our primary endpoint, lung function (FEV1) will be assessed using a portable desktop computerized spirometer (Vitalograph). All procedures will be performed in accordance with American Thoracic Society guidelines. Predicted volumes will be calculated according to the global lung index guidelines to adjust for ethnicity.[20] As Forced Expiratory Flow $(FEF)_{25-75}$ is a volume dependent measurement, decreases in forced vital capacity (FVC) following exposure to air pollutants affect this parameter. It has been demonstrated however that isovolume adjustments to this parameter provide a useful means of accessing intrinsic narrowing of the peripheral airways.[21] We therefore propose to correct the measured flows to the participants predicted total lung capacity, using the forced expiratory flow at 50 and 60% of predicted total lung capacity (TLC): $FEF_{50\%TLC}$ and $FEF_{60\%TLC}$ respectively.[22] The area under the forced expiratory flow curve will also be determined, as this has been suggested to be a sensitive indicator of bronchoconstriction. Fractional Expired Nitric Oxide and impulse oscillometry and asthma symptoms will also be collected before, during and after the exposure tasks in accordance with European Respiratory Society guidelines.

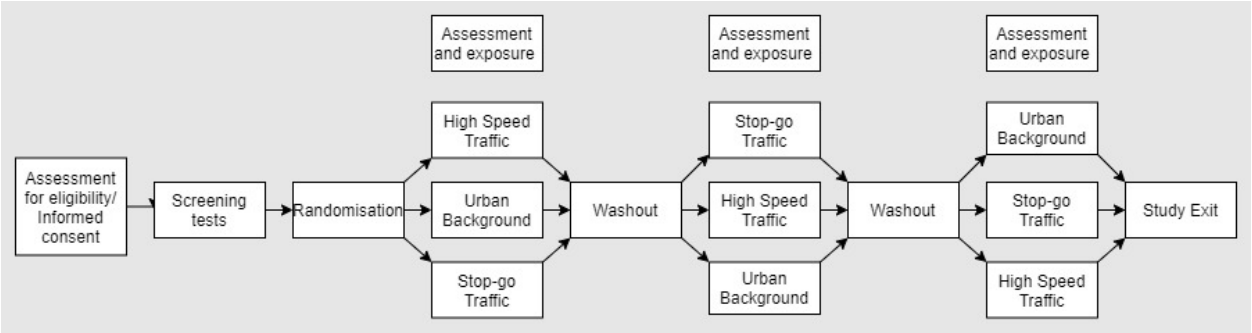


*Figure 3. Flow of participants through IONA.*

### 9.9. Abstaining from medication.

Participants will continue to use all their routine asthma medication during the study. In agreement with ERS spirometry guidance participants will be asked to abstain from using reliever medication for six hours prior to performing spirometry. If participants need to use their medication in the six hours prior to the first spirometry testing of an assessment visit they will be offered an opportunity to reschedule the assessment visit. If participants need to use their inhaler during the testing protocol the testing will end immediately. If a participant wishes to continue with the study, they will be offered another testing date, after a brief discussion with Griffiths (GP specialising in Asthma). If testing is stopped after discussion with Griffiths participants will still receive all expenses, and this will be made clear to the participants at the start of the study.

### 9.10. Exercise and exposure protocol:

To control for variations in air quality, exposure will occur for two-and-a-half hours between 11:00am and 13:30pm on weekdays. Between pre- and post- health assessments, participants will be asked to cycle on a static exercise bike at a watt equivalent to 60% of estimated heart rate maximum for six exercise bouts of 15 minutes separated by fifteen minute rest periods. This exercise intensity was chosen to maximise inhaled doses of PM and gaseous pollutants over the exposure period while remaining below the first ventilation threshold to reduce variation in ventilatory drift, and minimising discomfort for the participants. Physiological drift will be monitored by Ratings of Perceived Exertion questionnaire and real-time monitoring of the participants heart rate and estimated breathing rate.

### 9.11. Participant withdrawal

Participants will be informed and reminded that they can withdraw from the study at any time. Participants will be able to choose to start cease their involvement with the study or to cease their involvement and have their data removed from the study.

Consent forms and information sheets will tell participants their last time opportunity to have their data removed from the study.

### 9.12. End of study definition

The end of this study is defined as the date on which the last data is collected.

### 9.13. Study Timeline

| GANTT CHART | | Responsibility | Sep-22 | Oct-22 | Nov-22 | Dec-22 | Jan-23 | Feb-23 | Mar-23 | Apr-23 | May-23 | Jun-23 | Jul-23 | Aug-23 | Sep-23 | Oct-23 | Nov-23 | Dec-23 | Jan-24 | Feb-24 | Mar-24 | Apr-24 | May-24 | Jun-24 | Jul-24 | Aug-24 | Sep-24 | Oct-24 | Nov-24 | Dec-24 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pilot Project | Pilot Study | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Student set up | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Data collection | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Data analysis | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| IONA Project Management | Set up study meeting/ teams | JS | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Confirm protocol | JS | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Study Sponsorship | JS | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Research ethics | JS | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Study documents | JS | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Statement of work | JS | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | QA/QC plan | JS/HH | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Confirm budget | CG | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Develop risk assessment | JS | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | ID field testing requirements | JS | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Recruit study team | JS | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | PPI group meeting | CG | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Set up ISC | IM | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Ten month progress report | CG | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Final report | CG | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PDRA support | QA Manager 0.05 24 months | CG | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | QM PDRA (8 months 100%) | JS | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Max Priestman (6 months 100%) | DG | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Amber Vaughan (0.8 11 months) | IM | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Exposure study | Pilot program | JS | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Recruitment | JS | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Participant exposure period | JS | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Health data processing | JS | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Data linkage | JS/HH | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Analysis | KK/HH | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Write up | CG | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Air quality monitoing | Site preparation | DG | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Data collection | DG | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Data preparation | DG | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Data linkage | JS/HH | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Sample processing | Consumable purchases | IM | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Sample processing | IM | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Data linkage | JS/HH | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

## 10. Statistical considerations

### 10.1. Sample size

Since we will randomize the participants to assign a sequence for their exposures to the three sites we will have six sequences (ABC/ BCA/ CBA/ ACB/ BAC/ CAB), a 6X3 Williams crossover inequality test of paired differences.[17] For a significance level of 0.05, a Δ (Difference between the reduction in FEV1 between 2 sites) of 4.69 with a standard deviation of 6.4 [23] and a total of 30 participants (5 per sequence) we have a power of 91.54%. However, assuming a dropout rate of 20 to 30% and taking into account that specific exposures to contrast are not available yet and will be assessed when the exposure methods are developed, we intend to recruit 48 participants in total. We will define 12 groups of 4 individuals and then assign a group to each participants. Each two groups will have a same sequence.

### 10.2. Descriptive analysis

After data quality check, we prepare data description tables including number of observations, mean and standard deviation of variables. We will use graphical methods (e.g. scatterplots) to get insight in the correlation between different exposure variables and the associations between specific exposures and health endpoints as well.

### 10.3. Statistical modelling

We will use mixed effect regression model to estimate the effects of different pollutant sources ( tailpipe or non-tailpipe) on the change in health point, which will be defined by difference of various factors such as FEV1 and FVC between pre-exposure and post-exposure (0, 2, or 18 hours). For each endpoint, one key time post-exposure has been selected, based on the time of maximal effect in previous controlled exposure studies, for example for FEV1 this is 2 hours post exposure.[23] In this model, when measurements and pollutant characterization become available, the exposure covariates will be the integrated average concentration of the various pollutants and /or sources during the exposure period. We will take the results of the source apportionment and feed them into the model. The method will provide estimates of exposure due to tailpipe emissions (Black Carbon and Organic Aerosol) and non-tailpipe, specifically break wear and tyre & road wear, emissions. We will include these exposure types in the model. We will start with a single-exposure covariate models (for every source type), then we can decide how to develop a model with all of the significant source types. Levels (eg. Integrated average concentration) of each source (tailpipe/non-tailpipe) will be included in the model. The effects of different pollutants will be compared based upon statistical significance and the effect size for selected exposure contrasts, such as a pre-defined change of the concentration of the pollutant or interquartile range. Two pollutant models will be specified as well to evaluate which pollutant has the strongest association with a certain health endpoint.

In our models we will account for potential confounders which may be present as the exposure contrast cannot be entirely predicted by the design: we will include meteorological variables such as temperature, relative humidity and wind direction during the exposure time; as well as individual characteristics such as age, gender, severity of asthma and allergies.

We will use R (version 4.1.3 or newer; R Development Core team) for the application of mixed models using functions from the lme4 and nlme packages.

## 11. Ethics

The study will be sponsored and managed by QMUL and supported by Imperial College London, according to MRC guidance: https://www.mrc.ac.uk/publications/browse/good-research-practice-principles-and-guidelines/

This study has received University Research Ethics Committee approval (QMERC22.170.). The study will be carried out according to the principles of the Helsinki Agreement.

### 11.1. Risks to participants: Asthma attack or unwell

There are risks associated with exposing people living with asthma to high air pollution via exercise protocols. Such activities may increase the likelihood of accidental events, with asthma attacks being the most likely.

### 11.2. Risk mitigation: Asthma attack or unwell

In order to mitigate potential accident events during the study we will take the following precautions:

- The study will only proceed after University Ethics Committee approval and sponsor indemnity
- Our protocol has been devised with people living with asthma and asthma specialist clinicians and researchers from Asthma and Lung UK public and patient involvement (PPI) group.
- Participants will only be recruited if they have mild - moderate stable asthma, as defined by British Thoracic Society Guidelines
- Participants will be screened by a physiologist (Dr James Scales) and a second opinion may be sought from Griffiths, a clinician with specialist knowledge in asthma, if required.
- Usual medication will be continued
- The exercise intensity and locations have been selected to be highly representative of day to day exposures, as such participants will not be exposed to unusually high air pollution.
- Participants will be further screened for unstable asthma on the day of exercise exposures
- Basic rescue asthma medication (albutarol and large volume spacer) will always be available at assessment sites, with researchers trained in its use and administration
- Participants will be closely monitored during the exercise protocol and will be repeatedly encouraged to provide feedback about their physical state during the exercise.
- Prof Chris Griffiths or a nominated respiratory clinician will be available during all exposure testing
- A risk register and an adverse events register will be maintained for the duration of the study

- Accidents will be discussed with the Chief Investigator and reported promptly to the Sponsor as per Good Clinical Practice regulations, and reviewed at study Project Management Group

### 11.3. Response to Asthma attack or unwell participants:

This section should be read alongside SOP 16 and the field testing risk assessment. If a participant has an asthma attack during the protocol they will be encouraged to use their rescue medication as advised by their physician. Salbutamol inhalers and spacers will be available at the testing site if needed. A researcher on site will be trained in appropriate first aid. Each site has facilities available to make participants comfortable, including toilets, blankets, isolated room, water and food.

In the event of a participant feeling unwell the research team will:

- Calmly reassure the participant and encourage the participant make themselves suitably comfortable.
- The participant will be observed for any signs of distress or breathing difficulty.
- Place a pulse oximeter on the participant and assess oxygen saturation and pulse.
- If required, first aid will be administered by trained researchers.
- If first aid fails to address the situation the team will seek emergency advice.
- All three sites, have been chosen to have unblocked access for emergency vehicles.
- A report will be completed within 48 hours to be discussed with the project management team.

### 11.4. Risks to participants: Accidental personal data release

As with all human participant research there is a risk of inadvertent release or loss of personal data through incorrect storage of electronic or hardcopy data or through incorrectly transferring or handling data.

### 11.5. Risk Mitigation: Accidental personal data release

To mitigate this we shall follow best practice guidelines provided in Standard operation procedures (SOP) by our Clinical Trials Unit. Paper records will be stored securely in locked filling cabinets in password locked rooms in the pass-protected Centre for Primary Care and Public Health. Electronic records will be stored in a password-protected study database on a secure server, in the Centre for Primary Care and Public Health. In the study database, personal details (name, address,

date of birth) will be kept separate from research data, which will be identified by a unique study reference number. In tables of data, participants will only be identified by number not by initials or name. Data management procedures will be completed in compliance with the GDPR and trial regulations. Survey reported data will be stored in the QMUL data safehaven, where data will be held in a UK server and access will be facilitated by two factor authentication. Survey software with integrated data validation checks and audit trails will be used to record study data. Any data transfers between QMUL and Imperial College London will be completed via encrypted Secure File Transfer Protocol. All data will be backed-up weekly to ensure data is safeguarded from accidental loss.

### 11.6. Annual Safety Reporting

The CI will send an Annual Progress Report to the REC and the sponsor using the Health Research Authority (HRA) template on the anniversary of the REC "favourable opinion".

## 12. Public involvement

This proposal was designed with input from the Asthma and Lung UK Centre for Applied Research PPI group. Examples of impact the PPI group has already had include:

- Highlighting the need for breaks during the two hour walking task.
- Highlighting the need to make sure roads are matched for traffic flow to mitigate possible confounders of noise and anxiety.
- Discussed acceptability of respiratory tests. PPI group highlighted the peak expiratory flow, FeNO and spirometry will be familiar tests to most potential participants

As the study progresses the PPI group will be invited to project management group (PMG) meetings. The group will collaborate as advisors and partners in the study and are invited to provide unsolicited advice. To ensure the PPI group can provide meaningful advice and guidance the CI will regularly keep them informed of study developments via email. The meetings will follow the EUPATI (European Patients Academy on Therapeutic Innovation) roadmap (https://bit.ly/3bugCMk) discussing themes of: Research design and planning, research conduct and operations and dissemination and communication.

## 13. Data handling and record keeping

### 13.1. Data management

Survey Lab will be used to store electronic case report forms all data will be held on GDPR compliant databases with integrated data validation checks and audit trails. All collected data will be held on backed up encrypted servers. Any data transfers between ICL and QMUL will be performed via Secure File Transfer Protocols. Paper records, CRFs and consent forms and recruitment logs will be held locally in double locked locations in Yvonne Carter Building 58 Turner St E12AB (QMUL).

Data Sharing: we will comply with HEI policy on data sharing. A copy of study data will be held on the HDRUK BREATHE secure data hub https://www.breathedatahub.com. BREATHE's mission is 'Better respiratory health through better connected data'.

### 13.2. Confidentiality

We shall follow best practice guidelines provided in SOPs by our Clinical Trials Unit. Paper records will be stored securely in locked filling cabinets in password locked rooms in the pass-protected Centre for Primary Care and Public Health. Electronic records will be stored in a password-protected study database on a secure server, in the Centre for Primary Care and Public Health. In the study database, personal details (name, address, date of birth) will be kept separate from research data, which will be identified by a unique study reference number. In tables of data, participants will only be identified by number not by initials or name. Data management procedures will be completed in compliance with the GDPR and trial regulations. Survey reported data will be stored in the QMUL data safe haven, where data will be held in a UK server and access will be facilitated by two factor authentication. Survey software with integrated data validation checks and audit trails will be used to record study data. Any data transfers between QMUL and Imperial College London will be completed via encrypted Secure File Transfer Protocol. All data will be backed-up weekly to ensure data is safeguarded from accidental loss.

### 13.3. Record retention and archiving

In accordance with the UK Policy Framework for Health and Social Care Research, research records will be kept for 20 years after the study has completed while personal records will be stored for one year after the study has been completed. At the completion of the study data will be moved to a trusted archive centre. At the end of the retention period data will be destroyed in accordance best practice guidelines at the time of destruction.

## 14. Laboratories

### 14.1. Central and local laboratories

The air quality supersites are major field observatories owned and managed by David Green's team at ICL. They are funded by Natural Environment Research

Council and UK government and provide >100m$^2$ of indoor and outdoor laboratory space in contrasting traffic and urban background locations and are ideally suited to understanding the sources of pollution affecting the urban environment. They are equipped with high time resolution instrumentation that operates continuously reporting data to national and international air quality networks.

The mobile measurement trailer is a mobile laboratory owned and managed by DR David Green's team at ICL. It carries an identical instrument setup to the two supersites and can be used independently or part of a measurement triplicate as in this experiment. It has been used in a number of field campaigns (e.g. [24,25] and has recently be deployed to Manchester (UK) and Birmingham (UK) and Barcelona (ES) to quantify NTE.

The laboratories at ICL have a dedicated room for the storage of human tissues under the provisions of the UK's Human Tissue act.

### 14.2. Sample preparation and collection

Nasal mucus samples will be collected via nasal lavage. Nasal lavages will be performed at three time points, pre and 2-hour post at the field testing sites while the 18 hours-post exposure measure will be collected in QMUL physiology laboratories. Samples will be biobanked in 5 mL aliquots at -80$^o$C at ICL under Human Tissue Act licence 12521 (local Human Tissue Act lead, Dr Ian Mudway).

In addition to the nasal lavage sample 4x10mL of whole blood from a single draw *via* venepuncture from a vein in the lower arm will also be collected pre-, 2- and 18-hours post exposure. Blood plasma from these samples will be biobanked in 1 mL aliquots at -80$^o$C at ICL under Human Tissue Act licence 12521 (local Human Tissue Act lead, Dr Ian Mudway).

Samples will be collected in disinfected facilities at the field-testing site. Screens will be used to ensure participant privacy.

### 14.3. Laboratory procedures

Samples across the interval pre- to 1-hour post exposure will be used to examine metal deposition in the nasal airways and the acute release of endogenous constitutively expressed native damage-associated molecular patterns (DAMPs) and alarmins, such as high mobility group box 1 (HMGB1) S100 proteins, myeloperoxidase-DNA complexes (MPO-DNA), reflecting both cellular injury and NETosis induction, plus IL-33 released from damaged epithelial cells and Thymic stromal lymphopoietin (TSLP). Here we hypothesise that metal deposition in the nasal airways, reflecting brake (Cu, Ba and Sb), tire (Zn) and road wear (Fe, Al), causes oxidative stress, triggering cell injury, DAMP/alarmin release resulting in an allergic skewing of the airway. This will be examined across the interval pre- to 18-

hours post exposure, through examination of the concentration of panels of Th2 and Th17 cytokines using Cytometric bead array (CBA) technology. This will allow us to examine the immune and physiologic responses observed in the exposed volunteers in relation to: (a) the site classification, (b) the daily variation of ambient metals/metalloid across the sites; and (c) daily variation in the nasal deposited dose of source-specific metals, including tailpipe tracers (V. Ni and Cr). Nasal lavage samples will also be retained as a downstream resource for the quantification of tire wear tracers, which is outside available budget, but aligns with ongoing work within the MRC Centre for Environment and Health at ICL. Urine samples will be retained as a downstream source for the quantification on analgesic use and tire wear tracers, which is outside of available budget but also aligns with the MRC Centre for Environment and Health at ICL

### 14.4. Sample storage and transfer

Samples will be transferred from the collection sites in London and Luton to ICL in cooler boxes (4$^{o}$C) using authorized courier services. Upon arrival samples will be stored with HTA approved facilities at -80$^{o}$C. All transport, storage and disposal protocols will adhere to the College's SOPs, which can be found on the ICL Governance site.

## 15. Safety reporting

Accident events occurring between visits/questionnaires/sample collections will not be recorded or reported as they are not the aim or focus of this study.

### 15.1. Urgent Safety Measures

The CI may take urgent safety measures to ensure the safety and protection of the study subjects from any immediate hazard to their health and safety. The measures should be taken immediately. In this instance, the approval of the REC prior to implementing these safety measures is not required. However, it is the responsibility of the CI to inform the sponsor and Main Research Ethics Committee (*via* telephone) of this event immediately.

The CI has an obligation to inform both the Main REC in writing within 3 days, in the form of a substantial amendment. The sponsor (Joint Research Management Office [JRMO]) must be sent a copy of the correspondence with regards to this matter.

**16. Monitoring and auditing**

For further explanation of monitoring and auditing this section should be read along with the documents:

1) Hei-form-forQAQC plan (IONA) V5SOP
2) SOP 01 Spirometry V1.0. 23.08.2022
3) SOP 02 Height Weight V1.0. 23.08.2022
4) SOP 03 FeNO V1.0. 23.08.2022
5) SOP 04 Nasal Lavage V1.0. 23.08.2022
6) SOP 05 Venepuncture V1.0. 23.08.2022
7) SOP 06 Exercise Testing V1.0. 23.08.2022
8) SOP 07 Data Management and QC V1.0. 23.08.2022
9) SOP 08 Automatic Urban and Rural Network (AURN) LSO Manual 01.10.2021
10) SOP 09 Air Quality Data Validation and Ratification Process
11) SOP 10 ERG Data Ratification Instruction Manual V1.14 01.02.2012 **CONFIDENTIAL**
12) SOP 11 UK Airborne Particulate Concentration and Numbers LSO Manual Aerosol Chemical Speciation Monitor (ACSM) V1.14 **CONFIDENTIAL**
13) SOP 12 UK Airborne Particulate Concentration and Numbers LSO Manual Xact 625i Ambient Metals Monitor, Cooper Environmental **CONFIDENTIAL**
14) SOP 13 UK Airborne Particulate Concentration and Numbers LSO Manual AE33 Aethalometer **CONFIDENTIAL**
15) SOP 14 Particle Concentrations & Numbers & the Black Carbon Networks in the UK - QA/QC Procedures **CONFIDENTIAL**
16) SOP 15 Oscillometry 28.02.2023
17) SOP 16 Asthma response 28.02.2023

The Sponsor or delegate retains the right to audit any study, study site or central facility. In addition, any part of the study may be audited by the funders where applicable.

Dr James Scales will be responsible for the data management, storage, record keeping, coding, data linkage, and metadata generation. All researchers have up to date GCP and Data Governance training. All data will be managed according to European Union General Data Protection (GDPR) guidelines.

QMUL studies adhere to the NHS Research Governance, Framework for Health and Social Care, the Data Protection Act and the Human Tissue Act. We follow QA and QC protocols are developed by the PCTU.

We shall hold a trial Master file recording all key study documents and study discussions, ethical review board reports and staff training logs. Detailed meta-data will be kept. Methodologies and analyses will be summarised in readme files, including information on variables names, missing data labels, definition of acronyms, etc. For example, sample tracking records, SOPs and information on QA/QC procedures, stored as pdfs on secure servers with metadata tables linking relevant documentation to datasets. For new data acquisitions/ collations data

dictionary/metadata describing data content will be created embedded in the data or stored separately. Methods and analysis techniques will be documented and stored in relevant study folders.

# 17. Study committees

## 17.1. Project Management Group (PMG)

Monthly project management meetings will be chaired by Dr James Scales and will consist of the co-investigators. An open invite will be available to AUKCAR PPI group representative. Queen Mary University London will lead the health assessment and analysis with Dr James Scales having day-to-day involvement project managing the programme. Imperial College London will lead the exposure data collection and processing and supervise the statistical protocols and analysis, with ongoing statistical support of the QMUL (junior statistician).

## 17.2. Independent Scientific Group (ISC)

We will convene an ISC to meet three times during the two year study. Its role is to monitor and advise on study conduct and progress on behalf of the Sponsor and HEI. Meetings may be by teleconference at the discretion of the Chair. The ISC composition is designed to provide expertise in all relevant facets of the study design and conduct. ISC meetings will include at least one member of the AUKCAR PPI group.

# 18. Finance and funding

This work is funded by Asthma + Lung UK as part of the Asthma UK Centre for Applied Research [AUK-AC-2012-01 and AUK-AC-2018-01] and The Health Effects Institute(USA)

# 19. Insurance and indemnity

The insurance that Queen Mary has in place provides cover for the design and management of the study as well as "No Fault Compensation" for participants, which provides an indemnity to participants for negligent and non-negligent harm.

# 20. Dissemination of research findings

Dissemination is a standing item on the PMG agenda, ensuring interim study findings are rapidly and effectively communicated. Our PPI group will co-write or review all

study outputs for dissemination via traditional and social media throughout the study.

This project is designed as a feasibility study to inform applications to further funding. As such data will be presented in applications to NIHR, MRC and Wellcome trust.

Specific dissemination tools will include:

1) Webinars on websites of our institutions, to provide more detailed summaries of results, with downloads of key documents.

2) Presentations, especially to London partner organisations including the GLA, councils and Health and Wellbeing Boards.

4) Presentations at national and international conferences, including the European Respiratory Society and the American Thoracic Society.

3) Peer reviewed publications targeted at the world's leading medical journals.

## 21. References


1. Murray CJ, Abbafati C, Abbas KM, et al. Five insights from the global burden of disease study 2019. *The Lancet.* 2020;396(10258):1135-1159.
2. Delamater PL, Finley AO, Banerjee S. An analysis of asthma hospitalizations, air pollution, and weather conditions in Los Angeles County, California. *Science of the Total Environment.* 2012;425:110-118.
3. Liu Y, Pan J, Zhang H, et al. Short-term exposure to ambient air pollution and asthma mortality. *American journal of respiratory and critical care medicine.* 2019;200(1):24-32.
4. Levy ML. National review of asthma deaths (NRAD). *The British Journal of General Practice.* 2014;64(628):564.
5. Selley L, Schuster L, Marbach H, et al. Brake dust exposure exacerbates inflammation and transiently compromises phagocytosis in macrophages. *Metallomics.* 2020;12(3):371-386.
6. Steenhof M, Janssen NA, Strak M, et al. Air pollution exposure affects circulating white blood cell counts in healthy subjects: The role of particle composition, oxidative potential and gaseous pollutants - the RAPTES project. *Inhalation Toxicology.* 2014;26(3):141-165.
7. Strak M, Hoek G, Godri KJ, et al. Composition of PM affects acute vascular inflammatory and coagulative markers - The RAPTES project. *PLoS ONE.* 2013;8(3):e58944.

8. Steenhof M, Mudway IS, Gosens I, et al. Acute nasal pro-inflammatory response to air pollution depends on characteristics other than particle mass concentration or oxidative potential: The RAPTES project. *Occupational and Environmental Medicine.* 2013;70(5):341-348.
9. Strak M, Hoek G, Steenhof M, et al. Components of ambient air pollution affect thrombin generation in healthy humans: the RAPTES project. *Occup Environ Med.* 2013;70(5):332-340.
10. Strak M, Janssen NA, Godri KJ, et al. Respiratory health effects of airborne particulate matter: the role of particle size, composition, and oxidative potential-the RAPTES project. *Environ Health Perspect.* 2012;120(8):1183-1189.
11. Steenhof M, Gosens I, Strak M, et al. In vitro toxicity of particulate matter (PM) collected at different sites in the Netherlands is associated with PM composition, size fraction and oxidative potential--the RAPTES project. *Part Fibre Toxicol.* 2011;8:26.
12. Strak M, Steenhof M, Godri KJ, et al. Variation in characteristics of ambient particulate matter at eight locations in the Netherlands – The RAPTES project. *Atmospheric Environment.* 2011;45(26):4442-4453.
13. Font A, Tremper AH, Priestman M, et al. Source attribution and quantification of atmospheric nickel concentrations in an industrial area in the United Kingdom (UK). *Environ Pollut.* 2022;293:118432.
14. DfT. Department for Transport Road traffic statistics. https://roadtraffic.dft.gov.uk/#6/55.254/-6.053/basemap-regions-countpoints. Published 2022. Accessed 11/05/2022.
15. Fussell JC, Franklin M, Green DC, et al. A review of road traffic-derived non-exhaust particles: emissions, physicochemical characteristics, health risks and mitigation measures. *Environmental Science & Technology.* 2022.
16. USEPA. AP42 Emission factor Documentation for AP-42. In: Agency USEP, ed. Vol Section 13.2.1 Paved Roads. Washington DC, USA2011.
17. Wang B-S, Wang X-J, Gong L-K. The Construction of a Williams Design and Randomization in Cross-Over Clinical Trials Using SAS. *Journal of Statistical Software, Code Snippets.* 2009;29(1):1 - 10.
18. Nathan RA, Sorkness CA, Kosinski M, et al. Development of the asthma control test: a survey for assessing asthma control. *J Allergy Clin Immunol.* 2004;113(1):59-65.
19. Martineau AR, Hanifa Y, Witt KD, et al. Double-blind randomised controlled trial of vitamin D3 supplementation for the prevention of

acute respiratory infection in older adults and their carers (ViDiFlu). *Thorax.* 2015;70(10):953-960.
20. Graham BL, Steenbruggen I, Miller MR, et al. Standardization of Spirometry 2019 Update. An Official American Thoracic Society and European Respiratory Society Technical Statement. *American Journal of Respiratory and Critical Care Medicine.* 2019;200(8):e70-e88.
21. Weinmann GG, Bowes SM, Gerbase MW, Kimball AW, Frank R. Response to acute ozone exposure in healthy men. Results of a screening procedure. *Am J Respir Crit Care Med.* 1995;151(1):33-40.
22. Blomberg A, Mudway IS, Nordenhäll C, et al. Ozone-induced lung function decrements do not correlate with early airway inflammatory or antioxidant responses. *Eur Respir J.* 1999;13(6):1418-1428.
23. McCreanor J, Cullinan P, Nieuwenhuijsen MJ, et al. Respiratory effects of exposure to diesel traffic in persons with asthma. *N Engl J Med.* 2007;357(23):2348-2358.
24. Font A, Tremper AH, Lin C, et al. Air quality in enclosed railway stations: Quantifying the impact of diesel trains through deployment of multi-site measurement and random forest modelling. *Environmental Pollution.* 2020;262:114284.
25. Font A, Tremper AH, Priestman M, et al. Source attribution and quantification of atmospheric nickel concentrations in an industrial area in the United Kingdom (UK). *Environmental Pollution.* 2022;293:118432.