Advancing Tools for Human Early Lifecourse Exposome Research and Translation

ATHLETE

Bradford Protocol for the Adolescent Follow-Up of the HELIX Subcohort and the Co-Production of Interventions to Improve the Urban Exposome

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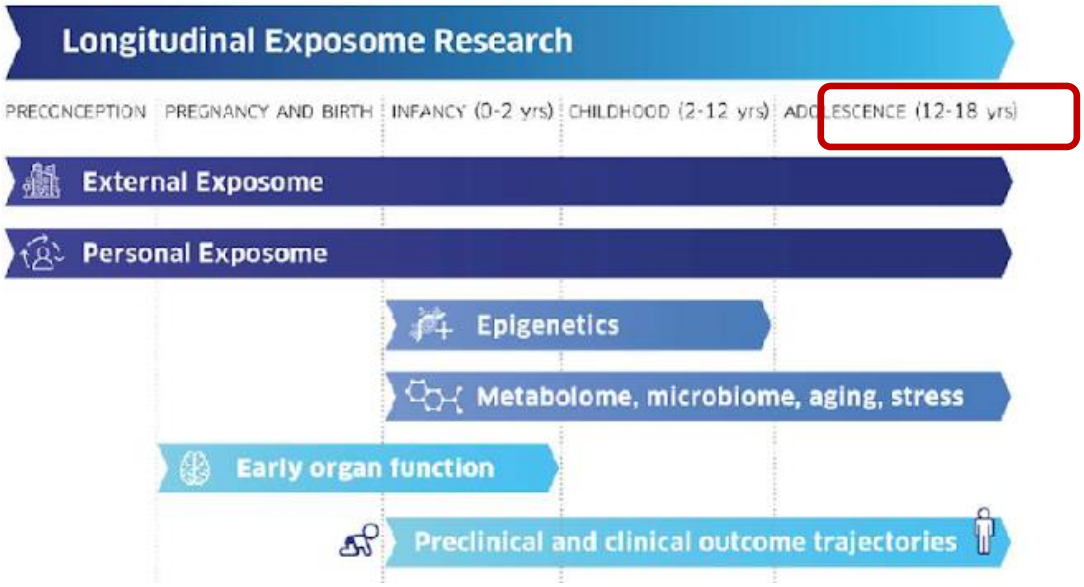

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Abbreviations

ALT	Alanine transferase
AST	Aspartate transferase
BiB	Born in Bradford
BIHR	Bradford Institute for Health Research
BTHFT	Bradford Teaching Hospitals NHS Foundation Trust
CBCL	Child Behaviour Check List
CI	Chief Investigator
DPO	Data Protection Officer
EDEN l'Enfant	Étude des Déterminants pré et postnatals du développement et de la santé de
FFQ	Food Frequency Questionnaire
GDPR	General Data Protection Regulation
GGT	Gamma-glutamyl transferase
GIS	Geographical Information System
HDL	High-density lipoprotein
IgE	Immunoglobulin E
INMA	INfancia y Medio Ambiente
INSERMInstitut	National de la Sante et de la Recherche Medicale
ISGlobal	Barcelona Institute for Global Health
KANC	Kaunus cohort
MoBa	Norwegian Mother and Child Cohort Study
NIPH	Norwegian Institute of Public Health, Division of Environmental Medicine
NO_2	Nitrogen dioxide
OP	Organophosphate
PAHs	Polycyclic aromatic hydrocarbon
PEM	Personal exposure monitors
PFASs	Perfluoroalkyl sulfonates
PIS	Participant Information Sheet
RF	Research Fellow
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus
SPN	Spain
UoC	University of Crete
UK	United Kingdom
VDU	Vytauto Didziojo Universitetas
WP	Work Package

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1. Background and objectives

1.1 Background

Environmental hazards account for a probably large, but not well characterised, proportion of disease burden. Known environmental risks (air pollution, lead, occupational) are estimated to contribute around 25% of non-communicable diseases (NCDs). However, these estimates are likely to represent only the tip of the iceberg as the full impact of pollution, especially chemical pollution, on NCD development is not known, but is almost certainly undercounted due to the absence of sufficiently robust evidence for many exposures and health outcomes

Priority setting for regulation and policy requires a holistic approach to multiple environmental hazards. In encompassing the totality of our environmental exposures, the exposome's main advantage over traditional 'one exposure one disease' approaches is that it provides an unprecedented conceptual framework for the study of multiple environmental hazards (urban, chemical, lifestyle, social) and their combined effects. As such, the exposome provides input into priority setting and into a wide range of policy issues covering more than one exposure at once.

First exposome studies have provided important groundwork to develop next-generation exposome tools: they have made remarkable progress in measuring many environmental exposures in relatively large sample sizes, and in understanding how multiple exposures correlate and co-exist, how the exposome varies geographically between European countries and over time, how we may explore associations between multiple exposures and health, and how we may agnostically search for early perturbation in the biological pathways leading to disease. This work has clearly outlined the next set of exposome-related challenges, paving the way for the development of next generation tools and data, and for better translation of knowledge into the policy and regulatory field.

The early part of the life course presents important windows of opportunity for prevention. Health and disease are full life course processes and it is well recognised that the early parts of the life-course, from conception and even pre-conception onwards, are particularly vulnerable to environmental influences with life-long consequences. The most common NCDs have at least part of their origin in the first 18 years of life, and prevention during these periods will not only improve child health, but also improve life-long health and disease trajectories. Building exposome tools and data for the future thus needs to start in the early stages of the life course.

The ATHLETE project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 874583. ATHLETE aims to develop a toolbox of advanced, next-generation, exposome tools and a prospective exposome cohort, which will be used to systematically quantify the effects of a wide range of community-level and individual-level environmental risk factors on mental, cardio metabolic, and respiratory health outcomes and associated biological pathways during the first 2 decades of life, to implement acceptable and feasible exposome interventions, and to translate the resulting evidence to policy recommendations and prevention strategies.

ATHLETE will set up a prospective Europe-wide exposome cohort covering the first 2 decades of the life course, which will integrate data on the external, chemical, physical, behavioral, and social domains of the exposome, as well as on health outcomes and biological omics

responses, from preconception until adolescence. ATHLETE will bring together the key European pregnancy/birth cohorts with the most comprehensive already existing exposome data (Figure 1). As part of ATHLETE, we will follow up a unique existing exposome cohort into adolescence (the HELIX Subcohort) and also integrate other adolescent cohorts and "new" birth cohorts with improved in-depth exposome data.

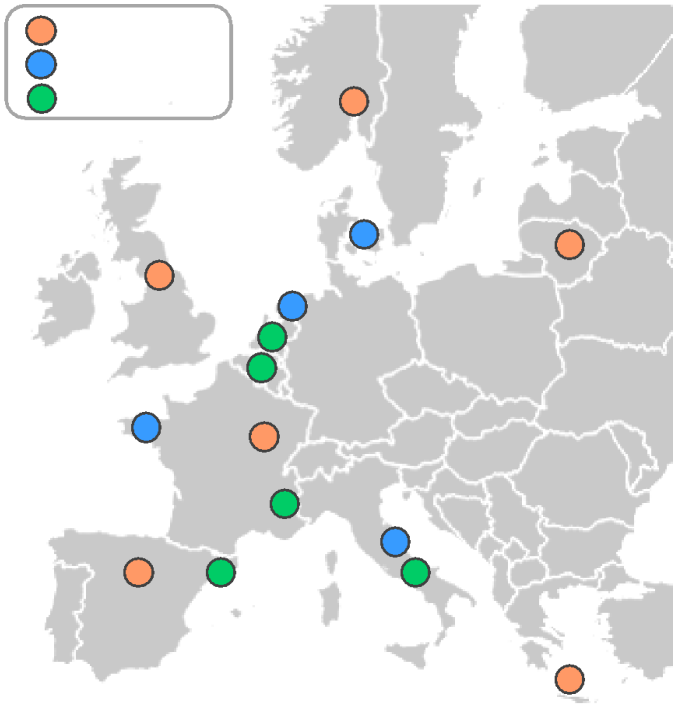


Figure 1. The ATHLETE cohorts

ATHLETE will also demonstrate the development of co-produced effective and scalable interventions to reduce personal exposure to the harmful effects of the urban exposome. By developing interventions in close partnership with communities and key stakeholder, ATHLETE will ensure that the co-produced interventions are both acceptable and feasible, increasing the likelihood of rapid translation into practice.

1.3 Protocol Structure

Born in Bradford are involved in fieldwork activities for two work-packages of ATHLETE. Work-package one (WP1) is a follow-up of BiB adolescents previous recruited to the BiB HELIX cohort. Work-package seven (WP7) will involve co-production, implementation and evaluation of interventions to reduce urban exposome exposure in primary school aged children. These work-packages will progress in parallel. The remainder of this protocol is structured into section one which covers WP1 activities and section two which covers WP7 activities.

2. SECTION ONE: Adolescent Follow-up of the HELIX Subcohort

The Adolescence Follow-up of the HELIX subcohort consists in following the 1,300 children who participated in the HELIX subcohort when they were between 6 to 11 years of age (Maitre et al. 2018; Vrijheid et al. 2014). In this new follow-up, these children will be examined 7 years later, in the adolescence period, when they will be between 12 to 18 years. Within HELIX, these children and their mothers were fully characterized for the external and internal exposome, including exposure biomarkers and 'omics' biomarkers, during pregnancy and at

the age of 6-11 years. This new follow-up will generate new data on exposures (personal, chemical, external), omics, and health outcomes. A new follow-up of the HELIX subcohort children represents a unique opportunity to systematically characterise the effect of the early-life exposome, repeatedly assessed over time (from pregnancy till adolescence), on health outcomes into adolescence. In Bradford we plan to follow up all 231 children who were recruited into the Bradford Helix subcohort between 2014-2015 (ethical approval reference: 14/YH/0013), now that they are 13-14 year old. The 231 children to be approached for ATHLETE consist of 205 children with previous successful measurement of exposure markers and 26 children without.

The main **aim** of the present protocol is to **implement common data collection protocols for the new follow-up of the HELIX cohort into adolescence.** These protocols will consider the specific age of the children and fully involve them in obtaining informed consent and answering questionnaires. Specific objectives include:

1. To assess adolescent growth and obesity, blood pressure, neuro-behaviour, mental health, and psychosocial issues, asthma, allergies, and respiratory health, and pubertal development using harmonized clinical assessment protocols, spirometry, cognitive computer testing, and questionnaire information;
2. To obtain personal monitoring data on physical activity, location, mobility, sleep, and air pollution;
3. Collect usual routes of commuting by using geographic information system (GIS) software (qGIS);
4. To collect biological samples (fasting blood, repeated urine samples, hair, and stool samples) for a complete and accurate assessment of the chemical exposome, the biological pathways, and the gut microbiota;
5. Collect extensive behavioral, lifestyle, social and psychological factors, and exposure sources in a harmonized questionnaire from the participating adolescent and their parents;

2.1 Methods

2.1.1 Overview of the HELIX Subcohort Adolescence Follow-up

The HELIX Subcohort Adolescence Follow-up will use the six European population-based longitudinal birth cohort studies participating in HELIX (Figure 1): BiB (UK), EDEN (France), INMA (Spain), KANC (Lithuania), MoBa (Norway), and RHEA (Greece). The HELIX Subcohort includes a subsample of BiB (N=205), EDEN (N=198), INMA (N=223), KANC (N=204), MoBa (N=272), and RHEA (N=199) (Table 1). In total, 1,301 children were followed (Annex 29). We expected that, out of the 1,301 participants at age 6-11 years, at least 85% will participate again (N~1100). This estimate is based on adolescent participation rates in the INMA cohort and on the fact that only families with high interest in the study were selected for participation in the HELIX Subcohort. In HELIX Subcohort (age 6-11 years), further subjects were invited and examined (n=322) following the same protocols for clinical examination and sample collection, and the same questionnaires, but these were not included in the measurement of exposure biomarkers for the HELIX study (Maitre et al. 2018) (Table 1) These extra subjects (26 in Bradford) will also be invited in the new follow-up.

Briefly, from the corresponding entire cohorts, the HELIX Subcohort mother-child pairs were selected based on the following criteria: (a) age 6–11 years at the time of the visit, with a preference for ages 7–9 years if possible; (b) sufficient stored pregnancy blood and urine samples available for analysis of prenatal exposure biomarkers; (c) complete address history

available from first to last follow-up point; (d) no serious health problems that may affect the performance of the clinical testing or impact the volunteer's safety (e.g., acute respiratory infection). In addition, the selection considered whether data on important covariates (diet, socioeconomic factors) were available (Maitre et al. 2018). In this new follow-up, no inclusion criteria will be applied because all HELIX Subcohort children (and extra subjects) will be invited to participate in the new follow-up.

Table 1. *Characteristics of the cohorts participating in the HELIX Subcohort (extracted from Maitre et al. 2018)*

Cohort	Years of birth	Region covered by HELIX	No. of births in HELIX Subcohort	No. of extra subjects	Age in the HELIX follow-up (2013-2016)	Age in the HELIX adolescence follow-up (2021)
BiB, UK	2007-2010	Bradford	205	26	6.6 years	13-14 years
EDEN, France	2003-2006	Poitiers	198	7	11.0 years	17-18 years
INMA, Spain	2003-2008	Sabadell	223	266	8.8 years	15-16 years
KANC, Lithuania	2007-2008	Kaunas	204	3	6.4 years	12-13 years
MoBa, Norway	1999-2008	Oslo	272	19	8.4 years	14-16 years
RHEA, Greece	2007-2008	Heraklion	199	1	6.5 years	13-14 years

BiB, Born in Bradford; EDEN, Étude des Déterminants pré et postnatals du développement et de la santé de l'Enfant; INMA, INfancia y Medio Ambiente; KANC, Kaunus cohort; MoBa, Norwegian Mother and Child Cohort Study.

To obtain comparable follow-up data, protocols will largely repeat the common HELIX protocols from age 6-11 years. The following data will be collected from these 1,301 mother-child pairs.

Table 2. *Overview of the tasks that should be done in the next follow-up.*

Clinical examination	Biological samples	Sensors	Questionnaires
☐ Anthropometry	☐ Hair	☐ GENEActiv	☐ Address history
☐ Bioimpedance	☐ Urine	☐ ExpoApp3	☐ Home environment
☐ Blood pressure	☐ Stool	☐ Actigraph	☐ Socio-economic status
☐ Spirometry	☐ Fasting	☐ NO_2 diffusion	☐ Noise

blood tubes

- Neurodevelopment
  - N-Back
  - Roulettes task
  - Raven
- qGIS
- Outdoor environment
- Tobacco exposure
- Physical activity
- Diet
- Sleeping patterns
- Psychological distress
- Asthma and allergies
- Child Behaviour Checklist (CBCL)
- Pubertal development
- Medication
- Medical history

In previous follow-ups questionnaires were administered to the parents. In this new follow-up, given the age of the participants, most of the questions will be asked to the adolescents instead of their parents.

2.1.2 Inclusion and exclusion criteria

Inclusion criteria:

All participants (parents and children) that have consented and contributed to data collection in HELIX (n=231 families)

Exclusion criteria

Any participant that has consented and contributed to data collection in HELIX, but has withdrawn from the Born in Bradford cohort since or wishes to withdraw when approached for this follow up.

2.1.3 Fieldwork organization

The fieldwork is divided into two sections: an adolescent's and a parent's one.

Adolescent's section

- Clinical examination (anthropometry, bioimpedance, blood pressure, spirometry)
- Neurodevelopment computer testing
- qGIS questionnaire
- Questionnaires at home or in clinic
  - Main questionnaire
  - Food Frequency Questionnaire (FFQ)
  - Puberty questionnaire
- Collection of hair, stool, urines (2 urines during 6 consecutive days) and fasting blood
- 7 consecutive days (minimum) of personal exposure monitoring including

- Wearing a GENEActiv
- Wearing a Smartphone
- Wearing an Actigraph (only in a subsample for validation of physical activity data collected by GENEActiv)
- Carrying a NO_2 diffusion tube on the backpack (or place it in the living room in case they do not want to carry it)

- Sleep and physical activity diary during 7 consecutive days

Parent's section

- Short questionnaire (phone interview)
- Main questionnaire at home or at the study visit

The fieldwork organization is going to be decided by each cohort depending on the characteristics of each cohort and the available resources and preferences. **FOLLOW-UP OF ALL CHILDREN (EXPECTED 85% OF PARTICIPATION) NEEDS TO BE FINISHED IN MARCH 2022.**

IMPORTANT CONSIDERATIONS FOR THE ORGANIZATION OF THE FIELDWORK:

1. All HELIX children should be included in the visit (see Annex 29 for the list of IDs HELIX kids) – even if they do not want to do one part (e.g. blood, stools). The follow-up priorities are as follow:

	PRIORITIES (1= high priority to 4 = low priority)	
Clinical exam		
Anthropometry	1	**The clinical outcomes (and outcome questionnaires such as CBCL/SDQ, asthma/allergy) are our main priority. The clinical visit is essential.**
Blood pressure	1	
BIA	1	
Spirometry	1 (if possible)	
Neuro tests	1	
Devices		
GENEActive	3	**Monitoring can be left out if this makes the difference between participation or not.**
NO2 tubes	3	
ExpoApp3	3	
Actigraph	3	
Questionnaires		
qGIS	1	We need address data
Diary	3	If they do not do the monitoring devices (above), then this is not needed either
Adolescent main	1	
Adolescent FFQ	3	Can be done by parents
Adolescent puberty	2	
Parents phone	1	Parents' questionnaires should not be a problem (not important for convincing the kid)
Parents home	1	
Biological samples		

12 urines	2	Could be reduced if this makes the difference between participating or not
Blood	2	PaxGene optional
Feces	2	
Hair	Optional	

IMPORTANT NOTE: all the items above are important and all are included in the main protocol. Priorities should only be used if the adolescent would otherwise not participate at all. We can also ask if we can contact the family again next year if they do not want to do it now. We can ask the child which part he/she does not want to do.

2. Devices available per cohort (this allows to follow 6 children/week):
    - 7 GENEActiv
    - 7 Smartphone
    - 1 Actigraph (for the validation of GENEActiv in a subsample)
3. The monitoring week starts on Day 1 and finishes on the day of blood drawn (see Table 3 below) and must include a minimum of 7 consecutive days (24-hours period).
4. The blood drawn MUST be performed at the end of the monitoring week (from Day 8 to Day 10 both included, see Table 3 below).
5. The blood drawn MUST be performed after a FASTING period of at least 8 hours (not eating or drinking, except water). ONLY in the EXCEPTIONAL CASE in which fasting blood cannot be collected due to fieldwork constraints, non-fasting blood will be collected ensuring at least 2 hours fasting time since the last meal.
6. Blood MUST be processed in a maximum of 6 hours after collection (e.g. a blood sample collected at 8.00h should be processed before 14.00h).
7. The clinical examination and blood collection can be done in separate days.
8. Flexibility of the visits: the clinical examination can be done within 0-15 days after the delivery of the devices, so 7 days before the start of the monitoring week or 7 days after the blood collection (see Table 3).
9. Quarantine for COVID-19: if a child who has started the monitoring week needs to be in quarantine in the middle of the week, it is not necessary to repeat the monitoring week in case he/she has done at least 3 normal days. During quarantine the child will not wear the devices but he/she will collect the urines anyway. Blood should be collected from day 8 to 10; if the child is still on quarantine we recommend to take blood at home.
10. Summer holidays: children can be followed during summer holidays if we ensure that they can carry the devices for at least 3 days doing normal activities (the same as being in quarantine).
11. Hair collection and PaxGENE are optional.

IMPORTANT CONSIDERATIONS FOR THE ORGANIZATION OF THE FIELDWORK:

1. All HELIX children should be included in the visit – although they do not want to do one part (e.g. blood, stools) (see Annex 29).
2. Devices available per cohort:
   - 7 GENEActiv
   - 7 Smartphone
   - 1 Actigraph (for the validation of GENEActiv in a subsample)
3. The monitoring week should be of 7 consecutive days (24-hours period) minimum (Table 3).
4. The blood drawn should be performed at the end of the 7 days of the monitoring week.
5. The blood drawn MUST be performed after a FASTING period of at least 8 hours (no eating or drinking, except water).
6. Blood MUST be processed within a maximum of 6 hours after collection (e.g. a blood sample collected at 8.00h should be processed before 14.00h).
7. The clinical examination and blood collection can be done in separate days.

Table 3. *Flexibility of the visits: the clinical examination can be done within 0-15 days after the delivery of the devices and blood extraction can be done from day 8 to day 10.*

	Day -7	Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17
Hand out material								x																	
Monitoring									x	x	x	x	x	x	x	x	x	x							
Blood drawn																x	x	x							
Clinical examination	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x

Considering the large number of tests, questionnaires, and devices that need to be completed and carried during 1 week, our suggestion is to organize the visit on <u>two different days</u>:

Option 1

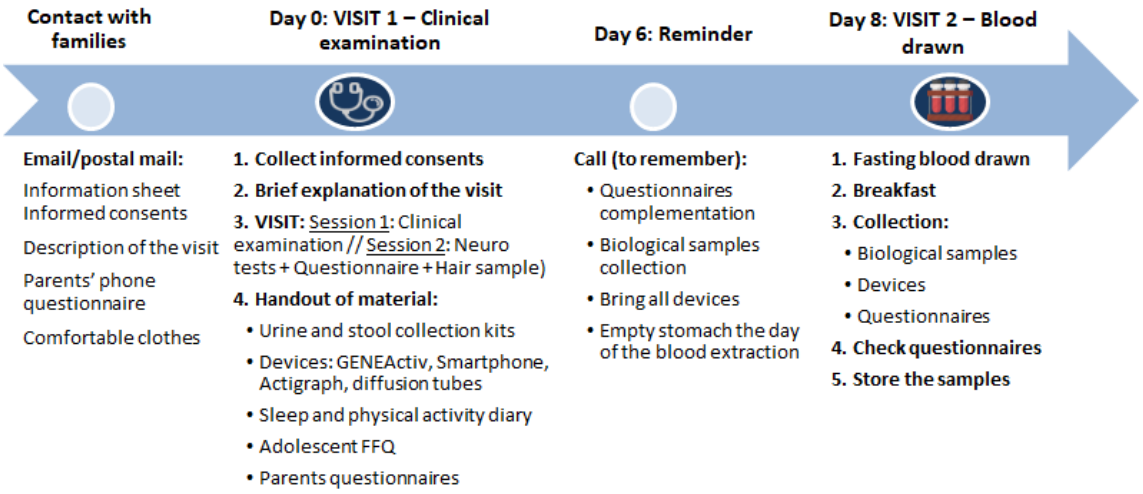
- Visit 1 (Day 0): clinical examination and hand out of the material for the 7-days personal monitoring and the biological samples collection kits;
- Visit 2 (Day 8): blood collection early in the morning and collection of all the 7-days personal monitoring material, and stools and urines samples.

Option 2

- Visit 1 (Day 0): hand out of the material for the 7-day personal monitoring and the biological samples collection kits (this can be done at home, at the study centre, or sent it by postal mail);
- Visit 2 (Day 8): blood collection early in the morning followed by the clinical examination and collection of the 7-days personal monitoring material, and stools and urines samples.

2.1.3.1 Option 1 – clinical examination and blood drawn on two days

Figure 2. *Suggested organization of the visit on two different days*



CONTACT WITH FAMILIES

First contact: We will make the first contact with the families by phone (or post if we are not able to contact by phone) and inform them about the new visit corresponding to the adolescence period. The cover letter, information sheet and the consent forms (parents and adolescent) (Annexes 1 and 2) will be sent via email, postal mail, or given by hand.

Few days before the visit: families will be telephoned or visited about a week before the first visit to:

- Go over the information sheet, describe the whole visit, and answer possible questions that parents and the adolescent may have
- Remind them to bring the signed informed consents on the day of the visit (Annexes 1 and 2)
- Remind the adolescent that they should wear **COMFORTABLE CLOTHES for the clinical examination** on the day of the visit

Day 0: VISIT 1 - CLINICAL EXAMINATION

- **Time of the day: can be in the morning or in the afternoon (record the time of the visit on the clinical data sheet)**
- **Duration: 90 minutes** (Session 1: 35 min clinical examination + 3 min qGIS + 5 min hair / Session 2: 28 min neuro tests + 15 min hand out material)
- **Place: clinical research facility, home**

1. **Collection of the informed consents.** Check that they are signed and completed.
2. **Brief explanation of what the visit will consist of**
3. **THE VISIT:** It will consist of two sessions of 45 minutes each + an explanation about the different devices that they will use during the week. One possibility, if there are two nurses/fieldworkers, is to visit two adolescents in parallel (see Table 4) in two separate spaces. If it is not possible to have two different spaces, a bulkhead can be installed to preserve the privacy and, likewise, avoid distractions during the neurodevelopment tests.

Table 4. *Outline of the visit*

Time	Adolescent 1	Adolescent 2
8:00 - 8:10	Welcome	
8:15 - 9:00	Session 1	Session 2
9:05 - 9:55	Session 2	Session 1

Sessions details:

- **Session 1:**
  1. Clinical examination**:** the clinical examination will include: anthropometric measurements, bioimpedance, blood pressure, and spirometry. Note: it is very important to follow the SAME ORDER during the clinical examination - see section 2.14. (*35 minutes*)
  2. Complete the qGIS questionnaire (see section 2.1.5.2) (*3 minutes*)
  3. Collect a lock of hair (see section 2.1.7.1) (*5 minutes*) - OPTIONAL

- **Session 2:**
  1. Neurodevelopment computer tests (see section 2.1.6) (*25 minutes*)
  2. Hand out of all material (*15 minutes*) and explanation of each device and biological sample collection kits. List of material:
     - URINE COLLECTION KIT:
       - 12 urine containers and a special box to maintain the temperature
       - Urine collection instructions and questionnaire (Annex 3)
     - STOOL COLLECTION KIT:
       - 1 collection tube for the stools.
       - Stool collection instructions and questionnaire (Annex 3)
     - MONITORING DEVICES (to be worn/carried during at least 7 consecutive days): Please note for the BiB cohort: we expect that some adolescents will require two subsequent Saturday appointments due to their school commitments. In order to achieve 7 full days of measuring, we will drop off the devices at the home address and explain via phone or, if requested, for example via ZOOM call.
       - Instructions (Annex 3)
       - A GENEActiv that the adolescent will wear to record physical activity and sleeping patterns (see section 2.1.8.1).
       - A smartphone with the ExpoApp3 installed, to be carried in a pouch provided by the fieldworkers, to record mobility (see section 2.1.8.2).
       - An Actigraph accelerometer that the adolescents will wear to record physical activity (only in a subsample) (see section 2.1.8.3).
       - A NO_2 diffusion tube that will be placed on the outside of their backpack to measure the personal NO2 exposure levels (see section 2.1.8.4). If they do not want to carry it, we can offer the possibility of placing the NO2 in the living room.
       - Incidence form: to report any issues with devices

- SLEEP AND PHYSICAL ACTIVITY DIARY that the adolescent will fill in every day, during 7 consecutive days, when he/she wakes up and before going to sleep (see section 2.1.5.3).
- INFORMATION SHEET with a detailed explanation of what the adolescent has to do during the monitoring week.
- ADOLESCENT Food Frequency Questionnaire (FFQ) to be completed at home (see section 2.1.5.1)
- PARENTS QUESTIONNAIRE to be completed at home including the Child Behaviour Checklist (see section 2.1.5.1).

Day 6: REMINDER

Adolescents and their families will be contacted by phone to:

- Perform the parents' short questionnaire (phone interview)
- To remind them:
  - To complete the questionnaires and bring them to the visit.
  - To collect stool samples on Day 6 or Day 5 To bring the urine and stool samples and the corresponding questionnaires
  - To bring all the devices: GENEActiv, (Actigraph), NO_2 diffusion tube and the corresponding incidences sheet.
  - To bring the sleep and physical activity diary.
  - That the adolescent is required to FAST, consuming no food or liquids other than water, for at least 8 hours before the second visit to perform the blood drawn.

Day 8: VISIT 2 - BLOOD DRAWN

- **Time of the day: in the morning after minimum 8 hours fasting**
- **Duration: 75 minutes** (15 min blood drawn + 15 min breakfast + 45 min questionnaire)
- **Place: clinical research facility, home**

- Perform the **BLOOD DRAW** (*15 minutes*)
- After the blood drawn, teenagers will be left for *15 minutes* for breakfast and, if necessary, go to the toilet.
- The nurses/fieldworkers will
  - Collect
    - the biological samples (12 urine samples and 1 stool sample), label them and ensure that the corresponding questionnaires are completed (Annexes 5 and 8).
    - the different devices and the corresponding incidence sheet.
    - the sleep and physical activity diary.
    - the adolescent's and parent's questionnaires
  - Check that all questionnaires/forms are completed.
  - Store the blood samples in the fridge until processing (**for a maximum of 6 hours**).

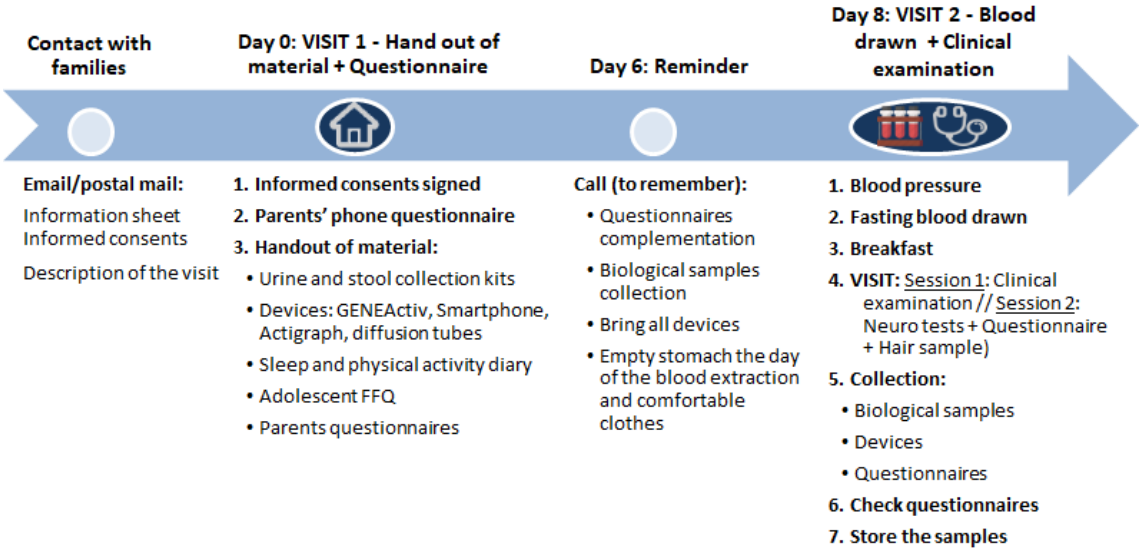
- **Adolescent's main questionnaire (see section 2.1.5.1) (*45 minutes*)**

  The adolescent main questionnaire takes 45 minutes approximately. Record on the clinical data sheet if the adolescent answered the questionnaire alone or with help from fieldworkers (Annex 18). It should be answered by the adolescent alone, but fieldworkers should be available to answer questions the adolescent might have:

- to provide a specific timeframe to the adolescent to fill it in (to increase the completeness of the questionnaire)
- to ensure support from fieldworkers to the adolescent in case of doubts
- to guarantee a semi-private environment for the adolescent

2.1.3.2 Option 2 – clinical examination and blood drawn on one day

Figure 3. *Suggested organization of the visit on one day*



CONTACT WITH FAMILIES

First contact: make the first contact with the families by phone and inform them about the new visit corresponding to the adolescence period.

Day 0: VISIT 1 - HAND OUT OF MATERIAL + ADOLESCENT'S QUESTIONNAIRE

- **Time of the day: not important**
- **Duration: 75 mins** (10 min visit description + 15 min hand out material + 45 min questionnaire)
- **Place: clinical research facility, home**

Families can be visited at home or at the study centre. If material is sent by postal mail, families will be telephoned.

It is PREFERABLE that the families are visited at home or at the study centre to:

- **Ensure that the consent forms are signed before the start of the visit (Annexes 1-2);**
- **Have the adolescent answering the main questionnaire**

If families are not visited they can send back the signed consent forms by postal mail.

NOTE: As COVID-19 pandemic are easing home visits may be carried out for example to collect consent or fasting bloods. . The situation will be monitored closely and official and local guidelines followed

The following will be done during the family visit:

- Go over the information sheet, describe the whole visit, and answer possible questions that parents and the adolescent may have (*10 minutes*).
- Sign the **consent forms** (parents and adolescent) (Annexes 1 and 2).
- **Hand out of all material (*15 minutes*)**: the personal exposure monitoring devices, the urine and stools collection kits, the diary, the information sheet and the incidences form are handed out to the adolescent following a detailed explanation about each one of them:
    - URINE COLLECTION KIT:
        - 12 urine containers and a special box to maintain the temperature
        - Urine collection instructions and questionnaire (Annex 3)
    - STOOL COLLECTION KIT:
        - 1 collection tube for the stools.
        - Stool collection instructions and questionnaire (Annex 3)
    - DEVICES (to be worn/carried during at least 7 consecutive days):
        - Instructions (Annex 3)
        - A GENEActiv that the adolescent will wear to record physical activity and sleeping patterns (see section 2.1.8.1).
        - A smartphone with the ExpoApp3 installed, to be carried in a pouch provided by the fieldworkers (see section 2.1.8.2).
        - An Actigraph accelerometer that the adolescents will wear to record physical activity (only in a subsample) (see section 2.1.8.3).
        - A NO_2 diffusion tube that will be placed on the outside of their backpack to measure the personal NO_2 exposure levels (see section 2.1.8.4). If they do not want to carry it, we can offer the possibility of placing the NO2 in the living room.
    - SLEEP DIARY that the adolescent will fill in every day, during 7 consecutive days minimum, when he/she wakes up and before going to sleep (see section 2.1.5.3).
    - ADOLESCENTSQuestionnaires (via link or paper based) adolescent questionnaire, puberty questionnaire and FFQ to be completed at home or at study centre (see section 2.1.5.1)
    - PARENTS QUESTIONNAIRE Parents questionnaires to be completed at home or at study centre including the CBCL (see section 2.1.5.1).
- **Adolescent's main questionnaire (see section 2.1.5.1) (*45 minutes)***

  (Note: To be completed ONLY in the case the adolescents are seen at their home or at the study centre, otherwise should be completed during the visit on day 8, **after the neuro tests**)

  The adolescent main questionnaire takes 45 min approximately. It should be answered by the adolescent alone, but fieldworkers should be available to answer questions the adolescent might have. Record on the clinical data sheet if the adolescent answered

the questionnaire alone or with help from fieldworkers. It MUST be answered when the fieldworkers visit the participant's at home or when the adolescent is at the study centre:

- to provide a specific timeframe to the adolescent to fill it in (to increase the completeness of the questionnaire)
- to ensure support from fieldworkers to the adolescent in case of doubts
- to guarantee a semi-private environment for the adolescent

Day 6: Reminder

Adolescents and their families will be contacted by phone /test or WhatsApp to:

- Perform the **parents' short questionnaire** (can be phone interview)
- Remind them
  - To complete the questionnaires and bring them to the visit.
  - To collect stool samples on Day 7 or Day 6 in case they usually have constipation.
  - To bring the urine and stool samples and the corresponding questionnaires.
  - To bring all the devices: GENEActiv, (Actigraph), NO_2 diffusion tube and the corresponding incidences sheet.
  - To bring the sleep and physical activity diary.
  - The adolescent is required to FAST, consuming no food or liquids other than water, for at least 8 hours before the second visit to perform the blood drawn, and she/he should wear COMFORTABLE CLOTHES for the clinical examination.

Day 8: VISIT 2 - BLOOD DRAWN + CLINICAL EXAMINATION (if not already completed)

- **Time of the day: in the morning after minimum 8 hours fasting**
- **Duration: 2 hours** (15 min blood drawn + 15 min breakfast + 90 session 1 and 2). Note: Add 45 min in the case the adolescent's questionnaire is answered during this visit.

- Perform the **BLOOD DRAW (10 minutes).**
- After the blood drawn, teenagers will be left for 15 minutes for breakfast and, if necessary, go to the toilet.
- Brief explanation of what the visit will consist of.
- **THE VISIT**: It will consist of two sessions of 45 minutes each. One possibility, if there are two nurses/fieldworkers, is to visit two teenagers at the same time (see Table 4) in two separated spaces. If it is not possible to have two different spaces, a bulkhead will be installed to preserve the privacy and, likewise, avoid distractions during the neurodevelopment tests.

Table 4. *Outline of the visit*

Time	Adolescent 1	Adolescent 2
8:00 - 8:10	Welcome	
8:15 - 9:00	Session 1	Session 2
9:05 - 9:55	Session 2	Session 1

Session details:

- **Session 1:**
    1. Clinical examination (it is very important to follow the SAME SEQUENCE during the clinical examination - see section 2.1.4). The clinical examination will include: anthropometric measurements, bioimpedance, blood pressure, and spirometry (35 minutes).
- **Session 2:**
    1. Neurodevelopment computer tests (see section 2.1.6) (25 minutes)
    2. Complete the qGIS questionnaire (see section 2.1.5.2) (3 minutes)
    3. Collect a lock of hair (see section 2.1.7.1) (5 minutes)
- **Session 3 (applies only in the case the adolescent's main questionnaire cannot be answered on Day 0 – see above for details) (45 minutes)**
    1. Adolescent's main questionnaire

       The adolescent main questionnaire takes 45 min approximately. It should be answered by the adolescent alone, but fieldworkers should be available to answer questions the adolescent might have. Record on the clinical data sheet if the adolescent answered the questionnaire alone or with help from fieldworkers. It MUST be answered when the fieldworkers visit the participant's at home or when the adolescent is at the study centre:

       - to provide a specific timeframe to the adolescent to fill it in (to increase the completeness of the questionnaire)
       - to ensure support from fieldworkers to the adolescent in case of doubts
       - to guarantee a semi-private environment for the adolescent while answering

- The nurses/fieldworkers will:
    - Collect
        - the biological samples (12 urine samples and 1 stool sample), label them and ensure that the corresponding questionnaires are completed (Annexes 5 and 8).
        - the different devices and the corresponding incidence sheet.
        - the sleep and physical activity diary.
        - the adolescent's and parent's questionnaires.
    - Check that all questionnaires/forms are completed. Record on the clinical data sheet if the adolescent answered the electronic or paper version and if help was needed from parent or fieldworker needed
    - Store the blood samples in the fridge until processing (**for a maximum of 6 hours**).

2.1.4 Clinical examination

Clinical examination will be conducted by trained nurses and MUST FOLLOW the following order (before the visit, provide an opportunity to go to the toilet).

Preparing for the visit:

- Remove heavy clothes (jumper, coat) and shoes and outer clothes impeding measurements (2 minutes)

Standing position:

- Anthropometric measurements: height, weight, and waist circumference (5 minutes)

Supine position:

- Resting in supine position (5 minutes)
- Bioimpedance (1 minutes)

Sitting position:

- Resting in sit position (5 minutes)
- Blood pressure (5 minutes)
- Spirometry (10 minutes)

IMPORTANT: blood pressure must be measured before the spirometry test in order to not interfere with the blood pressure results.

Getting dressed (2 minutes)

All results must be recorded in the clinical data sheet (Annex 18). It is important to record the time taken in each part in the clinical data sheet (Annex 18) in order to improve the scheduling of next follow-ups.

2.1.5 Questionnaires, qGIS, and diaries

2.1.5.1 Questionnaires

In this follow-up visit, given the age of the participants, there are questionnaires for both the adolescents and their parents.

1) The adolescent's main questionnaire includes the following sections:
   - Physical activity
   - Diet (not the FFQ)
   - Psychological distress
   - Tobacco exposure
   - Pubertal development (including Tanner Scale with drawn pictures of the corresponding sex. If parents are not happy for pictures to be used, the corresponding text boxes will be used only. We will validate this section of the puberty questionnaire in a proportion of children where we can include the drawings. The text only questionnaire will be used at first visit and repeated at the second visit (one week in between) with pictures included.
   - Sleeping patterns
   - Light exposure before going to sleep
   - Outdoor environment (green spaces, noise)

2) The adolescent's Food Frequency Questionnaire (FFQ)

3) The parent's questionnaires contain the following sections:
   - Address history
   - Home environment (cooking and heating)
   - Socio-economic status (family affluence scale, subjective wealth, income)
   - Noise
   - Psychological distress

- Medical history
- Information about their child:
    - Asthma and allergies
    - Medication use
    - Diet
    - Sleep and physical activity diary
    - Perception of physical activity

Adolescents MUST complete the longer questionnaire by their own during one of the two visits, and fieldworkers will be available to help them with difficult questions if needed. Fieldworkers will record on the clinical examination data sheet if the questionnaire was filled in by the adolescent alone, or with their help (Annex 18). Adolescent's FFQ will be completed at home together with their parents. In the instance where the online link is used to complete the questionnaires, all questionnaires can be completed at home and the fieldworkers can check that everything was completed correctly at the next visit. Parent's questionnaire will be divided in a short one administered by phone and a long one that will be completed at home between visit 1 and 2.

2.1.5.2 qGIS

During the visit, questions related to the adolescent's main address(es) and usual commuting routes will be collected by the fieldworkers using the qGIS software (Annex 6). This enhances the accuracy of mobility data collection and ensure separation of these sensitive personal data from the health data (3 min).

2.1.5.3 Sleep and physical activity diaries

The adolescent will be asked to complete the sleep and physical activity diary every day during seven consecutive days between visit 1 and visit 2 (5 min/day approximately).

2.1.6 Neurodevelopment tests

We will assess a variety of neurobehavioral outcomes that include several functional domains and clinical phenotypes sensitive to environmental exposures. These outcomes will be assessed by internationally standardized tests and questionnaires. The cognitive functions assessed will be non-linguistic and culturally blind.

The tests will be administered through laptops and no more than 25 minutes duration will be required. Each fieldworker can assess a maximum of one participant. Ensure that the room used is quiet and the tests are done with minimal interference. Headphones will be used and fieldworkers will be trained to instruct the adolescents in a standardized way. The instructions of the tests (Annex 3) are explained by the fieldworker before starting each test. Once testing is complete, the data is automatically stored on laptops. After each day, a backup to a USB is made. There will be a record of the general conditions for the administration of the tests (Annex 18). Parents will additionally complete the CBCL (Annex 19) without any interference from the fieldworkers (except if the parent needs help/clarification). All fieldworks must be trained by an expert examiner (fieldworker from ISGlobal) before starting the cognitive examinations.

We intend to assess three main functional domains (by 3 computer tests*) and one clinical phenotype (by parental questionnaire)

- <u>Functional domains (computer tests):</u>
    - Non-verbal, abstract and cognitive function: Raven (Annexes 13 and 14) (10-15 minutes).

- Attention and working memory: N-Back test (Annex 15) (5 minutes)
- Hot Executive function: hot cognition employs emotional influence on decision making: Cup Task - Roulette Version (Annexes 16 and 17) (8 minutes)

- Clinical phenotype: by behavioural questionnaire completed at home by the parents.
  - Child behavioural and emotional problems: CBCL (Annex 19) (15 minutes)

2.1.7 Biological sample collection

Table 5 shows the biological samples to be collected from each of the adolescents during this follow-up and their purposes:

- One hair lock will be collected by the fieldworkers after the clinical examination of the adolescent (in visit 1) following procedure in Annex 18.
- 12 urine samples (morning and night samples from 6 consecutive days) and 1 stool sample will be collected by the adolescent between visit 1 and 2. In visit 2, participants will bring them to the centre and the fieldworkers will store the urines in the fridge or the freezer depending if they are processed the same day or not (see Annex 21) (**maximum 6 hours**). **Stool samples MUST be directly stored at -80ºC.**
- **If adolescent does not bring the urine samples with them, a new sample will be collected at this point**.
- Fasting blood will be collected by trained nurses (in visit 2)

Table 5. *Biological samples to be collected in new follow-up of HELIX Subcohort*

Type of sample	**Tube/Bag**	**Sample processing**	**Sample quantity required**	**Purpose**
Hair	Zip- lock bag	Hair	-	Reserve
Urine	Virtex Vacusence **112510** plastic polypropylene 70/10mL (collected at 12 time points)	Weekly pool (3 aliquots)	0.35 mL	Phthalates
			0.5 mL	Phenols
			0.5 mL	OP pesticides
			0.5 mL	Other pesticides (metabolites of pyrethroids, 2,4-dichlorophenoxyacid, boscalid and imazalil)
			0.2 mL	Cotinine
			0.5 mL	Glycol ethers
			0.5 mL	Polycyclic aromatic hydrocarbon
			0.5 mL	Creatinine
			1.75 mL	Exogenous metabolomics
		1 aliquot of each urine + 6 aliquots of the weekly pool	1.8 mL x 18 aliquots	Reserve
Blood (19.5 mL)	6 mL silica (clot activator) vacutainer **368815**	Serum	0.5 mL	Endogenous metabolomics
			0.25 mL	Glucose, total and HDL cholesterol, triglycerides, phospholipids, ALT, AST,

				GGT
			0.1 mL	Reserve - Antibodies against SARS-CoV-2 + inflammatory proteins
			0.45 mL	Reserve - IgEs
			<1 mL	Reserve
	5 mL silicone coated glass vacutainer **367614**	Serum	0.5 mL	Exogenous metabolomics
			0.5 mL	Exogenous metabolomics
			<1.5 mL	Reserve
	6 mL K2EDTA (trace elemento determination) **368381**	Whole blood	0.9 mL	Metals and elements
			0.5 mL	Reserve
		Buffy coat	0.5 mL	Telomere length
		Plasma	0.2 mL	PFASs
			0.5 mL	Endogenous metabolomics
			<1 mL	Reserve
		Red cells	1.5 mL	Reserve
	2.5 mL PaxGene **762125** OPTIONAL	RNA	2.5 mL	Transcriptomics
Stool	1 Zymo tube **R1101**	Bacterial DNA	1 Zymo tube	Microbiome

2.1.7.1 Hair sample collection

A lock of hair, small enough to not affect the adolescent's appearance, will be collected and put in a zip-lock. Details on collection, labelling, and storage of hair samples can be found in the Hair Collection SOP (Annex 20). Collection of hair is OPTIONAL.

2.1.7.2 Urine sample collection

Urine samples will be collected by the adolescent at home during 6 consecutive days. A morning and night sample will be collected each day. Twelve urine collection kits will be handed out to the adolescent in visit 1 and he/she will bring back all urine samples in visit 2. Urines will be collected in a 70 mL propylene container and then transferred to a 10 mL tube (Annex 3). A wide range of chemicals will be determined in urine (see Table 4). Further details on processing, labelling, and storage of urine samples can be found in the Urine Collection SOP (Annex 21).

2.1.7.3 Stool sample collection

A stool sample will be collected by the adolescent at home, preferably in the morning of the day before visit 2. The adolescent will bring the stool sample to the ATHLETE fieldworkers on visit 2. Stool samples will be collected for an accurate assessment of the gut microbiota. The protocol for collection of the stool samples by the adolescent can found in Annex 3, while the protocol on labelling, and storage of stool samples can be found in the Child Stools Collection SOP (Annex 22).

2.1.7.4 Blood sample collection

Fasting blood samples will be collected by trained nurses early in the morning for subsequent determination of glucose, insulin, lipids, hepatic enzymes, metals, perfluoroalkyl compounds, and telomere length (see Table 4). No more than 19.5 mL will be drawn. Details on collection, processing, labelling, and storage of blood samples can be found in the Blood Collection SOP (Annex 23).

2.1.8 Smartphones and sensors

2.1.8.1 GENEActiv

Adolescents will wear the wrist-worn accelerometer GENEActiv during at least 7 consecutive days between visit 1 and 2, during daytime and at night to assess their sleeping patterns and their physical activity levels. Instructions for fieldworkers can be found in Annex 24.

2.1.8.2 Smartphone

A smartphone app, called ExpoApp3, designed to obtain positioning/mobility and physical activity will be used. Information obtained from ExpoApp3 will be integrated into the spatial models to generate built environment data (e.g. air pollution, green spaces). The ExpoApp3 will be installed on a smartphone belonging to ISGlobal. Adolescents will carry the smartphone during daytime (i.e. when they are awake) in a pouch during 7 consecutive days between visit 1 and 2. Instructions for fieldworkers can be found in Annex 25.

2.1.8.3 Actigraph

In order to validate data on physical activity from the GENEActiv and the ExpoApp3, a subset of adolescents of each cohort (around 30-35 adolescents per cohort) will carry an Actigraph. The Actigraph measures physical activity and it will be carried on the frontal part of the right hip attached to be phone pouch during daytime (i.e. when they are awake). Instructions for fieldworkers can be found in Annex 26.

2.1.8.4 NO_2 diffusion tubes

NO_2 diffusion tubes will be used to measure personal exposure to NO_2. The NO_2 diffusion tube will be placed on the outside of the adolescent's backpack. Instructions for fieldworkers can be found in Annex 27.

In case the adolescent does not want to carry the diffusion tube on the backpack, it can be placed at home in the living room. To compare the personal and indoor measurement, give two tubes to a subsample of children (1 every six): one tube should be placed on the backpack add the other in the living room for the whole week.

The adolescents will receive printed instructions on how to use and take care of the smartphone, the devices and the NO_2 tubes (Annex 3).

2.2 Ethical Issues

Ethical issues and health risks arising within the new follow-up of HELIX Subcohort concern:

a) The collection of information about behavioral, lifestyle, social and psychological factors, exposure sources, and other habits in adolescents.

b) The collection of 12 urine samples, 1 stool sample, 1 hair sample and 1 blood sample (19.5 mL) in adolescents. Urine, stool, and hair samples are considered non-invasive

and do no present any particular concern risk. Blood collection is kept to an absolute minimum.

c) To carry the GENEActiv and the Actigraph accelerometers (Actigraph only a subsample of each cohort) during 7 consecutive days. These devices do not present any particular risk for the participants.

d) To carry a smartphone with the ExpoApp3 application to record geolocation/mobility during 7 consecutive days. The smartphone will not use a SIM card.

e) To carry a NO_2 diffusion tube during 7 consecutive days to measure personal exposure to air pollution. This diffusion tube does not present any particular risk for the participants.

f) Collection of biological samples for future studies on genetic variation (transcriptomics and epigenomics (DNA methylation and miRNA)), through sequencing or microarrays. Potential incidental genetic findings with actionable health consequences need to be reported back to the families and a protocol should be developed in each cohort.

g) The storage and transfer of data, including geocodes, to central data warehouse.

Research studies in countries participating in the work are regulated by both national and international legal and ethical rules.

2.3 Timeline

Due to the COVID-19 pandemic, the follow-up period has been extended to March 2022

			2020				2021			
	Start	End	1st T	2nd T	3rd T	4th T	1st T	2nd T	3rd T	4th T
Task 1.5. ATHLETE Adolescence Follow-up	Jan 2020	Dec 2021								
Protocol										
Development	Jan 2020	July 2020								
Translation	June 2020	July 2020								
Obtain permission from local ethics commission	July 2020	Dec 2020								
Questionnaire										
Development	March 2020	July 2020								
Translation	Aug 2020	Oct 2020								
Software development	July 2020	Sept 2020								
Installation	Sept 2020	Oct 2020								
Testing by cohort	Sept 2020	Oct 2020								
Obtain permission from local ethics commission	July 2020	Dec 2020								
Execution of Fieldwork										
Material preparation	June 2020	Oct 2020								
Contact with families	Sep 2020	Dec 2020								
Pilot	Oct 2020	Dec 2020								
Visit	Jan 2021	Dec 2021								
Transfer of data for storage	Jan 2021	Dec 2021								

3. SECTION TWO: Co-production of interventions to reduce urban exposome exposure in primary school children

ATHLETE WP7 will demonstrate the development and evaluation of co-produced effective and scalable interventions to reduce personal exposure to the harmful effects of the urban exposome. We will focus on primary school-aged children as children are particularly vulnerable to their urban environment, a source of physical, chemical and behavioural exposures (e.g. pollution, lack of green space, noise, physical activity) all of which have been associated with a variety of health outcomes including asthma, mental health, obesity and cognitive development (de Bont et al. 2019; Khreis et al. 2017; McEachan et al. 2018; Stansfeld and Clark 2015). Schools are often urban exposome 'hotspots' located in areas of high pollution or noise, compounded by high levels of car use during the 'school run'; within the UK, around half of children are driven to school (Department for Transport 2014). Interventions will be developed in close partnership with communities and key stakeholders ensuring that our co-produced interventions are both acceptable and feasible, thus increasing the likelihood of rapid translation into practice.

We aim to characterize urban exposures and co-produce interventions to reduce personal urban exposure amongst primary school-aged children in inner city Bradford, UK and Barcelona, Spain. This protocol includes details of the study design and sample size at both sites, but the application relates to Bradford fieldwork only.

Our objectives are to:

1. Explore when and where key exposures occur
2. Understand barriers and enablers to improving exposure to the urban environment
3. Co-produce interventions to reduce personal urban exposure.
4. Evaluate the impact and explore the sustainability of the co-produced interventions.

3.1 Method

3.1.1 Design

There are three phases to the research design:

1. Baseline data collection and co-production of acceptable and feasible interventions to reduce the urban exposome amongst primary school age children. (Autumn 2021)
2. Pre-test-post-test evaluation of the co-produced interventions. (Spring 2022)
3. 6 month follow-up to explore the sustainability of the interventions. (Autumn 2022)

The following methods will be repeated in all three phases of the research:

a) Static air pollution monitoring in schools;
b) Personal urban exposure monitoring with primary school age children to quantify their exposure to the urban exposome; and
c) A questionnaire given to primary school age children and their parents to gather data on school travel modes and preferences, play and physical activity, self-assessed physical and mental health, and support for proposed interventions.

Additional qualitative methods will be used in phase 1 to gather evidence for co-production:

d) Walk along interviews with parent and child dyads to explore their perceptions of the urban exposome on the route from school, and their perceived barriers and enablers to reducing the harmful urban exposome.
e) Photovoice discussion groups with children, using photos taken during walk along interviews as the basis for group discussion and community mapping of the urban exposome.

The data collected in phase 1 will be used to co-produce interventions to reduce the urban exposome with children, parents, teachers and local 'healthy place decision makers' (e.g. from local and municipal authorities).

Additional qualitative methods will be used in phase 3 to explore the sustainability of the interventions:

f) Focus groups with parents and children.
g) Interviews with teachers in participating schools.

3.1.2 Recruitment

- We will recruit N=4 schools (2 schools in Bradford, UK and 2 in Barcelona, Spain).
- Within these schools we will recruit N=100 children (25 per school) and their parents to complete a travel questionnaire, which will be repeated in all three phases of the research.
- A sub sample of 40 children (20 in Bradford and 20 in Barcelona; 10 in each school) will be recruited to be citizen scientists. These children will carry air pollution monitoring devices for 1 week at baseline, 2 weeks pre- and post-intervention, and 1 week at 6 month follow up. These children will be recruited from those who complete the survey.
- A sub sample of 30 parent child dyads (15 in Bradford and 15 in Barcelona; 7-8 per school) will be recruited to take part in walk along interviews. These children will also be recruited from those who complete the survey.
- The 30 children who take part in walk along interviews will also be recruited to take part in a Photovoice discussion group with other children from their school.
- From the sub sample of 40 citizen scientists, we will invite children and their parents to take part in a focus group discussion at 6 month follow up.
- We will recruit teachers from participating schools (n=6 total) to take part in an interview at 6 month follow up.

3.1.3 Eligibility

Schools:

- Within areas of high deprivation
- Within areas where there is a high concentration of NO_2
- Willing to install 2 static monitors (one inside classroom and one in the outside grounds) for 1 year, and NO_2 diffusion tubes during fieldwork weeks.
- Able to commit to facilitating children with data collection and device management

Children:

- Children aged 9-11 at participating schools
- Parents able and willing to give informed consent to take part
- Child able and willing to give verbal assent to take part

Parents:

- Parent with child aged 9-11 at participating school participating in the study.
- Able and willing to give informed consent to take part.

Exclusions:

- Schools that are taking part in an existing air quality study or in a school environment intervention study.
- Participants who are unable to give informed consent due to lack of capacity or if they speak a language that we are unable to facilitate through our resources.

3.1.4 Screening for WP7

A shortlist of eligible primary schools will be identified based on local authority NOx exposure data and location in areas of high deprivation. We will approach schools from this shortlist, prioritising those within areas with the highest concentrations of NOx.

Eligible year group/classes of children will be identified through discussions with the recruited schools. It is anticipated that all children within a particular class in each school and their parents will be offered the opportunity to complete the travel survey. This survey will include questions to help us identify:

i. those participants most interested in changing their travel behaviour and monitoring personal exposure as citizen scientists; and
ii. those participants who usually walk home from school, who would be eligible to take part in a walk along interview.

The commitment required from teachers, parents and children will be explained to interested schools as part of the recruitment and screening process.

3.1.5 Consent

Schools: Schools will be provided with the head teacher participant information sheet (PIS) (Annex 31) and a school's consent form (Annex 32). Informed consent will be taken from participating schools for the installation of static monitors and NO_2 tubes and for the school to facilitate children's use of mobile PM sensors, NO_2 diffusion tubes and the EXPOApp3.

Pupils and parents: Parents will be given a parent PIS (Annex 33) and a parent consent form (Annex 34). Participating children will also be given a child PIS (Annex 35) and verbal assent will be taken prior to data collection. The parent PIS and consent form will cover the following:

- Consent to take part in three waves of the parent and child travel questionnaire. (The phase 1 questionnaire for parents to complete with their children will be sent home with the PIS.)

- Expression of interest and consent for their child to take part (if eligible and selected) in personal exposure monitoring as a citizen scientist.
- Expression of interest in and consent to take part (if eligible and selected) in a parent and child walk along interviews, and for children to take part in a Photovoice workshop.

Teachers: Teachers invited to take part in interviews will be given a teacher PIS (Annex 36) and a teacher consent form (Annex 37).

3.1.6 Withdrawal

All participants will be free to withdraw without giving any reason at any time during the course of the study. Any data collected up to that point will be retained for analysis unless otherwise specified by the participant.

3.1.7 Sample size (Bradford only)

- **Schools:** 2 schools in areas of high deprivation and high levels of pollution
- **Questionnaire:** 50 children (25 per school) target sample size, based on inviting a whole class to take part.
- **Personal monitoring**: A sub sample of 20 children will be selected, based on interest in changing and monitoring their travel behaviour.
- **Parent/ child dyads:** A sub sample of 15 parent child dyads (7-8 per school) will be selected based on children who walk home from school to take part in the walk along interviews.
- **Photovoice discussion groups:** 15 children will be invited to take part in workshops, based on those who took part in walk along interviews.
- **Co-production workshops:** All participants will be invited to take part in a workshop to discuss the findings from the research in their school.

3.1.8 Equipment

1) Static air quality sensors
2) Portable air quality sensors (Atmotube Pro)
3) NO_2 diffusion tubes
4) Smartphones with ExpoApp3 and Atmotube Pro app
5) Tablet (to be provided by researcher on the day for walk along interview) with PicVoice app to allow parent/ child dyads to take photos on their journey.

3.1.9 Data collection

3.1.9.1 Static monitoring

2 static particulate matter monitors (Figure 3) be placed in each of the 2 schools for up to a year, to cover the baseline data collection period, implementation and evaluation of the interventions One monitor will be placed indoors at a key location (for example, classroom) and one will be outside (in playground, close to the classroom). The precise locations may differ between the schools depending on operational considerations. All monitors will measure

particulate matter (PM), temperature (T) and relative humidity (RH), some will additionally measure CO (carbon monoxide), NO (nitrogen monoxide), NO2 (nitrogen dioxide), VOCs (volatile organic carbon) and O3 (ozone).

Figure 3: Optical particle counter (OPC) sensor to measure particulate matter. Produced by (and purchased from) Alaphasense Ltd.

Schools and the research team will be able to access the recorded particulate matter data and (if available) choose to display their data on a public website.

3.1.9.2 Personal Exposure Monitoring

In addition to the static monitors, 3 pieces of equipment will be used to assess personal exposure to the urban exposome on children's journeys to and from schools: portable monitors, NO_2 diffusion tubes and smartphones with the ExpoApp3 installed. Children will carry these devices for 1 school week (Monday to Friday). Children will also be given a Citizen Scientist log book that includes information about each device, an incident monitoring sheet and a daily travel diary for completion (Annex 38).

Portable monitors

School pupils will be given portable Personal Exposure Monitors (PEM) to wear which will allow quantification of the urban exposome children are exposed to on their journey to and from school. We plan to use the Atmotube PRO wearable device, which children will attach to the outside of a bag provided by the project, using a carabiner clip. This device measures concentrations of particulate matter (PM1, PM2.5, and PM10) and Volatile Organic Compounds (VOCs), plus atmospheric pressure, temperature, and humidity. Children will also be asked to carry a research smartphone with the Atmotube app installed. This smartphone will be carried in a bag provided by the project. Data from the Atmotube Pro will sync with the smartphone app throughout the study week.

Figure 4: Atmotube Pro portable air quality monitor

Care will be taken with data from the PEM monitors to ensure anonymity of the participants. Data taken in, or close to, a child's home will only be displayed without latitude / longitude / school / participant information so as to ensure it is anonymous. This distance will be increased in any instance where this is insufficient to anonymise the data (e.g. houses in remote / easily identifiable locations). Data from within the buffer zone will only be seen by the research team.

NO_2 diffusion tubes

Children will also be asked to wear an NO_2 diffusion tube during the study week. They should attach this to their outer clothing using a clip supplied with the tube (Figure 5). This will monitor NO_2 concentration.

As NO_2 diffusion tubes do not collect time series data (they only provide an average exposure for the duration of the study week), to estimate NO_2 exposure on children's journeys to/from school, NO_2 readings will also be taken from the school and home environment.

- NO_2 tubes will be installed in indoor and outdoor locations in schools – ideally the same locations as the static monitors – for the duration of Personal Exposure Monitoring fieldwork.
- Children will be given 2 NO_2 tubes to measure exposure at an indoor and outdoor location in their home for the duration of the study week (Figure 6). The tubes and clips/straps will be put in the same bag as the PEM monitor to take home on the 1st day of data collection, along with written instructions on home installation in their Citizen Scientist log book (Annex 38).

Figure 5: NO_2 diffusion tube (wearable)

Figure 6: NO_2 diffusion tube (static)

ExpoApp3

We will provide all children with a research smartphone with the ExpoApp3 installed (Annex 25). The app is designed to measure location and physical activity. Children will be required to carry the smartphones with them throughout the data collection period in a bag provided by the research team. The smartphones will only be enabled with WiFi and GPS functionality and will not have SIM cards that will allow children to make or receive calls. The smartphone will also have the Atmotube app installed; other than this no other apps will be downloaded on the phone. Data from the ExpoApp3 will be sent to the 'ExpoHub' (see Annex 25) when connected to WiFi. No personal data will be displayed on the devices and children will be assigned with a pseudo anonymised identification number.

Weekly monitoring routine for children

Children will complete a travel diary on each school day and use the following devices.

Device	**1st Friday**	**Weekend**	**Mon-Thurs**	**2nd Friday**
Atmotube Pro	Wear on journey home from school. Switch off at 7pm, then charge it.	Leave switched off in the kit bag.	Switch on at 7am. Wear on journey to and from school and during school. Switch off at 7pm, charge it.	Switch on at 7am. Wear on journey to school, then hand in to BiB.
Smartphone	Carry home from school in kit bag. Charge it at 7pm.	Leave in the kit bag.	Carry on journey to and from school in the kit bag. Charge it at 7pm.	Carry on journey to school in the kit bag, then hand in to BiB.
Wearable NO_2 tube	Wear on journey home from school. Put it in a safe place in the living room after 7pm.	Leave in the living room.	Wear on journey to and from school and during school. Put it in a safe place in the living room after 7pm.	Wear on journey to school. Close tube and place in kit bag to when told to at school.
Home indoor NO_2 tube	Carry home from school in the kit bag. Place and open as instructed in the living room.	Leave in place.	Leave in place.	Close tube and place in kit bag as instructed to hand into BiB.
Home outdoor NO_2 tube	Carry home from school in the kit bag. Place and	Leave in place.	Leave in place.	Close tube and place in kit bag as instructed to

	open as instructed outside your front door.			hand into BiB.

Daily monitoring routine for children

Approx. Time	Location	Actions	Comments
7.00	**In Home**	● Equipment left in the living room overnight. (Atmotube Pro and Smartphone must be charged.) ● Unplug Atmotube Pro and Smartphone . Put chargers away in backpack. ● Switch on Atmotube Pro at 7am.	Atmotube Pro doesn't need to monitor overnight and can be switched off daily between 7pm and 7 am.
	Travel to school	● Child travels to school as normal, carrying the equipment. ● Atmotube Pro should be clipped to backpack strap. ● Personal monitoring NO_2 diffusion tube should be clipped to coat/outer clothing.	
9.00	**Start of the school day**	● Child carries the equipment with them throughout the school day. ● Personal monitoring NO_2 diffusion tube can be left attached to a coat if the child wears one to school.	
15.30	**End of the school day**	● Class teacher and child checks that they are taking home the: - Smartphone - Atmotube Pro - NO_2 diffusion tube	If child attends an after school club, equipment should be taken there.
15.30 to 19.00	**Travel from school**	● Child travels home from school as normal, carrying the equipment. ● Atmotube Pro should be clipped to backpack strap. ● Personal monitoring NO_2 diffusion tube should be clipped to coat/outer clothing.	
19.00	**In home**	● On arrival at home, child places equipment in the living room. ● Switch off Atmotube Pro at 7pm. ● Plug in Atmotube Pro and	

		Smartphone to charge overnight.	
After school	**In home**	● Child completes a daily travel diary (in Citizen Scientist log book, Annex 38)	This should take no more than 5 minutes each day.

3.1.9.4 School travel questionnaire

Children and their parents will be invited to complete a school travel questionnaire (Annex 39) on three occasions over the course of the project:

1. At baseline data collection in Autumn 2021
2. During the intervention implementation week in Spring 2022
3. At 6 month follow up in Autumn 2022

This child section of the questionnaire will collect data about the children and how they feel about the environment around their home and school, school travel modes and preferences, physical activity, and self-assessed physical and mental health. The parent section of the questionnaire will collect data about the parent, their child's health, reflection on their child's school travel, and support for proposed interventions.

All three waves of the questionnaire will be printed and provided to the children at school. Children can complete their portion of the questionnaire at school or at home depending on the preferences of the teachers and parents. The Parent Information Sheet and Consent From and the Child Participant Information Sheet (Annexes 33, 34, and 35) will be provided at the same time as the first questionnaire. A pre-paid envelope will be included for these documents to be returned directly to the Born in Bradford project office.

3.1.9.5 Walk along interviews

A sub sample of 30 parent and child dyads (15 per site) will be invited to participate in walkalong interviews with a researcher. A researcher will meet the parent and child at school at the end of the school day and accompany them on their journey home. Children and parents will be given a research tablet with the PicVoice app which they will both use to take pictures and record notes of urban exposure on their journey (Figure 6). The app allows users to take pictures and record a voice note to accompany the image.
Further information on this can be found at:
https://play.google.com/store/apps/details?id=com.edukunapps.picvoice&hl=en_GB.

The researcher will carry out a semi-structured interview with the parent/ child dyad, following the walk along interview SOP (Annex 40) and aided by an interview guide (Annex 41) and a participant handout (Annex 42). The interviews will be audio recorded for transcription. The researcher will also complete a brief field note diary as soon as possible after the interview (Annex 43). In the event that audio recording of the walk along interview is not possible or poor quality (e.g. due to social distancing), the field note diary will be used to capture additional comments from parents and children that are not recorded by the PicVoice app.

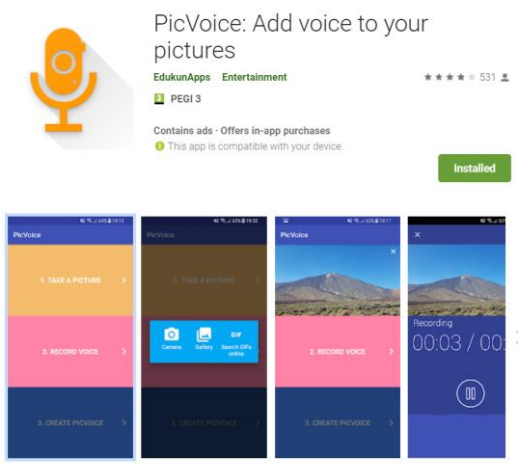


Figure 6: Illustration of PicVoice App

3.1.9.6 Photovoice discussion groups

A sub sample of 7-8 children per school (those who take part in the walk along interviews) will be invited to participate in a Photovoice discussion group with a researcher. This will take place on school premises during or after the school day depending on participant preferences. This discussion group will be aided by a discussion guide (Annex 44), and the photos that the children took during their walk along interviews will be used as visual prompts. During the discussion group, children will take part in a mapping exercise, using different coloured stickers to represent unhappy and unsafe places, and safe and favourite places, on their walk to and from school. These discussion groups will be audio recorded and transcribed.

3.1.9.7 Co-production workshop with teachers, parents, and children

A co-production workshop will be held in each school including Year 5 children, teachers, parents, local policymakers, and researchers. This will take place on school premises during or after school hours, depending on participant preferences. The workshops will be aided by an outline of potential activities (Annex 45) using photos taken by children from the walk along interviews and data from the personal monitors. The co-production workshop will use these and discussions to come up with ideas to make the school travel healthier and safer.

3.1.9.8 Focus groups with parents and children

Approximately 6 months after the implementation of the inventions in schools, parents and children will be invited to attend a focus group to discuss their experience of taking part in the project, and the perceived impact and sustainability of the intervention. We will organise 1 or 2 focus groups per school, depending on the number of families who wish to take part. Focus groups will take place on school premises during the school day or after school by arrangement. These focus groups will be aided by a discussion guide (Annex 46) and will be audio recorded and transcribed.

3.1.9.9 Teacher interviews

Approximately 6 months after the implementation of the inventions in schools, teachers of pupils in participating classes will be invited to take part in an interview to discuss the school's experience of taking part in the project, and the perceived impact and sustainability of the

intervention. These interviews will take place on school premises during the school day or after school by arrangement. The interviews will be aided by a discussion guide (Annex 47) and will be audio recorded and transcribed.

3.1.10 Analysis

3.1.10.1 Sensors

The **static sensor data** will be analysed to understand the levels and variability of air pollutants children attending that school are exposed to and identify modifiable sources of exposure.

The **personal exposure** data (PEM data from N=10 children per school) will be used to build a picture of the typical exposure to air pollution that children encounter in their daily life. All visualisations of the data will ensure that the location of the children's homes is not shown. Most map-based analysis will focus on gridded data, which further anonymises it. Through this analysis we aim to identify exposure "hot spots" and clean areas and explore whether different routes would reduce exposure.

3.1.10.2 NO_2 diffusion tubes

The NO_2 exposure data will be used to determine the average concentration of compounds that were present in the air: (i) at school; (ii) at the child's home; and (iii) on journeys to and from school over the monitoring period. The results are reported in parts per billion (ppb) and micrograms per meter cubed (µgm-3) to allow comparison with health guideline levels.

3.1.10.3 ExpoApp3

The ExpoApp3 integrates real-time information from geo-location and geographic information systems to calculate time spent in microenvironments (such as at home, in-transit, and at school) and environmental exposures and doses (such as to green spaces). Data on mobility and location will be used to characterize in real-time where the participants spend their time and where they are exposed, and will be used in conjunction with data gathered from the PEM to characterize where and when exposures to the urban exposome are occurring.

3.1.10.4 Questionnaire

Questionnaire data will be inputted into a database for descriptive analysis. Each participant will be assigned a unique ID to link their survey responses in phases 1, 2 and 3.

3.1.10.5 Interviews, Photovoice discussion groups, and focus groups

Data from the walk along interviews, Photovoice discussion groups, parent and child focus groups and teacher interviews will be transcribed and coded in NVIVO and analysed using thematic analysis. The pictures and audio notes from walk along interviews will be analysed using the autophotography method (Glaw et al. 2017).

3.2 Quality assurance

3.2.1 Citizen science

All participating pupils and teachers will be given training by a researcher on how to use the sensors and other equipment to collect data. Pupils will also be given a Citizen Scientist log book with information about all of the data collection equipment and how to use it (Annex 38). This book will also contain an exposure monitoring form and an incident log. Teachers will remind the pupils daily to carry their sensors and to charge them whilst at school.

3.2.2 Sensors

Live data fed in from the static sensors to the database will be regularly monitored by researchers to ensure the devices are working correctly and to identify and rectify any issues. Colleagues at IS Global in Barcelona will undertake a weather station co-location pilot with the Atmotube Pro sensors to validate their performance against a high spec model. During fieldwork, data from these sensors will be downloaded from the smartphone device at the end of each study week and shared with IS Global via a secure cloud API. This will enable regular monitoring and identification of any issues. The cloud is hosted on an EU server and no personal data will be stored or shared there – it operates using de-personalised MAC addresses and associated readings.

3.2.3 Qualitative data

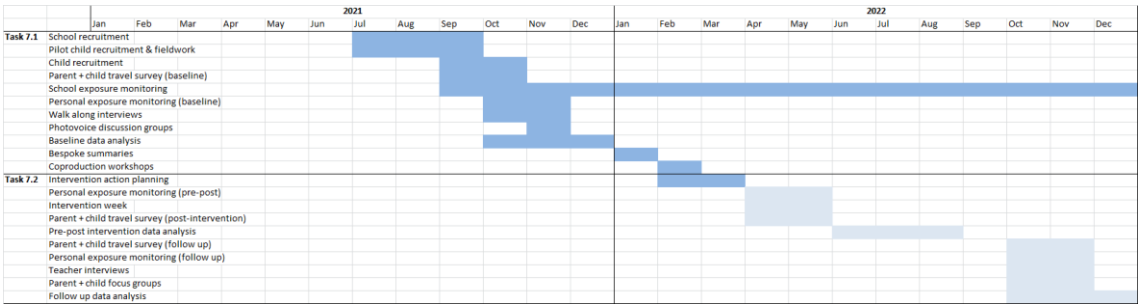
The study will be conducted in accordance with the principles of the current MRC Good Clinical Practice (GCP) guidelines. All data collection staff will be trained before collecting data. An up-to-date list of personnel certified to collect data will be maintained within the BIHR. 10% of the interview and focus group discussions (when transcribed) will be coded by 2 researchers. They will then meet to discuss the labels and agree on a set of codes to apply to subsequent transcripts and inform a coding framework. A code for 'other' will be included to avoid missing data that does not fit the agreed framework. The researchers will meet again when all the transcripts have been coded and agree on any new codes that have emerged.

3.3 Ethical issues

If schools chose to have aggregated pollution data displayed on a public website, no data will be shared from within the buffer zone. Personal details (such as home addresses) will only be available to the core research team and will be stored securely at BiB.

Lone worker policy: Researchers will follow the Bradford Teaching Hospitals NHS Foundation Trust policy for working alone. The researcher will take a mobile phone on all fieldwork visits. S/he will have a study contact based in BiB. The researcher will inform the study contact of when s/he is conducting a walk along interview, will arrange an agreed contact time, and will provide the study contact with the participant's details (name, phone number and address). The researcher will telephone the study contact when the visit has been completed. If the researcher does not contact the study contact by the agreed time, the study contact will first try to contact the researcher via mobile phone. If the study contact is unable to contact the researcher s/he will contact the participant and then, if necessary, contact the police.

3.4 Timeline



4. Informed consent

Informed consent will be sought before the start of the follow-up visit (see Annexes 1 and 2, 33 and 36). Informed consent forms and information sheets will be in a language and terms understandable to the participants of the study. There will be some variation between countries given the differences in language, requirements of the specific ethics committee within each country, and some variation in population group and specifics of the protocols (e.g., location of research centre, arrangements of participant travel). Signed consent forms will be kept in a locked cabinet with restricted access. A copy of the signed consent form will be provided to the consenting parent/guardian. Participants are free to change their mind about participation and to withdraw from the study at any time. Whatever the participants decide, their healthcare will not be affected in any way and their participation in BiB is also not affected. If a participant wishes to withdraw from the study, their samples (WP1) and personal exposure monitoring and survey data (WP7) may be retained and used unless the participant specifically requests that their samples are destroyed (WP1) or their data is deleted (WP7), in which case we will make every effort to ensure that no further analysis is conducted on their samples data.

Each cohort has a policy for recording incidental findings related to the results of research diagnostic tests and recording adverse events.

5. Personal data processing

Adequate measures to ensure personal data protection and confidentiality will be taken, according to the Regulation (EU) 2016/679 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data. National regulations on personal data protection will be implemented to guarantee the highest standards in personal data management.

The following principles will be applied when processing personal data: lawfulness, fairness and transparency; purpose limitation; data minimization (necessary and proportionate for the research objective); accuracy; storage limitation and integrity and confidentiality.

General procedures to be included in the research protocol to safeguard the privacy of study subjects:

- Written consent will be obtained from all the participants in the study to use their personal data. Consent forms include a specific clause on personal data protection informing the study participants how their data is going to be treated and stored, the research purpose, the Data Protection Officer (DPO) contact and their rights.
- Pseudonymization will be implemented as a general standard, meaning that all material obtained in the framework of the project, including questionnaires and biological material, will be identified through a code, the name and/or other personal data that could allow the identification of the participant will never be indicated. This unique identifier will link all basic data required for the study. The master key file linking the center's study numbers with personal identifiers will be maintained in a password protected file with limited access. Whenever possible, anonymization will be applied.
- All files containing personal data will be stored in encrypted and password-locked files. Access to these files will be limited to authorized project personnel.
- In the case of tracking participants by geo-localization techniques, the geo-localization data will be store separately from the other participant's data (health, etc).
- All audio recorded data will immediately be copied on to a password protected computer at BIHR and deleted from the device. Once the recordings have been transcribed, they will then also be deleted.
- Only researchers linked to the project will have access to personal data.
- Personal data will not be transferred, except in the cases considered by law.
- Reported study results will pertain to analyses of aggregate data. No individual's name will be associated with any published or unpublished report of this study.
- All project personnel will be trained in the importance of confidentiality of individual records and required to sign a confidentiality agreement.

6. Governance and oversight

McEachan (Chief Investigator) will oversee the ATHLETE project in Bradford. An operational group meeting (chaired by Yang, Principal Investigator) is conducted monthly with key research staff across WP1 and WP7 to oversee implementation of the research project. Quarterly meetings between the Bradford Institute for Health Research in Bradford, UK and ISGlobal in Barcelona, Spain to oversee progression of WP1 (chaired by Maribel Casas, ISGlobal) WP7 (chaired by McEachan). The ATHLETE Executive group (comprising work package leaders) will meet monthly to ensure consortium is progressing against key milestones. A school's group will meet biannually to oversee the work carried out in schools.

7. References

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8. Appendix

8.1 Annex 1: ____n/a_____

8.2 Annex 2: ____n/a_____

8.3 Annex 3: Instructions for adolescents /see separate document

8.4 Annex 4: Adolescent's Main questionnaire /see separate document

8.5 Annex 5: Adolescent's FFQ /see separate document

8.6 Annex 6: SOP for Geocoding/ see separate document

8.7 Annex 7: Parents Short Questionnaire

8.8 Annex 8: Parent Main Questionnaire

8.9 Annex 9: SOP Anthropometry/not included

8.10 Annex 10: SOP Bioimpedance /not included

8.11 Annex 11: SOP Blood pressure /not included

8.12 Annex 12: SOP Spirometry /not included

8.13 Annex 13: SOP Raven/ see separate document

8.14 Annex 14: Raven Annex/ not included

8.15 Annex 15: SOP N-Back/ see separate document

8.16 Annex 16: SOP Roulettes Task / see separate document

8.17 Annex 17: Roulettes Task Annex/not included

8.18 Annex 18: Clinical data sheet /see separate document

8.19 Annex 19: CBCL validated (Child Behavior checklist)/see separate document

8.20 Annex 20: SOP Hair /not included

8.21Annex 21: SOP Urine /not included

8.22 Annex 22: SOP Stool /not included

8.23 Annex 23: SOP Blood /not included

8.45 Annex 45 Coproduction workshop outline / see separate document

8.46 Annex 46 Focus group guide/ see separate document

8.46 Annex 47 Teacher interview guide/ see separate document