INMA-Sabadell cohort
Protocol 9 years

V3, 15 July 2013

Coordinator: CREAL (Centre for Research in Environmental Epidemiology)

Funding:

- **HELIX** (The Human Early-Life Exposome – novel tools for integrating early-life environmental exposures and child health across Europe): EC Exposome Programme (FP7-ENV-2012-308333)
- **EXPOsOMICS**(Enhanced exposure assessment and omic profiling for high priority environmental exposures in Europe) : EC Exposome Programme (FP7-ENV-2012-308610)
- **ANSES 2012** (Impact de l'exposition aux particules ultrafines sur la fonction ventilatoire et les symptômes respiratoires chez l'enfant d'âge scolaire: étude dans les cohortes PARIS et INMA)**:** Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail (2012-2-147)
- **MINECO** (Epigenetics in the early life exposure to air pollution and neurodevelopment and behaviour disorders within families in the INMA Sabadell birth cohort): Ministerio de economía y competitividad (SAF2012-32991), Plan Nacional 2012
- **GERoNiMO** (Generalised EMF Research using Novel Methods – an integrated approach: from research to risk assessment and support to risk management), FP7-ENV-2013-603794

CREAL personal contacts of people involved in the INMA-Sabadell 9-years

Investigator	Task	Specific task	e-mail
Jordi Sunyer	PI INMA-Sabadell	Coordination	jsunyer@creal.cat
Martine Vrijheid	HELIX coordinator	Coordination	mvrijheid@creal.cat
Manolis Kogevinas	EXPOSOMICS coordinator	Coordination	kogevinas@creal.cat
Mark Nieuwenhuijsen	EXPOSOMICS coordinator	Coordination	mnieuwenhuijsen@creal.cat
Susana Gros	INMA Project manager	Coordination	sgros@creal.cat
Jordi Júlvez	Coordinator INMA-Sabadell	Fieldwork coordination – Subcohort A	jjulvez@creal.cat
Maribel Casas	HELIX Children Panel Study coordinator	Fieldwork coordination – Subcohort B and C Biobank INMA-Sabadell	mcasas@creal.cat
Laia Font	EXPOSOMICS fieldwork coordinator	Fieldwork coordination – Subcohort B and C	lfont@creal.cat
Oliver Robinson	HELIX subcohort coordinator	Fieldwork coordination – Subcohort A	orobinson@creal.cat
Silvia Fochs	Nurse INMA-Sabadell	Clinical examination Samples collection	sfochs@creal.cat
Nuria Pey	Nurse INMA-Sabadell	Clinical examination Samples collection	npey@creal.cat
Muriel Ferrer	Psychologist	Neurodevelopment assessment	mferrer2@creal.cat
Pau Pañella	Technician INMA-Sabadell	Fieldwork – Subcohort B and C	Start in October
Cecilia Persavento	Technician INMA-Sabadell	Samples storage Biobank INMA-Sabadell	mpersavento@creal.cat
David Donaire	Personal exposure monitoring coordinator	Smartphones, UVR, and ar pollution sensors coordinator	ddonaire@creal.cat
Montserrat de Castro	GIS technician	GIS data	mdecastro@creal.cat

Abbreviations

CAP	Primary Care Center
CBCL	Child Behaviour Check List
CREAL	Centre for Research in Environmental Epidemiology
DDE	Dichlorodiphenyldichloroethylene
GIS	Geographical Information System
HCB	Hexachlorobenzene
Hg	Mercury
INMA	Environment and Childhood
OP	Organophosphates pesticides
miRNA	microRNA
PBDE	Polybrominated diphenyl ethers
PCB	Polychlorinated biphenyl
PFAS	Perfluoroalkyl sulfonates
UVR	Ultraviolet radiation
VOCs	Volatile Organic Compounds

Content

Appendix

- **Annex 1: Protocol INMA Sabadell - Subcohort A**
  - Annex 1.1: Instructions for families
  - Annex 1.2: Consent forms
  - Annex 1.3: HELIX Questionnaire
  - Annex 1.4: Child Behaviour Checklist
  - Annex 1.5: Address history questionnaire
  - Annex 1.6: (Conners CRS-R short form)
  - Annex 1.7: SOP Anthropometry
  - Annex 1.8: SOP Bioimpedence
  - Annex 1.9: SOP for blood pressure measurement
  - Annex 1.10: SOP Fractional exhaled NO
  - Annex 1.11: SOP Spirometry
  - Annex 1.12: Clinical Examination Data Sheet

- **Annex 2: Protocol INMA Sabadell - Subcohort B**
  - Annex 2.1: Handout Monitoring Days
  - Annex 2.2: Consent forms
  - Annex 2.3: Incidence sheet

- **Annex 3: Protocol INMA Sabadell - Subcohort C**
  - Annex 3.1: Handout Monitoring Week 1st period
  - Annex 3.2: Handout Monitoring Week 2nd period
  - Annex 3.3: Consent forms
  - Annex 3.4: Personal UVR Dosimeter
  - Annex 3.5: Short questionnaire on recent exposures
  - Annex 3.6: Incidence sheet
  - Annex 3.7: Home/Street characterization form
  - Annex 3.8: Sample Data Sheet

- **Annex 4: Protocolo 9 años Estudio de Hermanos (MINECO)**
  - Anexo 4.1: Consentimientos informados
  - Anexo 4.2: Cuestionario general madres
  - Anexo 4.3: Cuestionario de capacidades y dificultades (SDQ)
  - Anexo 4.4: Toma de muestra de epitelio bucal

- **Annex 5: Founding projects**
  - Annex 5.1: HELIX
  - Annex 5.2: EXPOsOMICS
  - Annex 5.3: ANSES 2012
  - Annex 5.3: MINECO

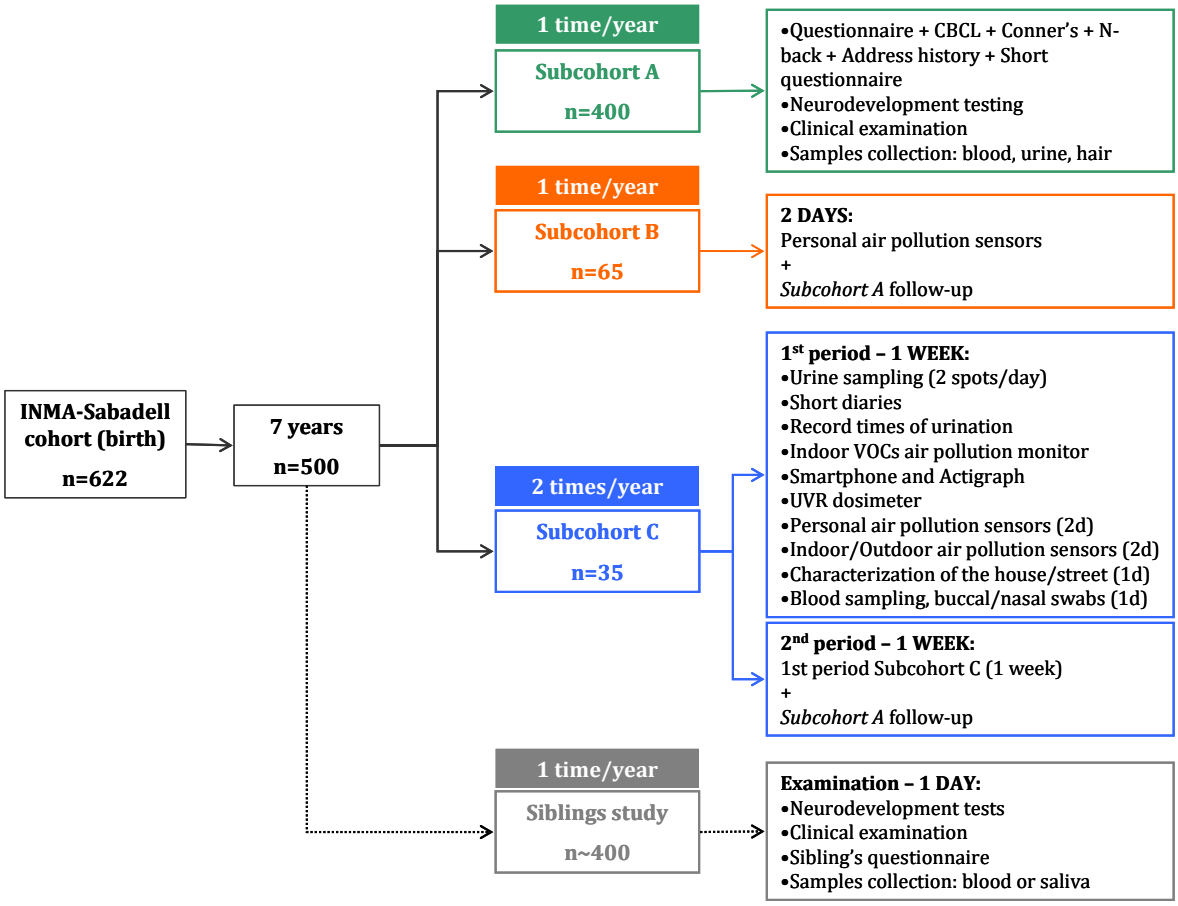
A. Introduction

The birth cohort INMA-Sabadell began in June 2004 and is planning a new follow-up in 2014, when children will be 9 years of age. This new follow-up will be conducted as part of five different projects. The main objectives of these projects are the following:

- HELIX (The Human Early-Life Exposome): to use new technologies to characterise early-life exposure to a wide range of environmental hazards, and integrate and link these with data on major child health outcomes including growth and obesity, and neurodevelopment, thus developing an "Early-Life Exposome" approach.
- EXPOSOMICS(Enhanced Exposure Assessment and omic Profiling for High Priority Environmental): to predict individual disease risk related to the environment, by characterizing the external and internal *exposome* for common exposures (air and drinking water contaminants) during critical periods of life, including *in utero*.
- ANSES (Impact de l'exposition aux particules ultrafines sur la fonction ventilatoire et les symptômes respiratoires chez l'enfant d'âge scolaire: étude dans les cohortes PARIS et INMA): to study the impact of ultrafine particles exposure on lung function, and allergic and respiratory symptoms in 8-9 years-old children included in two birth cohorts (PARIS in France and INMA in Spain.
- MINECO (Epigenetics in the early life exposure to air pollution and neurodevelopment and behaviour disorders within families in the INMA Sabadell birth cohort): to asses the impact of prenatal exposure to air pollutants on neurodevelopment/behavioural disorders and on epigenetic marks (DNA methylation changes) by using/comparing sibling pairs.
- GERoNiMO (Generalised EMF Research using Novel Methods – an integrated approach: from research to risk assessment and support to risk management): to evaluate possible health effects (cognitive and behavioural development, reproductive effects) of exposure to radiofrequency in children.

This new follow-up will integrate four different study-designs, drawing on different study populations for different levels of data collection:

Study design and study populations of the INMA-Sabadell 9-years follow-up



A.1) Objectives

The main objectives of this new follow-up are the following:

1. Collect extensive data on individual behaviours, diet, social characteristics, exposure sources and mobility throughout a questionnaire (*Subcohort A*);
2. Collect blood, urine and hair samples in suitable conditions to assess biomarkers of persistent and non-persistent organic pollutants, metals, cotinine, metabolic profiles, DNA methylation, free miRNAs, mRNAs, and proteins (*Subcohort A*);
3. Assess postnatal growth and obesity, neurodevelopment and asthma and respiratory function in the children using harmonized clinical assessment protocols, spirometry, cognitive computer testing and questionnaire information (*Subcohort A*);
4. Obtain personal measurements on air pollutants to study the impact on respiratory and allergic symptoms, to quantify the contribution of different areas of life (home, school, transport) to air pollutants total exposure, and to validate models (*Subcohort B and C*);
5. Collect very detailed information on short-term temporal variability in exposures and 'omics' biomarkers, on individual behaviours (such as diet and physical activity), and validation data (*Subcohort C*);

6. To assess the impact of prenatal exposure to air pollutants on neurodevelopment/behavioural disorders and on epigenetic marks (DNA methylation changes) by using/comparing sibling pairs (*Siblings study*).

B. Methods

B.1) Overview

All children participating in the previous INMA follow-ups will be invited to participate. Three different study designs will be conducted:

1) **Subcohort A (n=400):** this follow-up requires:

- Completion of a questionnaire by the mother;
- Completion of a short cognitive computer test (N-back) by the mother;
- Completion of three short questionnaires (Conner's, Child Behaviour Check List (CBCL), Address history) by the mother;
- Collection of child urine samples at home the night before and on the morning of the visit;
- Neurodevelopment testing by the child;
- Clinical examination of the child;
- Collection of biological samples (blood, hair) from child.

Participation in these studies will not represent any cost for families (transport expenses will be reimbursed). In addition they will receive a gift (of approximately 30€ value) in gratitude for providing blood and participating in the study.

2) **Subcohort B (n=65):** children will carry personal air pollution sensors during two days and the following day they will conduct the *Subcohort A* follow-up.

Participation in these studies will not represent any cost for families (transport expenses will be reimbursed). In addition they will receive a gift (of approximately 30€ value) in gratitude for providing blood and participating in the study.

3) **Subcohort C (n=35):** children will be followed intensively during 1 week (7 days) in 2 different periods in one year; at the end of the week of the second period they will conduct the *Subcohort A* follow-up. These 2 intensive weeks requires:

- All week:
  - Collect two urines every day: the first morning and the last of the day
  - Completion of short diaries by the mother with information on meal times, cosmetics, pesticides, and medication use
  - Record of times of urination by the child (if possible)
  - Carry a smartphone (with GPS, accelerometer, and noise application) and Actigraph
  - Carry a ultraviolet radiation (UVR) dosimeter
  - Have an indoor air pollutants sensor in the living room (Volatile Organic Compounds (VOCs))

- Two last days of the week:
  - Carry personal air pollution sensors
  - Have air pollution sensors to measure $PM_{2.5}$ concentrations placed indoors (in living room) and outdoors at each residence
  - Completion of a short questionnaire about air pollution exposures
  - Characterization of the house and street by the technician
- End of the week (at CAP Sant Fèlix):
  - Collect the last urine void of the day and the first morning one

  1st period:
  - Completion of a short questionnaire about recent exposures by the mother
  - Collection of blood from the child using a local anesthetic
  - Collection of buccal/nasal swabs from the child

  2nd period:
  - Conduct the Subcohort A follow-up at the end of the week (clinical examination + questionnaire + blood, hair, buccal and nasal swabs collection)

During all the study period a $PM_{2.5}$ air pollution sensor will be placed in a reference site in the city of Sabadell.

Participation in these studies will not represent any cost for families (transport cost will be reimbursed). Additionally, they will receive compensation (100€ cash for both weeks).

4) Siblings study (n~400): this follow-up requires:

- Completion of a questionnaire by the mother;
- Neurodevelopment testing by the child;
- Physical examination of the child;
- Collection of blood or buccal scrape samples (if blood is not possible).

B.2) Fieldwork organisation

1) Subcohort A (n=400)

Families will be telephoned and informed about the new INMA-follow-up. If they agree to participate, prior to the visit they will receive by post:

- consent forms (general and genetics);
- an information pack detailing what to expect from the visit and specific instructions;
- two urine collection containers in specially made cool box;
- Child Behaviour Check List (CBCL) to complete and bring to visit;
- Conner's questionnaire (for ADHD evaluation);
- Address history questionnaire to complete and bring to visit.

Families will be telephoned about a week before visit to go over instructions and to ask if they have signed consent forms. Families will be asked to collect a urine specimen from the child the night before the visit and the first morning void sample on the day of the visit. They will be asked to record what the children ate for breakfast and/or last

meal prior to visit. On arrival to the CAP Sant Fèlix they will be welcomed to the centre and their personal details confirmed and the self-complete questionnaires checked and collected. Nurses will check the consent forms and pick up the two urines and questionnaires completed. The children will be taken to a separate room to carry out the neurodevelopment tests on a computer, which will take up to an hour. Meanwhile the mothers will complete a computer-aided version of the questionnaire assisted by a nurse. The mothers will then also complete a much shorter version of the cognitive computer test given to the children. On completion of their respective tasks, the mothers and children will be reunited. Clinical examination of the child will then be conducted including anthropometry (weight, height, waist circumference, skinfolds), bioimpedance, spirometry and blood pressure measurement; this will take about 45 minutes. Finally a hair and blood (and urine if they did not bring samples with them*)* sample will be collected. The blood is collected at the end of the visit to ensure an approximate 2 hour fasting time since last meal.

Organization of the fieldwork is flexible; all visits can be done in only one visit at the CAP Sant Felix or in different visits. In that case, mother will be telephoned to ask if they would like to participate and if they accept they will be cited to the CAP Sant Felix (without the child). In the CAP, mothers will sign the consent forms and carry out a computer-aided version of the questionnaire. This will take approximately 1 hour and a half. After it, nurses will give them the three short questionnaires (CBCL, Conner's, and Adress history) to complete at home and the two urines containers. No more than one week after this fist visit, nurses will meet the child at school, together with more INMA children from the same school, and carry out the neurodevelopment tests on a computer and the clinical examination. This will take 1.5 hours, approximately. The afternoon of the school visit, mothers will come to the CAP with the child and nurses will take them blood and hair samples. Finally, mothers will complete a short questionnaire about recent exposures. This will take 30 minutes.

Detailed explanation can be found in: "Annex 1: Protocol INMA Sabadell - Subcohort A"

2) Subcohort B (n=65)

Two children will be followed per week (assuming that two personal air pollution sensors will be available), in total eight children per month. The technician will meet the mother and the child at home and explain them the protocol. Mothers will sign the consent forms and receive:

- personal air pollution sensors
- instructions
- urine kit: two urine containers and cool box
- CBCL, Conner's, and address history questionnaires

During two days, children will carry the personal air pollution sensors in a back bag. They will charge the battery of the sensors at midday and at night. As in *Subcohort A*, we will ask mothers to collect the last urine the night before and on the morning of the visit (day after the two days of the monitoring period). The day after the 2 monitoring days, they (mother and child) will go to the CAP Sant Fèlix and carried out the *Subcohort A* follow-up:

- Child:

  - Neurodevelopment test
  - Clinical examination
  - Blood and hair collection
- Mother:
  - Complete questionnaire

Detailed explanation can be found in: "Annex 2: Protocol INMA Sabadell - Subcohort B"

3) **Subcohort C (n=35)**

These children will be followed during 1 week (7 days) in 2 different seasons. At the end of the second season they will conduct the *Subcohort A* follow-up. Two children will be followed per week (assuming that four smartphones, four UVR dosimeters, and two personal air pollution sensors will be available), in total eight children per month. One or two days before starting the monitoring week, the technician will meet the mother and child at home and explain them the protocol carefully. Mother will sign the informed consent form and receive:

- an information handout detailing what to expect from the visit and specific instructions;
- smartphone with a belt that will collect data on mobility
- actigraph that will collect data on physical activity
- ultraviolet radiation (UVR) dosimeter
- short diaries (included in the handout)
- urine kit: 15 urine containers, freezer and fridge box, ice pack and cooler
- CBCL, Conner's, and address history questionnaires (only 2nd period)

The technician will install the indoor VOCs air pollution sensor in the living room. During all the week, children will carry the smartphone, the actigraph, and the UVR and charge them every midday and night. We will ask families to collect the first and the last urine voids of the day during the whole week. Urines will be placed in the freezer, but in case that there is not enough space in the freezer, the technician will pick up urines every two days. Mother will have to complete short diaries about the meal times, the times of urination, and the use of cosmetics, medication and pesticides. On day 5 of the monitoring week, the technician will come to their home and bring them the personal air pollution sensors and place a sensor inside and outside home. The technician will install an indoor and outdoor air pollution sensor and do a characterization of the house and street of subject. During two days, children will carry the personal air pollution sensors in a back bag and charge them at midday and at night. We will ask mothers to change the filter of the Cyclone Pump sensor on Day 7 to collect 24 hours air pollution measurements and to answer few questions on air pollution exposures during these 2 days. We will ask them to collect the last urine of day 7 and the first urine of day 8 (as in *Subcohort A and B*) but store them in the fridge.

On Day 8 of the first period, the mother and the child will go to the CAP and bring back all material (smartphone, actigraph, UVR, indoor and outdoor air pollution sensor, personal air pollution sensors, handout with diaries completed, all urines). Mothers will also complete a short questionnaire about recent exposures including physical activity and environmental tobacco smoke, and about what the child ate for breakfast and/or last meal prior to visit. Nurses will collect blood and buccal scrape from child.

On Day 8 of the second period, the mother and the child will go to the CAP and carry out the *Subcohort A* follow-up:

- Child:
  - Neurodevelopment test
  - Clinical examination
  - Blood, hair, and buccal/nasal swabs collection
- Mother:
  - Complete questionnaire

Detailed explanation can be found in: "Annex 3: Protocol INMA Sabadell - Subcohort C"

4) **Siblings study (n~400)**

Siblings' follow-up will follow the same scheme as *Subcohort A* follow-up. Mothers will receive the informed consent form at home and in the CAP they will complete the sibligns' questionnaire at the same time as the *Subcohort A* questionnaire. Meanwhile, the psychologist will conduct the neurodevelopment evaluation of the sibling. After it, nurses will take blood from the child. If this is not possible, they will collect a buccal scrape sample.

Detailed explanation can be found in: "Annex 4: Protocolo 9 años Estudio de Hermanos"

B.3) Questionnaires and diaries

Study	Questionnaire	Completion place	Annex
Subcohort A	Child Behaviour Checklist	Home	1.4
Subcohort B	Conner's	Home	1.6
	Address history questionnaire	Home	1.5
	HELIX Questionnaire	CAP	1.3
Subcohort C	Diaries (meal times, cosmetics, medication, pesticides use, and air pollution exposures for Day 6 and 7)	Home	3.1 & 3.2
	Records time of urination	Home	3.1 & 3.2
	Short questionnaire on recent exposures	CAP	3.5
	HELIX Questionnaire	CAP	1.3
Siblings study	Sibling's questionnaire	CAP	4.2

B.4) Neurodevelopment testing

Subcohort A + **Subcohort B** + **Subcohort C** → see all Annex 1

Siblings study → Annex 4.3

B.5) Clinical examination

Subcohort A + Subcohort B + Subcohort C:

- Anthropometry: Annexes 1.7
- Blood pressure measurement: Annex 1.9
- Spirometry: Annex 1.11
- Clinical Examination Data Sheet: Annex 1.12

Siblings study:

- Anthropometry: Annexes 1.7
- Spirometry: Annex 1.11
- Clinical Examination Data Sheet: Annex 1.12

B.6) Smartphones and sensors

All instructions, including instructions for the smartphones, Actigraph, ultraviolet radiation sampler, indoor VOCs air pollution sensor, indoor and outdoor air pollution sensors (2 days), personal air pollution sensors (Cyclone Pump, DiSCmini, and microAeth), and urine collection and storage are included in Annexes 2.1 and 3.1/3.2.

B.7) Biological samples collection

Subcohort A (n=400) + Subcohort B (n=65)

Type of sample	N	Tube	Sample processing	Sample quantity Required, mL or mg	Purpose
Urine	465 (2x100 mL)	High quality polypropylene 100 mL collection containers (collected at two time points)	Urine	1,75 0.35 0.5 0.5 1.0 0.2 0.5	Metabonomics Phthalates Phenols OP Pesticides Metals Cotinine Creatinine, specific gravity
Blood	465 (18 mL)	4 mL silica vacutainer	Serum	0,6 0,2	Metabonomics HDL, Cholesterol, trigycerides, glucose
		5mL silica glass vacutainer	Serum	2.0	PCBs, DDE, HCB, PBDE
		6 mL EDTA (trace metal tube)	Whole blood Blood smear DNA Plasma	0.9 0.1 2.5 0.5 1.0 0.2	Heavy metals Cell count Methylomics Proteomics miRNA PFASs
		3 mL Tempus	RNA	3	Transcriptomics

Hair		Zip- lock bag	hair	50-100 mg 20 mg	Hg Nicotine / Cotinine

Subcohort C (n=35)

1st period

Type of sample	N	Tube	Sample processing	Sample quantity Required, mL	Purpose
Urine	35 (15 x 100 mL)*	High quality polypropylene 100 mL collection containers (collected at two time points)	Urine	1,75	Metabonomics
				0.35	Phthalates
				0.5	Phenols
				0.5	OP Pesticides
				0.5	Creatinine, specific gravity
Blood	35 (13 mL)	6mL silica vacutainer	Serum	0,6	Metabonomics
				0,2	HDL, Cholesterol, trigycerides, glucose
				0.5	Adductomics
				0.125	Cytokines screen
		4 mL EDTA	Blood smear	0.1	Cell count
			Plasma	0.5	Proteomics
				1.5	miRNA
			DNA	2.0	Methylomics
		3 mL Tempus	RNA	3	Transcriptomics
Buccal and nasal swabs	35	---			RNA-expression DNA-methylation

*Number of urine samples analyzed will be discussed
This scheme is integrating HELIX and EXPOSOMICS blood collection schemes.

2nd period

Type of sample	N	Tube	Sample processing	Sample quantity Required, mL or mg	Purpose
Urine	35 (5 x 100 mL)*	High quality polypropylene 100 mL collection containers (collected at two time	Urine	1,75	Metabonomics
				0.35	Phthalates
				0.5	Phenols
				0.5	OP Pesticides

		points)		1.0	Metals
				0.2	Cotinine
				0.5	Creatinine, specific gravity
Blood	35 (20 mL)	5mL silica glass vacutainer (no additive)	Serum	2.0	PCBs, DDE, HCB, PBDE
		6mL silica vacutainer	Serum	0.6	Metabonomics
				0.2	HDL, Cholesterol, trigycerides, glucose
				0.5	Adductomics
				0.125	Cytokines screen
		6 mL EDTA (trace metal tube)	Whole blood	0.9	Heavy metals
			Blood smear	0.1	Cell count
			DNA	2.5	Methylomics
			Plasma	0.5	Proteomics
				1.0	miRNA
				0.2	PFASs
				1.0	miRNA
		3 mL Tempus	RNA	3	Transcriptomics
Hair	35	Zip- lock bag	hair	50-100 mg	Hg
				20 mg	Nicotine / Cotinine
Buccal and nasal swabs	35	---	---	---	RNA-expression DNA-methylation

*Number of urine samples analyzed will be discussed
**In the second period, and as part of the subcohort follow-up, more blood will be collected to measure persistent organic pollutants, metals, lipids, etc.
This scheme is integrating HELIX and EXPOSOMICS blood collection schemes.

Germans

Type of sample	N	Tube	Sample processing	Sample quantity required, mL or mg	Purpose
Blood	400 (8 mL)	4 mL silica	Serum	3x0.5 mL	Backup
		4 mL EDTA	Whole blood	2 mL	DNA extraction
			Plasma	3x0.5 mL	Backup

C. Personnel tasks

Nurses:

- phone contact and recruitment
- family coordination

- neurodevelopment tests (*Subcohort A, B, and C*)
- mother's questionnaire
- clinical examination
- blood and hair collection

Nurse assistant: samples processing

Technician:

- *Subcohort B* and *C* monitoring periods coordinator
- devices' quality control and calibration
- setting and data uploading and cleaning
- home visits
- pick up urines at home (if necessary)

Psychologist: neurodevelopment tests (*Siblings Study*)

D. Ethical issues

Ethical issues and health risks arising within the ***Subcohort A*** study concern:

a) The collection of information about diet and other habits;

b) The collection of 2 urine samples, 1 hair sample and 1 blood sample (18 mL). Hair and urine samples are considered non-invasive and do not present any particular risk. Blood collection is kept to an absolute minimum.

c) Collection of genetic information (including genomics (sequencing or genotyping), transcriptomics (RNA sequencing) and epigenomics (DNA methylation and miRNA sequencing). This may produce incidental health findings with health consequences (such as identification of highly penetrant alleles conferring disease).

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

Ethical issues and health risks arising within the ***Subcohort B*** study concern:

a) to carry a personal air pollution sensor kit for two days in two periods to measure outdoor air pollutants;

b) The collection of information about dietary and other habits;

c) The collection of 2 urine samples, 1 hair sample and 1 blood sample (18 mL). Hair and urine samples are considered non-invasive and do not present any particular risk. Blood collection is kept to an absolute minimum.

d) Collection of genetic information (including genomics (sequencing or genotyping), transcriptomics (RNA sequencing) and epigenomics (DNA methylation and miRNA sequencing). This may produce incidental health findings with health consequences (such as identification of highly penetrant alleles conferring disease).

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

Ethical issues and health risks arising within the ***Subcohort C*** concern:

a) the collection of information about meal times, cosmetics, medication, and pesticides use;

b) the collection of 15 urine samples, two blood samples, and two buccal swabs samples (separated in 2 periods/seasons). Urine, buccal and nasal swabs samples are considered non-invasive and do not present any particular risk. Blood collection is kept to an absolute minimum. From our experience, ethics committees would not allow frequent blood sampling in children so we are limiting this to one collection of 13 mL and another one of 20 mL;

c) to carry a smartphone with applications to record physical activity, mobility, and noise data and a Actigraph for one week in two periods. Although no health risks has been revealed linked to smartphones, we recommend that children carry them in the small pouch provided;

d) to carry a UVR sampler for one week in two periods;

e) to carry a personal air pollution sensor kit for two days in two periods to measure outdoor air pollutants;

f) the installation inside home of a passive sampler to measure BTEX concentrations (indoor air pollutants), in two different seasons. The installation does not present any particular risk but may lead to a slight noise in the living room during the day;

g) the installation outside home of a passive sampler to measure $PM_{2.5}$ concentrations (outdoor air pollutants) during two days in two different seasons. The installation does not present any particular risk but may lead to a slight noise during the day;

h) the storage and transfer of data to central data warehouse.

Ethical issues and health risks arising within the ***Siblings Study*** concern:

a) The collection of information about diet and other habits;

b) The collection of 1 blood sample (8 mL). Blood collection is kept to an absolute minimum. In case that blood cannot be collected, a saliva sample (non-invasive) will be taken.

c) Collection of genetic information. This may produce incidental health findings with health consequences (such as identification of highly penetrant alleles conferring disease).

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

Concerning *Subcohorts B and C*, in case of withdrawal of a volunteer during the recruitment period, another mother/child pairs will be recruited. In case of withdrawal after the first monitoring period, the informed consent and all the information collected (questionnaires, biological samples,) will be destroyed if the family requires.

All INMA activities will be carried out according to existing guidance in ethics as indicated in the Universal Declaration on Bioethics and Human Rights adopted by UNESCO (19/10/2005); the Council of Europe Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine (1997) and its additional protocol on biomedical research (2005); the Helsinki Declaration (2008) and relevant EU laws (European Parliament and Council Directive 2001/20/EC).

We are aware of further relevant guidance and codes, including:

- Recommendation Rec 4 (2006) of the Committee of Ministers to member states on research on biological materials of human origin (Council of Europe) as the main international guidelines for medical research;
- The Nuremberg Code (1947) addressing volunteer consent and proper acting;
- The charter of Fundamental rights of the EU Directive 95/46/EC of the European Parliament and of the Council of 24 October 1995 on the protection of individuals with regard to processing of personal data and on the free movement of such data;
- Convention of the Council of Europe on Human Rights and Biomedicine signed in Oviedo on 4 April 1997, and the Additional Protocol on the Prohibition of Cloning Human Beings signed in Paris 12 January 1998;
- UN Convention on the Rights of the Child;
- Universal Declaration on the human genome and human rights adapted by UNESCO (1997);
- Opinions of the European Group of Advisers on the Ethical Implications of Biotechnology (1991-1997) and the European Group on Ethics in Science and New Technologies (as from 1998).

We have read the guidance available on the web: http://cordis.europa.eu/fp7/ethics_en.html.

D1) Informed consent

Informed consent will be sought before the start of the follow-up visit. The consent form is given and will be kept in the study subject's personal file. A copy of the signed informed consent form will be provided to every subject/subject's legally authorized representative. Alternatively, the samples can be destroyed at the study volunteer´s request if they do not wish their samples to be used in further research after the study; the samples would then be destroyed in the appropriate manner after a specified time. The study subject may always change his/her opinion in the course of the study for any reason at any time without penalty or loss of benefits to which they are otherwise entitled. Details on the possibility of study participants to request removal of their coded data from the study will be included in the informed consent information form. Arrangements for the procedures in the case of incidental findings will be decided by individual cohorts.

Insurance/indemnity arrangements will be in place prior to the commencement of the study in order to provide adequate protection of the subjects. This information will be provided to the local ethical committee with the protocol as well as indicated in the information to participants.

D2) Storage of the data

In order to safeguard the privacy of study subjects:

- 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;
- Where personal information is used, including questionnaires and biological material it will be safety stored in secure facilities, and names will be replaced by unique study numbers, and stored separately. Primary databases and analysis files will be stored on computers with personal identifiers removed;
- Geocodes will only be linked to unique study numbers and no other information including health data. The geocodes will be used for the sole purpose of obtaining individual exposure estimates. Only these exposure estimates, and not the geocodes themselves, will be linked to any other information.
- Subjects will be identified by a unique study number assigned by staff of the partner organizations. This unique identifier will link all basic data required for the study. The master key file linking the centre's study numbers with personal identifiers will be maintained in each centre;
- All files containing personally identifiable information, including the master key file, will be stored in password protected computer files. Access to these files will be limited to authorized project personnel;
- Hard copy records or computer generated records containing personally identifiable information will be stored in locked cabinets in an office with limited access;
- Personal information is never transferred between countries;
- All project personnel will be trained in the importance of confidentiality of individual records and required to sign a confidentiality agreement;
- During the project life-span information will be accessed and handled by members of the research teams only, unless specific permission has been given by the INMA executive committee.

E. Time line

The overall fieldwork, considering the four study designs, will take 24 months. We are planning to start in November 2013 and finish in December 2015.

Subcohort A

6 children/week (approximately)

November 2013 – December 2015

Subcohort B

2 children/week

November 2013 – September 2014

Subcohort C

2 children/week

1st period: November 2013 – March 2014

2nd period: April 2014 – September 2014

Siblings Study

*number of children examined per week will depend on *Subcohort A* visits

November 2013 – December 2015