OFFICIAL TITLE OF THE STUDY: Understanding and preventing the impact of endocrine disruptors on the hypothalamus-pituitary axis in sensitive populations

NCT NUMBER: Not available

DATE OF THE DOCUMENT: 21ST AUGUST 2025

Hypiend - Clinical study to evaluate the effectiveness of a multicOmpoNent behavioural intervenTion to reduce endocrINe disrUptor exposure during the pErinatal period in women and their offspring (CONTINUE).

Protocol- Hypiend Perinatal Study

ÍNDEX

1. BACKGROUND AND JUSTIFICATION

1.1 Title, acronym, unique identifier of the clinical study

Clinical study to evaluate the effectiveness of a multic**O**mpo**N**ent behavioural interven**T**ion to reduce endocr**IN**e disr**U**ptor exposure during the p**E**rinatal period in women and their offspring (CONTINUE).

1.2 Study rationale

1.2.1 Extent and evaluation of current knowledge directly linked to the scientific question(s) to be answered by the clinical study.

Human epidemiological data suggest that EDC exposure during the perinatal period can negatively affect infant growth trajectory and neurodevelopment and possibly play an important role in the rapid epidemiological growth of obesity and diabetes.[1,2,3] As an example, one study carried out in 460 mother–infant pairs from Korea showed that Mental and/or Psychomotor Developmental Indices of the Bayley Scales of Infant Development were inversely correlated in 6-month-old males with the maternal urinary levels of the phthalate metabolites MEHHP, MEOHP and MBP at the third trimester of pregnancy.[4] Very recently, it was reported that mothers with overweight displayed increased levels of the highly persistent environmental chemicals polychlorinated biphenyls in breast milk 2-weeks postpartum and that there was a negative association between some of these compounds and the head circumference-for-age, weight-for-age, and weight-for-length z-scores of the infant at the age of 6 months.[5]

However, there are discrepancies among findings, which can be attributed to differences in the temporal window of EDC exposure assessment (i.e., early versus late gestation or lactation), in the infants' age at which the measurements were carried out or to a misclassification of EDC exposure due to a single-point analyses of their exposure. In fact, one of the limitations of many epidemiological studies is the use of single spot urine samples at one time-point to estimate the exposure to EDCs. The limitation relies on the short biological half-lives of many of these chemicals and their quick excretion in urine, properties that cause a demand for multiple time point for a precise estimation of exposure assessment.[1,2,3] Although different epidemiological studies have found an association between higher exposure to EDCs and neuroendocrine and neurodevelopmental alterations in toddlers and children[1,6], as far as we know, no clinical interventions have been carried out to elucidate whether reducing the exposure to EDCs can contribute to counteract/ameliorate these harmful effects.

So far, very few human intervention studies focused on reducing the exposure to EDCs during the perinatal period have been conducted, some carrying out educational approaches and others focused on dietary or other changes.[7,8] Relevantly, most of them had small sample sizes (<100 people) and analysed a maximum of two different types of EDCs and quite a few were carried out without control groups. Moreover, the level of adherence in this type of interventions is low in general, reflecting the need to implement adequately behavioural change techniques to foster long-term lifestyle habit improvements.

1.2.2 Outcomes (efficacy, safety) Of completed and number of ongoing clinical studies utilizing the same intervention in the same indication (including review of public registers)

Recently, the PREVED project was carried out to improve knowledge, to enhance risk perception and to change exposure behaviour regarding EDCs of 268 women during pregnancy and up to 14 months after birth[10]. The authors reported a significant increase in the evolution of risk perception score and overall psychosocial score in the two intervention groups that received 3 workshops during pregnancy, one group in neutral location (leaflet on EDCs and collective workshops in a meeting room), and the other group in contextualized location (leaflet on EDCs and collective workshops in the real-life pedagogical apartment) when compared with a control group (leaflet on EDCs). However, no differences in consumption of canned food and in percentage of women having a decrease in bisphenol A or parabens concentrations in urine were found between the control and the two intervention groups. In another RCT carried out in 51 women in Korea, a 4-week web-based behavioural intervention reduced the urinary concentrations of BPA, different phthalate metabolites, and parabens in mothers with young children[18]. Moreover, an ongoing study aims to determine if a personalized mobile intervention is able to reduce the exposure to EDCs in adults of child-bearing age (ClinicalTrials ID: NCT05780047). Despite some promising results, these examples illustrate the need to carry out larger multicomponent clinical and community-based intervention studies focused on minimize the exposure to EDCs at short, medium, and long time to obtain results that are more conclusive.

1.2.3 Level of evidence related to the mechanism of action of the intervention in the planned clinical study population

EDC-Mix-Risk and ENDpoiNTs are two outstanding Horizon 2020 projects that combined the experimental research in both cells and animals with epidemiological data. Researchers of both projects and others nicely showed using a statistical model for multivariate regression with data obtained in the SELMA pregnancy study that different EDCs (including phthalates, alkyl phenols, and perfluoroalkyl substances) analysed in the urine and serum of women at week 10 of pregnancy were associated with language delay of their children at 2.5 years of age.[19] Afterwards, the identified EDCs were mixed and tested in human brain organoids as well as in *Xenopus leavis* and *Danio rerio* to elucidate the molecular and functional impact of exposure. Finally, the authors integrated both experimental and epidemiological data and carried out a risk assessment approach, finding increased odds of language delay in up to 54% of the offspring who had prenatal exposures above experimentally derived levels of concern.[19] Despite these very relevant findings, a limited number of studies carried out in humans have shed light on the mechanisms underlying the EDC-mediated dysfunctions on HP axis, including the induction of epigenetic changes in key genes involved in foetal development, neuroendocrine regulation and early puberty as well as the alterations of the inflammatory response and the gut microbiota (dysbiosis). EDCs may increase the risk of childhood neurodevelopmental disorders by interfering with early life estrogenic and thyroid hormone signalling or metabolism.[1,6,20] In this sense, in humans, it has been shown that some phthalates may reduce thyroxine and triiodothyronine concentration in pregnant women and children and that the exposure to phthalates produced oxidative stress and can also affect the health of the offspring through epigenetic re-programming of the foetus and placenta. In another study, maternal exposure to heavy metals affected progeny neurodevelopment, and changes in DNA methylation of genes controlling neurodevelopment were observed in cord blood, but not the blood collected at mid-childhood[21]. Analysis of exposure-outcome identified differentially methylated CpG on DAB Adaptor Protein 1 (DAB1) gene as a marker of the effect of prenatal polycyclic aromatic hydrocarbon

(PAH) exposure on children's mental development.[22] Increased exposure to PAHs during pregnancy was positively correlated with the methylation of insulin-like growth factor IGF1 and 2, key factors in human growth and development that are maternally imprinted[23]. Incorrect methylation of those genes during early development relates to reduced birth weight and increased predisposition to metabolic problems, like obesity, cardiovascular diseases or diabetes.[24] However, despite this evidence, as far as we know, no human intervention studies aimed at reducing the exposure to EDCs have analysed the molecular impact that can accompany these changes in EDC exposure. Further research is needed to shed more light on these issues, including molecular and omics-based analyses in humans to elucidate whether human intervention studies aimed at reducing EDC exposure can favourably modulate these molecular changes.

Different molecular and omics approaches will be carried out to shed more light in the framework of the HP axis on the epigenetic drivers most susceptible to disruption, the genes and the pathways most differently affected, the interplay between intestinal microbiota and the impact of EDC exposure on systemic inflammation. These analyses will be carried out in samples obtained in the study considering our results obtained in preclinical models exposed to EDC mixtures trying to resemble the real human exposure, to increase the chance to obtain robust and reliable results using a targeted approach. Specifically, in women the analyses will be carried out during pregnancy and after delivery at different time points and will be focused on the analyses of HP axis-related hormones and cytokines, intestinal microbiota, methylation of different CpG sites of key genes involved in HP axis as well as the expression of these genes. In their offspring, in addition to these analyses, the circulating levels of kisspeptin will be also analysed at birth (cord blood) and at the age of 18 months (capillary blood/dry blood spots –DBS-). Overall, these analyses will allow us to go into depth on the mechanisms by which EDCs exert their harmful effects as well as how intervention studies aimed at reducing the exposure to these chemicals can modulate these biological pathways and the molecular biomarkers of exposure identified. As far as we know, this approach has not yet been carried out in large human intervention studies in the framework of EDCs.

2. OBJECTIVES

2.1 Main Objective:

The general objective of HYPIEND is to understand the effects of EDC co-exposure on the function and epigenetic programming of the HP axis to delineate intervention strategies for minimizing exposure and neuroendocrine consequences during the perinatal and pre-pubertal stages.

The primary objective of this clinical study is to demonstrate that a multicomponent intervention implemented in health care centres from three European countries is effective in reducing the levels of EDCs in different body fluids of pregnant women, breastfeeding and formula feeding women as well as in their infants up to 18 months of age, improving at the same time the level of HAPA constructs (psychological determinants of behaviour) and the knowledge about these chemicals at family level.

2.2 Secondary Objectives:

a. To demonstrate that this multicomponent intervention designed to reduce the exposure to EDCs is effective for preventing some of the harmful effects related to HP disruption in infants up to the age of 18 months (weight, growth, and neurodevelopmental related alterations).
b. To provide a better understanding on the relationship between the concentrations of EDCs in different fluids and the levels of HP-related hormones, cytokines, DNA methylation and gene expression patterns in blood and gut microbiota composition, shedding more light on the mechanisms by which EDCs can exert their harmful effects.

c. To explore through multivariate analyses and health risk assessment the predictors of concentrations of EDCs in the body fluids (urine, blood, and milk).

3 RESEARCH METHODS

3.1 Type of Study

Randomized Controlled Trial (RCT):

1. Group intervention, consisting of a multicomponent intervention (digital tool aimed at promoting lifestyle habits to reduce EDC exposure by providing personalized recommendations + telephone monitoring + workshops about environmental health education) during pregnancy and the first 18 months after delivery (Figure 1).
2. Group control (standard of care). The control condition consists of a single online education module/written information about EDCs addressed to reduce their exposure. They will also have access to the digital tool to answer the questionnaires, but this group will not receive any recommendations or missions from the professionals concerning EDCs along the study (Figure 1). After finishing the intervention, the participants of the control group will be offered access to all the developed material (workshops…) and to use the app. Participants from different environmental areas will be recruited to account for different exposures to EDCs.

3.2 Design

3.2.1 Theoretical framework behind the intervention

HYPIEND aims at evaluating whether a multi-centric, multicomponent interventional trial (digital tool aimed at promoting lifestyle habits to reduce EDC exposure by providing personalized recommendations + phone calls + workshops about environmental health education) carried out in 810 women from pregnancy until their children are 18 months old is able to reduce the exposure to different EDCs.

This intervention will be designed considering the gradual introduction of small changes (i.e., by using goal setting), carrying out a family-based approach, in a personalized manner, considering targeting different routes of exposure and providing to the participants frequent feedback and support. With this design, we expect to promote a behavioural change to achieve long-lasting effects in reducing the exposure to EDCs. The effects will be compared with a control intervention group, which will only receive a digital printable booklet with information on EDC hazards and on strategies to limit their exposure. Partners will be encouraged to join women during the medical visits and the environmental health education workshop.

The stages of change[9] will be followed as a general framework to organize the order of information transmitted to participants to increase adherence to the intervention. We expect that participants will initially be uninformed of the problem of EDCs, and thus unlikely to actively look for information on this topic. Therefore, at recruitment, information about the effect of EDCs on their babies and their own health will be provided to motivate them to engage with the issue (moving from the pre-consideration to the consideration stage). Next, a workshop will aim to build a solid motivation to change their exposure to EDCs (moving from consideration to the decision stage). Finally, when the motivation to change is strong enough, the digital tool will be offered as it helps bridge the intention-behaviour gap with progressive tasks to be implemented in daily life. Attention will also be focused on building habits for participants to move from action to maintenance stage, as the study runs for 24 months.

The existing behaviour change interventions aiming at reducing exposure to EDCs among pregnant women have mainly focused on risk awareness among the population using the Health Belief Model[10]. However, the socio-cognitive literature has criticized the Health Belief Model for its weak predictive validity[11]. Globally, motivational models (including the Health Belief Model) are criticized because they assume that everybody holding an intention will translate it into behaviour.[12] Indeed, the literature showed that around 50% of intentions are not translated into action.[13]

The Health Action Process Approach (HAPA) suggests that the adoption, initiation, and maintenance of health behaviours must be explicitly conceived as a process that consists of at least a motivation phase and a volition phase. The latter might be further subdivided into a planning phase, action phase, and maintenance phase. It is claimed that perceived self-efficacy plays a crucial role at all stages along with other cognitions. Relying on HAPA, techniques will be selected to create motivation among pre-intenders and then help them translate their intention to change into action. Furthermore, the intervention will use the richness of behaviour change technics (BCT taxonomy, Intervention Mapping Taxonomy, Theory and Technique Tool) to maximize the probability of effective behaviour change.

The intervention is conceived to target the psychological determinants of behaviour according to the HAPA model. As presented in the Figure 1, among preintenders (i.e., people that do not have the intention to change yet), motivation must be built through three constructs: risk perception, outcome expectancies, and task self-efficacy. In the context of EDCs, which translates respectively to being aware of the threat posed by the substances, believing that changing behaviour will lead to preventing the negative consequences, and perceiving being able to reduce exposure to EDCs. Increasing those three beliefs will lead to an intention to change, i.e., taking the decision to reduce one's family exposure to EDCs. For this intention to be translated into action, planification of how the actual behaviour will be performed in the daily life, and anticipating barriers and how to surmount them is necessary, as well as self-efficacy regarding maintaining the change in the long run, and recovery in case of failure to maintain the change in lifestyle.

Intervention components are highlighted in colours on the Figure 1. Green represents the aspects that the workshops will cover, and orange represents the aspects the app will target.

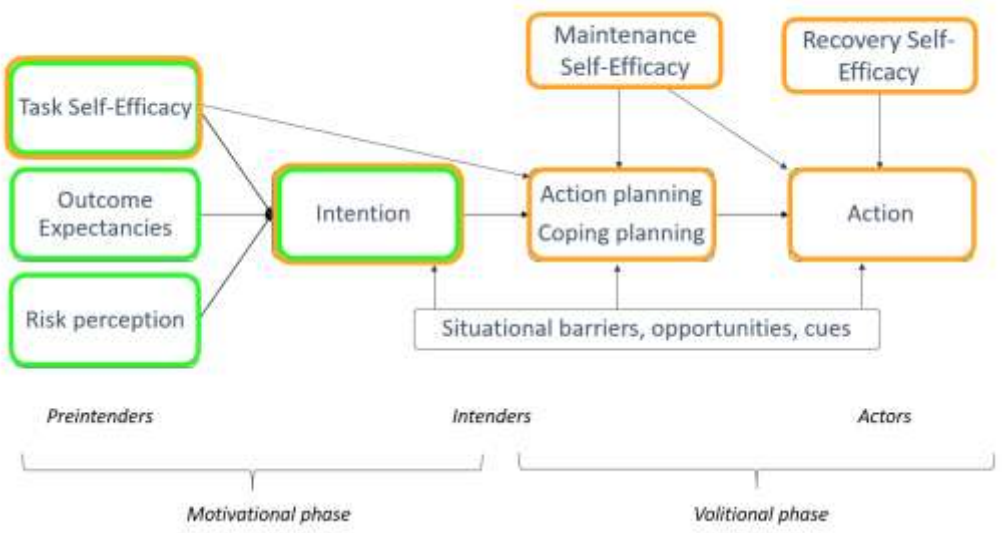


Figure 1. HAPA model (adapted from Schwarzer, 2014) and the intervention components

Furthermore, the study will be carried out in three different countries, including countries with a reported lower (Belgium and Spain) and higher (Poland) exposure to EDCs In HYPIEND we will evaluate the concentration of the exposure biomarkers of different families of EDCs in maternal urine at the first trimester of pregnancy (baseline), at delivery and 18 months after delivery, and in their offspring, at 4-7 weeks after birth and at the age of 18 months. EDC will be also quantified in different fluids in which these pollutants that are more persistent can be found: 1) cord blood, which is a highly valuable fluid because the levels of EDC are a proxy for in utero exposure and can allow us to evaluate the exposure to EDC in mother–infant pairs[14,15]; 2) breast milk at 4-7 weeks post-partum, as a source of non persistent[16,17] and persistent EDC. 3) maternal peripheral blood at the first trimester of pregnancy, at delivery and 18 months after delivery.

So far, this multi-fluid approach at different time points in large mother-child intervention studies at the family level have not been carried out and could strongly contribute to properly understand the real exposure to EDC and their impact on child health and development. The relationship of prenatal and postnatal exposure to these pollutants in relation to hypothalamic-pituitary (HP) disruption and body weight, growth, behaviour, and neurodevelopment-related alteration of infants at different time points until the age of 18 months will be also evaluated. This could also allow the consortium to establish a cause-effect relationship between reduction in the exposure to EDCs and the prevention of harmful health effects related to HP disruption. Human health risk assessment in relation to exposure to EDCs will be also evaluated in women and their offspring of both groups including all the data obtained in the different fluids (urine, peripheral blood, cord blood, and breast milk) at different time points.

3.2.2 Characteristics of the study population (size, age group, sex distribution, inclusion and exclusion criteria)

- Site: This is going to be a multicentric study, including three countries with different reported levels of exposure to EDCs
    1. Low exposure level: Spain and Belgium
    2. High exposure level: Poland

- Centres for recruitment:
    1. In Spain:
        - The Germans Trias University Hospital
        - Hospital Universitario San Cecilio (HUSC) de Granada.
    2. In Belgium: University Hospital Leuven (KUL), Belgium.
    3. In Poland: Centre of Postgraduate Medical Education (Centrum Medyczne Kształcenia Podyplomowego, CMKP)

3.2.3 Sample size and power calculation

Total N: 810 pregnant women (270 pregnant women per country: Spain, Belgium and Poland).

Clinical analyses:

- Main outcome: urinary concentration of mono(2-ethyl-5-oxohexyl) phthalate (MEOHP): 624 pregnant women (312 from control group/ 312 from intervention group) at the corresponding study points. 104 control participants/ 104 intervention participants per country. This sample size is based

on the sample size calculated to obtain statistically significant differences among groups (n=624 women excluding the estimated 30% of dropouts; for the sample size calculation, we included an extra number of participants considering a 30% of drop-out during the study, which yields a total of 810 women to be recruited).

- Other EDCs in urine, peripheral blood, cord blood and milk. 522 pregnant women at the corresponding study points (261 from control group/ 261 from intervention group) at the corresponding study points. 87 control participants/ 87 intervention participants per country.

- Biochemical, molecular and omics analyses: 200 pregnant women (100 from control group/ 100 from intervention group) at the corresponding study points. 33 control participants/ 33 intervention participants per country.

Very few RCTs have been conducted to evaluate the effects of interventions focused at reducing exposure to EDCs in the perinatal period (during pregnancy and in the early postnatal period). In one study[22], 62 mothers with young children were enrolled in a web-based behavioural intervention, and significant changes were observed after 1 month in almost all the compounds analysed. Ouazzani et al.[10] evaluated the effects of an educational intervention deployed during pregnancy in 268 women but failed to detect significant results on the concentration of urinary EDCs. With this data, we estimate a small effect size of 0.25 in the reduction of the urinary concentration of mono(2-ethyl-5-oxohexyl) phthalate (MEOHP) (main outcome) between the control and the intervention groups in our study. We would expect to detect a 25% decrease in the urinary levels of MEOHP in the intervention group vs control group at the end of the study.
Thus, considering 80% power and a two-tailed α of 0.05, we calculate that 624 pregnant women will be needed (312 per group) to detect significant differences in the main outcome (urinary concentration of MEOHP). Thus, to allow for a 30% drop out rate (estimated to be high due to the nature of the study: we expect a high drop-out in the postpartum), 405 subjects per group will be needed to be recruited (total N, 810). Therefore, 270 women will be recruited in each of the three countries, including women from different environmental areas

The sample size was calculated choosing as a main outcome the urinary concentrations of MEOHP, which is an oxidative metabolite of MEHP and, therefore, it is a secondary metabolite of Di(2-ethylhexyl) phthalate (DEHP). This election was based on a scientific article in which was reported that mental and/or Psychomotor Developmental Indices of the Bayley Scales of Infant Development were inversely correlated in 6-month-old infants with the maternal urinary levels of MEHHP and MEOHP at the third trimester of pregnancy in a study carried out with 460 mother–infant pairs.[4] However, since during the initial phase of the project a predictive modelling approach will be carried out in order to select the EDC mixtures with high harmful effects to be evaluated in preclinical models, the definitive main outcome could be redefined once we obtain the predictive modelling results.

We would expect to detect a 25% decrease in the urinary levels of MEOHP in the intervention group vs control group at the end of the study.

3.2.4 Inclusion/exclusion criteria

Pregnant women attending the first visit of prenatal surveillance will be invited to participate according to inclusion/exclusion criteria

Inclusion criteria (*Participants should fulfil ALL these criteria)*

- Pregnant women with a viable pregnancy confirmed by ultrasound until 13 weeks and 0 days of gestation (recruitment between 6-11 weeks). Their partners will be invited to participate but this is not mandatory for inclusion.
- BMI at Visit 1 between 18.5-40 kg/m²
- Intention to breastfeed
- Being able to read the language of their respective countries (Dutch/French –Belgium-, Spanish and/or Catalan –Spain- and Polish –Poland)
- Being 18 years or older
- In possession of a smartphone. If participants do not have a smartphone because of socio-economic reasons, a smartphone will be at their disposal for the whole duration of the study

Exclusion criteria *(If a participant fulfils ANY of the following)*

- Unable to sign informed consent (cultural barriers, psychological conditions)
- Abuse of substances (alcohol, drugs)
- Chronic use (at least, for three months before pregnancy) of any medication that might affect the HP axis:
  - Antidepressants
  - Insuline.
  - Levothyroxine, Methimazole, Propylthiouracil.
  - Oral corticosteroids (topical and inhalation formulations will be allowed)
  - Arginine vasopressin (AVP)
  - Mifepristone.
  - Anticortisolic drugs: Metyrapone (Metopirone), Ketokonazole, Osilodrostat (Isturisa), Mitotane (Lysodren), aminoglutethimide (Cytadren) and Levoketoconazole (Recorlev).
- Multiple gestation
- Type 1 or 2 diabetes
- Pregnant women will not be consented into research by any HCP with whom they have a dependent relationship (Declaration of Helsinki) (I would remove this point).

3.2.5 Recruitment and Informed Consent

Women will be provided with oral as well as written information regarding the purpose of this study, the study design (including the randomization procedure and allocation to control or intervention group), the variables that will be studied and the distribution of visits throughout the follow-up period.

The participation of newborns/infants in this study has been included in the informed consent form, in which each mother, as the legal representative of the minor, exercises her right to provide informed consent by representation.

All the participants will receive an informative leaflet (Annex 1) with a general scheme and FAQs about participation in this trial. Additionally, they will be given an Informed Consent Format (Annex 2) that should be carefully read and fully understood.

Women will be encouraged to make as many questions as they need before signing this document. The signature of the Informed Consent is mandatory to enroll any participant and proceed with randomization.

3.2.6 Randomization

Once confirmed that the pregnant woman fulfils criteria to participate in the study and agrees to do so by signing the Informed Consent, we will allocate her in one of the two groups (control vs intervention) through a randomization procedure.

There will be an electronic eCRF (electronic Case Report Form) to be used to register the clinical data: REDCap, fully compliant with 21 CFR Part 11, GDPR, FISMA; and HIPAA. This is a secure web application specifically geared to support online and offline data capture for research studies and operations. The participant's name and other identifiers will be stored separately from the research database to ensure anonymity. Personal data in the database will not be identifiable, an automatically created patient id number is used.

The eCFR will be available for the four centers to manage the key aspects of random sequence generation. Real-time responses from such a system will enable assigning unique numbers for each participant (enrollment/randomization), independently of the center they belong to.

Based on randomization numbers, the allocation ratio will be 1:1 for the interventional group and the control group. Investigators in charge of the analysis of the data will be blinded for the condition in which the participants are randomized.
There will also be an emergency unblinding module in case of any serious adverse event either by subject number or by group assigned. This module can only be accessed by sponsors and statisticians.

4.2.7 Multicomponent behavioural intervention

A multicomponent behavioral intervention during pregnancy and up to the first 18 months after delivery, composed by:

1. Digital tool called "*App Hypiend Digital Tool*" aimed at promoting lifestyle habits to reduce EDC exposure by providing personalized recommendations
2. Telephone monitoring
3. Workshop about environmental health education

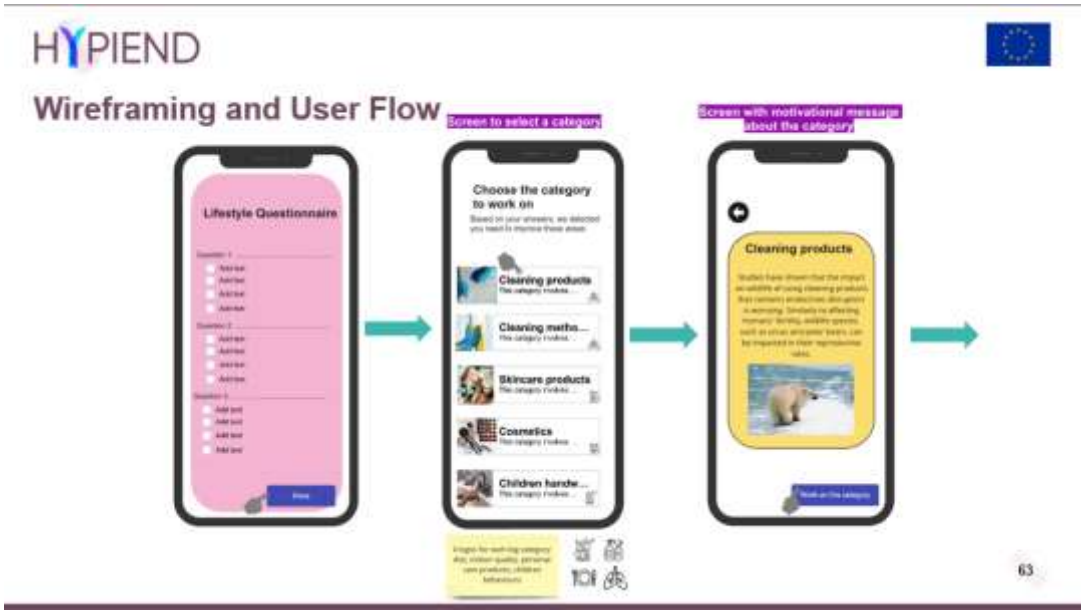
1. ***Digital tool ("App HYPIEND Digital Tool")***

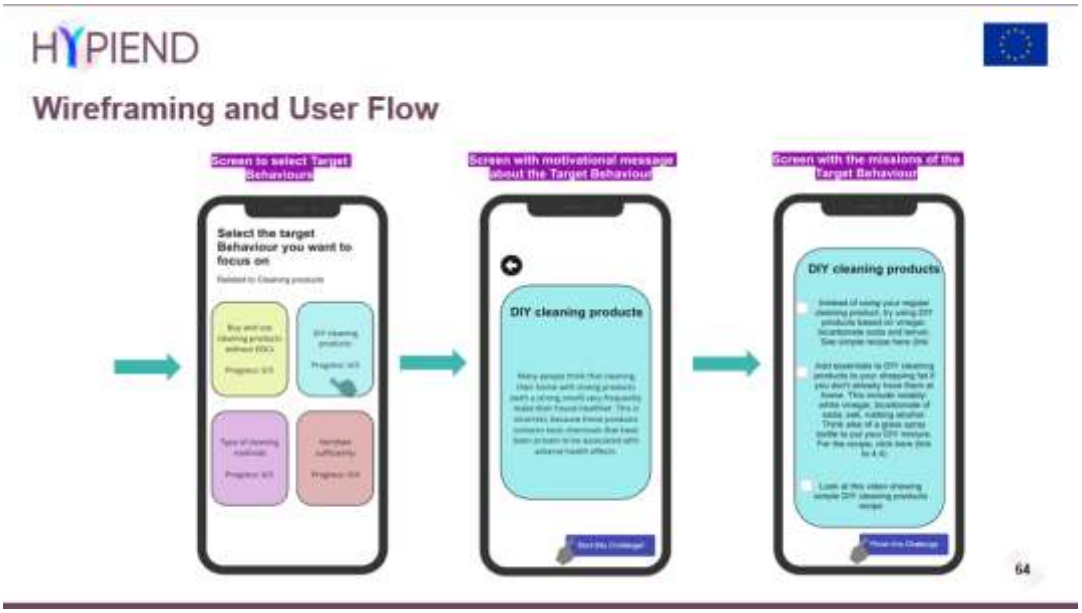
   Women will have available a mobile app developed by EURECAT and designed to limit the exposure to EDCs in women from pregnancy until their children are 18 months old in a personalized and gamified way. The content of the app is built by integrating the HAPA constructs using behaviour change techniques.
   After answering an initial questionnaire about lifestyle habits related to EDC exposure, the app will provide them with personalized missions considering those habits with a higher risk to expose them to EDCs. By selecting these missions successively, they will receive frequent recommendations, tips and motivational quotes that will help them to accomplish the selected mission. For example, graded tasks will be used to increase task self-efficacy (i.e., initiate the change). Providing tools to conserve mental resources will be used to increase maintenance self-efficacy (i.e., once the change started and needs to become a habit). Action planning and problem solving will be used to help intenders become actors.

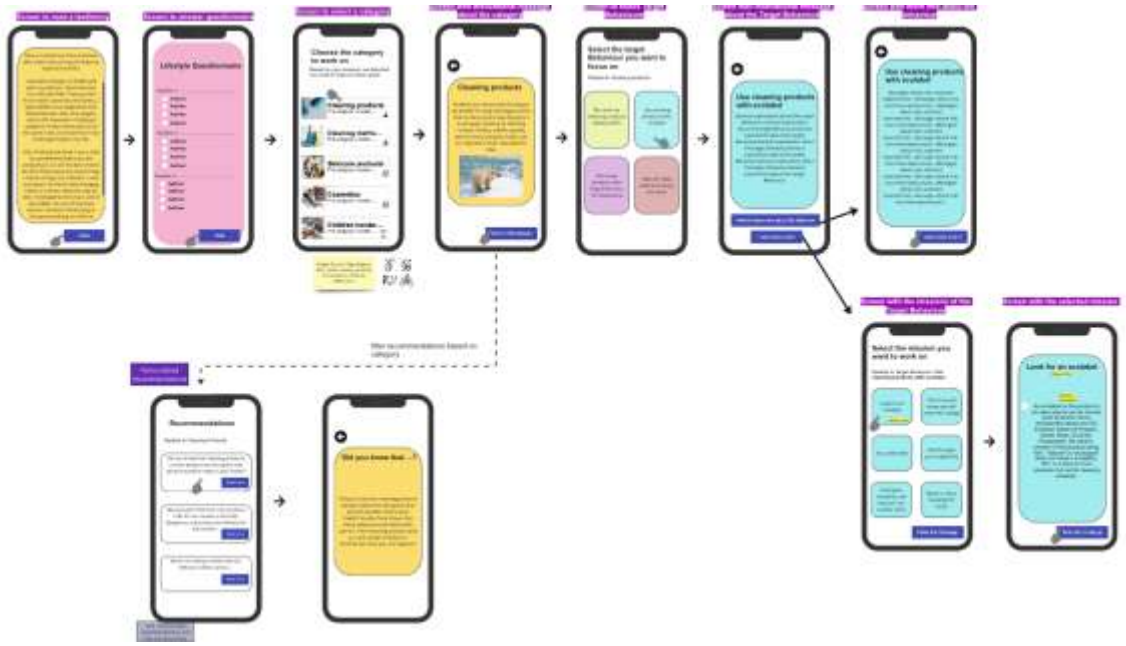
   The application has been developed with two different versions:
   - one for professionals/researchers/recruiters and
   - another for participants/end users.

The components of this application:

- Sign in up and log in
- Home page
- Questionnaires
- Missions (only the intervention group)
- Recommendations (only the intervention group)
- Push notifications
- Forum/chats







Components of the backoffice (exclusively for researchers):

- Questionnaires that the clinician must answer with the patient
- Visualization of questionnaire responses.
- Forum/chat
- Exportation of responses

2. ***Telephone monitoring:*** To provide personalized support and feedback
   - From 6 weeks postpartum will be contacted every two months.
   - Moreover, the researchers will contact them via phone call 6 months after delivery aimed at encouraging the adherence to the intervention and to solve potential doubts.
   - In addition, parents will be contacted to explain how they should fill out the neurodevelopment questionnaire, which they must send us in the coming days.
   - If a long period of inactivity is detected at any point of the study, the researchers will contact the families by phone.
   -

3. ***Workshops***

An interdisciplinary group of experts on EDCs (paediatric endocrinologists and environmental epidemiologists), psychologist expert on behaviour change, and nutritionists present in the consortium (University of Granada, Hospital Germans Trias i Pujol, University of Geneva, and Eurecat) will design and develop the content of the workshops.

Two onsite workshops will be conducted during the first half of pregnancy (before 20 weeks of gestation).

a) **Workshop 1:** Between 16-18 weeks of gestation. It will be focused on:
   a.1 definition of EDCs and their adverse health effects
   a.2 indoor air quality.

b) **Workshop 2**: Between 18- 20 weeks of gestation. It will contain:

b.1 dietary sources of EDCs.

Moreover, a third and a fourth session will be proposed to attend online at 30 weeks of pregnancy and around 6 weeks after delivery, respectively, covering personal care products and child's behavior and baby's products. They will also be recorded and sent as videos for those participants who did not attend.

The content of the workshops and the way they are conducted will be unified across the consortium. These workshops will be organized by experts on EDCs and by personnel of each institution that will be trained to give the workshop using the theoretical background of the HAPA frame. After conducting each workshop, the key messages of the session will be sent by phone as a reminder.

These workshops will be designed to reach several goals: increase knowledge on the hazards of EDCs for women and change psychological determinants (from the HAPA model) to result in a change in behavior that will lead to minimize the exposure of the women and their offspring to these chemicals. The workshops will not only provide information to raise awareness about the issue, but also include strategies to foster motivation to change (e.g., make the participants explain why it is important for them to change their habits), and facilitate action by increasing perceived capability.

Partners will be encouraged to join women during the medical visits and the environmental health education workshop.

CONTROL GROUP:
The control group will only receive a digital printable booklet with information on EDC hazards and on strategies to limit their exposure. After finishing the intervention, the participants of the control group will be offered access to all the developed material (workshops…) and to use the app with all the functionalities. Participants from different environmental areas will be recruited to account for different exposures to EDCs.

3.2.8 Follow-up and Timing of Visits

Visit 1: On-site, First trimester, from 6 weeks to 13 weeks and 0 days of gestation.

Visit 2: On-site, Third trimester, 28- 32 weeks of gestation.

Delivery/Birth

Visit 3: On-site, between 4^{th} and 7^{th} weeks after delivery.

Visit 4: On-site, at 18 months after delivery.

3.3 Study Variables

3.3.1 Maternal variables to be studied

A) Visit 1: On-site, First trimester, from 6 weeks until 13 weeks and 0 days of gestation.

- Anthropometric measurements: weight, height, and waist circumference according to standardized protocols.

- Questionnaires: at the first visit, the participant will be instructed to use the app "Hypiend Digital Tool" and how to self-administer questionnaires online through this app. The questionnaires are designed to explore different areas:

a. Socio-demographic data: urban/rural area of residence, work activity, economic background, level of education, personal and prenatal history.

b. Quality of life (WHOQOL-BREF) The World Health Organization Quality of Life is a quality-of-life assessment developed by the WHOQOL Group with fifteen international field centres, simultaneously, to develop a quality-of-life assessment that would be applicable cross-culturally.

c. Physical activity (IPAQ): The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health–related physical activity.

d. Dietary intake: a 3-day dietary record will be obtained through a specific digital tool called FASST (Food Assessment Tool), and a Food Frequency Questionnaire (FFQ).

e. Pregnancy-related Anxiety Questionnaire-Revised (PRAQ-R2)

g. The State-Trait Anxiety Inventory Test (STAI-T)

h. Lifestyle habits concerning exposure to EDC Questionnaire*:

i. EDC's knowledge and HAPA constructs Questionnaire*

* Based on existing tools particularly used to explore these domains.

A pilot study has been carried out with volunteers to estimate how long it will take for the participants to fill in each questionnaire. The time elapsed ranged from 2-15 minutes.

- Laboratory tests: At the first visit, we will also collect samples of blood, urine and faeces, according to the study protocol. See the Section "Biological samples and Clinical Analyses".

WS1 : air quality

WS2 : diet

Between 16 and 18 weeks of gestation, participants from the intervention group will receive an onsite workshop about air quality.

Around 18-20 weeks of gestation, a second onsite workshop will be organized for the intervention group, focusing on diet. The control group will not receive such information.

B)xVisit 2: On-site, Third trimester, 28- 32 weeks of gestation.

- Anthropometric measurements: weight, height, waist circumference and gestational weight gain according to standardized protocols.

- Questionnaires: The questionnaires will have been self-administered by the participants through the App Hypiend Digital Tool. The researchers will check their fulfilment at this visit. At visit 2, these are the questionnaires to fill out:

a. Socio-demographic data: They will only be evaluated just in case of any change.

b. Quality of life (WHOQOL-BREF)

c. Physical activity (IPAQ)

d. Pregnancy-related Anxiety Questionnaire-Revised (PRAQ-R2)

g. The State-Trait Anxiety Inventory Test (STAI-T)

h. Lifestyle habits concerning exposure to EDC Questionnaire*:

i. EDC's knowledge and HAPA constructs Questionnaire*

* Based on existing tools particularly used to explore these domains.

Workshop 3 (online): A video will be sent to the participants from the intervention group, around 30 weeks of gestation. This workshop will contain information regarding Personal care products.

C) Birth: During the active phase of labour, we will collect samples of peripheral blood and urine. Faeces will be collected at any time during the hospital stay for giving birth.

D) Visit 3: On-site, between 4th and 7th weeks after delivery.

- Anthropometric measurements: weight, height, waist circumference ~~and gestational weight gain~~ according to standardized protocols.

- Questionnaires: The questionnaires will have been self-administered by the participants through the App Hypiend Digital Tool. The researchers will check their fulfilment during this visit. At the third visit, the questionnaires used will be:

a. Socio-demographic data:

b. Quality of life (WHOQOL-BREF)

c. Dietary intake: a 3-day dietary record will be obtained through a specific digital tool called FASST (Food Assessment Tool).

d. The State-Trait Anxiety Inventory Test (STAI-T)

e. Lifestyle habits concerning exposure to EDC Questionnaire*:

f. EDC's knowledge and HAPA constructs Questionnaire*

* Based on existing tools particularly used to explore these domains.

- Laboratory tests: Participants will have collected 3 samples of breast milk during the days before the visit and they will carry them on.

Workshop 4 (online): A second video will be sent to the participants from the intervention group around 6 weeks after delivery. This video will contain information about child's behavior and baby's products.

Telephone Monitoring: From 6 weeks after delivery until 18 months postpartum, participants from the intervention group will be contacted bimonthly by WhatsApp or any other messaging app of their choice. The participants from the control group will not be contacted by the researchers.

E) Visit 4: On-site, 18 months after delivery.

- Anthropometric measurements: weight, height, waist circumference according to standardized protocols.

- Questionnaires: The questionnaires will have been self-administered by the participants through the App Hypiend Digital Tool. The researchers will check their fulfilment on this visit.

a. Socio-demographic data: Only in case of changes.

b. Quality of life (WHOQOL-BREF).

c. Physical activity (IPAQ)

d. Dietary intake: a 3-day dietary record will be obtained through a specific digital tool called FASST (Food Assessment Tool), and a Food Frequency Questionnaire (FFQ).

e. The State-Trait Anxiety Inventory Test (STAI-T)

f. Lifestyle habits concerning exposure to EDC Questionnaire*:

g. EDC's knowledge and HAPA constructs Questionnaire*

* Based on existing tools particularly used to explore these domains.

- Laboratory tests: Samples of blood, urine and faeces will be collected, according to the study protocol. See the Section "Biological samples and Clinical Analyses".

3.3.2 Study Variables in Children

A) Birth:

- Information regarding labour, and the physical condition of the baby will be recorded.

- Growth and anthropometric measurements: gestational age (weeks), sex, anogenital distance (AGD) weight-for-age (WFA), length-for-age (LFA), weight-for-length (WFL), head circumference-for-age (HCA), preterm birth, low birth weight, small for gestational age, large-for-gestational age. The anthropometric measurements will be plotted according to the WHO growth charts, except for premature children under 37 weeks, who will be assessed according to Fenton Charts up to 44 weeks of corrected age.

- At the time of birth, we will collect a blood sample from umbilical cord. Faeces will be collected at any time during the hospital stay.

B) Visit 3: On-site, between 4th and 7th weeks after delivery.

- Growth and anthropometric measurements at 6 weeks of age: gestational age (weeks), sex, anogenital distance (AGD) weight-for-age (WFA), length-for-age (LFA), weight-for-length (WFL), head circumference-for-age (HCA).

- A sample of urine from the baby will be collected during the visit. See the Section "Biological samples and Clinical Analyses".

C) Infant neurodevelopment will be assessed at the age of 6 months using the Ages and Stages Questionnaires, third edition (ASQ-3). The time frame for the test will be from 5 months to 6 months and 30 days of age. Parents will receive a phone call to explain how they should fill out the neurodevelopment questionnaire, which they must send us in the coming days.

D) Visit 4: On-site, at 18 months of age.

- Growth and anthropometric measurements: gestational age (weeks), sex, anogenital distance (AGD) weight-for-age (WFA), length-for-age (LFA), weight-for-length (WFL), head circumference-for-age (HCA).

- Infant neurodevelopment will be assessed at the age of 18 months using use the Ages and Stages Questionnaires, third edition (ASQ-3). The time frame for the test will be from 17 months to 18 months and 30 days of age.

- Laboratory tests: Samples of blood, urine and faeces will be collected, accordingly to the study protocol. See the Section "Biological samples and Clinical Analyses".

4. SAMPLE COLLECTION

	Visit 1	BIRTH	Visit 3	Visit 4
Mother	Blood Urine Faeces	Blood Urine Faeces	**Human milk**	Blood Urine Faeces
Baby		Cord blood Faeces	**Urine**	Blood** Urine Faeces

**** The obtention of blood from toddlers WOULD be done by using an emerging pain-free device (Tasso-plus®) as alternative to dried specimens for clinical analysis. https://www.tassoinc.com/tasso-plus**

OECD Guidelines:

Guidelines OECD 455, OECD 458, OECD150

Prior to starting any study, all materials (glass tubes, vessels, plastic ware) and reagents that will be used should be investigated, as defined in the protocol (6), for any possible interference with the measurements. OECD 458

The plastic-ware should be free of estrogenic activity. OECD 455

No recommendations of specific brands.

Collection Tubes:

Things to consider in the context of HYPIEND to avoid plastic cross contamination

Phthalates contamination detected by MS in samples collected in plastic tubes. For metabolomics analysis this should be considered.

Human samples will be collected in plastic tubes to obtain blood, milk and urine, but plasma, milk and urine should be storage in tubes that are certified free of Bisphenol A (BPA), Oleamide, DiHEMDA, and Phthalates. Some suggestions to be discussed:

Eppendorf tubes (Ref. 0030121023)

SuperClear® Centrifuge Tubes: https://www.labcon.com/tubessuperclearct.html

Eclipse™ Pipette Tips: https://labcon.com/tipseclipse.html

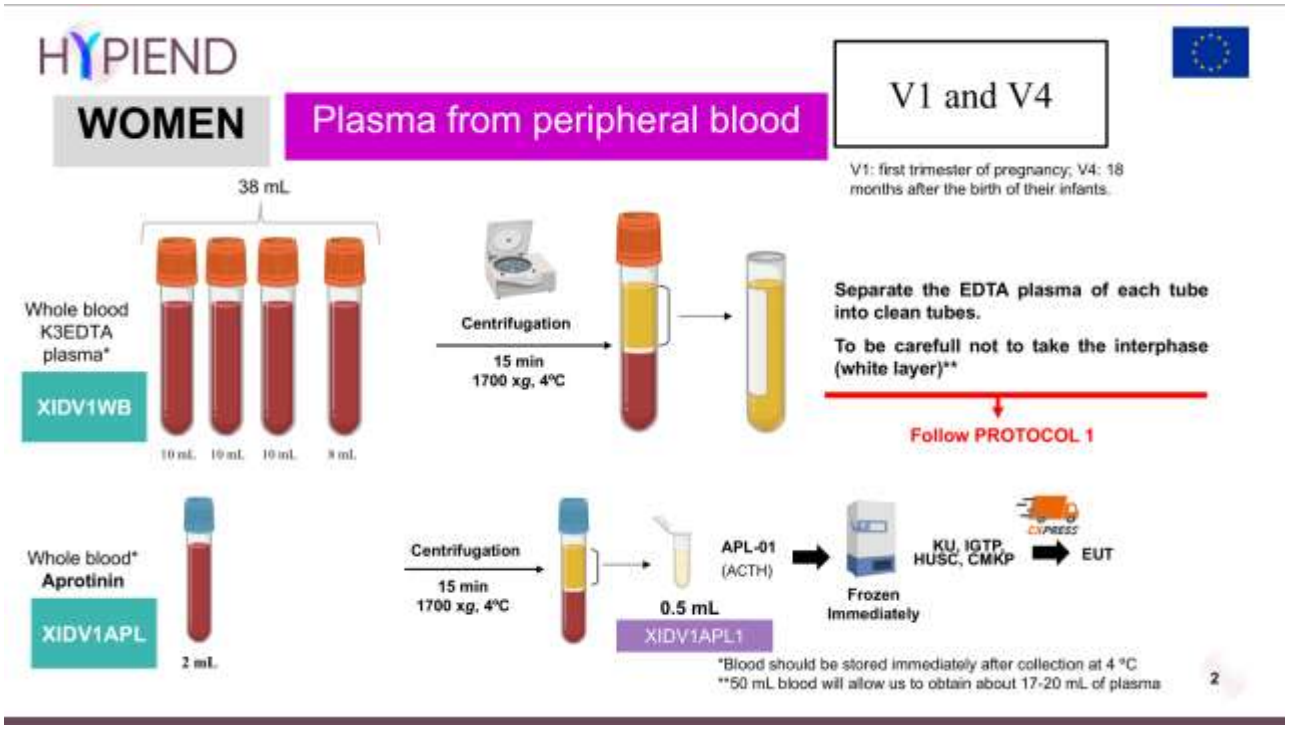
Maternal Blood:

At Visit 1 (first trimester) as well as at Visit 4 (18 months after delivery) maternal peripheral blood will be collected.

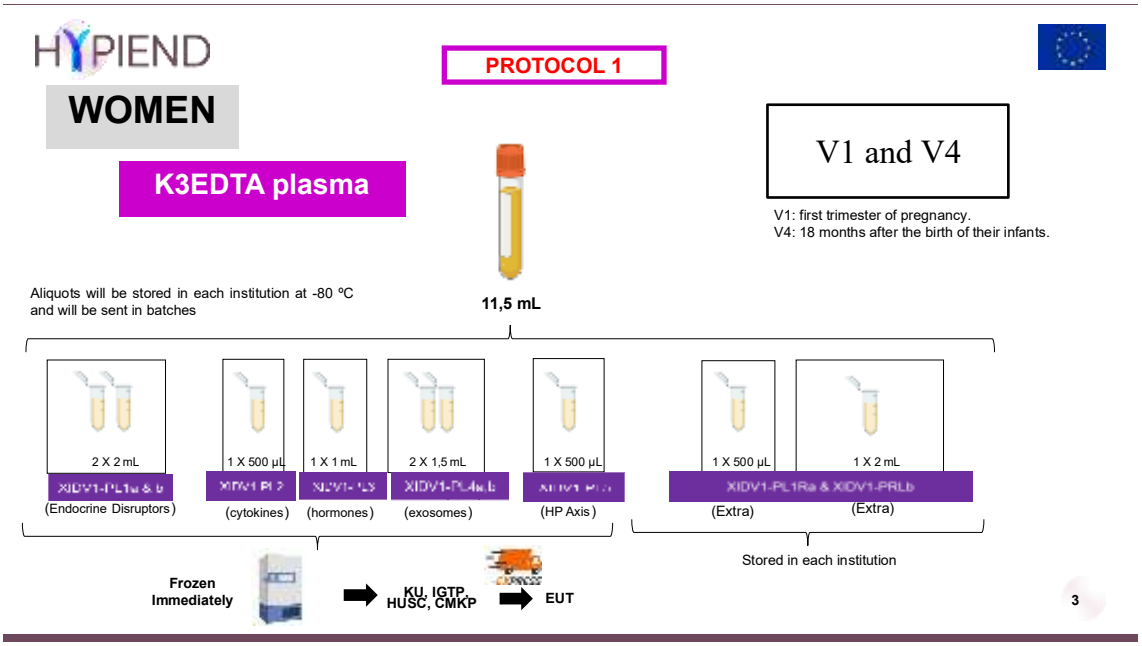
We will obtain:

- 4 tubes of 10 ml whole blood K3EDTA plasma (total 38 ml)

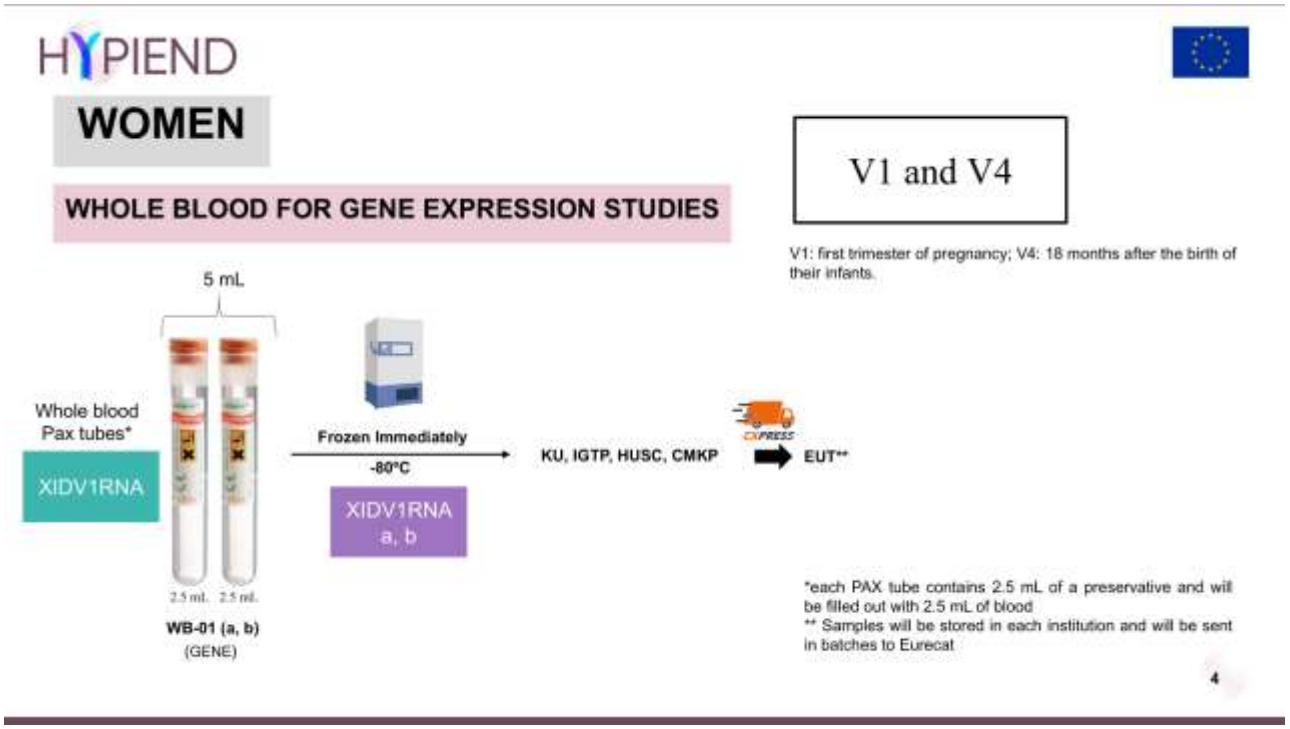
- 1 tube of 2 ml whole blood aprotinin
- 2 tubes of 2.5 ml whole blood Pax Tubes for gene expression studies
- 2 tubes of 2 ml: one for epigenetic analyses and one to be stored at biobank.



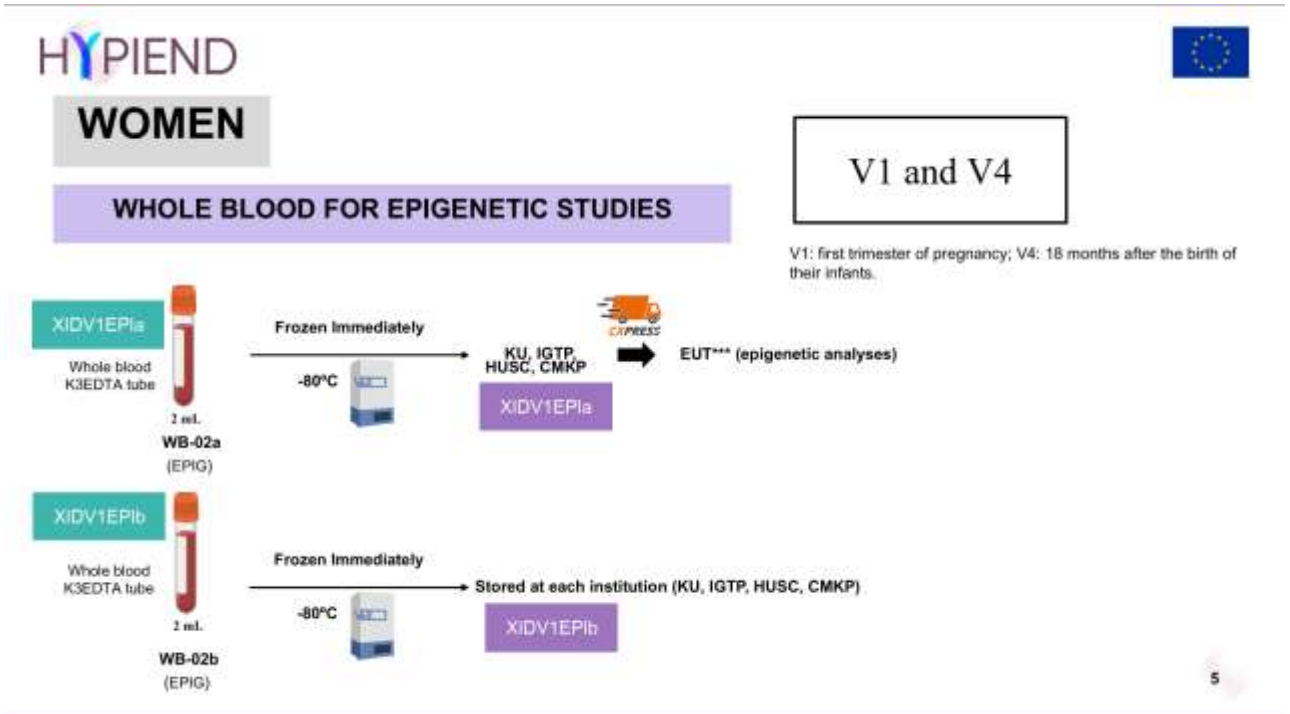
After centrifugation, we will get approximately 11,5 ml of plasma, that should be divided into aliquots as follows:



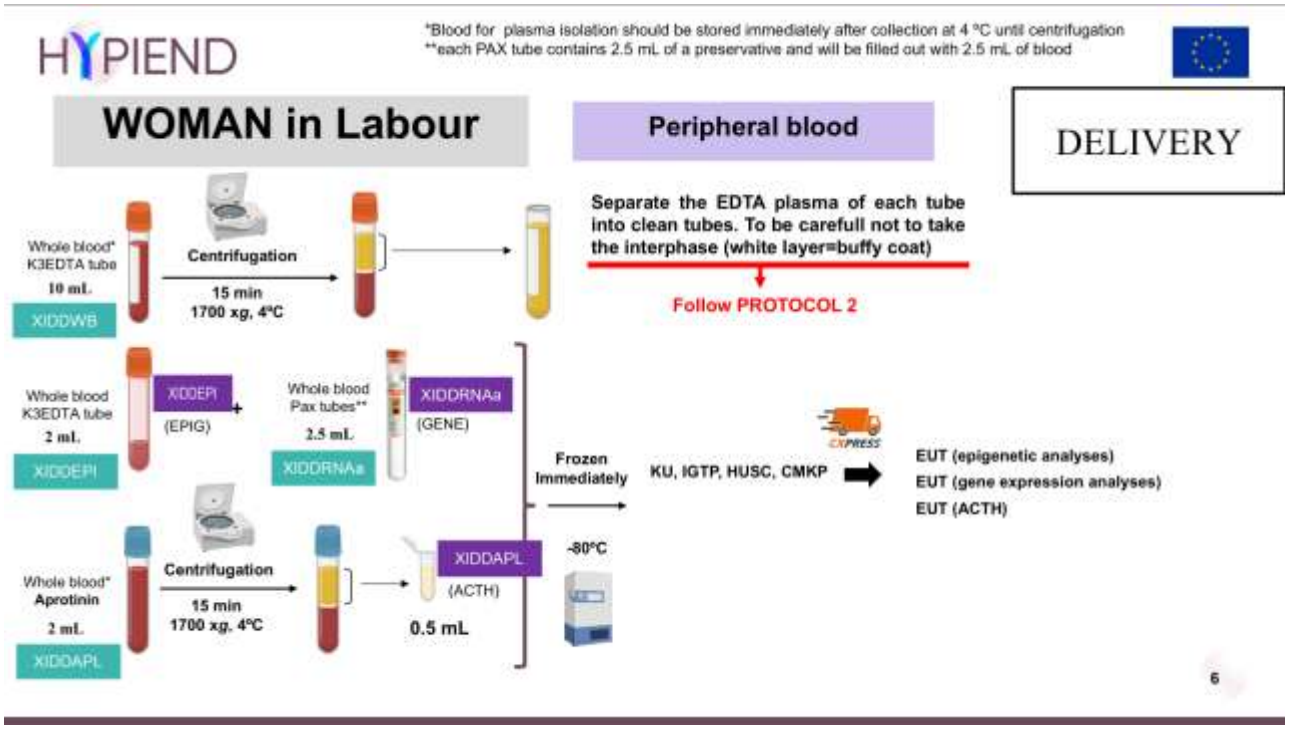
The 2 whole blood Pax Tubes of 2,5 ml will be frozen immediately at each institution:

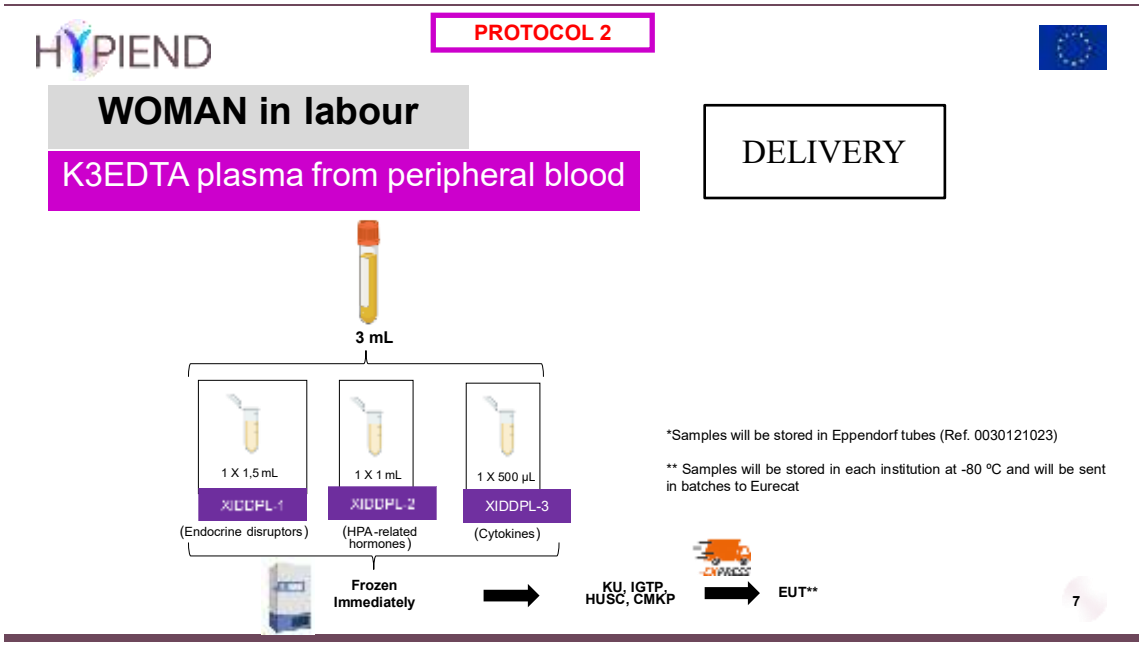


Eventually, the remaining 2 tubes of 2 ml of whole blood K3EDTA for epigenetic studies will be processed as follows:

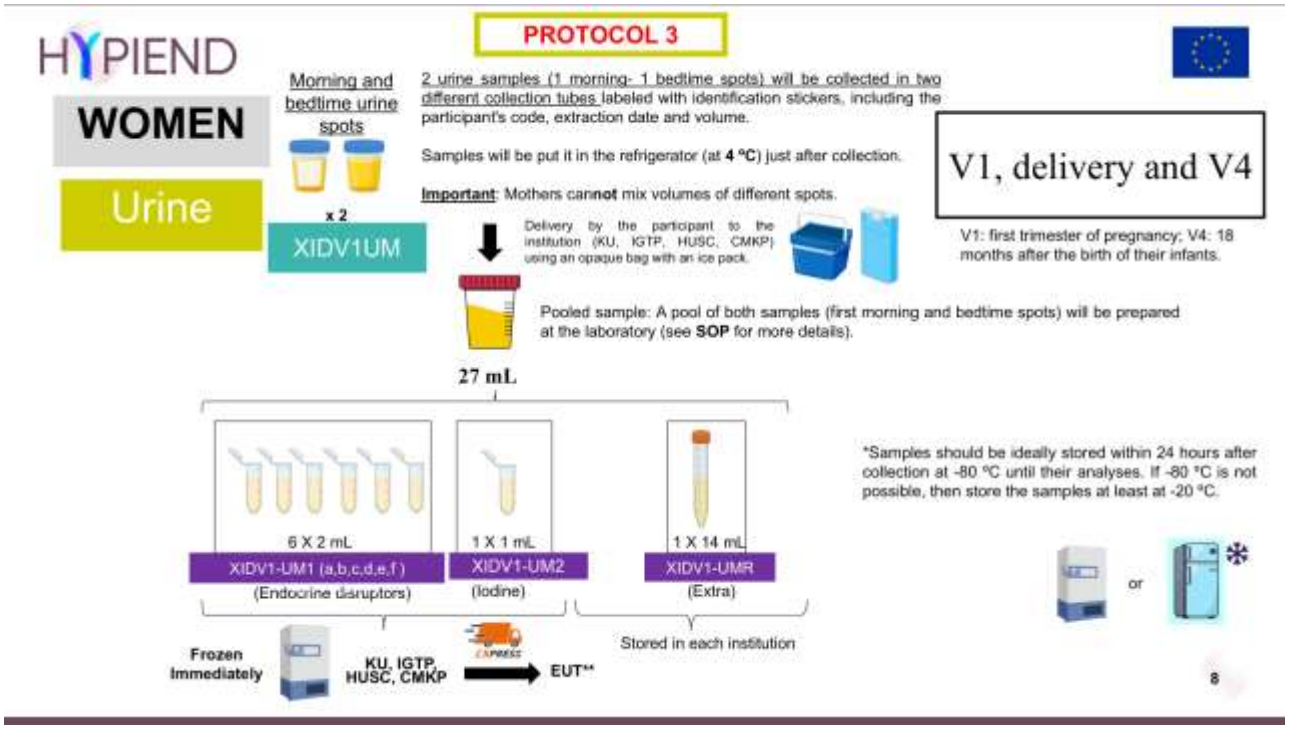


Maternal Blood at delivery:

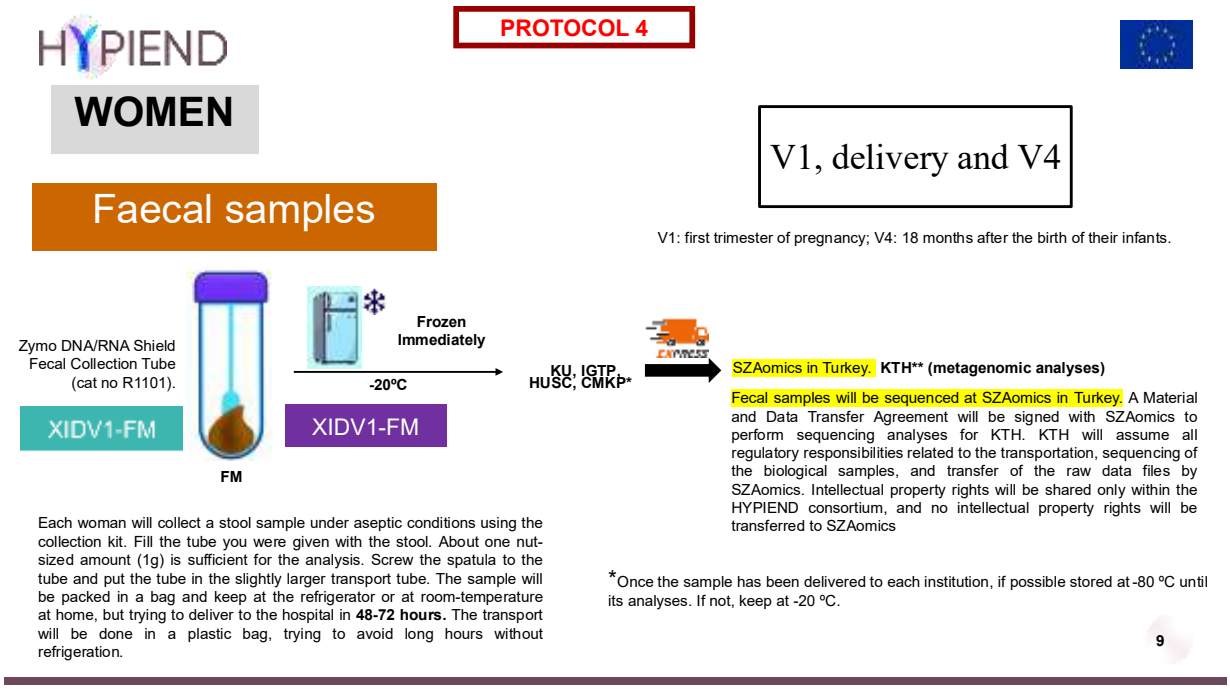




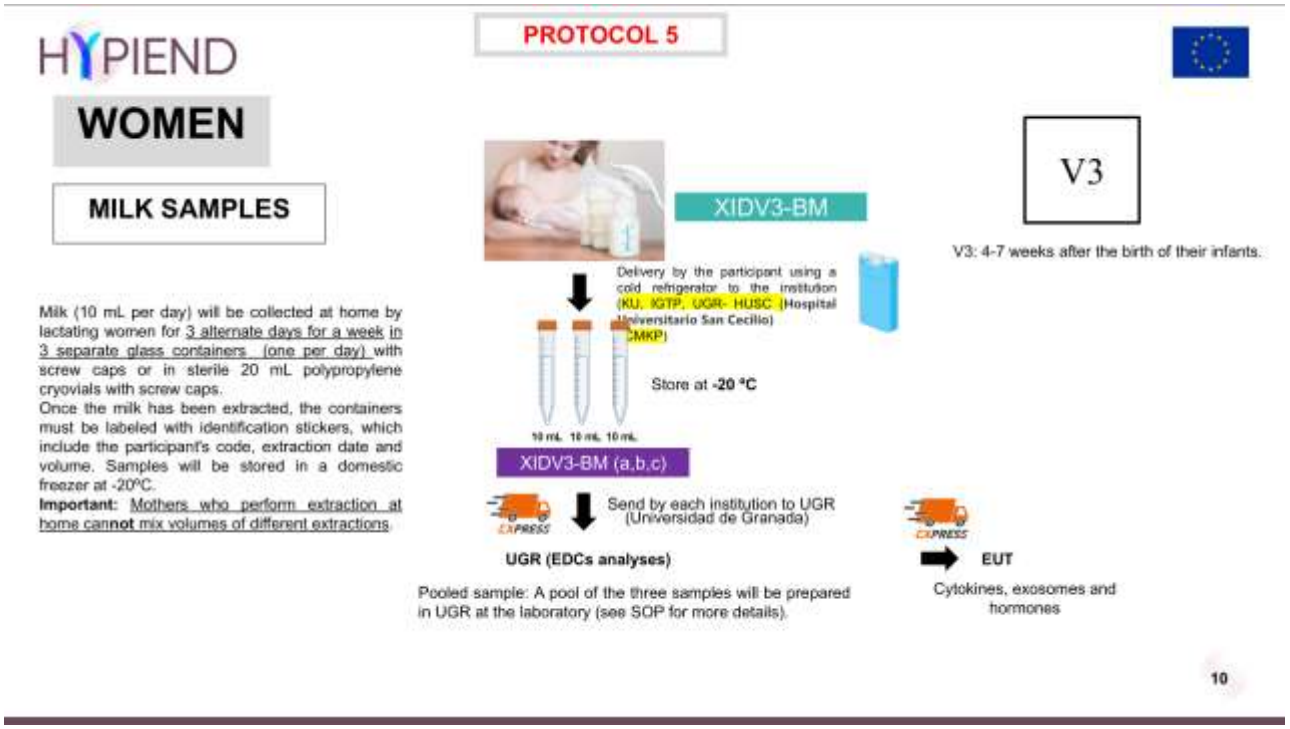
Maternal Urine:



Stool collection:



Human Milk:



Cord Blood: At the time of birth, 15 ml from venous cord blood will be collected.

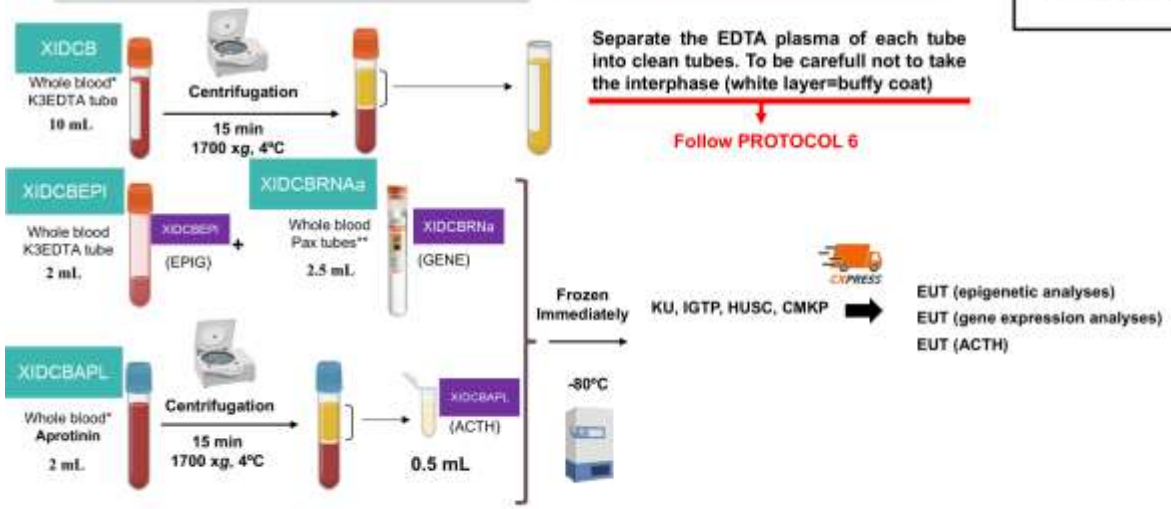
HYPIEND

*Blood for plasma isolation should be stored immediately after collection at 4 ºC until centrifugation
**each PAX tube contains 2.5 mL of a preservative and will be filled out with 2.5 mL of blood

INFANT-cord blood

VENOUS CORD BLOOD

DELIVERY



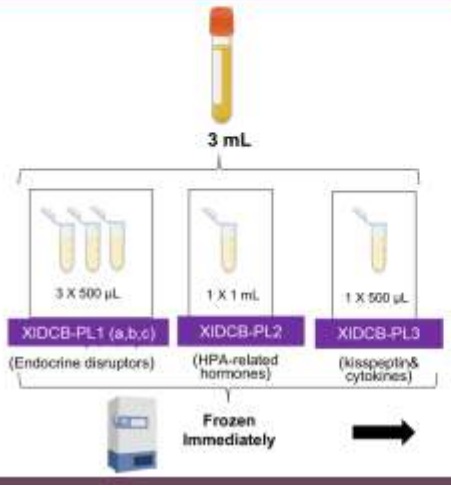
HYPIEND

PROTOCOL 6

INFANT-cord blood

K3EDTA plasma from cord blood

DELIVERY



*Samples will be stored in Eppendorf tubes (Ref. 0030121023)

** Samples will be stored in each institution at -80 ºC and will be sent in batches to Eurecat

HYPIEND

PROTOCOL 7

INFANTS

BLOOD SAMPLES

18 months

Collect **5** drop blood per volunteer in the collection paper (one drop inside per circle of 1.5 cm)

dry for 3-8 h in RT

3 DBS stored in 4°C
(zip bag + desiccant pack)

2 DBS stored in –80°C *
(zip bag + desiccant pack)

KU, IGTP, HUSC, CMKP

EUT (HP axis related hormones and Kisspeptin)

EUT (epigenetics)

* If not possible, keep at **-20 °C**.

** Samples will be stored in each institution and will be sent in batches to Eurecat. DBS for hormonal analyses will be sent with cold packs, and DBS for epigenomic analyses will be sent in dry ice.

HYPIEND

WAITING FOR A DECISSION

INFANTS

BLOOD SAMPLES

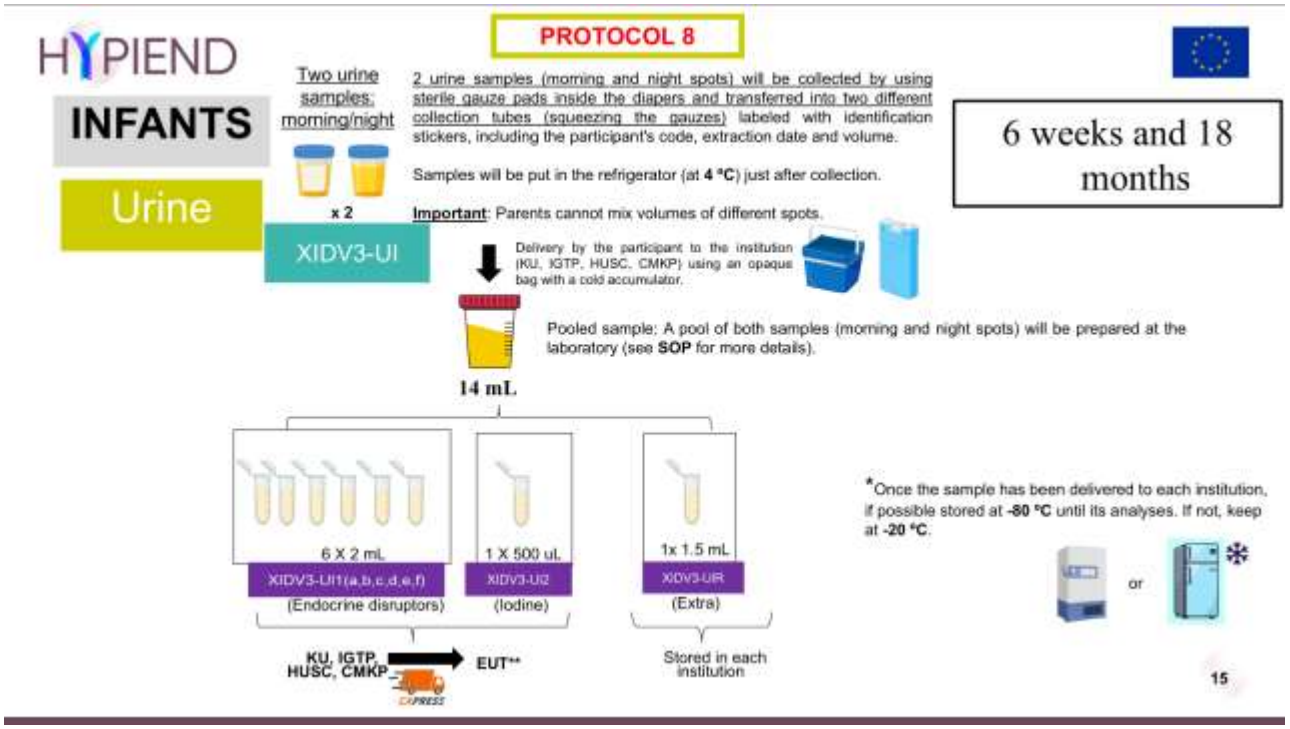
18 months

Infant blood: At 18 months of age, we will collect blood from children. To do so, we are planning to use a pain free device (Tasso®). We have already contacted the brand (https://www.tassoinc.com/solutions) to get information about two possible devices:

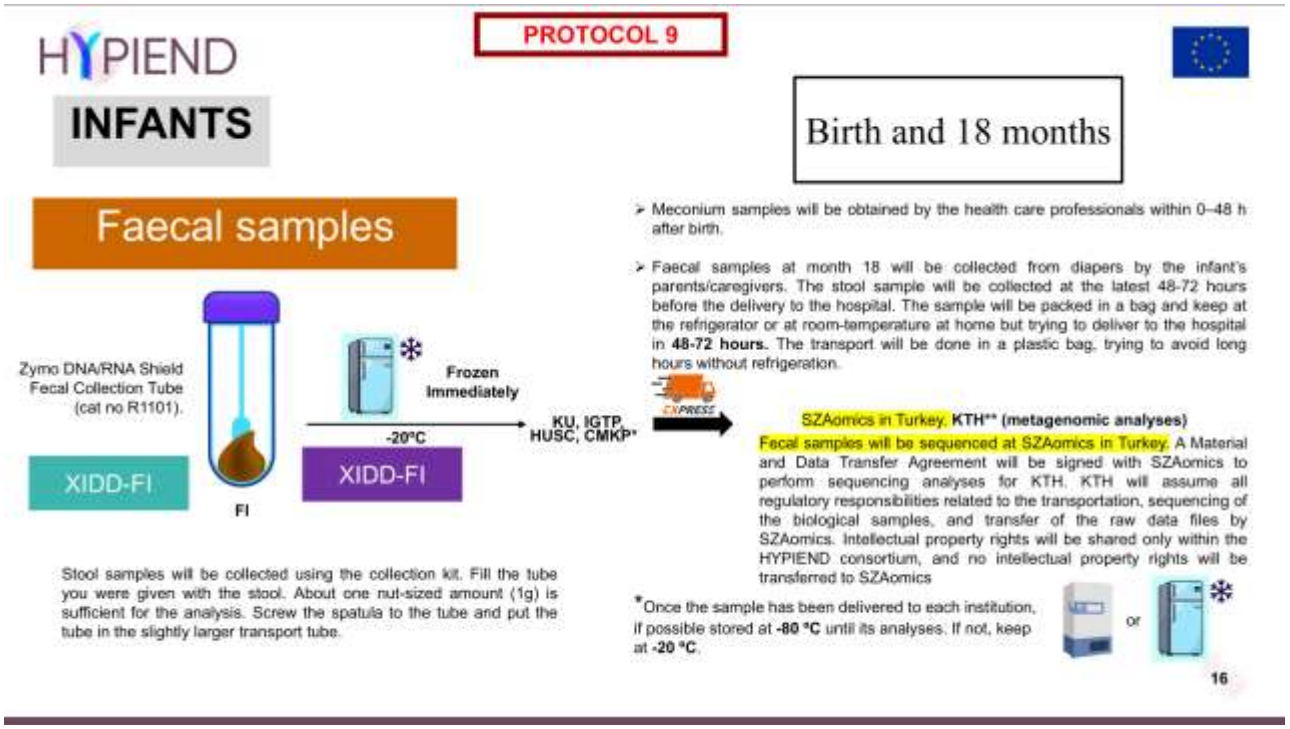
1. The Tasso+ device is a blood lancet that collects whole liquid blood samples. Multiple standard collection tubes are compatible. Tasso+ is CE Marked, MHRA registered, and available in Switzerland.
2. The Tasso-M20 device delivers whole dried blood samples from the patient to the lab. It can be used for a number of applications, including PK (pharmacokinetic) monitoring in patients enrolled in clinical trials. Subjects can successfully collect volumetrically precise samples regardless of access to trial sites. The Tasso-M20 is CE Marked and MHRA registered.

We will also decide how to proceed with clinical analysis according to the choice of such a device (whole blood versus dried blood spot).

Infant urine:



Infant faeces:



5. BIOCHEMICAL, MOLECULAR AND OMICS ANALYSES

5.1 Biochemical, molecular and omics analyses that will be carried out in biological samples obtained from women

VARIABLES	SAMPLE	TECHNIQUE	VOLUME/ AMOUNT	TIMELINE
EDC	Urine	Metabolomics by LC/GC-MS/MS[a]	12 mL *	V1, delivery, V4
	Plasma from peripheral blood		6 mL *	V1, V4
	Milk		10 mL	V3
Microbiota	Faeces	Shotgun sequencing	1 g	V1, delivery, V4
Cytokines, exosomes and hormones	Plasma from peripheral blood	Multiplexed immunoassays, cytometry	6 mL	V1, V4
	Milk		10 mL	V3
Gene expression	Whole peripheral blood	Q-PCR	5 mL	V1, V4
Epigenomics	Whole peripheral blood	DNA methylation of specific genes	3 mL	V1, V4
HP axis-related hormones	Plasma from peripheral blood	Immunoassays	1 mL	V1, V4
ACTH	Plasma–aprotinin from peripheral blood	Immunoassays	0.5 mL	V1, V4
Iodine	Urine	Colorimetric analysis	200 uL	V1, delivery, V4

V1: first trimester of pregnancy; V2: third trimester of pregnancy; V3: 4-7 weeks after the birth of their infants; V4: 18 months after the birth of their infants

a Analysis of EDC will be carried out using liquid (LC) or gas chromatography (GC) - tandem mass spectrometry (MS/MS). Specific instrument, columns, mobile phases, elution gradients and source parameters will be optimized for each EDC family.

5.2 Biochemical, molecular and omics analyses that will be carried out in biological samples obtained from infants

VARIABLES	SAMPLE	TECHNIQUE	VOLUME/ AMOUNT	TIMELINE
EDC	Urine	Metabolomics by LC/GC-MS/MS[a]	7 mL *	6 weeks &18 months
	Plasma from cord blood		1.5 mL	
				Birth
Microbiota	Faeces	Shotgun sequencing	1 g	Birth, 18 months
HPG-related hormones	Plasma from cord blood	Multiplexed immunoassays, cytometry	1 mL	Birth
	Peripheral whole blood		500µl	18 months
Cytokines	Plasma from cord blood		0,5 mL	Birth
Kisspeptin	Plasma from cord blood			Birth
	Peripheral whole blood		500µl	18 months
Gene expression	Whole cord blood	Q-PCR	2.5 mL	Birth
Epigenomics	Whole cord blood	DNA methylation of specific genes	3 mL	Birth

	Peripheral whole blood		500µl	18 months
ACTH	Plasma–aprotinin from cord blood	Inmunoassay	0.5 mL	Birth
Iodine	Urine	Colorimetric analysis	250 uL	Births, 18 months

a Analysis of EDC will be carried out using liquid (LC) or gas chromatography (GC) - tandem mass spectrometry (MS/MS). Specific instrument, columns, mobile phases, elution gradients and source parameters will be optimized for each EDC family.

* Volume may be different depending on the selected families of EDC

For each tube to be stored for further analysis, we have included a general label. Ex: Plasma from women for EDC analyses: PL-01 (a,b,c,d,e,f): PL=Plasma; 01= corresponds to the aliquots to be used for EDC analyses; a,b,c,d,e,f: 6 tubes are needed, one tube, one letter. When samples are collected, additional information will be included in the label: the number of visit and the code of the participant. Ex: PL-01a_V1_XXX

* Volume may be different depending on the selected families of EDC. Refer to Annex 1 (see folder WP·, perinatal_clinical_study) for more details.

STORAGE AND SHIPMENT OF SAMPLES

All samples collected will be processed according to specific protocols. Once labelled, they will be kept at the Biobank of the four centres (HGTiP, HUSC, CMKP; KUL).

Eurecat will be responsible for the shipment of samples to reference laboratories.

CLINICAL ANALYSIS PER VISIT

A.Variables to be analyzed at V1 (first trimester of pregnancy) in women.

VARIABLES	TOOL/TECHNIQUE
EDC (urine)	Metabolomics
EDC (plasma from peripheral blood)	Metabolomics
Microbiota (faeces)	Shotgun sequencing
Cytokines, exosomes and hormones (plasma)	Immunoassays
Gene expression (whole peripheral blood)	Q-PCR
Epigenomics (buffy coat-peripheral blood)	DNA methylation of specific genes
HP axis-related hormones (plasma)	Immunoassays
Iodine concentration (urine)	Colorimetric analysis

B. Variables to be analyzed at delivery in women.

VARIABLES	TOOL/TECHNIQUE
EDC (urine)	Metabolomics
EDC (plasma from peripheral blood)*	Metabolomics
Gene expression (whole peripheral blood) *	Q-PCR
Epigenomics (buffy coat-peripheral blood) *	DNA methylation of specific genes
HP axis-related hormones (plasma from peripheral blood) *	Immunoassays
Cytokines (plasma from peripheral blood) *	Immunoassays
Microbiota (faeces)	Shotgun sequencing
Iodine concentration (urine)	Colorimetric analysis

* Samples will be collected but there is no budget planned to carry out these analyses

C. Variables to be analyzed at V3 (between 4 and 7 weeks after the birth of their infants) in women.

VARIABLES	TOOL/TECHNIQUE
EDC (breast milk)	Metabolomics
Cytokines, exosomes and hormones (breast milk)	Immunoassays

D. Variables to be analyzed at V4 (18 months after the birth of their infants) in women.

VARIABLES	TOOL/TECHNIQUE
EDC (urine)	Metabolomics
EDC (plasma from peripheral blood)	Metabolomics
Microbiota (faeces)	Shotgun sequencing
Cytokines, exosomes and hormones (plasma)	Immunoassays
Gene expression (whole peripheral blood)	Q-PCR
Epigenomics (buffy coat-peripheral blood)	DNA methylation of specific genes
HP axis-related hormones (plasma)	Immunoassays
Iodine concentration (urine)	Colorimetric analysis

E. Variables to be analyzed at birth in infants.

VARIABLES	TOOL/TECHNIQUE
EDC (plasma from cord blood)	Metabolomics
Gene expression (whole cord blood)	Q-PCR
Epigenomics (buffy coat-cord blood)	DNA methylation of specific genes
HP axis-related hormones (plasma from cord blood)	Immunoassays
Kisspeptin (plasma from cord blood)	Immunoassays
Cytokines (plasma from cord blood)	Immunoassays
Microbiota (faeces)	Shotgun sequencing
Iodine concentration (urine)	Colorimetric analysis

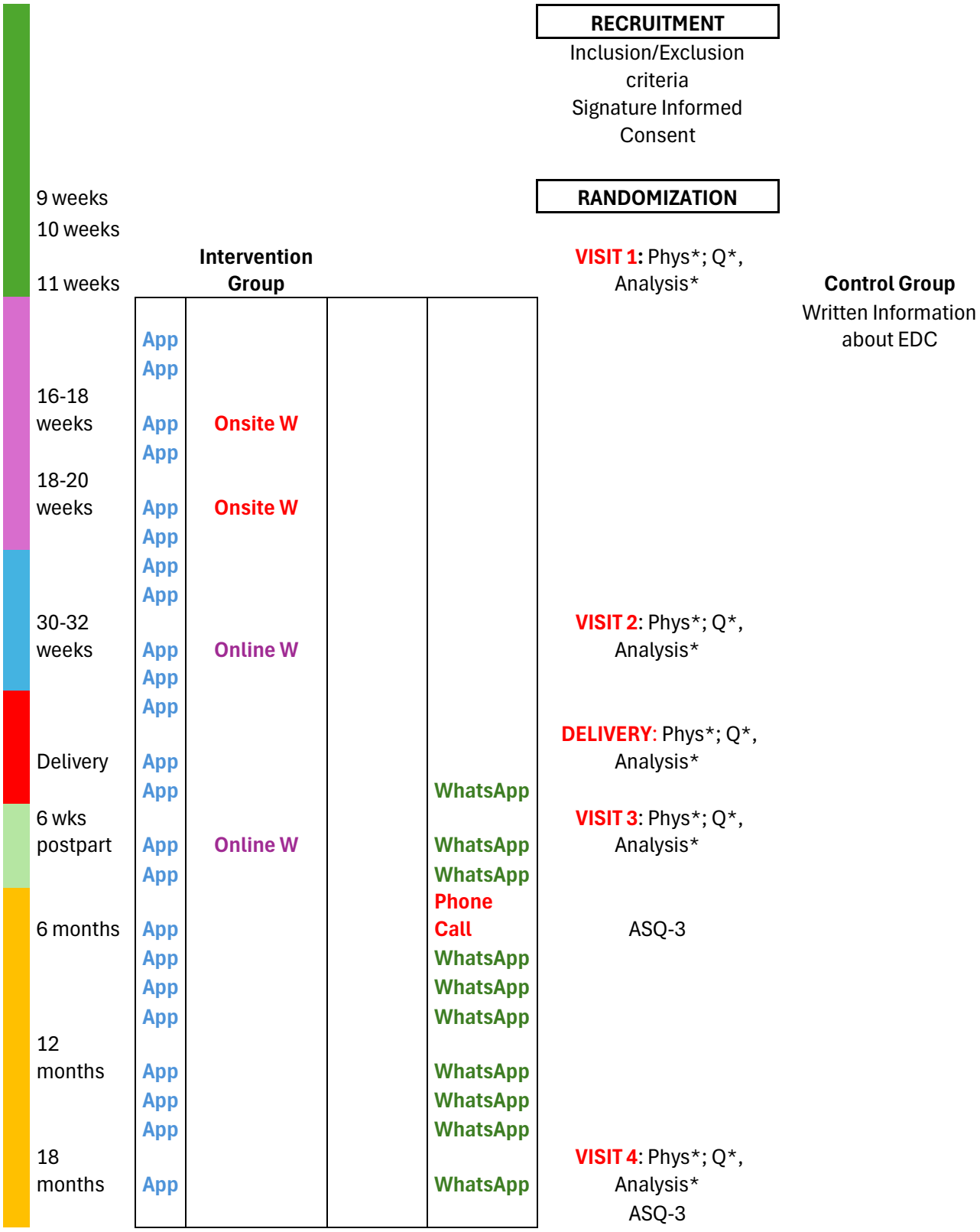
F. Variables to be analyzed at V3 (between 4 and 7 weeks after the birth of their infants) in infants.

VARIABLES	TOOL/TECHNIQUE
EDC (urine)	Metabolomics

G. Variables to be analyzed at 18 months in infants.

VARIABLES	TOOL/TECHNIQUE
EDC (urine)	Metabolomics
Microbiota (faeces)	Shotgun sequencing
Neurodevelopment	ASQ-3
Epigenomics (peripheral whole blood)	DNA methylation of specific genes
HP axis-related hormones (peripheral whole blood)	Immunoassays
Kisspeptin (peripheral whole blood)	Immunoassays

6. TRIAL FLOWCHART



App: Aplication Hypiend Digital Tool.
Onsite W: Presential Workshop about air quality (16-18 weeks of gestation)
Presential Workshop about Dietary sources of EDCs (18-20 weeks)

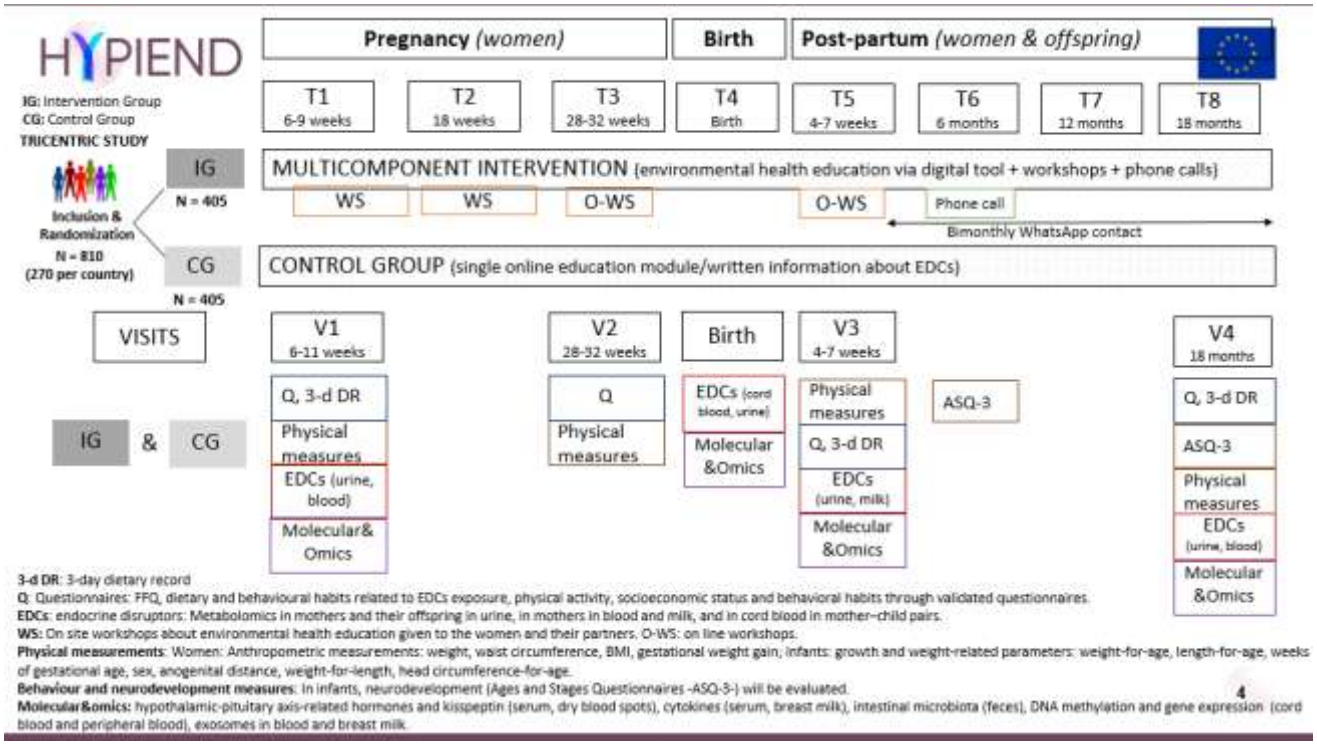
Online W: Video about Personal care Products (around 30 weeks of gestation)
Video about child's behavior and baby's product (6 weeks after delivery)

Phys*: Physical examination, anthropometric measurements.

Q*: Questionnaires regarding:

- General information
- Medical background
- Behaviors/Lifestyle habits
- EDCs' Knowledge
- Perceived exposure to EDC
- HAPA Constructs

Analysis*: Sample collection



7. STATISTICAL ANALYSIS

Descriptive statistics will be used to calculate central tendency measures and frequencies of sociodemographic characteristics and other covariates. EDC concentrations in the different biological matrices and outcome variables (anthropometric measures, IQ, hormone levels, etc.).

The statistical approach used to assess the effectiveness of the intervention will depend on the final distribution of the study variables, but we expect to compare the reduction in EDC levels in the two groups using paired parametric or non-parametric paired data tests (e.g., Student T/Wilcoxon signed-rank tests, McNemar/Wilcoxon signed-rank test for paired binary data), as well as mixed-effect regression models to account for recruitment center and repeated EDC measures. We will assess changes in mean/median pollutant levels and percentages of detection. We also consider including mixture analysis by using dimension reduction techniques to identify clusters of chemicals affected by the intervention with novel statistical methodologies such as Weighted Quantile Sum (WQS) Regression, G-Computation, or Bayesian Kernel Machine Regression (BKMR). The analyses will be done according to the intention-to-treat principle.

The identification of potential determinants of EDC exposure and the association between prenatal/postnatal exposure to EDCs and outcome variables in the intervention and control group as well will be evaluated using mixed-effect regression models (linear or logistic, depending on the nature of exposure and outcome variables), adjusted for confounders.

"The Statistical Analysis will describe the participant populations to be included in the analysis, as well as procedures and statistical methods for conducting the analysis. This section provides a summary of the planned statistical analysis of the primary and secondary endpoints (if applicable).
Describe the statistical methods to be employed. Stipulate the level of significance that is to be used in each Trial analysis, together with the procedure(s) for accounting for any missing, unused, and/or spurious data"

Endpoint	*Statistical Analysis Methods*
Descriptive variables, covariates, sensitivity analysis.	Descriptive statistics will be used to calculate central tendency measures and frequencies of sociodemographic characteristics and other covariates, EDC concentrations in the different biological matrices, and outcome variables (anthropometric measures, IQ, hormone levels, etc).
ANALYSIS OF EFFICACY VARIABLES INTER-GROUP EFFECTS (INTERVENTION VS CONTROL)	The analysis will be performed with the intention to treat (ITT) for all variables except for the main variable, which will also be analyzed by PP (per protocol). 1. VARIABLES COLLECTED IN DIFFERENT VISITS (except intestinal microbiota).

	- MIXED MODEL ANALYSIS (evaluate the effects over time between the two groups -intervention vs control-): Check the normality of the residues. Check if covariates should be added (for example age). Include intervention as a factor in addition to age and other covariates. • If we find a time effect, the variable changes over time regardless of the intervention. • If we find an intervention effect, the variable is different between interventions regardless of the moment (time) in which it is analyzed. • If the time x intervention interaction is statistically significant, it means that the intervention effect changes over time. Carry out a post hoc at each time (Bonferroni). Represent the variables in a line graph by groups. - ANCOVA ANALYSIS at each visit: analyze the intervention effect at each time using an ANCOVA for each visit. As covariates, values at baseline, age and if there are other covariates will be included. Post hoc Bonferroni. - ANCOVA ANALYSIS of the change: analyze the intervention effect at each time using an ANVOCA model with the dependent variable the value of the change in the variable vs baseline and the inter-subject factor the intervention. As covariates, values at baseline, age and if there are other covariates will be included. Post hoc Bonferroni. - STUDENT T TEST ANALYSIS: if in the previous analyses no significant changes are observed between interventions, try performing the test between groups to detect residual differences. Represent the variable change between visits in a bar graph.

INTRA-GROUP EFFECTS (WITHIN THE INTERVENTION GROUP AND WITHIN THE CONTROL GROUP)	The intra-group effects on the different variables between the different visits will be analyzed using a Student t test or Wilcoxon test (the latter, in the case of paired samples). Indicate the mean and SD values in a table for each group and indicate the significant values of the intra-group analyses.

8. DATA MANAGEMENT

Data management aspects (data standards, type of data capture, verification of data, central data collection, cleaning, analysis, reporting, security)

Approval for the proposed study will be requested from the medical ethical board of the four institutions. For participation in the study, informed consent must be signed, after receiving clear information about the study on paper and by an informative phone call in which study details are explained very carefully. All participants have the right to discontinue their participation in the study at any time without further explanation.

All data will be treated confidentially, and privacy of all participants will be protected by saving personal data separately from research data on encrypted files with identification numbers that can only be accessed by the main researchers working on this project. The file containing personal data will be destroyed after data collection has been completed. Data about the usage of the application will not contain IP address information and therefore it cannot directly identify its user and related personal information. Data management and agreements to transfer data/samples is dealt in WP6.

An electronic eCRF will be used to register the clinical data: REDCap, fully compliant with 21 CFR Part 11, GDPR, FISMA; and HIPAA. This is a secure web application specifically geared to support online and offline data capture for research studies and operations. REDCap can be used to collect virtually any type of data in any environment from anywhere in the world over a secure web connection with authentication and data logging.

The data compiled in REDCap are all those already characterized as maternal and infant variables aforementioned in this document.

The participant's name and other identifiers will be stored separately from the research database to ensure anonymity. Personal data in the database will not be identifiable, an automatically created patient id number is used.

9. DISSEMINATION PLAN

The HYPIEND Plan for Dissemination and Exploitation and Communication (P-DEC D5.1) comprises a set of measures and actions tailored per target groups aimed at:

►maximizing the outcomes, impacts and resonance of HYPIEND by disseminating.

► raising awareness and contributing to knowledge and capacity building and advancement of innovation in EU and to avoid duplication of research and demonstration work.

► paving a solid path for the continuous and future uptake of the results across the relevant European (and international) communities and beyond the HYPIEND market applications.

Specific dissemination activities for HYPIEND project are detailed in the table below.

A) External events like conferences and fairs

Key motivation: present scientific + technological concepts, approaches, results, and their impact through paper/poster presentations or workshop organizations.
Target audience: TG#1, 3, 4, 6 policy makers, industry, researchers incl. students.
Target events: European Congress of Endocrinology (ECE); European Society of Human Reproduction and Embryology (ESHRE), EUROTOX, Conference of the European Health Psychology Society, Zebrafish Disease Model Society, and/or the International Society for Fish Endocrinology; International Congress of Fish Biology; Obstetric congresses, The International Conference on Chemicals Management (ICCM), The International Conference on Environmental Science and Technology (ICEST), The European Endocrine Disruptors Exchange (EEDE), Gordon Research Conference on Environmental Endocrine Disruptors, The International Society of Environmental Epidemiology Conference (ISEE), The International Symposium on Environmental Pollution and its Impact on Life in the Mediterranean Region, The International Conference on Environmental Pollution and Health, The International Workshop on Environmental Toxicology (IWET), European Society for Environmental and Occupational Medicine (EOM) Conference, Society of Environmental Toxicology and Chemistry (SETAC) Europe Conference, European Congress on Endocrinology, International Symposius on Endocrine Disruptors.
KPIs:>30 events participated (talks, posters, display of materials) reaching **> 4,500 professionals**.
Post project: inclusion of HYPIEND results in >10 presentations in key fora after project's end.

B) Scientific Publications

Key motivation: exchange scientific knowledge, provide technical details and share open data; Publication of HYPIEND research into Zenodo and Research Gate.
Target audience: researchers and academics.
Target publications: Endocrinology; Neuroendocrinology; Lancet Diabetes & Endocrinology; Journal of Clinical Endocrinology &Metabolism; Nature Clinical Practice Endocrinology & Metabolism; Archives in Toxicology, Health Psychology, Behavioural Medicine, Applied Psychology, Health and Well-Being, Zebrafish, Frontiers in Physiology, Frontiers in Cell and Developmental Biology, Human Reproduction, Am J Obstet Gynecol, Eur J Obstet Gynecol Reprod, Journal of Clinical and Diagnostic Research, Environmental Health Perspective, Journal of Epidemiology and Community Health, Environmental Science & Technology, Journal of Environmental Science & Health Part C, EMBO Reports, Endocrine, Metabolic & Immune Disorders - Drug Targets, Toxicological Sciences, Environmental Pollution, Chemosphere, Environmental Toxicology and Pharmacology, Biomarkers, Journal of Applied Toxicology, Neurotoxicology, Journal of Clinical Endocrinology & Metabolism, Molecular and Cellular Endocrinology.
KPIs: **100% >20 peer-reviewed open-access** publications submitted. Via gold or green access. **>5 articles** in key specialised EU magazines.
Post project: **>5 papers** following or citing HYPIEND research.

C) Training, co-creation and knowledge transfer activities

Key motivation: co-organise webinars/workshops with other research groups, students and professionals of the endocrinology, toxicology, and molecular epidemiology sectors, provide detailed insights in key outcomes and methodologies to academic and analytical lab. professionals; inclusion of PROJECT results to existing training programmes.

KPIs: 2 e-learning lectures (M24-M26) targeting academia (researchers, students incl. PhDs and post-docs) on WP1 and WP2 - KUL & EUT-, **inclusion of project results in existing training courses/activities** - KTH, RAD, UGR - 5 **workshop with interventional studies target audience to raise awareness on EDC** - (M24 - SCI, IGTP, KUL, CPKE), **final event** (M60) engaging ≥100 professionals (ALL TG).

D) Dissemination towards policy makers and public authorities

Key motivation: workshops to bring together policymakers and scientists to discuss the project findings and implications for policy and action. This can help inform decision-making and ensure that project findings are integrated into policy and regulatory frameworks. Participate in policy and regulatory processes (public consultations and meetings to share project findings and recommendations with policy makers and regulators. This will support integration of HYPIEND results into policy debates.

Target audience: TG#1, 6 policymakers, external experts participating in regulatory and policy making;

KPIs: 9 dissemination actions with policymakers and public authorities, 27 policymakers and public authorities' feedback through feedback forms.

Post project: Keeping alive the policy permanent hub to maintain free access to results, and knowledge base generated in the project**.**

E) Clustering Activities and joint dissemination

Key motivation: exchange news and ideas in common outreach actions, network and share knowledge on research,clustering activities with other projects, usage of European Communication Channels (EC newsletters, Cordis, etc.), creation of community of interest around the HYPIEND to disseminate, collaborate and share knowledge.

Target audience: TG3, TG6 mainly: related EU Initiatives funded under the current call, industrial associations, academia and researchers;

KPIs: ~ 10 dissemination actions with EU initiatives and sister projects, contact at least 15 EU Initiatives/projects and making **cooperation agreement with at least 5 of them**, engaging with **+10 endocrine and nutrition associations** across Europe, **+4 informative newsletters**.

Post HYPIEND: participation in network, cluster, association events upon invitation, offering availability to be part of advisory boards or working groups.

10. BIBLIOGRAPHIC REFERENCES

1. O'Shaughnessy KL (2021). Perinatal exposure to endocrine disrupting chemicals and neurodevelopment: How articles of daily use influence the development of our children. Best Pract Res Clin Endocrinol Metab. 35(5):101568.
2. Heindel JJ (2022). Obesity II: Establishing causal links between chemical exposures and obesity. Biochem Pharmacol.199:115015
3. Schulz MC (2021). Inappropriately sweet: Environmental endocrine-disrupting chemicals and the diabetes pandemic. Adv Pharmacol. 92, 419-456.
4. Kim Y (2011). Prenatal exposure to phthalates and infant development at 6 months: prospective Mothers and Children's Environmental Health (MOCEH) study. Environ Health Perspect. 119(10):1495-1500.
5. Ellsworth L. (2020). Lactational exposure to polychlorinated biphenyls is higher in overweight /obese women and associated with altered infant growth trajectory: A pilot study. Curr Res Toxicol.1:133-140.
6. Braun JM (2017). Early-life exposure to EDCs: role in childhood obesity and neurodevelopment. Nat Rev Endocrinol. 13(3):161-173
7. Martin L (2022). Lifestyle interventions to reduce endocrine-disrupting phthalate and phenol exposures among reproductive age men and women: A review and future steps. Environ Int. 170:107576.
8. Sargis RM (2019). Interventions to Address Environmental Metabolism-Disrupting Chemicals: Changing the Narrative to Empower Action to Restore Metabolic Health. Front Endocrinol (Lausanne). 10:33.
9. Glanz, K. (2008). Health behavior and health education: theory, research, and practice. John Wiley & Sons.
10. El Ouazzani H (2021). Perinatal Environmental Health Education Intervention to Reduce Exposure to Endocrine Disruptors: The PREVED Project. International Journal of Environmental Research and Public Health;19(1):70.
11. Christopher J. Carpenter (2010) A Meta-Analysis of the Effectiveness of Health Belief Model Variables in Predicting Behavior, Health Communication, 25:8, 661-669
12. Christopher J. Armitage & Mark Conner (2000) Social cognition models and health behaviour: A structured review, Psychology & Health, 15:2, 173-189
13. Sheeran P (2016). The Intentation-Behaviour Gap. Social and Personality Compass, 10:9, 503-518
14. Rovira J. (2022) Mixture of environmental pollutants in breast milk from a Spanish cohort of nursing mothers. Environ Int 2022 Aug: 166:107375.
15. Iribarne-Durán LM (2022). Concentrations of bisphenols, parabens, and benzophenones in human breast milk: A systematic review and meta-analysis. Sci Total Environ. 806(Pt 1):150437.
16. Cao C (2021). Prenatal exposure to polycyclic aromatic hydrocarbons could increase the risk of low birth weight by affecting the DNA methylation states in a Chinese cohort. Reprod Biol. 21(4):100574.
17. Gingrich J (2020). Placenta Disrupted: Endocrine Disrupting Chemicals and Pregnancy. Trends Endocrinol Metab. 31(7):508-524.
18. Kim JH. (2021). Web-based behavioral intervention to reduce exposure to phthalate metabolites, bisphenol A, triclosan, and parabens in mothers with young children: A randomized controlled trial. Int J Hyg Environ Health; 236: 113798.
19. Caporale N. (2022). From cohorts to molecule: adverse impacts of endocrine disrupting mixtures. Science, 375-6582
20. Raja GL (2022). In utero exposure to endocrine disruptors and developmental neurotoxicity: Implications for behavioural and neurological disorders in adult life. Environ Res.203:111829.
21. Bozack A. K. (2021). Prenatal metal exposure, cord blood DNA methylation and persistence in childhood: an epigenome-wide association study of 12 metals. Clinical Epigenetics, 13-208
22. Guo J. (2022). Association Studies of Environmental Exposures, DNA Methylation and Children's Cognitive, Behavioral, and Mental Health Problems. Frontiers in Geneticics, 13-871820
23. Herbstman, JB. (2012). Prenatal Exposure to Polycyclic Aromatic Hydrocarbons, Benzo[a]pyrene–DNA Adducts, and Genomic DNA Methylation in Cord Blood. Environmental Health Perspectives, 120-5.
24. Heijmans BT (2008). Persistent epigenetic differences associated with prenatal exposure to famine in humans. Proc Natl Acad Sci USA;105(44):17046-9.

ANNEX 1: INFORMATIVE LEAFLET FOR PARTICIPANTS IN CLINICAL RESEARCH STUDIES

GUIDE FOR WRITING THE PATIENT INFORMATION SHEET AND INFORMED CONSENT FOR INTERVENTION STUDIES

FIP-CI_CEIC_V.1.0 (13/01/2021)

The Guide that is presented below is an adaptation of the "Guide for the correct preparation of a patient information sheet and informed consent (HIP / CI) model" that is used for clinical trials with drugs that are carried out in Spain"

To make it easier for the patient to read, the document must not exceed 15 pages (including consent forms or signature sheets). It is recommended to use a minimum font of 11 points, with a minimum interline spacing of 1.5, without reducing the margins of the document. For each section, the minimum essential content and recommendations on the appropriate content are indicated. Writing examples are included in specific sections.

A version and a date must be recorded at the foot of the document.

TITLE	Understanding and preventing the impact of endocrine disruptors on the hypothalamic-pituitary axis in sensitive populations Clinical study to evaluate the effectiveness of a multicomponent behavioral intervention to reduce exposure to endocrine disruption during the perinatal period in women and their offspring.
PROMOTOR	Institute of Research Germans Trias i Pujol (IGTP)
PROTOCOL CODE	HYPIEND- 101137440

PRINCIPAL INVESTIGATOR	Inés Velasco López		
PHONE	934978525	**MOBILE**	696914449

CENTRE	University Hospital Germans Trias i Pujol				
DEPARTMENT	Obstetrics % Gynecology				
CENTRE ADDRESS	Carretera de Canyet s/n				
CITY	Badalona	**ZIP**	08916	**PROVINCE**	Barcelona

1. Introduction

We are contacting you to inform you about a research study in which you are invited to participate. The study has been approved by a Research Ethics Committee. It is our intention that you receive the correct and sufficient information so that you can decide whether or not to participate in this study. For this, read this information sheet carefully and we will clarify any doubts that may arise. In addition, you can consult with the people you consider appropriate.

2. Voluntary Participation

We invite you to participate in the study because you are pregnant and belong to the group of patients without risk factors who are visited at Obstetrics consultations. You should know that your participation in this study is voluntary and that you can choose NOT to participate. If you decide to participate, you can change your decision and withdraw consent at any time, without this altering your relationship with your doctor or causing any harm to your medical care.

3. Study Objective

The general objective of HYPIEND is to understand the effects of exposure to substances known as endocrine disruptors (EDs) on maternal-child health (in general) and on hormonal functions (in particular), in order to outline intervention strategies for minimize exposure and consequences for the mother and her offspring during the perinatal period.

The main objective of this clinical study is to demonstrate that a multicomponent intervention implemented in care centers in three European countries is effective in reducing the levels of these disruptors in different body fluids of pregnant, lactating and formula-fed women, as well as in their offspring up to 18 months after birth, improving at the same time the level of knowledge of these chemical substances at family level.

The secondary objectives are:

a) Demonstrate that this multicomponent intervention designed to reduce DE exposure is effective in preventing some of the harmful effects related to disruption of the hypothalamic-pituitary hormone axis (HPH) in infants up to 18 months (weight, growth and alterations related to neurodevelopment).

b) To provide a better understanding of the relationship between the concentrations of endocrine disruptors in different fluids and the levels of EHH-related hormones, cytokines, DNA methylation and gene expression patterns in the blood and the composition of the intestinal microbiota, shedding more light on the mechanisms by which Des can exert their harmful effects.

c) Explore through multivariate analysis and health risk assessment the predictors of high levels of disruptors in body fluids (urine, blood and milk).

4. Description of the study

A detailed description of this study can be found as Appendix 1 to this document.

HYPIEND aims to evaluate a perinatal intervention in a 2-arm randomized controlled trial (RCT), with

a) An experimental group, which will receive a multicomponent intervention (digital tool aimed at promoting lifestyle habits to reduce exposure to disruptors through personalized recommendations + telephone follow-up + environmental health education workshops) during pregnancy and first 18 months after delivery (Figure 1).

b) A control group, whose participants will receive a single module of online education/written information about endocrine disruptors aimed at reducing their exposure, but this group will not receive any feedback from professionals about endocrine disruptors throughout the study (Figure 1).

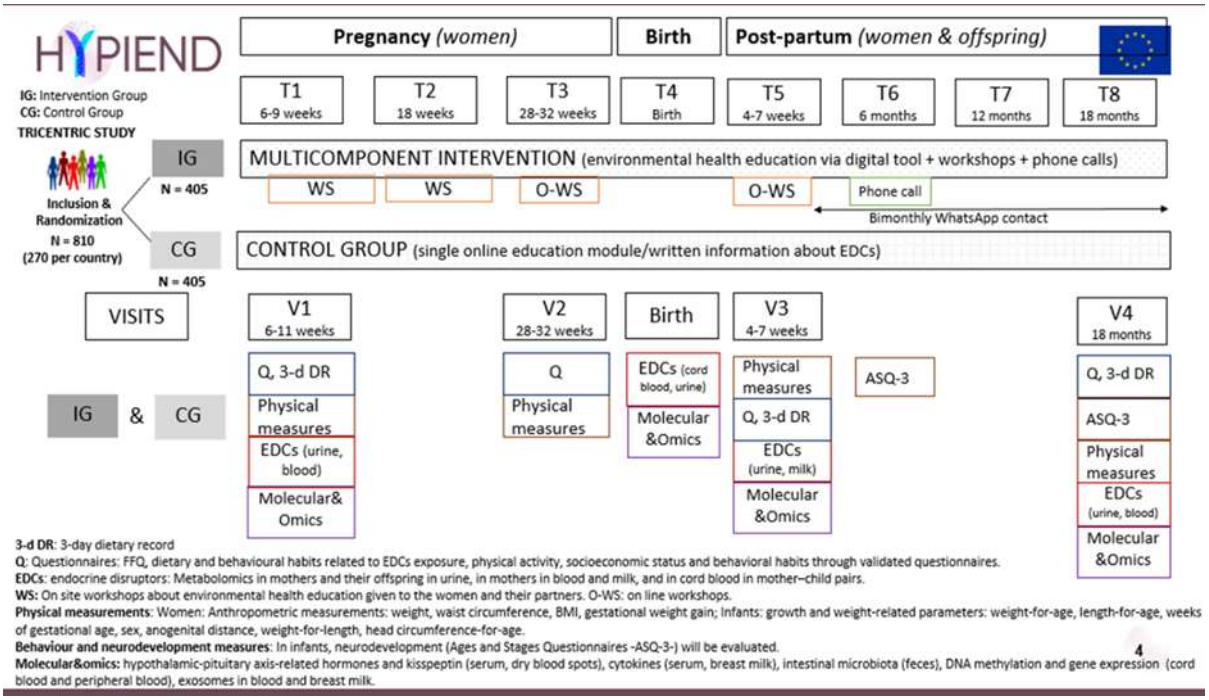


Figure 1: Study Design and Follow-up of participants

- <u>The participants in the study will be called at the times described in the protocol</u>, in order to verify the correct monitoring of the pregnancy in general and the research protocol in particular. All the variables and information about your pregnancy will be collected through surveys and questionnaires that you will fill out through the mobile application.

- We will not need access to your medical history to obtain data related to the pregnancy (age, weight, birth data) as we will collect them through visits and surveys. Likewise, the collection of biological samples (blood and urine) will be carried out by the health personnel who will attend each medical visit or hospital stay.

- This study will be carried out entirely at the Multipurpose Clinical Research Unit (UPIC) of the Germans Trias i Pujol University Hospital. The study will last 3 years with a recruitment period of 24 months for pregnant women and follow-up of pregnancy and childbirth, and of the newborn during the first 48 hours of life, as well as follow-up up to 18 months age of the boy/girl.

5. Study actions. What can your participation entail?

- Participation will not lead to any change in your treatment or in the rest of your medical care.

  The data will be included in a Data Collection Notebook in digitized format. All pregnant women, both those in the intervention group and those in the control group, will receive exactly the same healthcare from healthcare professionals: gynecologists and/or midwives.

6. Potential benefits

You should know that we cannot guarantee or promise that inclusion in the study will bring you any benefit, although scientific knowledge may be obtained that may benefir other people later.

All this data will be collected separately from the medical history and the "Pregnancy Card". The identity of pregnant women and their newborns is confidential and will only be known to the principal investigator and his collaborators, so all study information will be stored with sufficient safeguards. The data obtained in this study will be used exclusively to obtain scientific conclusions.

7. Expenses and financial compensation

Your participation in the study will not incur any additional costs. You will not be paid to participate.

8. Obtaining and using biological samples

In the event you give your authorization, this bilogical material will become part of the hospital's biobank of biological samples, called the IGTP-HUGTP Biobank. Even so, you or your family will be able to dispose of the samples when necessary for health reasons, as long as they are still available.

In any case, you (minors, through their guardian or legal representative) can exercise their rights of consultation, cancellation or opposition, and also obtain information about the research projects in which used their samples, going to:

Responsible for the IGTP-HUGTP Biobank file:

Fundació Institut d' Investigació en Ciències de la Salut Germans Trias i Pujol
Carretera de Can Ruti. Camí de les Escoles s/n
08916, Badalona. Espanya.
Phone 93 497 8653/8658
Electronic address: biobanc@igtp.cat ; igtp@igtp.cat

The donated samples may only be used for biomedical research purposes, in studies always approved by the Research Ethics Committee of the HUGTP and in accordance with current laws. The samples are coded for their use and the associated clinical data will be guarded in accordance with current legislation in order to guarantee confidentiality. In the event that the samples have not been anonymized, only the researchers and authorized persons who guarantee their confidentiality will have access to the personal data.

The Biobank may transfer data and samples to researchers from other centers, but always anonymously. The assignment must be approved by the Clinical Research Ethics Committee. The transfer of biological samples that you make to the Biobank is free and voluntary.

In some cases, genetic studies will be carried out with the samples given, from which information relevant to your health and that of your relatives can be obtained. In this case, we will contact you using the details in your clinical record.

Naturally, your right to decide not to be informed of the results of the research in which your samples have been used will be respected.

If you decide to sign this consent, you can also freely revoke it at any time by requesting it from the file manager of the IGTP-HUGTP Biobank or your referring doctor at the hospital. The revocation of consent has no negative impact on the health care you receive or could receive. In the event of revocation, your samples deposited in the Biobank and their associated data will be destroyed. In case of acceptance, they will be kept indefinitely until their termination.

9. Confidencialy and Data protection

Both the promoter and the health center will ensure that the confidentiality of your personal data collected during the study is maintained, in compliance with national and European data protection laws. For more information on the protection of personal data, see Appendix 2 to this document

10. Contact in case of doubts

If during your participation you have any doubts or need to obtain more information, please contact the study researcher at your center, at the telephone number listed at the top of this document.

APPENDIX 1: DESCRIPTION OF THE STUDY

Recruitment: Pregnant women attending the first visit of prenatal surveillance will be invited to participate according to inclusion/exclusion criteria

Inclusion criteria (Participants should fulfil ALL these criteria)

1. Pregnant women with a viable pregnancy confirmed by ultrasound until 11 weeks and 0 days of gestation (recruitment between 6-11 weeks). Their partners will be invited to participate but this is not mandatory for inclusion.
2. BMI at Visit 1 between 18.5-40 kg/m²
3. Intention to breastfeed
4. Being able to read the language of their respective countries (Dutch/French –Belgium-, Spanish and/or Catalan –Spain- and Polish –Poland-)
5. Being 18 years or older
6. In possession of a smartphone. If participants do not have a smartphone because of socio-economic reasons, a smartphone will be at their disposal for the whole duration of the study.

Exclusion criteria (If a participant fulfils ANY of the following)

1. Unable to sign informed consent (cultural barriers, psychological conditions)
2. Abuse of substances (alcohol, drugs)
3. Chronic use (at least, for three months before pregnancy) of any medication that might affect the HP axis:
   - Antidepressants
   - Insuline
   - Levothyroxine, Methimazole, Propylthiouracil.
   - Oral corticosteroids (topical and inhalation formulations will be allowed)
   - Arginine vasopressin (AVP)
   - Mifepristone.
   - Anticortisolic drugs: Metyrapone (Metopirone), Ketokonazole, Osilodrostat (Isturisa), Mitotane (Lysodren), aminoglutethimide (Cytadren) and Levoketoconazole (Recorlev).
4. Multiple gestation
5. Type 1 or 2 diabetes

Randomization: Once confirmed that the pregnant woman fulfils criteria to participate in the study and agrees to do so by signing the Informed Consent, we will allocate her in one of the two groups (control vs intervention) through a randomization procedure.

Based on randomization numbers, the allocation ratio will be 1:1 for the interventional group and the control group. Investigators in charge of the analysis of the data will be blinded for the condition in which the participants are randomized.

There will also be an emergency unblinding module in case of any serious adverse event either by subject number or by group assigned. This module can only be accessed by sponsor and statistician.

Intervention:

A multicomponent behavioral intervention during pregnancy and up to the first 18 months after delivery, composed by:

1. Digital tool called "Hypiend Digital Tool" aimed at promoting lifestyle habits to reduce EDC exposure by providing personalized recommendations
2. Telephone monitoring
3. Workshop about environmental health education

1. ***<u>Digital tool ("Hypiend Digital Tool")</u>***

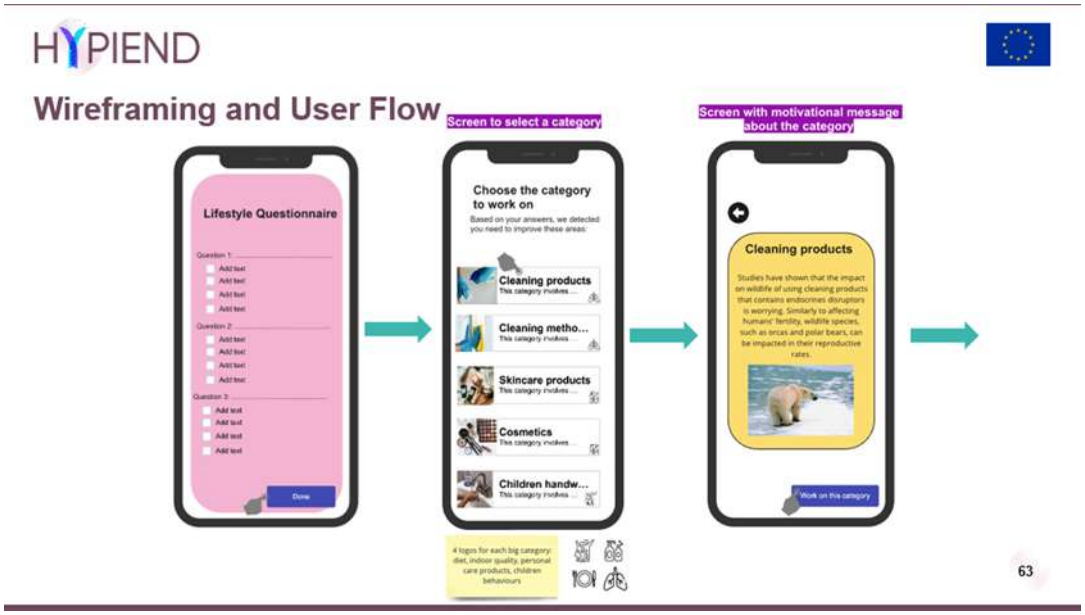
Women will have available a mobile app developed by EURECAT and designed to limit the exposure to EDCs in women from pregnancy until their children are 18 months old in a personalized and gamified way. Content of the app is built by integrating the HAPA constructs using behaviour change techniques. After answering an initial questionnaire about lifestyle habits related to EDC exposure, the app will provide them personalized missions considering those habits with a higher risk to expose them to EDCs. By selecting these missions successively, they will receive frequent recommendations, tips and motivational quotes that will help them to accomplish the selected mission. For example, graded tasks will be used to increase task self-efficacy (i.e., initiate the change). Providing tools to conserve mental resources will be used to increase maintenance self-efficacy (i.e., once the change started and needs to become a habit). Action planning and problem solving will be used to help intenders become actors.

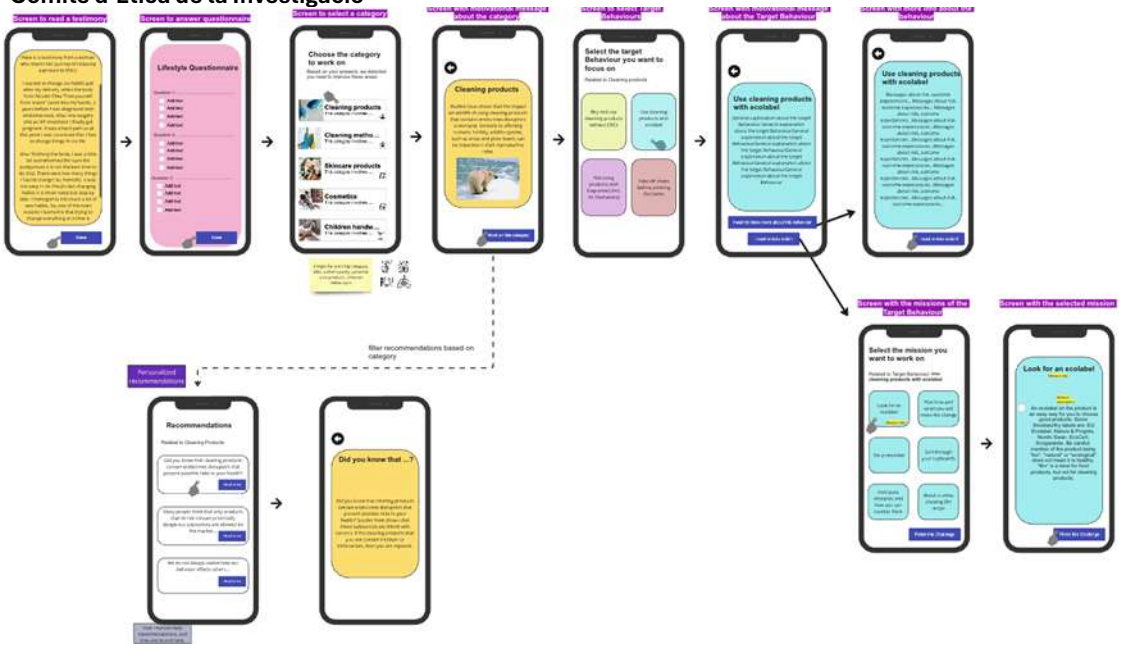
The application has been developed with two different versions:

- one for professionals/researchers and
- another for participants/end users.

The components of this application:

- Sign in up and log in
- Home page
- Questionnaires
- Missions (only the intervention group)
- Recommendations (only the intervention group)
- Push notifications
- Forum/chats





Components of the backoffice (exclusively for researchers):

- Questionnaires that the clinician must answer with the patient
- Visualization of questionnaire responses.
- Forum/chat
- Exportation of responses

2***. Telephone monitoring: To provide personalized support and feedback***

- From 6 weeks postpartum will be contacted every two months.

- Moreover, the researchers will contact them via phone call 6 months after delivery aimed to encourage the adherence to the intervention and to solve potential doubts.

- In addition, parents will be contacted to explain how they should fill out the neurodevelopment questionnaire, which they must send us in the coming days.

- If a long period of inactivity is detected at any point of the study, the researchers will contact the families by phone.

3. Workshops

An interdisciplinary group of experts on EDCs (paediatric endocrinologists and environmental epidemiologists), psychologist expert on behaviour change, and nutritionists present in the consortium (University of Granada, Hospital Germans Trias i Pujol, University of Geneva, and Eurecat) will design and develop the content of the workshops.

Two onsite workshops will be conducted during the first half of pregnancy (before 20 weeks of gestation).

Moreover, a third and a fourth session will be proposed to attend online at 30 weeks of gestation and 6 weeks after delivery, respectively. They will also be recorded and sent as videos for those participants who did not attend.

The workshops will be focused on four themes:

(i) definition of EDCs and their adverse health effects

(ii) dietary sources of EDCs,

(iii) indoor air quality, and

(iv) personal care products as a source of EDC exposure.

These workshops will be imparted by experts on EDCs and by personnel of each institution that will be trained to give the workshop using theoretical background of the HAPA frame. After conducting each workshop, the key messages of the session will be sent by phone as a reminder.

Partners will be encouraged to join women during the medical visits and the environmental health education workshop.

CONTROL GROUP:

The control group will only receive a digital printable booklet with information on EDC hazards and on strategies to limit their exposure. After finishing the intervention, the participants of the control group will be offered access to all the developed material (workshops…) and to use the app with all the functionalities. Participants from different environmental areas will be recruited to account for different exposures to EDCs.

FOLLOW-UP AND TIMING OF VISITS

Visit 1: On-site, First trimester, from 6 weeks to 11 weeks and 0 days of gestation.

Visit 2: On-site, Third trimester, 28- 30-32 weeks of gestation.

Delivery/Birth

Visit 3: On-site, between 4th and 7th weeks after delivery.

Visit 4: On-site, at 18 months after delivery.

Sample Collection

	Visit 1	BIRTH	Visit 3	Visit 4
Mother	Blood Urine Faeces	Blood Urine Faeces	**Human milk**	Blood Urine Faeces
Baby		Cord blood Faeces	**Urine**	Blood** Urine Faeces

APPENDIX 2. INFORMATION ABOUT PERSONAL DATA PROTECTION

Apx1 _PD_ceic_13/01/2021

TITLE	Understanding and preventing the impact of endocrine disruptors on the hypothalamic-pituitary axis in sensitive populations Clinical study to evaluate the effectiveness of a multicomponent behavioral intervention to reduce exposure to endocrine disruption during the perinatal period in women and their offspring.
PROMOTOR	Institute of Research Germans Trias i Pujol (IGTP)
PROTOCOL CODE	HYPIEND- 101137440

What data will be collected during the study and what will it be used for?

During your participation, the following data obtained from the questionnaires you fill in will be collected, as well as data related to the monitoring of the pregnancy, its evolution and the treatment you have received that we will collect at the visits. Only the data necessary to carry out the study will be collected.

The promoter will use this data exclusively to carry out the study. The promoter could also reuse these data for other research related to environmental exposure to endocrine disruptors under study, as long as they have a favorable report from an ethics committee or data protection expert. In this case, the researchers will contact the participants to inform them and ask for their permission.

Who is responsible for the processing of your data in this study?

In this study, both the Promoter and the health center are responsible for the treatment of your data. The controller's role is to ensure that your information is used correctly. The promoter and the center will comply with the data protection regulations:

-Regulation (EU) 2016/679 of the European Parliament and of the Council of April 27, 2016 (RGPD) relating to the protection of individuals with regard to the processing of personal data and the free movement of such data data
- The Organic Law 3/2018 of December 5, on the protection of personal data and guarantee of digital rights (LOPDPGDD)

How will your data be treated in order to maintain confidentiality?

During your participation you will be identified by a code. The list that relates the code to the data that identifies it (name, surname, medical history number, etc.) will be kept in your health center (guarded by the research team), and will not leave it. Specific security measures will be adopted to prevent re-identification and access by unauthorized third parties.

Access to your personally identifiable information will be restricted to the principal investigator of the study and his collaborators, to health authorities if necessary, to the Research Ethics Committee and to personnel authorized by the promoter, when necessary to verify the proper functioning of the study; but always maintaining confidentiality. Your identity could be revealed in exceptional cases, such as medical emergency situations for your health or legal requirement.

When there is an external Sponsor at the center: Neither the investigator nor the hospital will transfer any information to the Sponsor that can directly identify him. The Promoter will receive the pseudonymized data and will not have access to the code that allows you to relate your data to you.

How long will your data be kept?

Your data will be kept only for the time necessary to carry out the project. In addition, according to the Data Managment Plan of this study, there is an obligation to keep all the information generated by this study for a period of 10 years.

What rights do you have in relation to your data? Who can you contact?

You can ask at any time what data is being collected, who is using it and for what purpose (right of access). You can request a copy of your personal data for your own use or to pass it on to others (portability). You can correct and delete incorrect data (right of rectification and deletion), limit its use or oppose it (rights of limitation and opposition).

Some of these rights may be limited to ensure the validity of the research. You may have limited access to the data until the investigation is complete. If you withdraw your consent to the processing of the data, the data collected up to that point cannot be deleted and you may not be able to continue participating in the study.

You have the right to file a claim with the Data Protection Authority for any action by the Promoter or the Center that you consider infringes your rights.

If you wish, you can also contact the data protection officer (DPO) of your hospital, or the data protection officer of the promoter:

Center DPO contact details: Iris Bargalló Arraut
Responsable de Protecció de Dades
Germans Trias i Pujol Research Institute (IGTP)
Carretera de Can Ruti, Camí de les Escoles s/n
08916 Badalona, Barcelona, Spain
ibargallo@igtp.cat

Promotor DPO contact details: Catalan Health Institute (ICS)

dpd.ticsalutsocial@gencat.cat

Will your data be shared and transferred?

International transfers of data are not foreseen, but in the event that they occur, it will only be to countries that guarantee adequate compliance with data protection regulations for the existence of an adequacy decision or any other legally enabled mechanism.

ANNEX 2: INFORMED CONSENT FORM

TITLE	Understanding and preventing the impact of endocrine disruptors on the hypothalamic-pituitary axis in sensitive populations Clinical study to evaluate the effectiveness of a multicomponent behavioral intervention to reduce exposure to endocrine disruption during the perinatal period in women and their offspring.
PROMOTOR	Inés Velasco López Department of Obstetrics & Gynecology Hospital Universitari Germans Trias i Pujol
PROTOCOL CODE	HYPIEND- 101137440

I, ... (First and Last name)

I have read the information sheet that was given to me.

I was able to ask questions about the study.

I have received enough information about the study.

I have spoken to: ..... (Name and surname of researcher) I understand that my participation is voluntary.

I understand that I can withdraw from the study:

1st whenever I want

2º without having to give explanations

3º without this affecting my medical care.

I freely give my consent to participate in the study and I give my consent for the access and use of my data under the conditions detailed in Appendix 1. INFORMATION ON PROTECTION OF PERSONAL DATA.

Participant's signature	Researcher's signature
Date:	Date: