Some Title

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# Abstract

**Background**

**Objectives**

**Methods**

**Results**

**Discussion**

* Authorship must follow the ICMJE’s criteria. Author names should be listed in ScholarOne and author contributions should be detailed in the cover letter (“Author A…”). Neither names nor contributions should appear in the blinded manuscript. Do not include conflicts of interest.
* The use of the word “effect(s)” as a proxy for “association(s)” is discouraged.
* The length of the abstract must be less or equal than 200 words, and should be unstructured, stating the research questions, the methods used, and the results and conclusions of the research.
* The length must be <4,000 words, excluding abstract, references, tables, figure captions, acknowledgments, and Supplementary Material.
  + Use the AMA format.
  + In-text citations: full-sized Arabic numerals in parentheses within the sentence.
* Submission: SER members receive a 10% discount on the fees per page. This should be requested in the cover letter. Also 20% discount for Open Access charges. If uploading a single file (document, tables, figures, SM), designate the file as “Main document - anonymous”.

# Introduction

**Do not use “Introduction” as a heading**.

## Background and rationale

* Brief review of the literature to summarize current knowledge.
  + Acknowledge inconsistencies.
  + For each study, indicate whether it was observational or experimental, and note key characteristics of study populations or experimental models.
* Explain the scientific background and rationale for the investigation being reported.
  + Identify knowledge gaps addressed by the current study.
* Provide context for the study: include information on exposures and outcomes, and why they are relevant to environmental health.

## Objectives

* Provide a clear description of the study hypotheses/aims/objectives, and eventually an overview of the approach used to address them.

# Methods

## Study population and design

The Human Early-Life Exposome (HELIX) is an ongoing project which aims to characterize early-life exposures and their potential association with endogenous biomarkers and health outcomes[1](#ref-VrijheidSlamaRobinson:2014). It consists of six existing population-based birth cohort studies across Europe: BiB (Born in Bradford, UK)[2](#ref-WrightSmallRaynor:2013), EDEN (Study of determinants of pre- and postnatal developmental, France)[3](#ref-HeudeForhanSlama:2016), INMA (Environment and Childhood, Spain)[4](#ref-GuxensBallesterEspada:2012), KANC (Kaunas Cohort, Lithuania)[5](#Xd30c40380c9e99bac70b7fa3b0ada5ae8dec3e4), MoBa (The Norwegian Mother and Child Cohort Study, Norway)[6](#ref-MagnusIrgensHaug:2006), and Rhea (Mother–Child Cohort in Crete, Greece)[7](#ref-ChatziPlanaDaraki:2009), for a total of 32,000 mother-child pairs. A HELIX subcohort of 1,200 mother-child pairs was fully characterized for the external and internal exposome, including exposure and omics biomarkers during childhood. Eligibility criteria for inclusion in the HELIX subcohort included: a) age 6-11 years, with a preference for 7-9 years; b) availability of sufficient stored pregnancy blood and urine samples; c) availability of complete address history from first to last follow-up; d) no serious health problems, which might affect the results of the clinical testing. Further information can be found in[8](#ref-MaitreBontCasas:2018).

Ethical permission was obtained from the relevant authorities in the corresponding country.

## Variables

### Confounders

For each research question, defined by a specific type of exposure and outcome, the minimal set of covariates for inclusion in the analyses was selected on the basis of a directed acyclic graph (DAG) built with DAGitty[9](#ref-TextorvanderZanderGilthorpe:2016) and ggdag[10](#ref-Barrett:2023). The sets of covariates were selected to estimate the total effect of the exposure on the outcome. Further, each minimal adjustment set was *augmented* with precision covariates, defined as the set of parents variable of the outcome that are not parents of the exposure. The adjustment sets are provided in the Supplementary Material as text files compatible with DAGitty.

* For RQ1 I used creatinine values from HELIX. For RQ3 the ones from the steroids dataset. For RQ2, I included in the model both variables.

### Endocrine disrupting chemicals

Children were assessed between December 2013 and February 2016, and included neurological testing and urine collection. Urine samples of the night before and the first morning void on the day of the visit were combined to provide a more reliable exposure assessment. Non-persistent endocrine disruptors (EDCs) assessed in urine samples from children included phthalate metabolites, phenols, and organophosphate (OP) pesticide metabolites. A list of the environmental chemicals determined in urine samples and used for the present study is given in**?@tbl-info-chems**. The laboratory protocols for the analysis are described elsewhere[11](#ref-HaugSakhiCequier:2018).

### Corticosteroids

Urine samples of the night before the day of the visit were used to measure levels of the corticosteroids. These included glucocorticosteroids, glucocorticosteroid metabolites, glucocorticosteroid precursors, glucocorticosteroid precursor metabolites, androgens, and androgen metabolites. A list of the corticosteroids determined in urine samples and used for the present study is given in**?@tbl-info-mets**.

To assess the levels of corticosteroids and their metabolites, LC-MS/MS analysis was applied at the Applied Metabolomics Research Group, IMIM (Hospital del Mar Medical Research Institute). The laboratory protocols for the analysis are described elsewhere[12](#ref-MarcosRenauCasals:2014),[13](#ref-Gomez-GomezPozo:2020). Of the 1,004 urine samples, 980 children were matched to the HELIX subcohort.

Three additional markers, cortisol production, cortisol metabolism, cortisone production, and 11bHSD activity, were computed based on the following: cortisol production as the sum of cortisol and its metabolites (20aDHF, 20bDHF, 5bDHF, 5aTHF, 5bTHF, 6OHF, 5a20acortol, 5a20bcortol, 5b20acortol, 5b20bcortol), cortisol metabolism as the inverse of the ratio between cortisol and its metabolites, cortisone production as the sum of cortisone and its metabolites (20aDHE, 20bDHE, 5aTHE, 5bTHE, 6OHE, 5b20acortolone, b20bcortolone), and 11bHSD activity as the ratio between cortisone production and cortisol production. 11bHSD activity gives a measure of conversion of cortisone to cortisol.

### Neurodevelopment

Neurodevelopmental outcomes were assessed with standardized, non-linguistic, and culturally blind computer tests, including the Attention Network Test (ANT)[14](#ref-RuedaFanMcCandliss:2004). Further information can be found in[8](#ref-MaitreBontCasas:2018). Briefly, it is a computerized test that provides a measure of efficiency in three different functions of attention: alerting, orienting, and executive attention. The outcome of interest for the present study is the hit reaction time standard error (HRT-SE)[15](#ref-SunyerEsnaolaAlvarez-Pedrerol:2015), a measure of response speed consistency throughout the test. A high HRT-SE indicates highly variable reactions, and is considered a measure of inattentiveness.

## Statistical methods

### Data pre-processing

Concentrations of the corticosteroids were classified as quantifiable, below the limit of quantification (LOQ), possible interference or out of range, and not detected. For each metabolite, we computed the fraction of values below the LOQ and not detected, both within each cohort and overall. We proceeded to impute these values using half the value of the corresponding lower limit of quantification (LLOQ), for those metabolites that had less than 20% of missings within each cohort and 10% of missings overall. The remaining missing values were imputed using kNN from the VIM R package[16](#ref-KowarikTempl:2016), for those metabolites that had less than 40% of remaining missings within each cohort and 30% of remaining missings overall. We used 5 nearest neighbors. Values of cortisol production and cortisone production were expressed in nanograms per millilitre, whereas values of cortisol metabolism and 11bHSD activity were unitless.

Concentrations of the non-persistent EDCs were classified as quantifiable, below the limit of detection (LOD), possible interference or out of range, and not analysed. Concentrations below the LOD were singly imputed using a quantile regression approach for the imputation of left-censored missing data, as implemented in the impute.QRILC function from the imputeLCMD R package[17](#ref-lazar2015imputelcmd). Chemicals with more than 70% of observations below the LOD were not considered in the present study. Remaining missing values were imputed similarly using kNN. Values of the chemicals were expressed in grams per litre.

Missing values in the clinical outcome were imputed similarly using kNN. We further natural log-transformed them to improve model fit, assessed with posterior predictive checks. To do so, replicated data were simulated with the fitted models and compared to the observed data. We used the check\_predictions function from the performance R package using the default arguments[18](#ref-LudeckeBen-ShacharPatil:2021). Values of the clinical outcome were expressed in milliseconds (ms).

Missing values in the covariates were imputed similarly using kNN. Categorical covariates were imputed using the maxCat function, which chooses the level with the most occurrences. Creatinine values were expressed in grams per litre.

### Estimation of balancing weights

Stabilized balancing weights were estimated using the energy method available in the WeightIt R package[19](#ref-Greifer:2023). This methods estimates weights by minimizing an energy statistic related to covariate balance[20](#ref-HulingGreiferChen:2023), thus avoiding the need to specify a parametric model. Weights below the 0.1 and above the 0.9 quantiles were trimmed. Trimming might lead to decreased covariate balance and potentially change the estimand, but can also decrease the variability of the weights. Covariate balance was assessed using functionalities provided by the cobalt R package[21](#ref-Greifer:2023a). Specifically, we used *Love* plots to visualize covariate balance before and after adjusting.

### G-computation

* In RQ2, I included the logarithm of the denominator in the RHS. In RQ3, I used the logarithm of the ratio in the RHS.
* Describe all statistical methods with assumptions.
  + Description of outcome model, estimand.
  + Description of method used to estimate effects (e.g., g-computation).
  + Description of method used for SE and CI.
* Describe any methods used to examine subgroups and interactions (sub-group analysis or moderation analysis or analysis of effect-modification).
* Describe any sensitivity analyses.
* Names and version numbers for the used software packages, including non-data arguments if deviating from the default ones.

# Results

## Participants

* Give reasons for non-participation at each stage.

## Descriptive data

## Outcome data

## Main results

* All results on which study conclusions or inferences are based, including null findings and results of secondary or sensitivity analyses, must be reported. Use of sub-headings that describe the nature of the results (but no declarative statements).
  + Provide a clear and concise description of all findings without extrapolating beyond the study results.
  + Do not limit results to those *statistically significant* or that support the study hypotheses. Avoid using statistical significance testing as the sole or primary criterion for interpreting the obtained results. If significance testing or *p*-values are used, report numeric *p*-values, rounded to 1-2 digits, for all results. Use an uppercase italic letter “P”, and the values should not be bolded. Indicate whether are 1- or 2-sided.
* Give unadjusted and confounder-adjusted estimates and their precision (e.g., confidence interval). Make clear which confounders were adjusted for and why they were included. Include the number of observations for each analysis after accounting for missing data. Include numeric data within figures (e.g., forest plots), or provide tables with corresponding numeric data for all figures.
  + [marginaleffects tables](https://vincentarelbundock.github.io/marginaleffects/articles/tables.html).
* Report category boundaries when continuous variables were categorized.

## Other analyses

* Report other analyses done (e.g., analyses of subgroups and interactions, and sensitivity analyses).

# Discussion

## Key results

* Summarise key results with reference to study objectives.
* Provide a review of the relevant literature to put the study findings into context.
  + It should be complete and balanced, including inconsistent results.
  + It should include, for each source, sufficient details: study design, sample size, population, specific exposures and outcomes.

### What does the literature say?

* EDCs and neurodevelopment (ANT).
* EDCs and corticosteroids.
* Corticosteroids and neurodevelopment (ANT).

## Limitations

* Discuss limitations of the study, taking into account sources of potential bias or imprecision.
* Discuss both direction and magnitude of any potential bias.

Some limitations:

* Cross-sectional study.
* Chemicals measured in night and morning samples, whereas metabolites (the outcome) were measured only in night samples.
* Cortisol measured at night, when should be lowest.
* Change of estimand when trimming weights.
* Model misspecification.
* Mixtures effect.
* Residual confounding.
* Some confounders were not used since large percentage of missing values.
* Multiple comparisons.

## Interpretation

* End with a summary of the key findings and their implications for the study hypotheses, future research, and policy.
* Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.

## Generalisability

* Discuss the generalisability (external validity) of the study results.

# Funding

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