

Birth Cohort Study of Environmental Exposure and Childhood Development – PIPA Project Research Plan

1. Purpose

This study aims to investigate alterations in childhood development associated with exposure to environmental pollutants, from pregnancy until the age of 4. This is a prospective cohort study with the focus on children's health issues arising from environmental chemical exposures that occur since the conception. The study population will be all children born at the Federal University of Rio de Janeiro Maternity School, Rio de Janeiro/Brazil, for a period of 12 months. The study will collect social, demographic and health information, in addition to biological samples from parents and newborns. All newborns will be followed for 48 months and their landmarks of physical, neurological, psychological, and cognitive development recorded.

This project is a partnership between the units belonging to the Federal University of Rio de Janeiro (UFRJ), including the Faculty of Medicine and the Maternity School and units belonging to the Oswaldo Cruz Foundation (Fiocruz), University of Rio de Janeiro State (UERJ) and Evandro Chagas Institute (IEC/PA).

The project was initiated in June 2021, and the study population will be constituted for all children born at Maternity School (that parents have signed the Ethical term) until July 2022.

2. Rationale for a Birth Cohort study in Rio de Janeiro city, Brazil.

As a developing country, Brazil presents a wide range of environmental risks that can constitute hazards to children's health. According to the Brazilian Institute of Geography and Statistic, more than 80% of the Brazilian population lives in urban areas. In 6 metropolitan regions, the highest concentrations of particulate matter (PM) between 1995 and 2012 were above the international standard limits. The use of pesticides, although already high, is still increasing in both rural and urban areas of all country. For instance, between 2007-2013, there was a 90,5% increase in pesticide sales in Brazil, while crop area increased 19,5%. In urban areas, these compounds are used to control vector-borne diseases. Rio de Janeiro city is well known for the use of large amounts of pesticides to control vector-borne arboviral diseases, such as Dengue, Zika, and Chikungunya. Moreover, domestic pesticides, mainly pyrethrin derived compounds, are also widely used in Rio, which may pose an additional threat to pregnant women (and their fetus), but also to newborns and young.

3. Objective

To investigate individual and combined effects of environmental chemical pollutants, as well as the interactions between these exposures and the sociocultural environment and genetic patterns, on the children's development and health.

4. Hypotheses

1. "The exposure to environmental chemical pollutants determines fetus developmental alterations, causes birth adverse health effects, and harm the child's neuromotor and cognitive development".
2. "The effects of environmental pollutants on fetus and child health are modulated by interaction with the sociocultural environment and genetic patterns".

5. Methodology

This is a prospective birth cohort study of all children born at the Federal University of Rio de Janeiro Birthing Center, Rio de Janeiro/Brazil, for a period of 12 months. These children will be periodically evaluated since birth until the age 4 by clinical and laboratory exams, according to an established schedule.

5.1 Eligibility criteria

All children born live in the above defined conditions, at any gestational age, by normal or cesarean delivery, including gemelar gestations, from mothers of 16 years or older. There will not be exclusion of participants due to clinical intercurrents during the pregnancy or delivery.

OBS: In the occurrence of fetal loss, miscarriage or neonatal death an interview will be conducted with the parents to identify the conditions and factors that could have contributed to this outcome.

5.2 Enrollment

The Federal University of Rio de Janeiro Maternity School is one of reference hospitals monitoring high-risk pregnancies in Rio, but it is also a referral hospital for low-risk pregnancies in the public health system of Rio de Janeiro. High-risk pregnant women have their prenatal follow up conducted in the hospital, while low-risk pregnancies are followed in Family Health Centers close to their residences and, those living in the South Zone of the city, are referred for delivery to the Federal University of Rio de Janeiro Birthing Center.

The Hospital performs 2,000 to 2,200 deliveries/year, 50% of them being from high-risk pregnancies. All eligible pregnant women will be given the opportunity to participate in the study and will be asked to sign in a Consent Form.

Alongside the Birth Cohort, the project will develop a “Health, Environment and Child Development Educational Program” as a strategy of engagement of the mothers and their babies in the study. It will focus on educational activities related to the maternity, the fetus' development and the children’s health using digital interactive tools, including a web site and mobile app.

5.3 Sources of Data

The study protocol includes interviews, physical exams, and collection of biological samples at the 7th month of pregnancy, birth, and postnatal period until the age of 4 (Table 1).

a) Interviews

Parents will be interviewed in three different moments: during the gestation at the enrollment (gestation questionnaire); the following day after birth (birth questionnaire) and; during the postnatal pediatric visits for the monitoring of the newborns (follow up questionnaire) (Table 1).

b) Physical Examination and Testing

Eligible newborns will be periodically attended at the Federal University of Rio de Janeiro Birthing Center, according to the schedule shown in Table 1.

c) Hospital and other health records: prenatal examination records; immunization records.

d) Biological samples.

Table 1: Protocol for Data Collection

	Prenatal	Birth	3m	6m	12m	18m	24m	36m	48m
Clinical Exam									
Anthropometric		X	X	X	X	X	X	X	X
Physical Exam		X	X	X	X	X	X	X	X
NCPD Tests ¹			X	X	X	X	X	X	X
Biological Samples									
Umbilical Cord Blood		X							
Meconium		X							
Tissue samples ²		X							
Maternal milk			X	X					
Urine (child)			X	X	X	X	X	X	X
Urine (mother)	X								
Blood (mother)	X								
Questionnaires									
Gestation: 32 nd week	X								
Birth		X							
Follow up			X	X	X	X	X	X	X

¹Neuromotor, cognitive and psychologic Developmental Tests; ²umbilical cord and placenta

5.4 Variables of Study

- Exposure:

1. Information about sources of exposure, environmental (including domiciliary) and occupational, to the compounds listed in Figure 2.

- cleaning and hygiene products use in the home; pesticides used within and outside the home; occupations of household members; distance of home from industries and from hazardous waste sites; kind, type and frequency of foods consumed; access to drinking water; use of plastic cookware to heat food.

2. Collection and analyze of compounds concentrations in biological samples (Figure 2).

Figure 2: Environmental pollutants, type of samples and biological matrices

Biologic Matrix	Environmental Pollutants	Type of samples
Prenatal: 30th to 32nd week.		
Blood	Metals; pesticides; Organochlorine pollutants; Polycyclic aromatic hydrocarbon (PAHs); Bisphenol; polychlorinated biphenyls (PCBs); phthalates; polybrominated diphenyl ethers (PBDE); <i>Perfluoroalkyl e Polyfluoroalkyl</i> compounds	Parents
Urine	Metals; pesticides; Organochlorine pollutants; Polycyclic aromatic hydrocarbon (PAHs); Bisphenol; polychlorinated biphenyls (PCBs); phthalates; polybrominated diphenyl ethers (PBDE); <i>Perfluoroalkyl e Polyfluoroalkyl</i> compounds	Parents
Birth		
Umbilical Cord Blood	Metals; pesticides; Organochlorine pollutants; Polycyclic aromatic hydrocarbon (PAHs); Bisphenol; polychlorinated biphenyls (PCBs); phthalates; polybrominated diphenyl ethers (PBDE); <i>Perfluoroalkyl e Polyfluoroalkyl</i> compounds	Newborn
Urine	Metals; pesticides; Organochlorine pollutants; Polycyclic aromatic hydrocarbon (PAHs); Bisphenol; polychlorinated biphenyls (PCBs); phthalates; polybrominated diphenyl ethers (PBDE); <i>Perfluoroalkyl e Polyfluoroalkyl</i> compounds.	Newborn
Postnatal until 4 years		
Capillary blood	Metals	Infant / toddler
Urine	Metals; pesticides; Organochlorine pollutants; Polycyclic aromatic hydrocarbon (PAHs); Bisphenol; polychlorinated biphenyls (PCBs); phthalates; polybrominated diphenyl ethers (PBDE); <i>Perfluoroalkyl e Polyfluoroalkyl</i> compounds	Newborn / infant / toddler
Milk	Metals; pesticides; Organochlorine pollutants; Polycyclic aromatic hydrocarbon (PAHs); Bisphenol; polychlorinated biphenyls (PCBs); phthalates; polybrominated diphenyl ethers (PBDE); <i>Perfluoroalkyl e Polyfluoroalkyl</i> compounds	Mother

- Outcomes:

1. Birth: birthweight; birth length; head circumference at birth; fetal growth; miscarriage; fetal death; gestation duration at delivery neonatal health placental weight and dimensions; markers of birth asphyxia.

2. Postnatal: anthropometric measures (weight, length, height, head circumference, waist, abdomen and mid-upper arm circumferences; Body Mass Index); cognitive, motor, psychological and sensitive abilities and disabilities, short-term memory, speech and language, motor ability, attention, spelling, reading, executive function, non-word repetition);

vision and hearing disabilities; asthma; allergic sensitization; wheezing with whistling on the chest; coughing during the night.

- **Confounding and Effect Measure Modification factors or conditions:** prenatal and gestational morbidities; social drug use (alcohol, tobacco, and others); medications, dietary supplements and other remedies; sociodemographic characteristics (age, schooling, familiar income, household conditions, domiciliary density); prior pregnancies information; physical activity; maternal mental health; health perception; urban violence (local indicators such as death rate for homicide), domestic violence (mother's report).

5.5. Statistical analysis

Descriptive data analysis will be obtained from frequencies, measures of central tendency and variability. Then, we will use the multiple analysis techniques appropriate to each outcomes and exposures variable.

Considering that the main outcomes of interest are dichotomous, multiple binary logistic regression models will be adjusted taking into account potential confounders and mediators according to hierarchical models. To evaluate the quality of fit of the logistic regression models, diagnostic techniques will be used from the analysis of residues.

At times where repeated evaluations, specific techniques and models (GEE or mixed effects models, for example) are analyzed, will be incorporated into the analyzes, so as to consider the structure of internal dependence of the observations, characteristic of this situation.

Confounding

The hierarchical model will be developed to identify and differentiate possible confounding and modifying factors in different levels of causality. The modifying factors should be detected by specific statistical tests and the exposure variables adjusted accordingly. Among the potential modifying variables to be assessed, we'll include:

1. Pre-gestational and gestational morbidities;
2. Use of alcohol, tobacco and other drugs;
3. Use of medications, supplements and supplements
4. Exposure to extreme violence

Bias Control

Questionnaires and specific measures will be validated to avoid information bias. In addition, all professionals involved in data collection will be trained and periodically evaluated. This includes:

1. Application of questionnaires
2. Clinical and anthropometric evaluations
3. Collection, storage and analysis of biological samples.

We will assess measurement quality by repetition of interviews and anthropometric evaluations in a subsample of mothers and child. Kappa and intraclass correlation coefficients will be calculated according to each variable analysis.

Loss information will be better estimated during the pilot study planned, conducted in the second semester of 2017. In addition, according to the frequencies and types of follow-up losses that will be observed, sensitivity analyzes, or data imputation techniques may be used.

6. Pilot Study

A pilot study was conducted with all children born between October 1st, 2017 and March 31st, 2018 at the Maternity School of the Federal University of Rio de Janeiro. They were followed until 6 months. The Environmental Health Department of the Brazilian Ministry of Health financed the pilot study.

The Pilot study tested and evaluated the questionnaires, biological samples collection techniques, enrollment strategies and anthropometric, neuromotor, psychological and cognitive monitoring tools.

7. Publications related

1. Seefeld M; **Froes Asmus, C.I.R.**; Figueiredo ND; Câmara, V.M.; Luiz R.R.; Prata, A.; Martins M; Jacob, S.; S., Gobbo L.M., Vicentini, S.A.; Rezende JFF; Amim, J. Prenatal Exposure to Metals and Neurodevelopment in Infants at Six Months: Rio Birth Cohort Study of Environmental Exposure and Childhood Development (PIPA Project). *International Journal of Environmental Research and Public Health*, v.19, p.4295 - , 2022. <https://doi.org/10.3390/ijerph19074295>
2. Froes Asmus, C.I.R, Meyer, A; Cunha, A.J.L.; Damasceno, N.; Gomes, L. E.; Raggio R.; Rosa, A.C.S.; Couto S.J.; Barbosa, A.P.; Fernandes, TV.R.; Medronho, R.; Câmara, V.M. Multiple Environmental Exposure in Pregnant Women and Their Children in the City of Rio de Janeiro, Brazil, Rio Birth Cohort Study: PIPAProject. *Exposure and Health*, v.1, p.1, **2021**. <https://doi.org/10.1007/s12403-021-00394-9>
3. Naspolini, N.F., Meyer A., Moreira, J.C., Sun H., Fróes-Asmus, CIR., Dominguez-Bello MG. Environmental pollutant exposure associated with altered early-life gut microbiome: Results from a birth cohort study. *Environmental Research* 205 (2022) 112545. <https://doi.org/10.1016/j.envres.2021.112545>

4. Espindola Santos, A.; Meyer, A.; Dabkiewicz, V.E.; Câmara, VM; Fróes Asmus, CIR. Serum levels of perfluorooctanoic acid and perfluorooctanesulfonic acid in pregnant women: Maternal predictors and associations with birth outcomes in the Project. *J. Obstet. Gynaecol. Res.* Vol. 47, No. 9: 3107–3118, September 2021. <https://doi:10.1111/jog.14883>.
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6. Araújo M.S.A, Figueiredo N.D, Camara V.M, Froes Asmus, C.I.R. Maternal-child exposure to metals during pregnancy in Rio de Janeiro city, Brazil: The Rio Birth Cohort Study of Environmental Exposure and Childhood Development (PIPA project). *Environmental Research* 183 (2020) 109155. <https://doi.org/10.1016/j.envres.2020.109155>
7. Asmus CIRF, Barbosa AP, Meyer A, Damasceno N, Rosa ACS, Medronho R, Cunha AJL, Moreira JC, Fernandes TVRB, Martins M, Luiz RR, Câmara VM. Rio Birth Cohort Study on Environmental Exposure and Childhood Development – PIPA Project. *Annals of Global Health*. 2020; 86(1): 59, 1–10. <https://doi.org/10.5334/aogh.2709>.
8. Napolini, N.F.; Machado, P.P.; Fróes-Asmus, CIR.; Câmara, V.M; Moreira, J.C.; Meyer, A. Food consumption according to the degree of processing, dietary diversity and socio-demographic factors among pregnant women in Rio de Janeiro, Brazil: The Rio Birth Cohort Study of Environmental Exposure and Childhood Development (PIPA project). *Nutrition and Health*, v.1, 2020. (<https://doi.org/10.1177/0260106020960881>).
9. Figueiredo ND, Araújo MS, Luiz RR, Câmara VM, Jacob SC, Santos LMG, Vicentini AS, Froes Asmus CIR. Metal mixtures in pregnant women and umbilical cord blood at urban populations—Rio de Janeiro, Brazil. *Environmental Science and Pollution Research*, July 2020. <https://doi.org/10.1007/s11356-020-10021-w>

8. Financial Support

1. Environmental Health Surveillance Department / Brazil's Health Ministry (Process nº 733663/19-002)
2. Brazil Council for Scientific and Technological Development (CNPq) (Process nº 409275/2018-2).
3. Science and Technology Department / Brazil's Health Ministry (Process nº - 404168/2019-1).

4. Carlos Chagas Filho Foundation for Supporting Research in the State of Rio de Janeiro
(Process nº E-26/010.001894/2019).