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Ilaria Cosentini, Alessia Li Vigni, Noemi Aloï, Alessia Maria Sampino, Fabio Massimo Contarino, Paolo Colombo, Silvia Ruggieri, Gaspare Drago & Valeria Longo

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Prenatal Environmental Exposures and Distinct MicroRNA Patterns in Maternal and Cord Serum

Ilaria Cosentini^{1,*}, Alessia Li Vigni^{1,*}, Noemi Aloï^{1,2}, Alessia Maria Sampino¹, Fabio Massimo Contarino³, Paolo Colombo¹, Silvia Ruggieri^{1#}, Gaspare Drago^{1#} and Valeria Longo^{1#}

¹Institute for Biomedical Research and Innovation, National Research Council of Italy (IRIB-CNR), Palermo, Italy.

²Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Palermo, Italy.

³Department of Public Health, Siracusa Local Health Authority, Siracusa, Italy.

***Equally contributed to the manuscript**

#Corresponding Author 1: Valeria Longo

Institute for Biomedical Research and Innovation, National Research Council of Italy (IRIB-CNR), Palermo, Italy.

e-mail address: valeria.longo@irib.cnr.it

#Corresponding Author 2: Gaspare Drago

Institute for Biomedical Research and Innovation, National Research Council of Italy (IRIB-CNR), Palermo, Italy.

e-mail address: gaspare.drago@irib.cnr.it

#Corresponding Author 3: Silvia Ruggieri

Institute for Biomedical Research and Innovation, National Research Council of Italy (IRIB-CNR), Palermo, Italy.

e-mail address: silvia.ruggieri@irib.cnr.it

ABSTRACT

Background

Environmental exposures during pregnancy can influence crosstalk between the maternal and foetal compartments, eliciting distinct yet coordinated adaptive responses at the placental interface. MiRNAs act as key mediators, translating environmental stress into post-transcriptional regulatory signals.

Methods

This study investigates the effects of prenatal exposure, examining associations between essential elements (EEs), persistent organic pollutants (POPs), and circulating miRNA profiles in 95 maternal–cord pairs from the NEHO birth cohort. To this aim, single-chemical and bWQS models were performed in both maternal and fetal side. The miRNAs significantly associated with chemicals were used to perform pathway enrichment analysis.

Results

Single-chemical regression models identified distinct sets of miRNAs altered by EEs or POPs in the maternal and cord compartments, while mixture analysis (bWQS) confirmed these associations and revealed both shared and compartment-specific miRNA signatures. Pathway enrichment analysis of miRNA-target genes revealed a side characteristic modulation of key signaling and immune-related pathways.

Conclusions

These findings demonstrate that prenatal exposure to chemical mixtures modulates distinct maternal and foetal miRNA responses, highlighting the placenta's dual role as a selective barrier and active regulator of foetal programming.

Key words: birth cohorts; pollutants exposure; circulating miRNAs; multipollutant analysis; maternal-foetus interface

Plain Language Summary

During pregnancy, chemicals may pass differently from the mother to the developing foetus through the placenta. This study examined whether exposure to essential elements and persistent pollutants was associated with changes in circulating microRNA expression, small molecules that regulate gene activity. Blood samples from 95 mother–infant pairs enrolled in the NEHO birth cohort were analysed. Chemical exposures and microRNA patterns were measured in both maternal and cord blood, and the effects of individual analytes and mixtures were investigated. The results showed that only a few microRNAs responded similarly in both compartments, whereas most displayed distinct maternal and cord blood responses. These findings suggest that the placenta is not only a barrier, but also an active regulator of early-life development. This research may help to identify early warning markers of environmental effects during pregnancy.

Abbreviations

bWQS	bayesian Weighted Qantile Sum regression
BMI	Body Mass Index
Ct	Cycle threshold
DDE	Dichlorodiphenyldichloroethylene
DOHaD	Developmental Origins of Health and Disease
EE	Essential Element
FDR	False Discovery Rate
HCB	Hexachlorobenzene
miRNA	microRNA
LOQ	Limit of Quantification
NEHO	Neonatal Environment and Health Outcomes
NPCS	National Priority Contaminated Site
PCB	Poly-Chlorinated Biphenyl
PAH	Polycyclic Aromatic Hydrocarbon
POP	Persistent Organic Pollutant
OC	OrganoChlorine
SNV	Standard normal variate
TNC	Trans-Nonachlor

Introduction

In recent years, over 350,000 chemicals and chemical mixtures have been registered for production, resulting in the continuous exposure of the general population to multiple contaminants, including airborne particles, industrial chemicals, heavy metals, and persistent organic pollutants (POPs) ¹. These toxicants can enter the body via inhalation, ingestion, or dermal absorption and, once present in the circulatory system, they may interact with cells and tissues, interfering with essential biological processes. Such effects can be of particular concern during pregnancy, when maternal exposures can affect foetal development.

In this context, the Developmental Origins of Health and Disease (DOHaD) framework, also known as the Barker hypothesis, postulates that insults that occur during sensitive developmental windows can alter disease susceptibility across the lifespan ². According to the “two-hit hypothesis”, early-life exposures may induce latent biological differences that manifest later in life when a second environmental or physiological stressor occurs. Indeed, there are epidemiological studies which support this model, showing associations between prenatal exposure to environmental toxicants and adverse birth and childhood outcomes, including impaired growth, neurodevelopmental deficits, and immune dysfunction ^{3, 4, 5}. These findings highlight that foetal development is influenced not only by genetic determinants but also by modifiable environmental factors ⁶

A growing body of research indicates that epigenetic mechanisms mediate the long-term health effects of prenatal exposures. DNA methylation, histone modification, and gene silencing by microRNAs (miRNAs) are key processes linking environmental insults to altered gene regulation ^{7, 8, 9, 10}. MiRNAs, small non-coding RNAs that regulate gene expression and cellular homeostasis, are highly responsive to environmental stimuli ¹¹. Their stability in biofluids and detectability in blood make them promising non-invasive early biomarkers of exposure and biological impact ¹². Several studies have shown that maternal exposure to chemical mixtures is associated with differences in circulating miRNA expression, with potential consequences for offspring development and later disease risk ^{13, 14, 15}. Since many

of these molecular alterations likely originate at the maternal–foetal interface, the placenta emerges as a key organ of interest for the way it mediates this interface by ensuring nutrient and gas exchange and acting as a barrier to xenobiotics. However, evidence indicates that this barrier is not impermeable: POPs, polycyclic aromatic hydrocarbons (PAHs), and even particulate matter can cross into the foetal compartment ^{16, 17}. Such transplacental transfer raises concerns about direct foetal exposure to complex contaminant mixtures during critical windows of intrauterine development.

Despite increasing evidence linking pollutant exposure, miRNA dysregulation, and health outcomes, very few studies have simultaneously investigated both maternal and cord blood to assess maternal-foetal transfer and intergenerational effects ^{18, 19, 20}. In parallel, the importance of essential elements (EEs) during pregnancy is well established. Selenium (Se), zinc (Zn), and copper (Cu) are critical micronutrients whose physiological requirements increase during gestation to support foetal development. Their concentrations must remain tightly regulated, as both deficiency and excess have been associated with adverse maternal and foetal outcomes ²¹. Growing evidence also indicates their involvement in epigenetic regulation: Zn, Cu and Se have been shown to influence DNA methylation in non-pregnant population ^{22, 23, 24}, while their circulating levels have been associated with changes in miRNA expression in pregnant women, as previously observed in a birth cohort ¹⁵. In contrast, POPs, such as hexachlorobenzene (HCB), polychlorinated biphenyls (PCBs), and p,p'-DDE, are synthetic xenobiotics characterised by chemical stability and bioaccumulation through the food chain. POPs exert reproductive toxicity through multiple mechanisms, including endocrine disruption via interference with thyroid hormone signaling, impairment of steroidogenesis ²⁵, disruption of the hypothalamic-ovarian axis ²⁶, and associated alterations in circulating miRNA profiles, which may reflect or contribute to these biological changes ^{15, 27, 28}. Due to their lipophilic nature, POPs readily cross the placental barrier, contributing to adverse reproductive outcomes including pregnancy complications and altered fetal growth ²⁹. Their inclusion in the present study

reflects the specific exposure profile of pregnant women living in the Augusta-Priolo area, where dietary intake of local food, particularly fish, has been identified as an important route of contaminant transfer from environmental matrices to maternal serum ³⁰. Although both EEs and POPs have been linked to epigenetic alterations, including changes in miRNA profiles, they differ fundamentally in their biological nature: EEs are physiologically required nutrients subject to homeostatic regulation, whereas POPs are persistent toxicants with no essential biological function. Investigating these two classes of compounds within the same analytical framework may therefore provide complementary insight into the biological effects of environmental exposures during pregnancy. Nevertheless, few studies have concurrently examined EEs and POPs in both maternal and cord blood matrices.

To address this knowledge gap, birth cohorts enrolled in highly contaminated areas are a powerful tool for investigation. The Neonatal Environment and Health Outcomes (NEHO) cohort, established in southern Italy, provides such an opportunity. Conducted in the industrialized Augusta-Priolo area, recognized as a National Priority Contaminated Site (NPCS), NEHO was specifically designed to evaluate how environmental pollutants can affect the health of mothers and their children ^{29, 31, 32}.

For this study, we investigated the relationships between maternal exposure to environmental pollutants and circulating miRNA expression in maternal serum during the third trimester of pregnancy and in cord serum at birth. We i) assessed the concentration of selected inorganic and organic contaminants and their placental transfer, ii) examined their associations with miRNA expression in both maternal and foetal sides, and iii) explored the biological pathways potentially linking these molecular alterations to health outcomes. By integrating biomonitoring data, the evaluation of biomarkers, and questionnaire data, this work aims to advance our understanding of maternal-foetal health dynamics in polluted environments, as well as to provide insights which are relevant to public health interventions.

Methods

Study population

This study was conducted within the framework of the NEHO birth cohort, an ongoing longitudinal study designed to investigate the effects of environmental exposures on maternal and child health. A total of 95 mother-child pairs were selected from the NEHO cohort for the present analysis. Participants were recruited at the General Hospital of Lentini (Syracuse, Italy) and the Umberto I Hospital in Syracuse (Italy). The study involved mothers residing across a broad geographic area that included both municipalities located within the NPCS of Augusta-Priolo and its surrounding areas ³³.

The design and recruitment procedures of the NEHO cohort have been described in detail previously ³². In brief, between January 2018 and January 2020, 561 pregnant women were enrolled based on their continuous residence within the Augusta-Priolo area for at least 12 months prior to enrolment. Participants were recruited during prenatal visits at local healthcare facilities, and eligibility was confirmed based on standardized inclusion criteria, including residency, age ≥ 18 years, and the absence of pre-existing major chronic illnesses. Before enrolment, all participating mothers provided written informed consent for the collection, processing, and use of their personal data and biological samples for research purposes.

Data collection

Upon enrolment, detailed socio-demographic, lifestyle, medical, and environmental exposure information was obtained through structured web-based questionnaires. Follow-up assessments were conducted at 6, 12, and 24 months postpartum to gather longitudinal data on maternal health, infant development, and potential ongoing exposures. Clinical and delivery-related information, including gestational age at delivery, mode of delivery, and newborn anthropometric parameters (birthweight, length, and head circumference), was extracted from medical records by trained healthcare personnel at the time of birth. For this analysis, randomly selected mother-child pairs were included to ensure a sample representative of the general NEHO cohort inclusion criteria ³¹.

Blood sampling and handling

Blood samples were collected from all participants during the last trimester of pregnancy; cord blood samples were collected from the umbilical cord vein immediately after delivery. Serum samples were prepared as previously described³¹. After serum was separated by centrifugation, samples were temporarily stored at -20°C in each maternity unit and then transported on dry ice to the NEHO biobank for long-term storage at -80°C .

Analytical methods for trace metals and organic compounds

The analysis of POPs was performed at the Chemical Exposure Unit of the National Institute for Health and Welfare (Kuopio, Finland) using an Agilent 7000B gas chromatograph coupled to a triple quadrupole mass spectrometer (GC-MS/MS), as previously described¹⁵. Trace element analyses were conducted at the micropollutant unit of LERES (Laboratoire d'Étude et de Recherche en Environnement et Santé), at the French School of Public Health (EHESP, Rennes, France), as described in Davies et al.^{30, 34}. A total of 95 maternal and cord serum samples were analysed by inductively coupled plasma tandem mass spectrometry (ICP-MS/MS) after acid mineralization.

Certified reference materials and internal quality control samples (spiked blood and serum) were included in each analytical batch to ensure accuracy and reproducibility.

Maternal and cord serum concentrations of 15 chemicals were evaluated in this study: three Essential Elements—EEs (Se, Zn, and Cu), arsenic (As), mercury (Hg), three different organochlorine pesticides—OCs (HCB, Trans-Nonachlor TNC, and p'-p'-DDE) and seven polychlorinated biphenyls—PCBs (PCB-74, PCB-118, PCB-138, PCB-153, PCB156, PCB-180, PCB-183). Concentrations below the limit of quantification (LOQ) were imputed using a value equal to LOQ/2, following the recommendations of the statistical analysis plan developed within the HBM4EU project (<https://www.hbm4eu.eu/wp-content/uploads/2018/09/Deliverable-10.2-Statistical-Analysis-Plan.pdf>). Raw concentrations of POPs were normalized to total lipid levels by dividing both maternal and cord serum chemical concentrations by

total lipid levels³⁰. Both EEs and lipid-normalized concentrations of POPs were then log-transformed and further standardized using the standard normal variate (SNV) transformation to minimize data skewness and allow for meaningful comparison across analytes with differing chemical properties. Only chemicals for which less than 15% of samples had concentrations above the LOQ were included in the further analyses. Placental transfer was computed for each analyte by dividing cord serum concentrations by maternal serum concentration between matched samples. The ratios were calculated only when data for both matrices were available (LOQ < 15%).

Serum miRNA extraction and expression analysis

Total RNAs enriched with miRNAs were extracted from 200 µl of maternal or cord serum samples according to the miRNeasy Serum/Plasma Advanced Kit manufacturer's protocol (Qiagen, Milan, Italy). Reverse transcription reactions were performed using the miRCURY LNA RT kit (Qiagen, Milan, Italy), according to the manufacturer's protocol. Real-time analyses were performed using the Applied Biosystems StepOnePlus™ Real-Time PCR System for maternal samples, and the Applied Biosystems QuantStudio™ 3 Real-Time PCR System for cord samples; levels of 84 miRNAs (listed in Supplementary Data 1) were detected using the Human Serum/Plasma Small Focus (miScript) array panel (Qiagen, Milan, Italy, cat. YAHS-206ZC, formerly MIHS-106) and the miRCURY LNA Sybr Green PCR kit (Qiagen, Milan, Italy).

Raw cycle threshold (Ct) values were calibrated on internal spike-in controls using the Gene Globe platform (<https://geneglobe.qiagen.com/it/analyze>). Undetermined values of Ct were estimated as 40 Ct, which is the last cycle of the reactions. Delta Ct (ΔCt) was computed, correcting for endogenous controls, using the following equation (1):

$$(1) \Delta Ct = Ct_{miRNA} - \frac{\sum Ct_{end}}{4}$$

in which endogenous controls are indicated as end=hsa-miR-30c-5p, hsa-miR-103a-3p, hsa-miR-451a, hsa-miR-23a-3p.

MiRNAs with more than 30% undetermined values were excluded from the analysis.

Covariates

Covariates included self-reported information on maternal and child characteristics and behaviours; these were collected at enrolment using the “Baseline” questionnaire or at delivery through clinical birth records. Specifically, the following variables were collected at enrolment: maternal age (continuous, years), maternal pre-pregnancy BMI (continuous, kg/m²), maternal educational level (categorical, high/medium/low), parity (binary, primiparous/multiparous) and smoking during pregnancy (binary, yes/no). Additional data were extracted from hospital delivery records, including child sex (binary, male/female), type of delivery (binary, vaginal/caesarean) and gestational age (continuous, days). Alcohol consumption during pregnancy and lack of folate supplementation were excluded as covariates due to the low prevalence of self-reported cases (N=2;2.11% for both). A DAG was constructed to establish the minimum set of confounders (Figure S1).

Statistical analysis

Descriptive statistics were used to compare the main characteristics of the study sample (N=95) with those of the total sample (N=561), as well as to compare analyte concentrations in maternal and cord serum within the study sample. Categorical values were reported as absolute counts with percentages (N[%]). Continuous variables were summarized as median and interquartile range [Q1-Q3] for non-normally distributed data, and as means with standard deviation ($\pm$ SD) for normally distributed variables. Normality was assessed using the Shapiro-Wilk test. Differences between groups were evaluated using the Chi-square test for categorical variables and the Mann-Whitney U-test for non-normally distributed continuous variables. The paired Wilcoxon test was used to assess differences in analyte concentrations between maternal and cord serum. Pearson correlation coefficients were computed to assess linear relationships between analytes or miRNAs in the two groups.

Multivariable regression analyses were performed to investigate associations between each miRNA- Δ Ct value and single maternal and cord serum exposure concentrations. Multiple comparisons were corrected using the Benjamini-Hochberg false discovery rate (FDR) procedure³⁵ at an alpha level of 5%. Associations between explanatory variables and chemical exposures were examined using adjusted models, incorporating the covariates described in the previous section (and DAG in Figure S1). The type of delivery was included as confounders only for models with miRNAs in cord serum. Two separate analyses were conducted: in “Analysis 1”, each chemical was considered individually as the independent variable; in “Analysis 2”, a mixture analysis was performed. For Analysis 1, independent variables included each maternal or cord analyte, as well as the sum of selected PCBs in maternal serum (Σ PCB_{mat}(PCB-74,PCB-118,PCB-138,PCB-153,PCB-180)) and cord serum (Σ PCB_{cord}(PCB-138,PCB-153,PCB-180)), where the included PCBs had less than 15% of substituted values in each side. To assess the mixture effects of EEs (Se, Cu, Zn), POPs (p,p'-DDE, HCB, PCBs), and the total chemical mixture (TOTAL) on miRNA expression, Bayesian Weighted Quantile Sum (BWQS) regression was performed using the R package “bwqs”³⁶. The Hamiltonian Monte Carlo chain length was set to 1000, with no specified direction for prior distribution; all other parameters were kept at their default settings. Association with 95% credible interval excluding zero were considered significant. We set the chemical of concern weight threshold by dividing 1 by the number of chemicals considered for each analysis. The thresholds are reported in Table S1 of the supplementary materials.

The significant β coefficients estimated from both analyses were reported using a heatmap generated by the “pheatmap” function in the R package. Common associations between BWQS analyses and linear regressions were reported as Venn diagrams³⁷.

MiRNAs that showed significant associations with chemical exposures were selected for Reactome pathway enrichment analysis to identify key biological pathways which were differentially perturbed between the maternal and cord serum. Validated target genes for each selected miRNA were retrieved from three publicly available

databases of experimentally validated miRNA-target interaction: miRecords, miRTarBase, and TarBase. This was performed using the multiMir R package ³⁸. To ensure robustness, only validated target genes that were consistently reported across all the three databases were retained for further analysis. The final gene list was stratified according to the side of origin (maternal or cord), exposure mixture (POPs or EEs), and direction of association, based on miRNAs showing statistically significant associations in the bWQS models. Functional enrichment was performed using an over-representation analysis (ORA) approach implemented in the “compare Cluster” function of the cluster Profiler R package ³⁹. The background gene universe was defined as the set of all validated target genes identified for full panel of 84 miRNAs initially analyzed ⁴⁰. To reduce pathway redundancy, we first assessed similarities among statistically significant enriched pathways (adjusted p-value < 0.05) using the “pairwise_termsim” function. A similarity threshold of 0.2 was applied to construct a network in which each node represented a pathway, and the edges connected pathways with similarity values exceeding this threshold, with edge weights corresponding to the degree of similarity.

Community detection was then performed using the Louvain algorithm, and each resulting cluster was labelled based on the pathway with the highest degree (i.e., the most connected node within the community). The final results were visualized as a heatmap, highlighting the hub pathway. Only pathways comprising genes from at least four distinct miRNAs were included. For each pathway, we indicated whether the corresponding miRNAs were associated with EEs, POPs or TOT mixtures and specified the direction of the association (positive = red; negative = blue). The heatmap was organized by biological sample origin, with maternal-side results presented in the upper section and cord-side results in the lower section. All analyses were considered significant at a p level ≤ 0.05 and were conducted in R, version 4.5.2 ⁴¹.

Results

Characteristics of the study population

The NEHO cohort methods are described in detail elsewhere ³¹. Here, 95 mother/child pairs were randomly selected from within the larger context of the NEHO birth cohort. Table 1 shows a descriptive analysis comparing mothers belonging to the NEHO cohort living in the Priolo National Priority Contaminated Site (NPCS) (561 subjects) and the subset of these mothers analysed in this study (N=95; 16.9%). The analysis of demographic and gestational information (Table 1) shows that the two groups of mothers are homogeneous for age, pre-pregnancy BMI, marital status, educational levels, parity (Primiparous or Multiparous) and gestational length (days). No significant differences were observed between the two groups regarding pregnancy-related habits, such as folate supplementation, smoking, or alcohol consumption.

Focusing on the subpopulation being studied, these mothers had a median age of 31.0 years (IQR 28.0–34.0) and a median pre-pregnancy BMI of 23.1 kg/m² (IQR 20.4–25.1). Smoking during pregnancy was reported by 6 out of 95 mothers (6.32%), while 2 mothers (2.11%) reported alcohol consumption during pregnancy, and an equal proportion (2.11%) declared that they had not taken folic acid. All pregnancies reached full term, and all infants were born alive. The sex distribution of the children was balanced, with 47.4% females and 52.6% males.

Table 1. Descriptive characteristics of the study population: Descriptive table comparing mothers belonging to the NEHO cohort living in the Priolo NPCS (N=561) and the subset of these mothers analysed in this study (N=95).

	TOTAL N=561	SUBSET N=95	p-value
Mother's age (years) median[Q1-Q3] [§]	31.0 [27.0;34.0]	31.0 [28.0;34.0]	0.467
Mother's BMI (Kg/m ²) median[Q1-Q3] [§]	22.5 [20.3;25.5]	23.1 [20.4;25.1]	0.410
Marital Status - N(%) [#] :			0.164
Married	341 (61.0%)	65 (69.1%)	
Unmarried	218 (39.0%)	29 (30.9%)	
Educational level - N(%) [#] :			0.244
Low	158 (28.3%)	19 (20.0%)	
Medium	277 (49.6%)	53 (55.8%)	
High	124 (22.2%)	23 (24.2%)	
Parity - N(%) [#] :			0.948
Primiparous	246 (52.7%)	47 (51.6%)	
Multiparous	221 (47.3%)	44 (48.4%)	
Gestational age (days) - median[Q1-Q3] [§]	275 [270;282]	276 [270;282]	0.970
Folate supplementation - N(%) [#] :			0.269
no	5 (0.89%)	2 (2.11%)	
yes	555 (99.1%)	93 (97.9%)	
Smoking during pregnancy - N(%) [#] :			0.203
no	496 (88.7%)	89 (93.7%)	
yes	63 (11.3%)	6 (6.32%)	
Alcohol consumption during pregnancy – N(%) [#] :			0.625
no	553 (98.8%)	93 (97.9%)	
yes	7 (1.25%)	2 (2.11%)	
Sex – N(%) [#] :			0.916
Female	259 (46.2%)	45 (47.4%)	
Male	302 (53.8%)	50 (52.6%)	

[Q1-Q3] = [1st and 3rd quartile]; BMI = Body Mass Index; Educational level categorized as "High" (degree or higher qualification), "Medium" (upper secondary school diploma), and "Low" (secondary school diploma or lower);
[#] p values from Chi-squared test; [§] p values from Mann-Whitney *U* test; all statistical tests were two-sided.

Evaluation of metal and organic compound levels in maternal and cord serum

NEHO participants exhibited widespread exposure to a variety of environmental pollutants. Seven POPs, including HCB, p,p'-DDE, PCB-74, PCB-118, PCB-138, PCB-153, and PCB-180, and five trace elements, comprising three EEs (Cu, Zn, Se) and two non-essential elements known for their toxicity (Hg, As), were quantified in maternal and cord serum samples.

Supplementary Data 2 lists the concentrations of analytes in both maternal and cord serum, along with the percentage of values below the LOQ and the placental transfer ratio. All selected chemicals showed statistically significant differences between the two matrix concentrations ($p < 0.001$). Compounds with more than 15% of values below the LOQ were excluded separately for each matrix (maternal and cord). Consequently, the set of analysed compounds differed between matrices.

The placental transfer ratio provides insight into placental permeability and potential foetal exposure to environmental contaminants. A higher ratio indicates greater transfer efficiency from mother to foetus. A schematic representation of placental transfer ratio, for each chemical with LOQ percentage lower than 15% in both matrices, is shown in Figure 1. Among EEs, the placental transfer ranged from 0.13 for Cu to 0.76 for Se, with Zn being the only element exhibiting a median ratio above 1 [1.41 (1.17;1.59)]. In contrast, OC compounds displayed consistently low placental transfer ratios, with values ranging from 0.21 to 0.52, indicating limited permeability. Similarly, PCBs showed low transplacental transfer, with values ranging from 0.12 to 0.24 across individual congeners, reflecting their restricted transplacental movement.

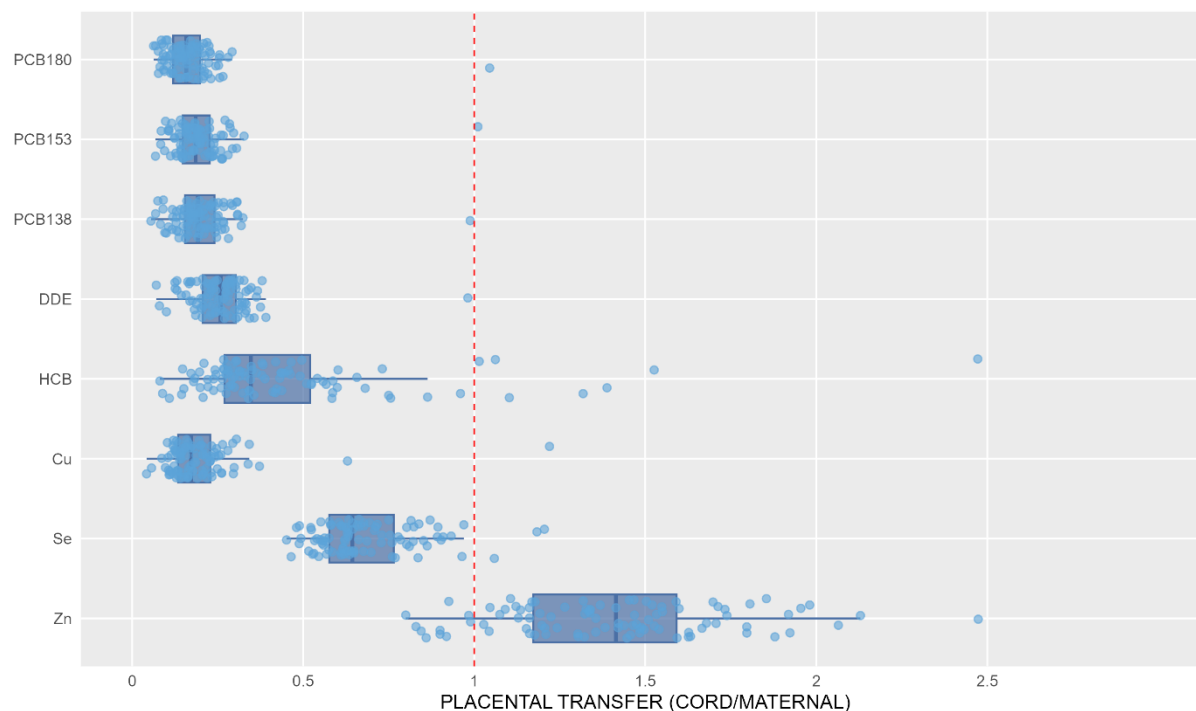


Figure 1. Placental transfer ratio: Boxplot reporting placental transfer ratio (cord/maternal- $n=95$) for each chemical which concentrations was below the LOQ in less than 15% of samples in both matrices.

To further investigate the distribution of analytes at the maternal-foetal interface, statistically significant correlations among analytes were evaluated separately within maternal serum (Figure 2A), and cord serum (Figure 2B). Correlations between maternal and cord serum were then assessed for each individual analyte (Figure 2C). For analytes with statistically significant maternal-cord correlations, scatter plots including the regression equation, R^2 , and p-value are shown (Figure 2D). Strong intra-group correlations were observed among substances with similar chemical characteristics in maternal serum (Figure 2A). EEs were weakly correlated with each other. Zn and Se did not show any significant inter-element correlations. Among OCs, HCB and p,p'-DDE were highly correlated ($r=0.79$). HCB also showed significant correlations with all PCB congeners; however, the strength of association showed an evident reduction in the Pearson correlation index with increasing chlorine substitution. Indeed, the correlation was strongest with PCB-118 ($r=0.68$,

five chlorine atoms) and lowest with PCB-180 ($r=0.33$, seven chlorine atoms). A similar trend was observed for p,p' -DDE, but only for the less chlorinated PCBs (PCB-74, PCB-118, PCB-138, and PCB-153). Finally, high inter-correlations were also found among PCB congeners, especially those with similar chlorination patterns.

In cord serum (Figure 2B), a strong correlation between Se and Cu ($r=0.66$) was observed. Both elements also exhibited weaker correlations with compounds having different chemical characteristics. Specifically, Se showed a slight positive correlation with PCB-138 ($r=0.22$) and a negative correlation with HCB ($r=-0.20$), whereas Cu was positively correlated with PCB-138 ($r=0.36$) and PCB-153 ($r=0.27$). OCs were not correlated with each other in cord serum but showed weak correlations with PCB congeners. As shown for the maternal side, the PCB congeners in cord serum remained strongly inter-correlated. Finally, several analytes showed moderate correlations between maternal and cord serum concentrations (Figure 2C), reinforcing the idea that, although placental transfer is generally limited, foetal exposure is more likely to be influenced by maternal levels. Although the placental transfer values of OCs and PCBs were very low, p,p' -DDE exhibited the highest correlated index between maternal and cord serum ($r=0.96$), followed by various PCBs. Among the EEs, although Zn was the only analyte showing placental transfer above 1, Se was the only one to show a slight maternal-cord correlation ($r=0.22$). The results are confirmed by the scatter plots in Figure 2D.

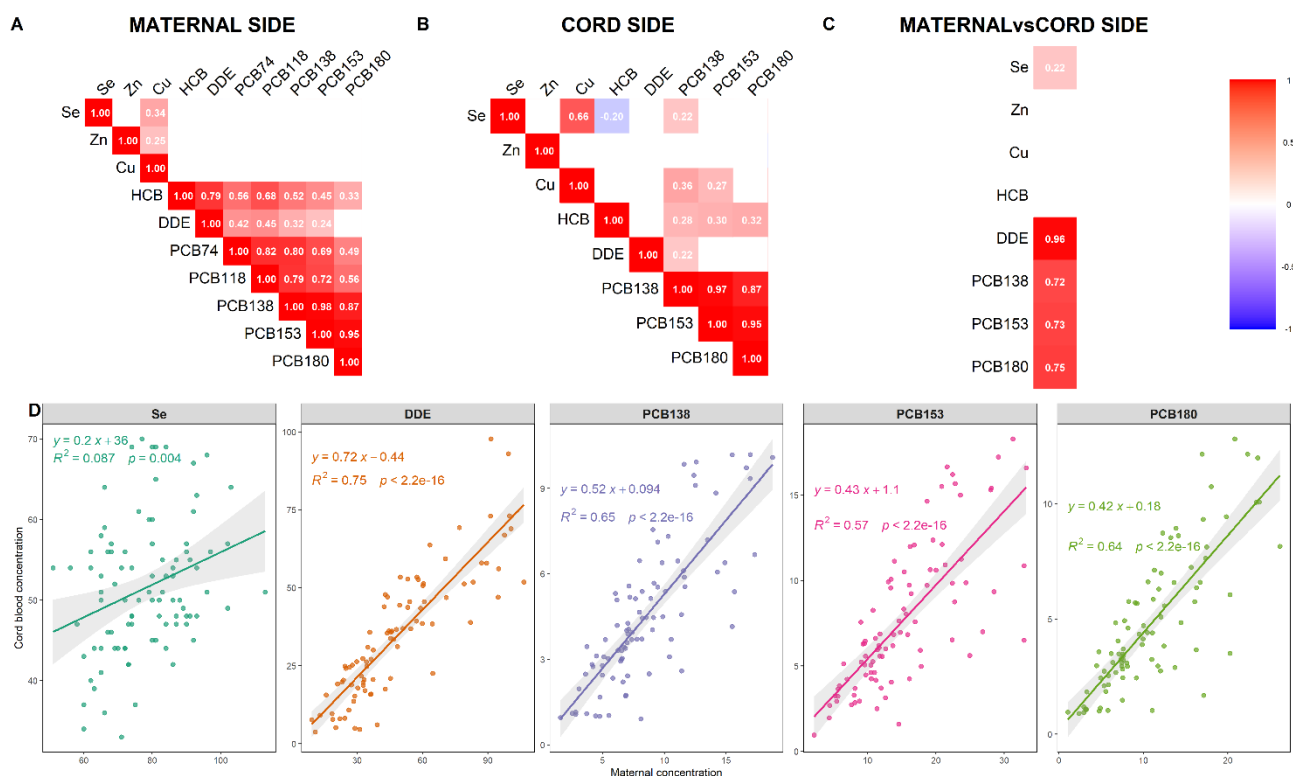


Figure 2. Intra- and inter-compartment correlations of persistent organic pollutant and essential elements: Statistically significant Pearson correlation indices are reported for analyte concentrations in maternal (A) and cord (B) serum. In (C) the results combining both maternal and cord samples are reported. Positive correlations (p -value < 0.05) are shown in red, and negative correlations (p -value < 0.05) in blue, with intensity proportional to the strength of the correlation. (D) Scatter plots report the associations of statistically significant correlated chemicals between cord and maternal side. Solid lines represent linear regression fits; shaded areas indicate the 95% confidence intervals.

Analysis of miRNAs in maternal and cord serum

A total of 84 miRNAs were analysed across maternal and cord serum samples. Among them, 75 from maternal serum and 79 from cord serum met quality thresholds ($Ct < 40$) in more than 70% of samples. The full list of miRNAs, along with their ΔCt values and the percentage of imputed values in each compartment, is reported in Supplementary Data 1. Five miRNAs were excluded from further analysis in both maternal and cord serum due to having more than 30% imputed values. Additionally, four miRNAs were removed from maternal serum because their

threshold Ct values were below 40 in fewer than 70% of subjects. We compared the median ΔCt values between maternal and cord serum to identify statistically significant differences in their expression levels. 31 miRNAs showed significantly lower ΔCt in maternal serum, indicating higher relative expression compared to the cord group. On the other hand, 35 miRNAs showed a higher median ΔCt value in maternal serum, reflecting greater expression in cord serum. The remaining 18 did not show significant differences between compartments.

These results suggest that specific circulating miRNAs are differentially expressed in maternal and cord blood, potentially reflecting foetal-specific gene regulation, maternal-foetal exchange, and exposure-related molecular signaling.

Exposures and miRNA expression associations

Statistically significant associations between individual chemicals, chemical mixtures, and circulating miRNAs are summarized in the heatmap shown in Figure 3. Results from maternal serum are displayed in pink, whereas those from cord serum are shown in light blue. Within each compartment, single-chemical (S-Cm) associations are presented first, followed by bWQS mixture associations. The left section of the heatmap reports miRNAs identified by both linear regression models and bWQS analyses, highlighting associations consistently detected across single-chemical and mixture-based approaches. The right section illustrates miRNAs identified exclusively through bWQS analyses. P-values were corrected for multiple testing using the False Discovery Rate (FDR), and only associations with adjusted $p < 0.05$ were presented. Three mixture models were tested: EEs, including only micronutrients; POPs, and the TOTAL mixture, including all exposures. The colour scale of the cells reflects the direction of the estimates, with positive associations shown in red and negative ones in blue. All models were adjusted for maternal age, maternal BMI, maternal educational level, gestational age (days), gender, smoking status, and parity. Type of delivery was additionally included in models including cord exposures as independent variables.

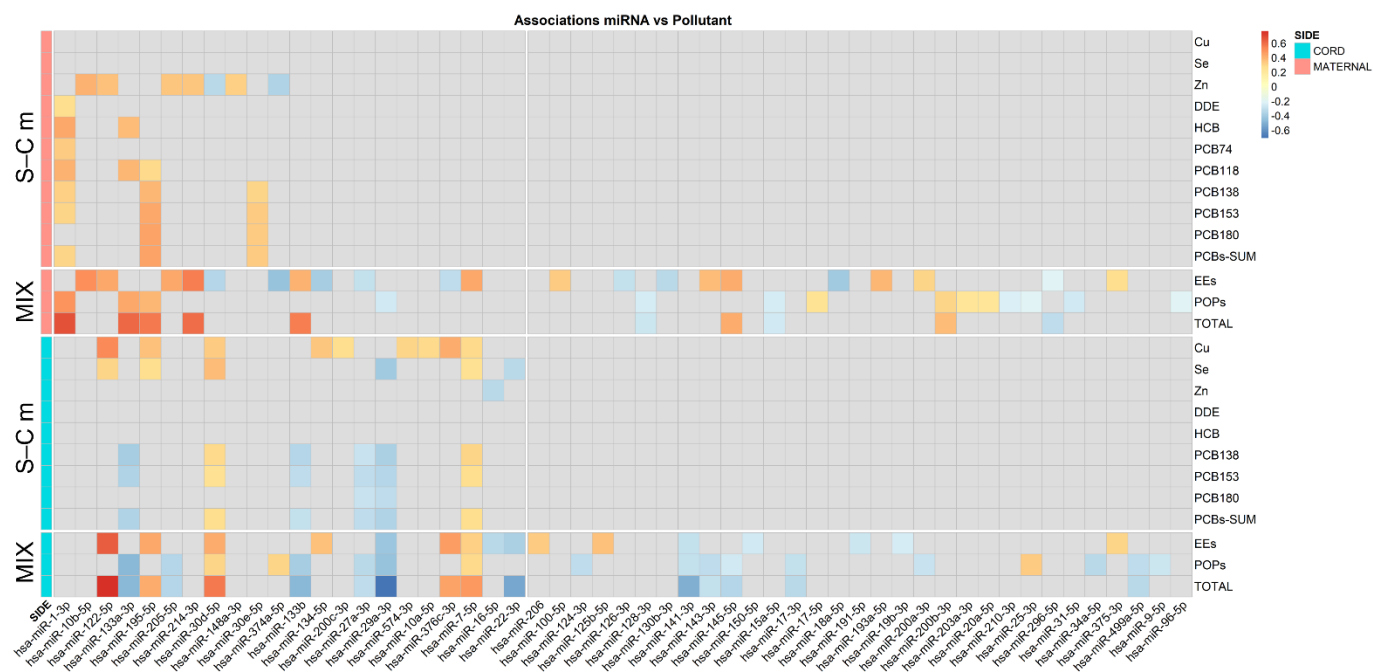


Figure 3. Associations of maternal and cord blood miRNAs with individual chemicals and mixtures: Heatmap showing statistically significant associations between chemicals and miRNAs. Pink results (left up) were obtained from the maternal side, whereas blue results (left bottom) were from the cord serum. S-C m refers to single-chemical models, while MIX corresponds to results from the *bWQS* models. The left side of the heatmap shows the results of single-component linear regression analyses (individual miRNA vs. individual chemical), including only associations with an FDR-adjusted p -value < 0.05 . In contrast, the right side reports the results of *bWQS* mixture models for three different chemical groups: EEs, POPs, and TOTAL (all chemicals combined). Cell colours represent the direction and magnitude of the estimates (positive associations in red and negative associations in blue). This layout highlights that the miRNAs displayed on the left were associated with individual chemicals in single-component analyses, whereas the miRNAs shown on the right were identified exclusively through mixture-model associations. MiRNA levels are expressed as ΔCt values; therefore, positive associations correspond to lower miRNA expression, whereas negative associations correspond to higher miRNA expression.

Single-chemical exposures associated with miRNA expression

Maternal Zn concentrations were positively associated with the higher ΔCt values (corresponding to lower expression levels) of five miRNAs, specifically miR-10b-5p, miR-122-5p, miR-148a-3p, miR-205-5p and miR-214-3p. In contrast, miR-30d-5p and miR-374a-5p were negatively associated with Zn levels (corresponding to higher expression levels) (Figure 3).

Regarding all considered POPs, maternal serum concentrations were positively associated with increased ΔCt values of miR-1-3p, with the only exception of PCB-180.

In addition, maternal HCB and PCB-118 concentrations were also associated with increased ΔCt values of miR-133a-3p. The highly chlorinated PCBs (PCB-138, PCB-153 and PCB-180) were positively associated with miR-30e-5p and miR-195-5p. These patterns were consistent with results obtained using the sum of maternal PCB concentrations: high concentrations of sum-PCBs were positively associated with miR-1-3p, miR-30e-5p and miR-195-5p (Figure 3). Detailed information on β estimates is reported in Table S2 and Figure S2.

Increasing cord serum concentrations of both Cu and Se were positively associated with higher ΔCt values of miR-7-5p, miR-30d-5p, miR-122-5p and miR-195-5p. Additionally, cord Cu concentrations were positively associated with higher ΔCt values of five miRNAs: miR-10a-5p, miR-134-5p, miR-200c-3p, miR-376c-3p and miR-574-3p. In contrast, higher Se concentrations in cord serum were associated with lower ΔCt values of miR-29a-3p and miR-22-3p. Moreover, miR-16-5p showed an inverse association with Zn concentrations (Figure 3).

All measured PCB congeners in cord serum (PCB-138, PCB-153, and PCB-180) were negatively associated with miR-27a-3p and miR-29a-3p. In addition, higher cord concentrations of PCB-138 and PCB-153 were positively associated with increased ΔCt values of miR-7-5p and miR-30d-5p and negatively associated with miR-133a-3p and miR-133b. These findings were consistent with analyses performed using the sum of PCBs (Figure 3). The β coefficients and confidence intervals for each significant association are reported in Table S3 and Figure S3.

Mixture effects of chemicals on miRNA Expression

In order to estimate mixture effects, we performed bWQS regressions to assess the effect of three different mixtures: EEs (Cu, Se, Zn), POPs (including p,p'-DDE, HCB, PCB-74, PCB-118, PCB-138, PCB-153, PCB-180), and the total mixture comprising both EEs and POPs. The number of statistically significant associations for each mixture and single-chemical regression are reported in Figure 4 (higher ΔCt values

are indicative of lower miRNA expression, while lower ΔC_t values reflect higher miRNA expression).

The bWQS analysis of maternal serum EEs identified twenty-one significant associations, six of which confirmed findings from the single-chemical regression models (Figure 3, and Figure 4 panel A). Among these, four showed a strong positive association, and two showed negative associations. In addition, fifteen associations not previously detected in the single-chemical models were identified (Figure 3 and Figure 4 panel-A). Overall, eight maternal miRNAs were positively associated with the EE mixture, while seven showed negative associations.

For POPs, the bWQS analysis confirmed three significant associations previously observed in maternal serum (Figure 3-and Figure 4 panel B). One association had been previously associated with HCB/DDE, one with HCB/PCBs and one with at least one PCB congener. Additionally, eleven miRNAs not previously identified in the single-chemical models were significantly associated with the POP mixture (Figure 3 and Figure 4 panel B), including four positive and seven negative associations.

When examining the total mixture, the bWQS model identified ten significant associations with maternal miRNA ΔC_t values (Figure 3 and Figure 4 panel B). Of these, three positive and one negative associations overlapped with those observed for the EE mixture, while four positive and two negative associations were consistent with those identified for the POPs. Detailed information about β estimates obtained by bWQS models in maternal serum are reported in Table S4 and Figure S4.

Nine out of eleven associations of the single-chemical analyses were confirmed through the bWQS analysis performed on the EE mixture in cord serum (Figure 3 and Figure 4 panel D). Specifically, four miRNAs identified by the bWQS analysis for the EE mixture had been previously positively associated in single-chemical models with both Cu and Se. An additional two miRNAs associated with the EE mixture had shown positive associations only with Cu in the single-chemical models, while two miRNAs previously negatively associated with Se in the single-chemical models were consistently negatively associated also in the mixture analysis. Finally, seven additional cord serum miRNAs were uniquely identified in the EE mixture analysis,

including three showing positive associations and four showing negative associations, which had not been detected in any single-chemical model.

Analysing the POP mixture in cord serum, six associations previously identified in the single-chemical regression were confirmed (Figure 3 and Figure 4 panel E). Of these, four miRNAs showed negative association, while two showed positive associations with at least one PCBs. Interestingly, two miRNAs identified in the POP mixture (miR-374a and miR-205-5p) had previously shown associations with maternal Zn in the single-chemical models, but in the opposite direction of effect. The bWQS analysis of the POP mixture revealed ten new associations, of which nine with a negative trend, reflecting an increase in expression.

The bWQS analysis of the total mixture in cord serum identified sixteen associations (Figure 3 and Figure 4 panel F). Among these, two negative and two positive associations overlap with those identified by both EE and POP mixtures. Four associations specifically overlapped with only EE mixture results, of which three were positive. In addition, eight negative associations emerged in common exclusively with those identified for the POPs mixture.

The β coefficients and confidence intervals for each significant association in cord serum are reported in Table S5 and Figure S5.

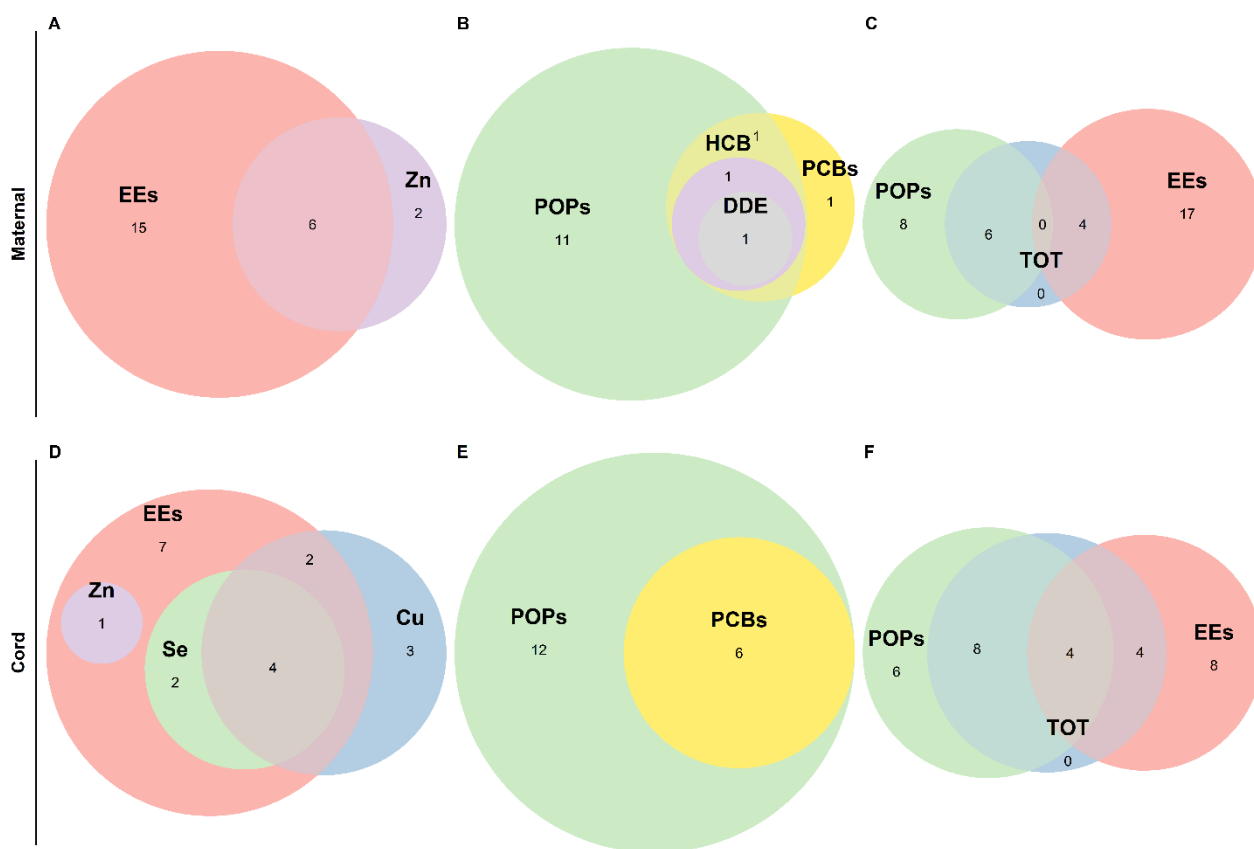


Figure 4. Shared and distinct miRNAs emerged from both single-pollutant and mixture analyses.: Venn diagrams showing overlaps between bWQS- and single-exposure-associated miRNAs in maternal (A-B) and cord (D-E) compartments. Shared miRNAs across the three mixtures are reported for maternal (C) and cord (F) side.

To further explore the drivers of the observed miRNA alterations, we examined the contribution of the individual components within pollutant mixtures to overall miRNA expression patterns on both the maternal and foetal side. The bWQS chemical weight thresholds are listed in Table S1.

As shown in Figure 5 (left side, EEs section), Zn exceeded the contribution threshold ($t_{EE_m}=0.33$) in 90% of the significant associations, followed by Cu (19%), and Se (1%) in maternal serum.

In the POPs section, HCB, PCB-74, and PCB-118 emerged as the most frequent contributors (50% of the significant associations), followed by p,p'-DDE (43%). PCB-153 exceeded the weight threshold in 36% of the associations, while PCB-138 and PCB-180 exceeded the 0.14 threshold ($t_{POP_m}=0.14$) in 28% of the associations.

In maternal serum, Se emerged as the predominant element across the total mixture, exceeding the weight threshold in 80% of the significant associations, followed by Cu (50%) as highlighted in Figure 5 (left side, TOTAL section). In contrast, Zn overcame the threshold in 40% of associations. Among the POPs, PCB-74 and PCB-118 were consistently above the threshold ($t_{TOTm}=0.10$) in 60% and 50% of the associations, respectively. HCB and PCB-138 showed weights above the threshold in 30% of the cases, while p,p'-DDE and PCB-153 exceeded the 0.10 threshold in 20% of the associations. PCB-180 was the least influential as 10% of associations reported PCB-180 as having weight above the threshold.

Regarding the cord serum, among the elements included in the EE mixture, Cu most frequently emerged as a key driver of the observed associations (50%), while Zn and Se showed a lower contribution frequency (37% and 31%, respectively) (Figure 5 right side, EEs section).

For the POPs section, as illustrated in Figure 5 (right side), PCB-138 emerged as the most recurrent contributor across the significant associations, exceeding the relevant threshold ($t_{POPc}=0.20$) in 78% of the cases. PCB-153 and HCB also demonstrated substantial contributions, with weights over the threshold in 67% and 61% of the significant associations, respectively. In contrast, PCB-180 exceeded the threshold in only 39% of the associations, while p,p'-DDE was the least frequent as having weight above the threshold in only 5% of the associations.

Overall, in cord serum, PCB-138 emerged as the most frequent component above the threshold, in 62% of significant associations (Figure 5 right side, TOTAL section), followed by HCB and PCB-153, each contributing in 50% and 44% of the cases. Cu and Se were involved in 37% of associations, while PCB-180 exceeded the threshold ($t_{TOTc}=0.125$) in 25% of associations. Finally, Zn and p,p'-DDE were the least represented, contributing both above the threshold in 19% of associations.

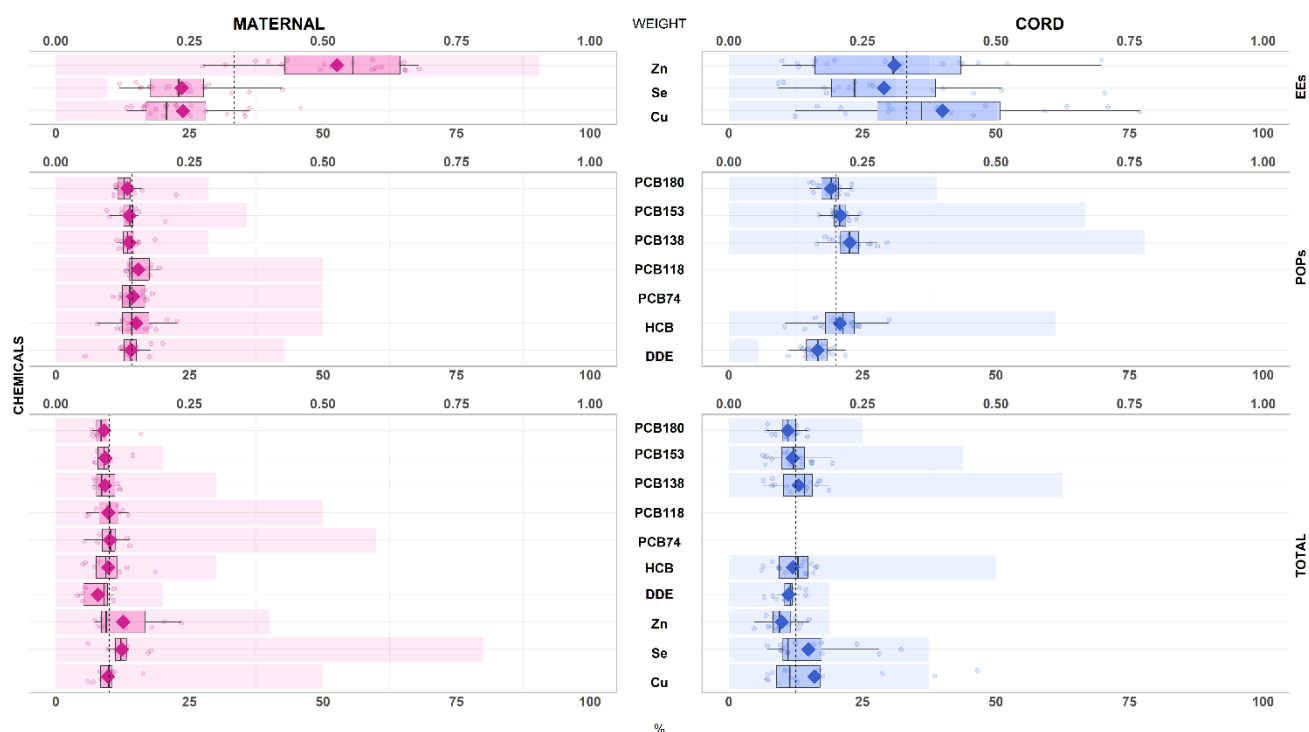


Figure 5. Mixture component (EEs, POPs and TOTAL) contributions to the outcome: The figure reports maternal information on the left ($n=45$ statistically significant miRNA-associated bWQS models) and cord results on the right ($n=50$ statistically significant miRNA-associated bWQS model). In each plot, bars correspond to the bottom axis and indicate how many times each chemical in the mixture showed a weight higher than threshold across statistically significant miRNAs. Box plot and circle points correspond to the upper axis (%). Individual data points indicate weights for each chemical. Box plots display the 25th, 50th, and 75th percentiles, where the mean weights are indicated by a rhombus. The dotted black lines indicate the threshold value.

Comparative miRNA-chemical associations in maternal and cord compartments

Associations between miRNAs and chemicals are summarized in Figure 6, which presents concordant and discordant associations identified in maternal and cord serum through the bWQS analysis. Overall, only a limited number of miRNAs exhibited consistent associations across the two matrices, both in terms of direction of the effect and mixture classes. For instance, miR-122-5p, miR-375-3p and miR-7-5p were positively associated with EEs in both maternal and cord serum, corresponding to higher ΔC_t values and therefore lower miRNA expression in both

matrices. Notably, miR-7-5p also showed a positive association with POPs in cord serum. Similarly, miR-29a-3p displayed consistent negative association with POPs in the maternal side and with both POPs and EEs in cord serum, corresponding to lower ΔC_t values and therefore higher miRNA expression. A second group of miRNAs exhibited concordant directions of associations but differed with respect to the mixture involved. MiR-27a-5p was negatively associated with EEs in maternal serum and with POPs in cord serum, while miR-195-5p displayed an opposite pattern, being positively associated with POPs in maternal serum and with EEs in cord serum.

In contrast, several miRNAs exhibited divergent patterns between maternal and cord compartments. Among these, five miRNAs showed opposite direction of association while remaining consistent in terms of mixture class. Specifically, miR-134-5p, miR-376c-3p and miR-30d-5p were negatively associated with EEs in maternal serum but positively associated in cord serum. Additionally, miR-30d-5p, showed a positive association with POPs in cord serum. Similarly, miR-25-3p and miR-133a-3p displayed an opposite direction of association with POPs between the two matrices: miR-25-3p was negatively associated in the maternal serum, and positively in the cord serum, whereas miR-133a-3p showed the opposite trend.

Finally, a subset of miRNAs displayed complete discordance, differing both in direction and mixture class across matrices. For example, miR-133b-3p, miR-143-3p, miR-145-5p, miR-200a-3p, and miR-205-5p were positively associated with EEs in maternal serum but negatively associated with POPs in cord serum. Conversely, miR-374a-5p showed a negative association with EEs mixture in maternal serum and positive association with POPs in cord serum. Detailed information is provided in Table S6, along with a complementary representation including total mixture results in Figure S6.

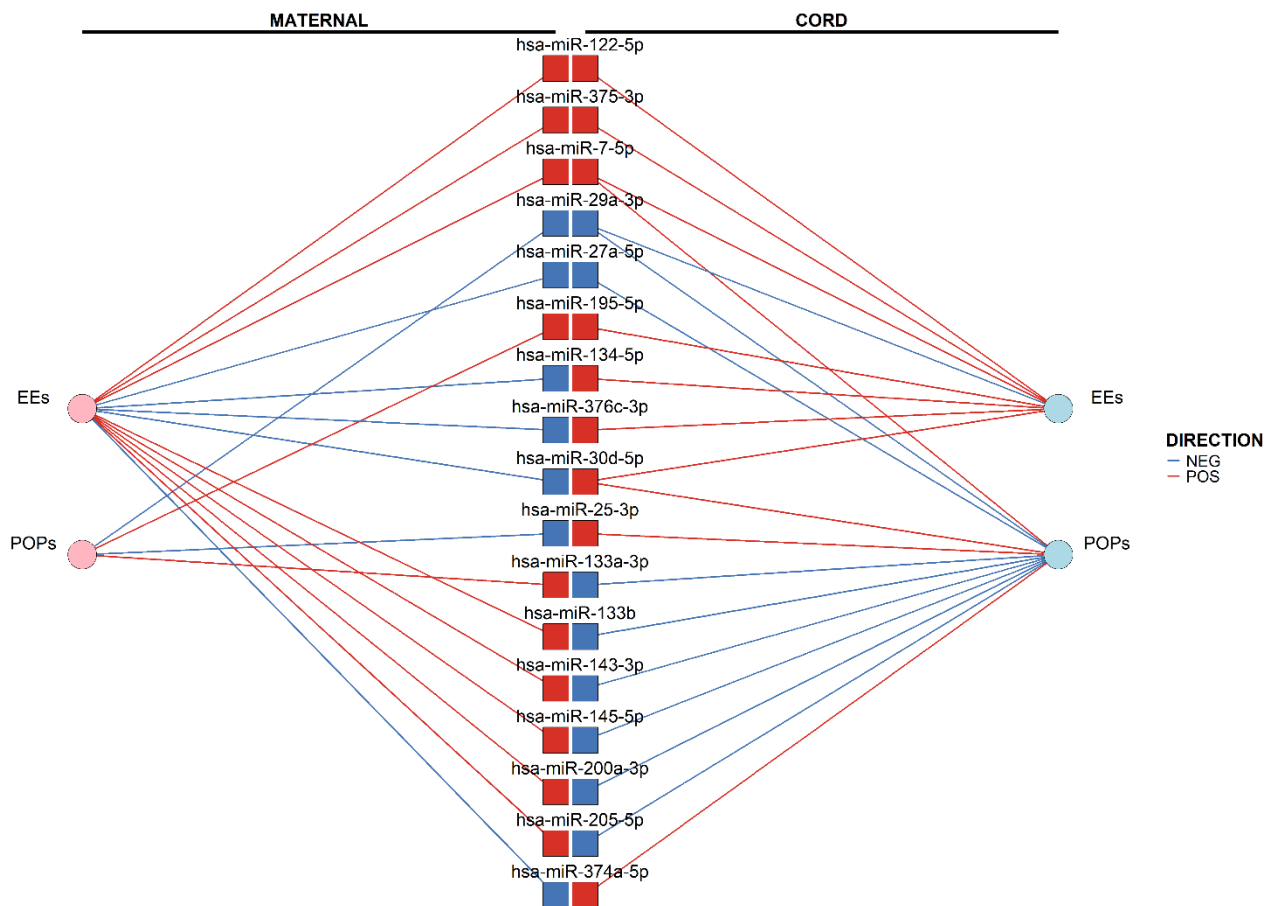


Figure 6. Concordant and discordant miRNA-mixture associations across maternal and cord compartments: Schematic representation of similarity/discrepancy associations between miRNAs and chemical exposure mixtures in maternal and cord compartments. Each miRNA is represented by a pair of adjacent squares (maternal on the left, cord on the right), coloured according to the direction of the association (red = positive; blue = negative). From each square, coloured edges connect to two circular nodes corresponding to the exposure mixtures: EEs, and POPs (pink-left = maternal mixture, light blue-right = cord mixtures). The colour of each edge matches that of the square, indicating the direction of the association for that specific mixture.

Pathway enrichment analysis

Pathway enrichment analysis was conducted on predicted targets of miRNAs associated with chemical exposures in maternal and cord serum. Overall, 494 validated target genes were identified for miRNAs significantly associated in the BWQS analysis. Among these, 144 genes were shared between the miRNAs identified in the two matrices, whereas 133 were uniquely targeted by maternal

miRNAs and 217 by cord serum miRNAs. The heatmap (Figure 7) illustrates differences in enrichment pathways across exposure groups and biological matrices, displaying the hub pathways targeted by at least four associated miRNAs. The miRNAs were grouped according to exposure type (EEs, POPs, or TOT mixtures), direction of the association (positive or negative), and biological matrix (maternal or cord serum) to identify clusters of enriched pathways.

The miRNAs detected in maternal serum and positively associated with the EE mixture were predominantly enriched in six key hub pathways with the lowest adjusted p-value: "Signaling by Receptor Tyrosine kinases", "Signaling by ERBB2", "Cell-cell communication", "MAP kinase activation", "Innate immune system", and "Axon guidance". These pathways were mainly represented by the genes *IRS2*, *ERBB3*, *EGFR3* and *PAK1*, which are also shared with miRNAs altered in the cord serum. In addition, the pathways "Disease of signal transduction by growth factor receptors and second messengers", "Cytokine signaling in immune system", "Signal Transduction" and "RAF/MAP kinase cascade" included also miRNAs that were negatively associated with the EE mixture. The "Generic transcription pathway" was the only pathway specifically enriched by miRNAs negatively associated with EEs in maternal serum. Key genes involved across this pathway include *PI3KR2*, which is specific to the maternal serum, and *ESR1* and *FOXO1* which were also identified as targets in cord serum.

Several pathways including "GPCR downstream signalling", "Signaling by Receptor Tyrosine kinases", "Adaptative immune system", "Epigenetic regulation of gene expression", "Infectious disease" and "RAF/MAP kinase cascade" were enriched exclusively by miRNAs negatively associated with POP mixture. The genes primarily involved in these pathways and specific to POPs-miRNAs association in the maternal side were *H3F3B*, *JUN* and *AGO1*. In contrast, pathways such as "Metabolism", "Cell-cell communication", "Cell cycle, Mitotic", and "Transcriptional Regulation by TP53", were enriched exclusively by miRNAs positively associated with the POP mixture. In addition to *H3F3B*, *CDKN1A*, *E2F1* and *HIST3CII* genes were identified as key genes involved in these pathways. On the other hand, pathways such as "Disease of signal

transduction by growth factor receptors and second messengers", "Signal Transduction", "Developmental Biology", "Signaling by NOTCH", "Cytokine signalling in Immune system", "Generic Transcription Pathway", and "Cellular response to stress" included both positively and negatively associated miRNAs.

When considering the total mixture, enriched pathways included "Signal transduction", "Cytokine signaling", and "Cell cycle-related processes", reflecting the combined contribution of different chemical classes to regulatory networks involved in immune signaling and cellular proliferation.

In cord serum, most pathways were enriched by miRNAs positively associated with EEs, including "Signal transduction", and "MAP kinase activation", as observed for maternal serum. However, specific pathways, such as "Cell cycle, Mitotic", and "Signaling by Rho GTPase", were identified as specifically enriched in cord side. Notably, the "Innate Immune system" pathway was enriched by miRNAs with opposite direction of association with EEs mixture compared to maternal side. Additional genes specifically targeted in cord serum included *PPP2CA*, *ERBB2*, *ELMO2*, and *CCND3* transcripts. The miRNAs associated with POPs were mainly negatively associated. Compared to maternal serum, the newly identified pathways include "MAPK kinase activation", "Innate immune system", "Signalling by Rho GTPases", "Signaling by WNT", "Signaling by NOTCH1 in cancer", "Disease of metabolism", "Oncogenic MAPK signaling", "KEAP1-NFE2L2 pathway" and "Adipogenesis". These pathways were mainly driven by genes specifically targeted by POPs associated miRNAs in cord serum, such as *RHOA*, *MAPK14*, *NOTCH1*, *NFKB1* and *RELA*. The enriched pathways identified for the total mixture were consistent with those obtained from separate mixture results. Finally, a subset of genes including *CCND1*, *IRS1*, *CDK6*, *EGFR* and *KRAS*, were consistently identified across biological matrices and mixtures. The detailed contribution of each miRNA to the statistically significant enriched pathways is reported in the Supplementary Data 14.

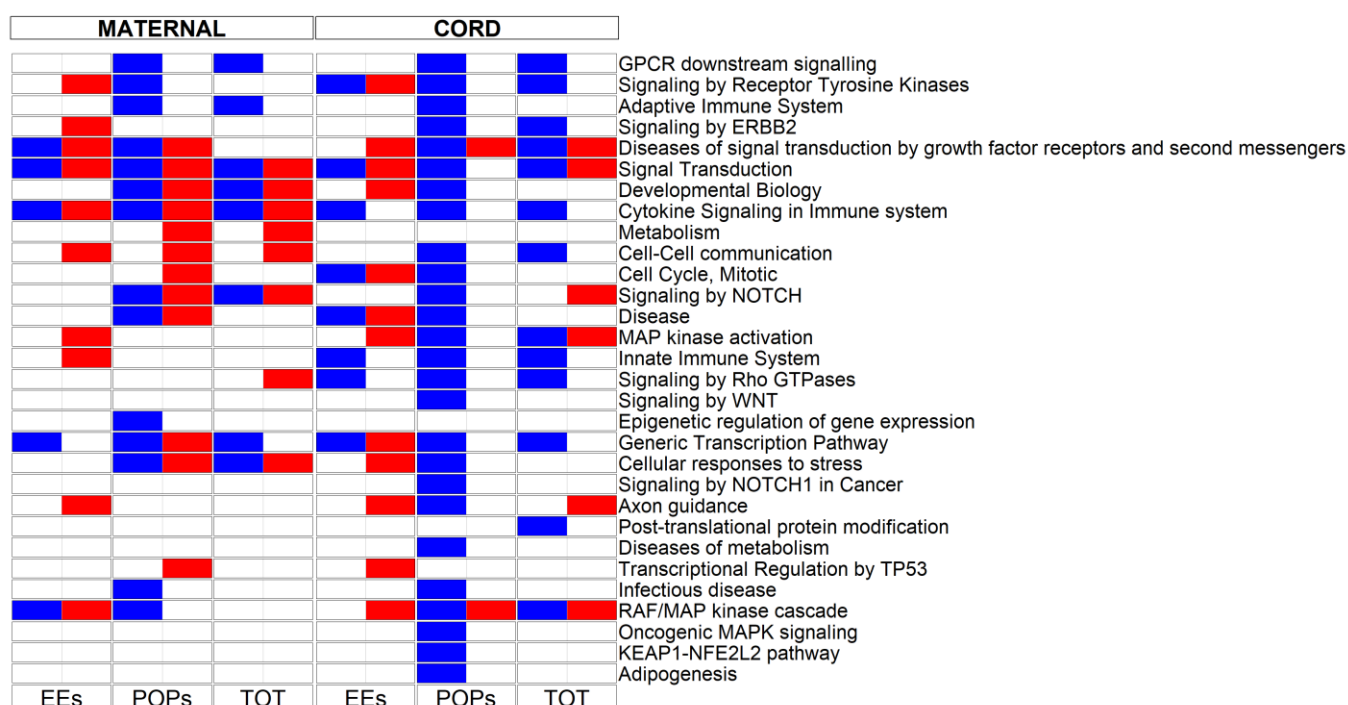


Figure 7. Reactome pathway enrichment analysis: Hub pathways are defined as those associated with at least four miRNAs. Red indicates pathways enriched in miRNAs positively associated in the bWQS analysis, whereas blue indicates enrichment in negatively associated miRNAs. Maternal associations are shown on the left, and cord blood associations on the right. For each matrix, results are presented separately for essential elements (EEs), persistent organic pollutants (POPs), and the total mixture (TOT).

Discussion

This study provides a characterization of epigenetic patterns associated with prenatal exposure to essential elements and organic environmental contaminants in a birth cohort recruited from a highly industrialized area, integrating exposure assessment with circulating miRNA profiling. The analysis was designed to explore i) the role of placental processes in pollutant transfer, ii) differences in circulating miRNA expression between maternal and foetal compartments, and iii) the relationships between chemical mixtures and miRNA expression in both compartments.

By analysing EEs and POPs in matched maternal and cord serum samples of the NEHO birth cohort, we provide evidence that the placental maternal–foetal interface acts as a selective chemical barrier. Placental transfer behaviour varied considerably across compounds. Among the EEs analysed, Zn showed a median transfer ratio greater than 1, consistent with active and regulated transport mechanisms supporting foetal development ^{42, 43}. In contrast, Se and Cu displayed moderate to low transfer efficiencies, while lipophilic POPs such as OCs and PCBs exhibited limited placental passage, with transfer ratios well below 0.5.

These findings align with prior reports which observed that the physicochemical properties of substances, particularly their hydrophobicity and protein-binding affinity, limit their mobility across the placenta ⁴⁴.

Strong intra-group correlations were found among chemicals with similar structures, while cross-group correlations were generally weak. Notable exceptions included Se with Cu and PCB-156 in maternal serum, and Se with Cu in cord serum. Strong correlations were observed between HCB and p,p'-DDE, as well as among PCB congeners, with correlation strength declining as the degree of chlorine substitution increased. These correlations highlight the placenta's role as a selective barrier that modulates, rather than simplistically prevents, pollutant transfer to the foetus. Furthermore, our data on essential elements demonstrated that they can be actively transported across the placenta through regulated mechanisms involving specific transporters, resulting in differential maternal–foetal distributions. This selective

transfer can be a key determinant of foetal exposure and may influence the observed associations between maternal element levels and cord blood miRNA profiles, highlighting the importance of considering placental pharmacokinetics when interpreting epigenetic outcomes.

Evidence from multiple cohorts (NEHO, MADRES, ECHO, ENVIRONAGE, and Asian studies) show that both essential and non-essential metals, especially when studied as mixtures, are associated with miRNA dysregulation, extracellular vesicle-mediated signaling changes, and broader molecular alterations linked to foetal development and growth^{15, 45, 46, 47, 48, 49}. Placenta-specific miRNA clusters, such as C19MC and C14MC, are known to play important roles in placental development and maternal-foetal communication^{50, 51}, showing strong tissue specificity and gestational age dependency⁵². However, these placenta-specific miRNAs were not included in the present analytical panel, preventing a direct evaluation of placental-specific epigenetic responses. Instead, the circulating miRNAs analysed here provide a broader, system-level representation of biological responses, capturing signals from multiple tissues and enabling association analyses with environmental exposures and chemical mixtures. However, this approach also presents some limitations, including the lack of tissue specificity and potential variability in circulating miRNA profiles across studies due to differences in cohort characteristics, exposure assessment, and analytical platforms.

This framework provides the basis for analysing associations between environmental pollutants and circulating miRNAs in the maternal and cord blood sides of the placenta in the NEHO birth cohort samples.

Essential trace elements appear to modulate miRNA profiles in a compartment-specific manner, with distinct patterns of expression observed between maternal and cord blood⁵³. In maternal serum, Zn showed both direct and inverse associations depending on the miRNA considered. In particular, higher maternal Zn concentrations were associated with lower ΔC_t values of miR-30d-5p and miR-374a-5p, corresponding to higher circulating levels of these miRNAs, suggesting a possible role of Zn in regulatory pathways involved in maternal-foetal

communication and pregnancy complications¹⁴. Conversely, higher Zn concentrations were associated with higher ΔCt values, corresponding to lower circulating levels, of several miRNAs involved in biological processes relevant to pregnancy, including trophoblast invasion, epithelial integrity, and immune tolerance (miR-148a-3p, miR-205-5p)^{54, 55}, metabolic homeostasis (miR-122-5p)⁵⁶, and vascular and placental function (miR-10b-5p, miR-206, miR-214-3p)^{57, 58, 59}. Given the established role of zinc in oxidative stress regulation, epigenetic enzyme activity and placental development^{60, 61}, these findings support the hypothesis that maternal zinc status may contribute to shaping circulating miRNA patterns linked to adaptive processes during pregnancy. In cord serum, Zn showed a different pattern compared with maternal serum, with higher Zn concentrations associated only with higher ΔCt values of miR-16-5p. In contrast, Cu concentrations were associated with lower ΔCt values of several miRNAs, including miR-7-5p, miR-10a-5p, miR-30d-5p, miR-122-5p, miR-134-5p, miR-195-5p, miR-200c-3p, miR-376c-3p, and miR-574-3p, indicating higher circulating abundance.

Selenium displayed heterogeneous association patterns depending on the miRNA considered, with higher Se concentrations associated with lower ΔCt values of miR-22-3p and miR-29a-3p, indicating higher circulating levels of these miRNAs. Overall, these observations suggest that several cord serum miRNAs may respond to neonatal metabolic conditions, supporting the concept that the foetal circulating miRNome is sensitive to environmental and physiological exposures during pregnancy⁶². Exposure to OCs, including p,p'-DDE, HCB, and different PCB congeners, was associated with coordinated miRNA patterns converging on pathways related to cardiovascular regulation and placental vascular function. In maternal serum, several OCs were associated with lower ΔCt values of miR-1-3p, indicative of higher circulating levels of this miRNA. Similarly, HCB and PCB-118 were associated with lower ΔCt values of miR-133a-3p. Both miR-1-3p and miR-133a-3p belong to the group of muscle-specific "myomiRs" involved in muscle development and cardiac function, suggesting that OC exposure may influence molecular pathways related to cardiovascular adaptation during pregnancy⁶³. PCB-

118 and PCB-138 showed broader association patterns, involving multiple miRNAs, whereas the more highly chlorinated congeners PCB-153 and PCB-180 were primarily associated with miR-195-5p and miR-30e-5p. These associations were also confirmed when considering the sum of PCB concentrations. Current evidence indicates that both miR-195-5p and miR-30e-5p play important regulatory roles during pregnancy. MiR-195-5p supports trophoblast proliferation, migration, and invasion and is involved in angiogenic and activin/Nodal signalling pathways, while its circulating levels have been linked to the anti-angiogenic imbalance characteristic of preeclampsia ⁶⁴. MiR-30e-5p has similarly been associated with vascular and immune-inflammatory adaptations, including preeclampsia trajectories, microvascular dysfunction, and late-pregnancy blood-pressure phenotypes ⁶⁵. Overall, these findings suggest that OC exposure may preferentially affect miRNA networks involved in cardiovascular, angiogenic, and placental regulatory processes. In cord serum, higher PCB concentrations were consistently associated with higher Δ Ct values of miR-29a-3p, indicating lower circulating levels of this miRNA across all measured congeners ⁶⁶. Previous studies have shown that miR-29a-3p, enriched in villus-derived exosomes, contributes to maternal-foetal immune tolerance by suppressing decidual NK-cell IFN- γ production, as reported in recurrent pregnancy loss ⁶⁷. In addition, PCB-138 and PCB-153 were associated with lower Δ Ct values of miR-7-5p and miR-30d-5p, suggesting higher circulating abundance, whereas the same congeners were associated with higher Δ Ct values of miR-133a-3p and miR-133b-3p, consistent with lower circulating levels. Together, these findings suggest that PCB exposure may disrupt miRNA networks involved in muscle and cardiovascular development. As a first approach, these single-pollutant models were very informative for identifying specific associations between exposures (chemicals) and biological responses (miRNAs), also showing qualitative and quantitative responses in both the maternal and foetal compartments. However, their use would oversimplify the real-world scenario in which individuals are simultaneously exposed to a complex mixture of contaminants.

Therefore, adopting multipollutant approaches provides a more realistic framework for analysing mixtures effects, enabling the identification of key drivers and better capturing the overall outcomes of environmental exposures on maternal and foetal health, particularly in highly industrialized areas with complex pollutant profiles.

By extending the analysis to include mixture models through bWQS regression, we identified several additional miRNAs associated with combined exposures to EEs and POPs, many of which were not detectable in the single-chemical models.

Our multipollutant analysis displayed both consistent and divergent associations between environmental mixtures and circulating miRNAs in maternal and cord serum, suggesting shared as well as compartment-specific regulatory responses. For instance, miR-7-5p, miR-122-5p and miR-375-3p were positively associated with essential element mixtures in both maternal and cord samples, indicating stable modulation by micronutrient-related uptake and supporting their role as common biomarkers of exposure across compartments. In contrast, several miRNAs demonstrated opposite directions of association between mothers and children, including miR-133b, miR-143-3p, miR-145-5p, miR-200a-3p and miR-205-5p, which may reflect differential maternal–foetal adaptive responses or compartment-specific regulation of gene expression in response to environmental stressors (consistent with the known roles of these miRNAs in trophoblast invasion, Epithelial–Mesenchymal Transition, endothelial signaling, and placental dysfunction) ⁶⁸.

Such divergent associations suggest that maternal and foetal systems develop distinct molecular programs to cope with environmental stress, reflecting compartment-specific priorities in maintaining pregnancy homeostasis. Notably, miR-195-5p showed consistent positive associations with both POPs and EEs mixtures in both compartments, probably due to its central role in placental and vascular biology ⁶⁹. By contrast, miR-30d-5p, miR-134-5p, and miR-376c-3p shifted direction depending on the exposure mixture considered, underlining the complexity of multipollutant effects and the necessity of integrated analytical approaches to capture them. Together, these findings suggest that, while some miRNAs act as robust biomarkers of shared maternal–foetal exposure, others may capture

regulatory programmes, underscoring the value of mixture-aware models in elucidating the molecular effect of environmental exposures during pregnancy.

Considering the placental transfer dynamics, our results illustrate how complex chemical exposures modulate compartment-specific miRNA responses. Overall, the bWQS regression demonstrated that, in the maternal serum, Zn emerged as the most frequent contributor to mixture-associated miRNA expression, whereas Cu dominated on the foetal side, emphasizing not only the chemical-specific but also the compartment-specific drivers of epigenetic regulation. Among POPs, PCB-74, PCB-118 and HCB were the most influential components in maternal serum, whereas PCB-138, PCB-153 and HCB were predominant in the cord compartment. These patterns suggest that the most biologically active or bioavailable pollutant components may differ across maternal and foetal systems, probably due to differences in toxicokinetic, metabolism, or placental transfer efficiency. Indeed, few miRNA-pollutant associations overlapped across compartments, despite strong correlations in pollutant levels. These divergences point to molecular responses influenced by tissue-specific regulation, chemical concentration, developmental context, and potential differences in the cellular composition of circulating carriers such as extracellular vesicles.

To further hypothesize the biological significance of this epigenetic modulation, we performed a pathway enrichment analysis on the predicted target genes of the identified miRNAs. To analyse the structures from these multi-dimensional signals in detail, miRNAs were grouped by exposure class [EEs, POPs, or total mixture (TOT)], direction of association in the bWQS models (positive vs negative), and biological origin (maternal vs cord). The general structure of the enriched pathways was organized into general hubs. Only pathways supported by at least four miRNAs are displayed, increasing confidence that the observed communities are not driven by single markers.

Pathway enrichment analysis suggests distinct yet partially overlapping molecular responses associated with environmental exposures in maternal and cord samples. Overall, both compartments show modulation of key signaling and immune-related

pathways, though with notable differences in directionality and pathway specificity. Notably, while some pathways (e.g., MAPK cascade, immune signaling) were enriched in both maternal and cord compartments, their differential activation patterns (positive vs negative associations) suggest compartment-specific regulatory responses. Such divergence may reflect differences in exposure kinetics, detoxification/biotransformation capacity, and developmental context between mother and foetus. When considering exposure categories, POPs appear to exert a broader and more pronounced effect, particularly in cord samples, where multiple pathways show consistent modulation. In contrast, essential elements display more selective effects, reflecting their dual role as nutrients and toxicants depending on concentration. Both excessive levels and deficiencies may result in adverse biological responses, as physiological homeostasis requires a tightly regulated range to support redox balance, immune function and hormonal activities^{70, 71}. Within this context, the present study highlights the interplay between placental transfer, environmental exposures, and compartment-specific miRNA regulation, demonstrating that both essential elements and persistent pollutants contribute to distinct maternal and foetal epigenetic responses.

The strength of this study lies in its integrative design, combining high-resolution chemical analysis with miRNA profiling in matched maternal and foetal serum, and applying both single-pollutant and advanced mixture modelling approaches. To the best of our knowledge, this combined approach represents a significant and original improvement in this field. However, some limitations must be acknowledged. First, we acknowledge the limitations of qRT-PCR-based miRNA quantification, including variability in normalization and sensitivity to technical factors, as well as the assumptions underlying miRNA-mRNA mapping and pathway enrichment analyses, which rely on existing databases and may lack context specificity. Indeed, the sample size, while sufficient for detecting robust associations, may have limited our ability to explore more specific interactions, and the cross-sectional design does not permit inference of causality. In addition, while circulating miRNAs are promising biomarkers, they may not fully capture the tissue-specific regulatory differences

occurring at the maternal–foetal interface or within target organs. Nonetheless, the consistency of key findings across models and the alignment with established biological pathways support their interpretive validity.

In summary, our data demonstrate that environmental exposures during the last trimester of pregnancy modulate distinct and compartment-specific miRNA responses in maternal and foetal circulation, regulated by individual chemical properties, complex mixture effects, and the placenta's filtering role. The identification of exposure-sensitive miRNAs involved in developmental, oxidative stress, and immune pathways provides mechanistic insight into how early-life exposures may contribute to long-term health trajectories. These results emphasize the importance of considering both the maternal and foetal compartments, accounting for chemical mixtures and integrating molecular biomarkers to better understand the developmental origins of health and disease. Furthermore, our findings highlight the pivotal role of the placenta as a selective barrier and an active regulator in mediating foetal exposure and shaping molecular responses to environmental stressors. However, whether the placenta actively sends distinct regulatory signals to the maternal and foetal sides or rather acts as a selective barrier that separates or integrates molecular cues, remains to be clarified and warrants further investigation.

Altogether, these results advance our understanding of how the interplay between environmental factors and placental function contributes to the regulation of gene expression in early development, setting the stage for a detailed discussion of the observed pollutant-specific transfer dynamics and miRNA expression profiles.

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Author contributions

IC involved in data analysis; ALV, NA, AMS involved in the generation of experimental data; PC responsible for the funding; FC responsible for human enrollement; SR, GD and VL supervised the project.

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Ethical Approvals

The research project was approved by the relevant Ethics Committee (Comitato Etico Catania 2, 11 July 2017, n. 38/2017/CECT2) and was conducted in accordance with the principles of the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013). Before enrolment, all participating mothers provided written informed consent for the collection, processing, and use of their personal data and biological samples for research purposes. All procedures complied with the European Union General Data Protection Regulation (EU 2016/679) and Italian data protection legislation. To protect participants’ privacy and personal data, questionnaire data and biological samples were pseudonymized through the use of unique ID tracking number.

Code Availability

All code used for data processing, statistical analyses, figure generation, and synthetic data creation is publicly available at Zenodo⁷². The repository also contains documentation describing the analytical workflow and instructions for generating and using the synthetic dataset.

Data Availability

The individual-level data analysed in this study cannot be made publicly available because they contain sensitive human subject information and are subject to privacy and ethical restrictions. To facilitate reproducibility, all input data underlying the figures and tables presented in this manuscript, together with the regression results, are provided in the Supplementary Data files. A synthetic dataset reproducing the structure and key characteristics of the original data, without containing identifiable participant information, is available in the project repository. The source data for the figures are reported in the Supplementary Data. The source data for Figure 1 is in Supplementary Data 3, the source data for Figure 2 is in Supplementary Data 4, the source data for Figure 3 is in Supplementary Data 8, the source data for Figure 4 is in Supplementary Data 9, the source data for Figure 5 is in Supplementary Data 10, the source data for Figure 7 is in Supplementary Data 11-12. Requests for access to the original data in the current study will be considered on a reasonable request basis to the authors responsible of the NEHO cohort, Dr. Gaspare Drago (gaspare.drago@irib.cnr.it) and Dr. Silvia Ruggieri (silvia.ruggieri@irib.cnr.it), including a brief description of the research use. Requests will be reviewed and a response will typically be provided within 4-6 weeks, subject to ethical approval, data protection regulations, and institutional data-sharing and use agreements. Access will be provided under a data use agreement restricting use of the data to the stated research purpose, prohibiting redistribution to third parties, and requiring appropriate citation of this study.

Declaration

The Authors declare not to have any conflict of interest.

Figure 1. Placental transfer ratio: Boxplot reporting placental transfer ratio (cord/maternal – $n=95$) for each chemical which concentrations was below the LOQ in less than 15% of samples in both matrices.

Figure 2. Intra- and inter-compartment correlations of persistent organic pollutant and essential elements: Statistically significant Pearson correlation indices are reported for analyte concentrations in maternal (A) and cord (B) serum. In (C) the results combining both maternal and cord samples are reported. Positive correlations are shown in red, and negative correlations in blue, with intensity proportional to the strength of the correlation. (D) Scatter plots report the associations of statistically significant correlated chemicals between cord and maternal side.

Figure 3. Associations of maternal and cord blood miRNAs with individual chemicals and mixtures: Heatmap showing statistically significant associations between chemicals and miRNAs. Pink results (left up) were obtained from the maternal side, whereas blue results (left bottom) were from the cord serum. S-C m refers to single-chemical models, while MIX corresponds to results from the bWQS models. The left side of the heatmap shows the results of single-component linear regression analyses (individual miRNA vs. individual chemical), including only associations with an FDR-adjusted p -value < 0.05 . In contrast, the right side reports the results of bWQS mixture models for three different chemical groups: EEs, POPs, and TOTAL (all chemicals combined). Cell colours represent the direction and magnitude of the estimates (positive associations in red and negative associations in blue). This layout highlights that the miRNAs displayed on the left were associated with individual chemicals in single-component analyses, whereas the miRNAs shown on the right were identified exclusively through mixture-model associations. MiRNA levels are expressed as ΔCt values; therefore, positive associations correspond to lower miRNA expression, whereas negative associations correspond to higher miRNA expression.

Figure 4. Shared and distinct miRNAs emerged from both single-pollutant and mixture analyses: Venn diagrams showing overlaps between bWQS- and single-exposure-associated miRNAs in maternal (A-B) and cord (D-E) compartments. Shared miRNAs across the three mixtures are reported for maternal (C) and cord (F) side.

Figure 5. Mixture component (EEs, POPs and TOTAL) contributions to the outcome: The figure reports maternal information on the left ($n=45$ statistically significant miRNA-associated bWQS models) and cord results on the right ($n=50$ statistically significant miRNA-associated bWQS model). In each plot, bars correspond to the bottom axis and indicate how many times each chemical in the mixture showed a weight higher than threshold across statistically significant miRNAs. Box plot and circle points correspond to the upper axis (%). Individual data points indicate weights for each chemical. Box plots display the 25th, 50th, and 75th percentiles, where the mean weights are indicated by a rhombus. The dotted black lines indicate the threshold value.

Figure 6. Concordant and discordant miRNA–mixture associations across maternal and cord compartments: Schematic representation of similarity/discrepancy associations between miRNAs and chemical exposure mixtures in maternal and cord compartments. Each miRNA is represented by a pair of adjacent squares (maternal on the left, cord on the right), coloured according to the direction of the association (red = positive; blue = negative). From each square, coloured edges connect to two circular nodes corresponding to the exposure mixtures: EEs, and POPs (pink-left = maternal mixture, light blue-right = cord mixtures). The colour of each edge matches that of the square, indicating the direction of the association for that specific mixture.

Figure 7. Reactome pathway enrichment analysis: Hub pathways are defined as those associated with at least four miRNAs. Red indicates pathways enriched in miRNAs positively associated in the bWQS analysis, whereas blue indicates enrichment in negatively associated miRNAs. Maternal associations are shown on the left, and cord blood associations on the right. For each matrix, results are presented separately for essential elements (EEs), persistent organic pollutants (POPs), and the total mixture (TOT).

References

1. Wang Z, Walker GW, Muir DCG, Nagatani-Yoshida K. Toward a Global Understanding of Chemical Pollution: A First Comprehensive Analysis of National and Regional Chemical Inventories. *Environmental science & technology* **54**, 2575-2584 (2020).
2. Barker DJ. The origins of the developmental origins theory. *Journal of internal medicine* **261**, 412-417 (2007).
3. Bouchard MF, et al. Prenatal exposure to organophosphate pesticides and IQ in 7-year-old children. *Environmental health perspectives* **119**, 1189-1195 (2011).
4. Grandjean P, Heilmann C, Weihe P, Nielsen F, Mogensen UB, Budtz-Jorgensen E. Serum Vaccine Antibody Concentrations in Adolescents Exposed to Perfluorinated Compounds. *Environmental health perspectives* **125**, 077018 (2017).
5. Grandjean P, et al. Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. *JAMA* **307**, 391-397 (2012).
6. Ward G, Correia Watts MP, Hansson SR. The unintended consequences of modernity: Pollution and its effect on reproductive, maternal and fetal health. *Pregnancy Hypertens* **40**, 101204 (2025).
7. Barouki R, et al. Epigenetics as a mechanism linking developmental exposures to long-term toxicity. *Environment international* **114**, 77-86 (2018).

8. Dieckmann L, Czamara D. Epigenetics of prenatal stress in humans: the current research landscape. *Clin Epigenetics* **16**, 20 (2024).
9. Goyal D, Limesand SW, Goyal R. Epigenetic responses and the developmental origins of health and disease. *J Endocrinol* **242**, T105-T119 (2019).
10. Jiang C, *et al.* Maternal oral exposure to low-dose BPA accelerates the onset of puberty by promoting prepubertal Kiss1 expression in the AVPV nucleus of female offspring. *Reproductive toxicology* **124**, 108543 (2024).
11. Chaiwangyen W, Khantamat O, Kangwan N, Tipsuwan W, de Sousa FLP. MicroRNA expression in response to environmental hazards: Implications for health. *Ecotoxicology and environmental safety* **300**, 118420 (2025).
12. Metcalf GAD. MicroRNAs: circulating biomarkers for the early detection of imperceptible cancers via biosensor and machine-learning advances. *Oncogene* **43**, 2135-2142 (2024).
13. Foley HB, *et al.* EV-miRNA associated with environmental air pollution exposures in the MADRES cohort. *Environ Epigenet* **10**, dvae019 (2024).
14. Dietrich B, Haider S, Meinhardt G, Pollheimer J, Knofler M. WNT and NOTCH signaling in human trophoblast development and differentiation. *Cell Mol Life Sci* **79**, 292 (2022).
15. Longo V, *et al.* A multipollutant low-grade exposure regulates the expression of miR-30b, Let-7a and miR-223 in maternal sera: Evidence from the NEHO cohort. *The Science of the total environment* **844**, 157051 (2022).
16. Agarwal P, Anand M, Chakraborty P, Singh L, Masih J, Taneja A. Placental levels of polycyclic aromatic hydrocarbons (PAHs) and their association with birth weight of infants. *Drug Chem Toxicol* **45**, 868-877 (2022).
17. Bove H, *et al.* Ambient black carbon particles reach the fetal side of human placenta. *Nat Commun* **10**, 3866 (2019).
18. Gaylord A, *et al.* An exploratory analysis of miRNAs measured in placental tissue and their associations with prenatal exposure to household air pollution. *Environ Epigenet* **12**, dvaf036 (2026).
19. Li Q, *et al.* Exploring the associations between microRNA expression profiles and environmental pollutants in human placenta from the National Children's Study (NCS). *Epigenetics* **10**, 793-802 (2015).
20. Tsamou M, Vrijens K, Madhloum N, Lefebvre W, Vanpoucke C, Nawrot TS. Air pollution-induced placental epigenetic alterations in early life: a candidate miRNA approach. *Epigenetics* **13**, 135-146 (2018).

21. Anicic R, Mihajlovic D, Kocic J, Jagodic J, Stojasavljevic A. Levels of Cu, Zn, and Se in Maternal and Cord Blood in Normal and Pathological Pregnancies: A Narrative Review. *International journal of molecular sciences* **27**, (2025).
22. Azimi Z, Isa MR, Khan J, Wang SM, Ismail Z. Association of zinc level with DNA methylation and its consequences: A systematic review. *Heliyon* **8**, e10815 (2022).
23. Eroglu B, *et al.* Trace element-linked DNA methylation sites and their association with type 2 diabetes and cardiovascular diseases: EPIC-Potsdam cohort study. *Clin Epigenetics* **17**, 172 (2025).
24. Long P, *et al.* Profile of copper-associated DNA methylation and its association with incident acute coronary syndrome. *Clin Epigenetics* **13**, 19 (2021).
25. Krawczyk K, *et al.* Persistent organic pollutants affect steroidogenic and apoptotic activities in granulosa cells and reactive oxygen species concentrations in oocytes in the mouse. *Reprod Fertil Dev* **35**, 294-305 (2023).
26. Brennan E, Butler AE, Drage DS, Sathyapalan T, Atkin SL. Serum polychlorinated biphenyl levels and circulating miRNAs in non-obese women with and without polycystic ovary syndrome. *Front Endocrinol (Lausanne)* **14**, 1233484 (2023).
27. Butz H, Kinga N, Racz K, Patocs A. Circulating miRNAs as biomarkers for endocrine disorders. *J Endocrinol Invest* **39**, 1-10 (2016).
28. Li Y, *et al.* Per- and polyfluoroalkyl substances, polychlorinated biphenyls, organochlorine pesticides, and polybrominated diphenyl ethers and dysregulation of MicroRNA expression in humans and animals-A systematic review. *Environmental research* **244**, 117832 (2024).
29. Cosentini I, *et al.* Influence of prenatal hexachlorobenzene, PCB and selenium levels on growth trajectories in the first year of life: Findings from the NEHO birth cohort. *Environment international* **195**, 109225 (2025).
30. Drago G, *et al.* Exposure profiles in pregnant women from a birth cohort in a highly contaminated area of southern Italy. *Scientific reports* **13**, 14815 (2023).
31. Ruggieri S, *et al.* Three contaminated sites in southern Italy. The Neonatal Environment and Health Outcomes cohort: protocol for a longitudinal birth cohort study. *BMJ Open* **9**, e029471 (2019).
32. Ruggieri S, Maltese S, Drago G, Cibella F, Panunzi S. The Neonatal Environment and Health Outcomes (NEHO) Birth Cohort Study: Behavioral and Socioeconomic Characteristics and Drop-Out Rate from a

Longitudinal Birth Cohort in Three Industrially Contaminated Sites in Southern Italy. *International journal of environmental research and public health* **18**, (2021).

33. Zona A, *et al.* [SENTIERI: Epidemiological Study of Residents in National Priority Contaminated Sites. Fifth Report]. *Epidemiologia e prevenzione* **43**, 1-208 (2019).
34. Davies S, *et al.* Pre-conception serum ferritin concentrations are associated with metal concentrations in blood during pregnancy: A cohort study in Benin. *Environmental research* **202**, 111629 (2021).
35. Benjamini Y, Yekutieli D. *The control of the false discovery rate in multiple testing under dependency* (2001).
36. Colicino E, Pedretti NF, Busgang SA, Gennings C. Per- and poly-fluoroalkyl substances and bone mineral density: Results from the Bayesian weighted quantile sum regression. *Environ Epidemiol* **4**, e092 (2020).
37. Wilkinson L. Exact and approximate area-proportional circular Venn and Euler diagrams. *IEEE Trans Vis Comput Graph* **18**, 321-331 (2012).
38. Ru Y, *et al.* The multiMiR R package and database: integration of microRNA-target interactions along with their disease and drug associations. *Nucleic Acids Res* **42**, e133 (2014).
39. Yu G. Thirteen years of clusterProfiler. *Innovation (Camb)* **5**, 100722 (2024).
40. Wijesooriya K, Jadaan SA, Perera KL, Kaur T, Ziemann M. Urgent need for consistent standards in functional enrichment analysis. *PLoS Comput Biol* **18**, e1009935 (2022).
41. Team RC. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. . (ed^(eds) (2025).
42. Brion LP, Heyne R, Lair CS. Role of zinc in neonatal growth and brain growth: review and scoping review. *Pediatr Res* **89**, 1627-1640 (2021).
43. Willekens J, Runnels LW. Impact of Zinc Transport Mechanisms on Embryonic and Brain Development. *Nutrients* **14**, (2022).
44. Jeong Y, *et al.* Placental transfer of persistent organic pollutants and feasibility using the placenta as a non-invasive biomonitoring matrix. *The Science of the total environment* **612**, 1498-1505 (2018).
45. Foley HB, *et al.* Extracellular vesicle-enriched miRNA profiles across pregnancy in the MADRES cohort. *PloS one* **16**, e0251259 (2021).

46. Howe CG, *et al.* Urinary metals and maternal circulating extracellular vesicle microRNA in the MADRES pregnancy cohort. *Epigenetics* **17**, 1128-1142 (2022).
47. Janssen BG, *et al.* Cohort Profile: The ENVIRonmental influence ON early AGEing (ENVIRONAGE): a birth cohort study. *Int J Epidemiol* **46**, 1386-1387m (2017).
48. Park CH, *et al.* How the Environmental Influences on Child Health Outcome (ECHO) cohort can spur discoveries in environmental epidemiology. *Am J Epidemiol* **193**, 1219-1223 (2024).
49. Zuo Y, Jiang Y, Li X, Tong J, Tao FB. Association of maternal exposure to trace elements and toxic metals with repeated measurements of foetal and early-childhood growth: a systematic review. *Occup Environ Med* **82**, 459-466 (2025).
50. Morales-Prieto DM, Ospina-Prieto S, Chaiwangyen W, Schoenleben M, Markert UR. Pregnancy-associated miRNA-clusters. *J Reprod Immunol* **97**, 51-61 (2013).
51. Paquette AG, Chu T, Wu X, Wang K, Price ND, Sadovsky Y. Distinct communication patterns of trophoblastic miRNA among the maternal-placental-fetal compartments. *Placenta* **72-73**, 28-35 (2018).
52. Smith MD, *et al.* Large-scale transcriptome-wide profiling of microRNAs in human placenta and maternal plasma at early to mid gestation. *RNA Biol* **18**, 507-520 (2021).
53. Grzeszczak K, Kwiatkowski S, Kosik-Bogacka D. The Role of Fe, Zn, and Cu in Pregnancy. *Biomolecules* **10**, (2020).
54. Shah KB, Chernausk SD, Teague AM, Bard DE, Tryggestad JB. Maternal diabetes alters microRNA expression in fetal exosomes, human umbilical vein endothelial cells and placenta. *Pediatr Res* **89**, 1157-1163 (2021).
55. Zhao L, Xiong M, Liu Y. Baicalin enhances the proliferation and invasion of trophoblasts and suppresses vascular endothelial damage by modulating long non-coding RNA NEAT1/miRNA-205-5p in hypertensive disorder complicating pregnancy. *J Obstet Gynaecol Res* **47**, 3060-3070 (2021).
56. Liu Y, *et al.* Abnormal miR-122-5p expression in decidual NK cells and its impact on trophoblast behavior: insights into unexplained recurrent pregnancy loss. *Int J Med Sci* **21**, 2824-2836 (2024).
57. Al-Rubaye S, *et al.* Aberrant expression of BAX, MEG3, and miR-214-3P genes in recurrent pregnancy loss. *Gynecol Endocrinol* **37**, 660-664 (2021).
58. Morales-Rosello J, Martinez-Hernandez AI, Scheel J, Loscalzo G, Garcia-Lopez EM, Murdoch C. Differential Expression of Micro-RNA in Maternal Blood of Pregnancies Affected with Late-Onset Fetal Growth Restriction. *Biomed Hub* **10**, 134-151 (2025).

59. Schlosser K, Kaur A, Dayan N, Stewart DJ, Pilote L, Delles C. Circulating miR-206 and Wnt-signaling are associated with cardiovascular complications and a history of preeclampsia in women. *Clin Sci (Lond)* **134**, 87-101 (2020).
60. Ding A, Cao Y, Li F, Yang Q, Zhou D, Dong X. Study on the Effects of Cadmium Exposure on Zinc Metabolism and FOXO3a Expression in the Preeclampsia Maternal-Fetal System. *Int J Womens Health* **17**, 2551-2559 (2025).
61. Wilson RL, *et al.* Zinc is a critical regulator of placental morphogenesis and maternal hemodynamics during pregnancy in mice. *Scientific reports* **7**, 15137 (2017).
62. Zaunschirm-Strutz J, Rieder A, Tocantins C, Diniz MS, Weiss E, Hiden U. MicroRNA profiling in umbilical cord plasma: links to maternal metabolism and neonatal metabolic and inflammatory traits. *J Physiol* **603**, 1663-1680 (2025).
63. Mitchelson KR, Qin WY. Roles of the canonical myomiRs miR-1, -133 and -206 in cell development and disease. *World J Biol Chem* **6**, 162-208 (2015).
64. Ghosh S, Thamotharan S, Fong J, Lei MY, Janzen C, Devaskar SU. Circulating extracellular vesicular microRNA signatures in early gestation show an association with subsequent clinical features of pre-eclampsia. *Scientific reports* **14**, 16770 (2024).
65. Timofeeva AV, Fedorov IS, Sukhova YV, Ivanets TY, Sukhikh GT. Prediction of Early- and Late-Onset Pre-Eclampsia in the Preclinical Stage via Placenta-Specific Extracellular miRNA Profiling. *International journal of molecular sciences* **24**, (2023).
66. Krauskopf J, *et al.* MicroRNA profile for health risk assessment: Environmental exposure to persistent organic pollutants strongly affects the human blood microRNA machinery. *Scientific reports* **7**, 9262 (2017).
67. Fang Z, *et al.* Increased levels of villus-derived exosomal miR-29a-3p in normal pregnancy than uRPL patients suppresses decidual NK cell production of interferon-gamma and exerts a therapeutic effect in abortion-prone mice. *Cell Commun Signal* **22**, 230 (2024).
68. Jin M, Xu Q, Li J, Xu S, Tang C. Micro-RNAs in Human Placenta: Tiny Molecules, Immense Power. *Molecules* **27**, (2022).
69. Zhou D, *et al.* MiR-195-5p facilitates the proliferation, migration, and invasion of human trophoblast cells by targeting FGF2. *J Obstet Gynaecol Res* **48**, 2122-2133 (2022).

70. Izquierdo Alvarez S, *et al.* Updating of normal levels of copper, zinc and selenium in serum of pregnant women. *J Trace Elem Med Biol* **21 Suppl 1**, 49-52 (2007).
71. Jain RB. Thyroid function and serum copper, selenium, and zinc in general U.S. population. *Biol Trace Elem Res* **159**, 87-98 (2014).
72. Ilaria, C. (2026). Hela06/NEHO-miRNAs: v1.0 (Version v1.0) [Computer software]. Zenodo. <https://doi.org/10.5281/zenodo.20814189>

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