

An integrated exposome risk prioritization strategy for disease-specific substance priority listing: Preeclampsia as a proof-of-concept

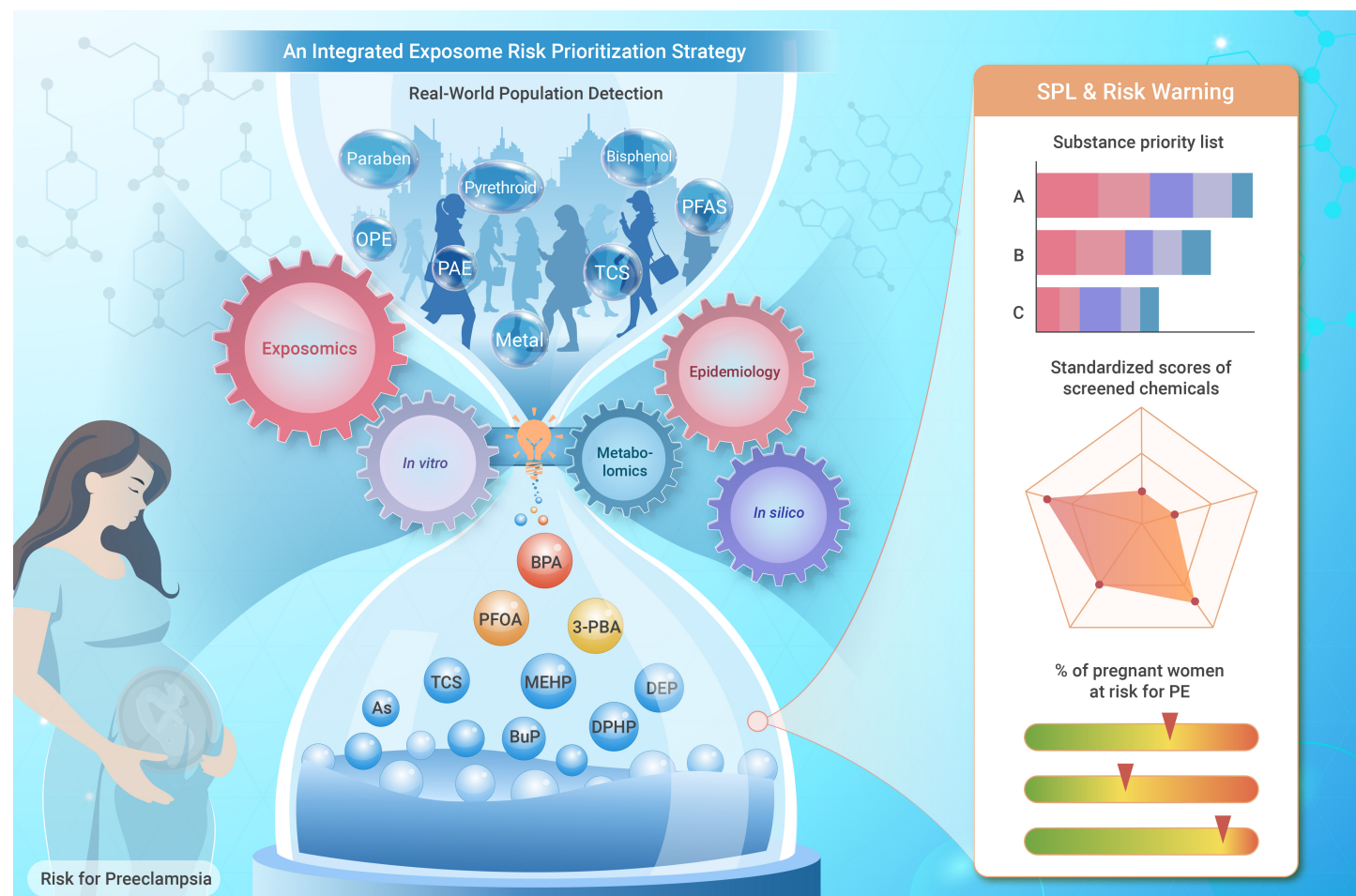
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GRAPHICAL ABSTRACT



PUBLIC SUMMARY

- The role of environmental causes in pregnancy complications is increasingly recognized.
- From exposomes to multi-omics, integrating research strategy for comprehensive chemical risk assessment.
- Bisphenol A, perfluorooctanoic acid and 3-phenoxybenzoic acid are the main risk factors for PE.
- Establishes chemical risk hierarchy for PE using exposure thresholds and mechanistical evidence.

An integrated exposome risk prioritization strategy for disease-specific substance priority listing: Preeclampsia as a proof-of-concept

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Environmental risk assessment and management policies are challenged by the ever-mounting number of chemicals, mandating effective substance priority list (SPL) for health risks. Hence, a comprehensive SPL tool considering evidence from varying levels is highly needed, particularly with disease-specific precision. Here, we constructed an integrated chemical risk ranking score (ICRRS) approach based on exposomic profiling, epidemiological analyses, *in vitro* cytotoxicity, metabolism-disruption, and *in silico* toxicity, to prioritize chemicals related to the risk of preeclampsia (PE). Using this approach, of the 99 chemicals measured, we shortlisted nine chemicals of concern that are most relevant to PE, including bisphenol A, perfluorooctanoic acid and 3-phenoxybenzoic acid, affecting up to 50% women. This multi-dimensional approach containing complementary evidence from various aspects, allowing a precision prioritization of chemicals in relation to PE risk. This model of SPL construction can be adapted for other diseases, assisting the identification of pivotal substances that should be placed under more strict regulations on production and usage for human health.

INTRODUCTION

The importance of environmental chemicals in disease etiology is increasingly recognized, necessitating urgent risk assessments.¹ These assessments include human evidence and toxicological investigations in various models, facilitating the understanding of chemical toxicity and potential threat to human health, guiding chemical management policy decisions, as well as offering mitigation strategies for prevention of diseases that arise therefrom. Methodologies for chemical risk assessment have thus far developed with diverse perspectives, such as epidemiological association analyses, animal experiments, alternative toxicological (AT) methods by the use of 2D, 3D cellular or organoid models and protein target screening techniques, and finally the application of toxicity database.² These single-sided evidence derived from human, experimental animal or cell studies may be less consistent in addressing the potential health effect of a given chemical substance. Given that risk assessments based on a single type of class of chemicals have been repeatedly conducted for most diseases, evaluating chemical risks from holistic perspectives adhering to the “kitchen sink” principle is crucial.^{3,4} However, current assessment methods are limited by their lack of extensiveness and thus are increasingly calling for real-life exposures, i.e., coverage of chemicals and mixture effects in multi-model assays.^{5–7} Therefore, a more comprehensive and integrative strategy is needed to assess the risks of real-life exposure to chemicals.⁸

Chemical risk prioritization has been a “holy grail” for many governmental agencies and authorities.^{9–11} The US Superfund Amendments and Reauthorization Act works together with US Center for Disease Control Agency for Toxic Substances and Disease Registry (ATSDR) and the Environmental Protection Agency (EPA) to announce their ATSDR 2022 Substance Priority List (SPL).¹² Likewise, the China’s first list of “emerging pollutants” for priority control was also issued by the Ministry of Ecology and Environment in 2023.¹³

Europe established the Human Biomonitoring for Europe (HBM4EU) project, focusing on priority substances in recent years.¹⁴ These SPLs offer quick lens for the governments in deciding on which chemicals are ranked higher in toxic effect on health of the public. These urgent needs for priority lists reflect growing concerns over the emergence of multitude of chemicals that may pose health threat.¹⁵ These existing SPLs, however, are generally generated from simple addition of toxicological data across many designs of studies, in varying animal and cellular models, for a wide range of health effects. It must be recognized that top prioritized substances for the risk associated with neurodevelopmental may not necessarily be placed at the same position for cardiovascular health. As such, it is in great need to develop disease-specific SPL for precision prioritization of chemicals across major diseases.

Chemical risk assessment is crucial for many diseases, especially complex multifactorial ones, due to their intricate molecular and cellular mechanisms influenced by various environmental factors. Preeclampsia (PE), a common pregnancy disease, characterized by placental malperfusion and diagnosed as hypertension with proteinuria after 20 weeks of pregnancy,¹⁶ exemplifies this need. The precise etiology of PE remains elusive, but environmental chemical exposure is increasingly recognized as a significant factor.¹⁷ For example, while the plasticizers bisphenol A (BPA) and di(2-ethylhexyl) phthalate (DEHP) have been positively associated with PE in some studies,¹⁸ other research has not corroborated these findings.¹⁹ Similarly, the associations of emerging pollutants per- and polyfluoroalkyl substances (PFASs) and the organophosphate esters (OPEs) flame retardants with PE remain controversial.^{20,21} The inconsistent results across studies make it challenging to draw definitive conclusions about the effects of environmental chemicals on PE. Identifying key pathogenic compounds is a major challenge in preventing PE. As current chemical risk assessments fall short in addressing the multifactorial nature and complex interactions of PE, developing new assessment strategies aiming at prioritization of environmental chemicals is necessary.

Here, using PE as a model health condition, we employed a multiple-dimensional, complementary approach to evaluate the chemical risk prioritization specifically in relation to the disease: starting with real-world mixed exposures and associations for significant risk factors in population, progressing to *in vitro* cell experiments, metabolomic analyses, and finally using *in silico* database calculations for comprehensive assessment. This strategy also involves chemical risk prioritization that was based on scores derived from the five domains, namely, exposure risk, human health risk, cytotoxicity, metabolomic disruption, and *in silico* toxicity. The integration of risk data generated through these disparate technologies will provide valid evidence to both minimize false discoveries and provide insights into mechanisms and adverse outcome pathways. The comprehensive modular framework provides an adaptive workflow for constructing disease-specific priority lists, establishes exposure thresholds via quantile-specific dose-response relationships, identifying pregnant women at elevated PE risk.

MATERIALS AND METHODS

Study workflow

We implemented a multi-dimensional strategy to assess the exposure risk

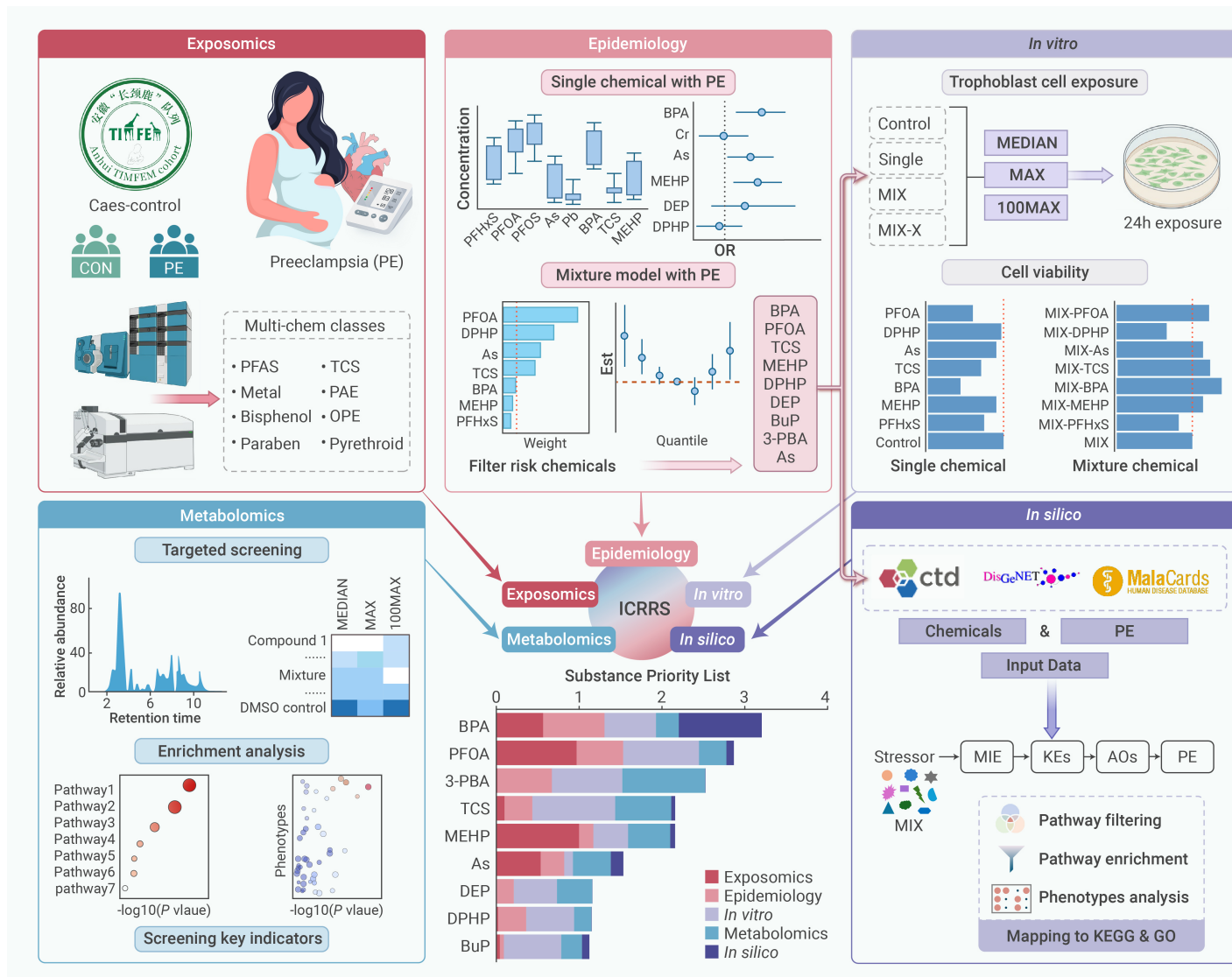


Figure 1. Overview of the integrated chemical exposome risk prioritization strategy, using PE as a model disease Study design: starting from real-world mixed exposures in population (exposomic and epidemiology), progressing to *in vitro* cell experiments, metabolomic analyses, and finally using *in silico* database calculations for comprehensive assessment, in which scores were given for each of the five risk dimensions, i.e., exposure risk, human health risk, cytotoxicity, *in vitro* metabolomic disruption, and *in silico* toxicity. The integrated chemical risk ranking score (ICRRS) approach was used to develop disease-specific SPL for precision prioritization of environmental chemicals. KEs, key events. MIE, molecular initial event. AOs, adverse outcomes.

of exogenous chemical mixtures to PE and prioritize the risk of chemicals based on adverse outcome pathway (AOP) strategy. Our workflow integrates real-world exposure of pregnant women, epidemiological analysis, *in vitro* experiment, metabolomic analyses, and *in silico* assessment (Figure 1). Maternal exposomic profile including 99 chemicals was analyzed using targeted monitoring. Associations between these exogenous chemicals and risk of PE, and relative importance of the chemicals in mixture were revealed through statistical modellings. Then, the population real-exposure concentrations of the selected key chemicals were used to assess the toxicity to trophoblast cells. Subsequently, *in silico* analyses were performed by extracting relevant chemical and disease-associated genes from the comparative toxicogenomics database (CTD), disease gene network (DisGeNET) and MalaCards human disease database, which constructed an AOP. This provides a comprehensive evidence chain for assessing the risk of chemicals to PE.

Dimension 1: exposure risk

Study population. This study with a case-control design, is based on the ongoing Towards Improved Maternal & Fetal health via multi-point Exposure Monitoring (TIMFEM) birth cohort study in Anhui, China. For current study, participants enrolled between January and December of 2022 were consid-

ered. This study was approved by the Ethics Committee of Anhui Medical University (YX2021-091F1), and all participating women provided written consents.²² Detailed information regarding the study design of TIMFEM, the diagnostic criteria of PE, and selection of covariates can be found in supplementary information (Text S1).

Exposure assessment. Maternal blood and urine samples were collected from the pregnant women within 48 hours prior to delivery, serum samples were separated after centrifugation and stored at -80°C and morning spot urine were kept at -20°C till analysis, respectively. Based on our previous published methods with minor modification,²³ we quantified the level of PFASs, BPs, PBs, TCS, PAEs, OPEs, and pyrethroids using liquid chromatograph/mass spectrometer (LC-MS/MS, Sciex 5500 Q Trap triple quadrupole mass spectrometer Sciex, Framingham, MA, USA),²² and metals were analyzed by the use of inductively coupled plasma mass spectrometry (ICP-MS, NexION 2000 ICP-MS, NexION®, PerkinElmer, USA). Details regarding sample processing, instrumental settings and quality control are available in Tables S1–S4 and Texts S2–S3.

Exposome ranking score. The concentration scoring of the chemicals was assessed by normalizing the median concentration of all chemicals. Normalizing to the median also facilitates the comparison of exposure levels across different chemicals and study groups, allowing for a more accurate assess-

ment of relative risk and the potential health impacts of the chemicals under investigation.

Dimension 2: human health risk

Statistical modeling. Only chemicals with a detection frequency (DF) greater than 50% were included for analysis. Values below the limit of detection (LOD) were replaced by LOD/√2 and natural logarithm was applied. Firstly, conditional logistic regression model was performed to explore the associations between individual chemicals and the incidence of PE (Table S5). Next, two mixture-effect models, generalized weighted quantile sum (gWQS) and Bayesian kernel machine regression (BKMR) were used to study the effects of co-exposure to the chemicals on PE. These two models were designed for investigating the potential cumulative and non-additive relationships between mixed chemical exposures and outcome, and representing a linear and non-linear responses, respectively. To evaluate the relative importance of individual chemical in the mixture, weight of chemical towards a positive direction and posterior inclusion probability (PIP) derived from gWQS and BKMR models were considered, respectively. All models were adjusted for covariates, including body mass index (BMI), alcohol consumption, occupation, annual house income, and education levels. Detailed definitions of covariates are given in Text S1 and Figure S1. The trend analysis of gWQS and the mixture analysis results of BKMR are shown in Figure S2. Based on the concentration of all chemicals and the model scores for each section, the substances with the highest scores in each category are selected after normalization (Tables S6–S7). The robustness evaluation of the gWQS and BKMR models is shown in Text S8.

Human health risk ranking score. The odds ratio (OR) from individual chemical analyses using conditional logistic regression, and the relative importance of each chemical in mixed-effect modeling were combined to generate an average score. This score was then normalized and ranked. The top-ranking chemicals in each category were subsequently selected for *in vitro* experiments to assess their cytotoxicity and potential for disrupting cellular metabolism.

Dimension 3: cytotoxicity

Viability of placental cells. Chemicals ranked top in each of their own category was then used in *in vitro* experiments to evaluate their cell toxicity and metabolism-disrupting features. *In vitro* experiments were performed to evaluate the toxicity of chemicals screened from epidemiological study on human trophoblast cell line HTR-8/SVneo (Table S8). To further elucidate the contribution of individual chemical towards a mixed effect as observed in population study, we used “leave-one-out” strategy with nine selected chemicals, including BPA, TCS, BuP, MEHP, PFOA, DPHP, DEP, 3-PBA, As.²⁴ Three dosages level were used, where MEDIAN, MAX and 100MAX represents the median, 95th percentile, 100 times of 95th percentile concentration measured in human population, based on data the 2018 population monitoring data of the US CDC.²⁵ Cell lines were treated in the chemicals for 24 hours before viability test was taken by Cell counting kit-8 (CCK-8) method. Details regarding cell culture conditioning, preparation of chemicals and performance of test can be found in the Text S4 and Figure S3.

Cytotoxicity ranking score. The cytotoxicity scoring of chemicals in *in vitro* experiments was based on individual exposure to 9 chemicals and a “leave-one-out” mixture exposure. In this study, this scoring criterion assessed the degree of stimulation or inhibition of cell growth by each chemical, using the CCK-8 assay to measure cell viability and proliferation. Positive growth rates reflected a stimulatory effect, while negative rates indicated inhibition. A preliminary toxicity score was assigned based on the relative growth rate of treated groups compared to controls.

Dimension 4: metabolomic disruption

Metabolism disruption. The mitochondrial and cytoplasmic metabolites of treated HTR-8/SVneo cells were extracted following our previous reported method.²⁶ A total of 388 metabolites, including amino acids and their derivatives, nucleotides and nucleosides, organic acids and derivatives, coenzymes, vitamins, lipids and their derivatives, carbohydrates and energy metabolites, fatty acids and their derivatives, neurotransmitters and related compounds, bile acids and their conjugates, peptides and peptide derivatives, was

analyzed by LC-MS/MS (AB Sciex 6500, AB Sciex Pte. Ltd. USA). Instrument information can be found in Tables S9–S10. Details of the cell preparation, metabolites extraction and instruments and setting, quality assurance and quality control procedures can be found in Text S5. Metabolites detected were annotated and identified by comparison with the human metabolome database (HMDB) standard metabolite database. Metabolite concentration data were normalized using z-score, and redundant or low-quality metabolites were removed, along with those missing in more than 80% of samples. Metabolites showing a significant difference between the treatment and control groups were identified based on a fold-change of 2 and a significance of $P < 0.05$, generating a list of differential metabolites. The selected metabolites were then subjected to multiple testing correction using false discovery rate. Differential metabolites were mapped to the Kyoto encyclopedia of genes and genome (KEGG) database for pathway annotation, and hypergeometric testing was employed to identify significantly enriched pathways. The enrichment results were ranked and visualized using bubble heatmaps. Pathway analysis was performed using MetaboAnalyst 6.0.

Metabolomic disruption ranking score. The the citric acid (TCA) cycle and amino acid metabolism, as the two key aspects of cell metabolism, were used for assessing the metabolic toxicity of chemicals. Significant changes in metabolite levels were used as indicators of metabolic interference. Both TCA and amino acid metabolism were scored and normalized based on the magnitude of these concentration changes, providing a quantification of the metabolic disruption caused by each chemical.

Dimension 5: *in silico* toxicity

Computational toxicity assessment. To explore potential interaction between chemical exposure and genetic inheritance and their collective effect on PE, we sought relevant information from CTD, DisGeNET, and MalaCards databases (Figure S5). Target genes were collected from three databases associated with PE (Table S11). We merged the genes from these three databases to form a comprehensive gene set related to PE to minimize the risk of missing critical genes. The target gene set linking nine chemicals (BPA, TCS, BuP, MEHP, PFOA, DPHP, DEP, 3-PBA, As) to PE consists of two parts: one part comprises genes collected from the CTD using the chemical names as keywords; the other part includes interaction genes from the databases (interaction genes are derived from extensive literature mining, data integration, experimental data, expert review, and community contributions). Through the analysis of a Venn diagram, these two sets of genes were ultimately integrated into the final target gene set. Following this, we employed gene ontology (GO) analysis and KEGG enrichment analysis to identify the target phenotypes and pathways (Tables S12–S14). Based on various chemicals, a chemical-gene-phenotype-disease path including target genes, target phenotypes, PE, and their interactions was constructed. From the chemical-gene-phenotype-disease path, phenotypes highly related to PE were selected as potential key events (KEs) based on phenotype definitions and literature evidence. Genes related to these overlapping phenotypes from were chosen as molecular initiating events (MIEs). By connecting different phenotype levels and physiological relationships, a computational AOP from chemicals to PE was constructed. The process of integrating databases and identifying PE and chemical genes is detailed in Text S6.

***In silico* toxicity ranking score.** We obtained an inference score based on the comprehensive research correlation of chemicals with genes and PE from the CTD database. The inference score for each chemical was calculated based on its weight in the MIEs and KEs stages, and then normalized.

Chemical risk prioritization

Monitoring the environmental concentrations of chemicals in the human body and the toxicity of their transformation products to special populations is a huge challenge. This study uses high-throughput mass spectrometry, omics analysis methods, bioinformatics analysis tools and computational models to complement each other. the risk score of chemicals for pe includes five dimensions (exposure risk, human health risk, cytotoxicity, metabolomic disruption, *in silico* toxicity), comprising a total of nine criteria: chemical concentration, conditional logistic regression model score, gWQS weight, relative weight in BKMR, target gene score, phenotypes score, pathways score, cytotoxicity score, TCA cycle score, and amino acid score (Text

Table 1. Epidemiological information of the study pregnancy participants.

Characteristic		Total population (n = 118)	Control (n=87)	PE (n=31)	P
Age, year, n (%)	≤27	56 (47.46)	43 (49.43)	13 (41.94)	0.612
	27~	51 (43.22)	37 (42.53)	14 (45.16)	
	≥34	11 (9.32)	7 (8.05)	4 (12.90)	
BMI, kg/m ² , n (%)	<18.5	17 (14.41)	15 (17.24)	2 (6.45)	0.008
	18.5~	75 (63.56)	59 (67.82)	16 (51.61)	
	≥24	26 (22.03)	13 (14.94)	13 (41.94)	
Gestational week, n (%)	<37w	80 (67.80)	60 (68.97)	20 (64.52)	0.816
	≥37w	38 (32.20)	27 (31.03)	11 (35.48)	
Alcohol consumption, n (%)	Never	98 (83.05)	78 (89.66)	20 (64.52)	0.003
	Ever	20 (16.95)	9 (10.34)	11 (35.48)	
Occupation, n (%)	Heavy physical work	51 (43.22)	37 (42.53)	14 (45.16)	0.663
	Light physical work	49 (41.53)	35 (40.23)	14 (45.16)	
	Others	18 (15.25)	15 (17.24)	3 (9.68)	
Annual household income, CNY, n (%)	<30 k	22 (18.64)	16 (18.39)	6 (19.35)	0.856
	30 k~	39 (33.05)	30 (34.48)	9 (29.03)	
	≥90 k	57 (48.31)	41 (47.13)	16 (51.61)	
Education, n (%)	High school or below	28 (23.73)	20 (22.99)	8 (25.81)	0.944
	Above high school	90 (76.27)	67 (77.01)	23 (74.19)	

Notes: n represents the number of samples. Occupation was grouped by based on the cumulative hours of physical activity involved in daily work, including heavy physical work (high-intensity), light physical work (light/moderate-intensity), others (low-intensity). Details on occupation definition can be found in Text S1. Differences between the control and PE groups were evaluated using the *Chi*-square test. Values in bold indicate statistically significant differences between control and PE groups, with $P<0.05$ denoting significance. Abbreviations: BMI, Body mass index. PE, preeclampsia.

S7, Table S15). The comprehensive score for each chemical was calculated through a weighted summation of normalized criteria scores, with weights reflecting the relative importance of each toxicological factor. For specific scoring details referring to Table S16. A generalized linear model based on the population's exposure to chemicals and the risk of PE was constructed. This model utilized restricted cubic splines to predict the trend of concentration and risk. The cutoff point corresponding to an OR of 1 was adopted as the threshold. The proportion of the population at risk above this threshold was calculated (Table S17). Finally, the process based on the above scoring system can assess the ability of these chemicals to have potential health effects. In addition, the rank consistency of each chemical was calculated by trend line in plot scatter diagrams. The relevant results are shown in Table S18.

Quantitative data in the experimental results were expressed as mean $\pm$ standard error of the mean (mean $\pm$ SEM). All of multiple comparisons were corrected using the Benjamini-Hochberg method to control the false discovery rate (FDR). All statistical analyses were conducted using two-sided tests, with $P\leq 0.05$ considered statistically significant. This study utilized R (Ver. 4.3.2) software for data analysis and graphs plotted.

RESULTS

Demographics and chemical exposure profile

The participants were part of an ongoing prospective birth cohort study, the TIMFEM study. PE and healthy mothers were matched by age (± 2 years) and gestational weeks at delivery (± 2 weeks) (Table 1). Majority (90.7%) of the participants were younger than 33 years of age, and 80% of whom delivered prematurely (<37 weeks' gestation). Body mass index (BMI) of the most included participants fall within the normal range between 18.5 and 23.9 kg/m². More women in the case group reported alcohol drinking before pregnancy than those of control group (38.48% versus 31.03%, $P=0.003$). No significantly differences were observed between the two groups in terms of occupational type, annual household income, and educational level ($P=0.663$, $P=0.856$, and $P=0.944$, respectively).

The exposomic profile of individual participant was detected using LC-MS/MS and ICP-MS, including 99 chemicals from eight broad categories, including PFASs (30) in serum, metals (24), bisphenols (BPs, 13), parabens (2), triclosan (1), phthalate metabolites (PAEs, 17), OPEs (11) and pyrethroid (1) in urine, detailed methodological and instrumental details are in Table S1. Distribution and DF of 99 chemicals are presented in Table 2. These results

Table 2. Concentration levels (ng/mL) of chemicals measured in the samples involved this study.

Chemicals	DF (%)	Min	25 th	Median	75 th	Max
<i>PFAS</i>						
PFBA	93.2	<LOD	0.31	0.52	0.92	2.80
PFPeA	94.1	<LOD	0.28	0.50	0.90	3.43
PFHxA	94.9	<LOD	0.16	0.33	0.67	1.77
PFHpA	75.4	<LOD	0.04	0.08	0.17	1.36
PFOA	94.1	<LOD	2.69	3.96	5.15	27.16
PFNA	94.9	<LOD	0.53	0.97	1.54	5.87
PFDA	94.9	<LOD	0.52	0.89	1.64	5.97
PFUoDA	94.9	<LOD	0.44	0.89	1.52	5.01
PFDODA	77.1	<LOD	0.06	0.10	0.20	1.41
PFTTrDA	81.4	<LOD	0.05	0.13	0.22	0.80
PFTeDA	36.4	<LOD	<LOD	<LOD	0.06	0.51
PFHXDA	22.0	<LOD	<LOD	<LOD	<LOD	0.60
PFODA	16.1	<LOD	<LOD	<LOD	<LOD	0.44
ADONA	44.1	<LOD	<LOD	<LOD	0.02	0.10
HFPO-DA	68.6	<LOD	<LOD	0.25	0.68	2.02
FOUEA	36.4	<LOD	<LOD	<LOD	0.03	0.30
PFBS	51.7	<LOD	<LOD	0.05	0.08	0.73
PFHxS	93.2	<LOD	0.50	0.89	1.91	11.57
PFHpS	57.6	<LOD	<LOD	0.05	0.10	0.71
PFOS	94.9	<LOD	2.88	5.54	8.27	23.58
6:2 Cl-PFESA	94.9	<LOD	0.61	1.18	2.14	7.57
PFDS	48.3	<LOD	<LOD	<LOD	0.03	0.24
6:2 FTS	69.5	<LOD	<LOD	0.08	0.21	0.74
8:2 FTS	50.8	<LOD	<LOD	0.02	0.05	0.45
FOSA	11.0	<LOD	<LOD	<LOD	<LOD	0.06
N-MeFOSA	26.3	<LOD	<LOD	<LOD	0.01	0.58
N-EtFOSA	22.9	<LOD	<LOD	<LOD	<LOD	10.16
N-MeFOSAA	30.5	<LOD	<LOD	<LOD	0.03	1.20
N-EtFOSAA	20.3	<LOD	<LOD	<LOD	0.01	0.19
8:2 diPAP	13.6	<LOD	<LOD	<LOD	0.01	0.28
<i>Metals</i>						
Li	97.5	<LOD	7.10	12.39	17.77	152.37
Be	0.8	<LOD	<LOD	<LOD	<LOD	0.10
Al	28.8	<LOD	<LOD	<LOD	0.45	42.63
V	97.5	<LOD	19.03	29.40	46.35	92.69
Cr	97.5	<LOD	20.87	56.76	84.50	246.54
Mn	36.4	<LOD	<LOD	<LOD	0.30	4.64
Fe	89.8	<LOD	13.99	44.49	93.90	1222.47
Co	97.5	<LOD	0.29	1.03	1.82	9.28
Ni	87.3	<LOD	1.96	6.04	11.46	50.86
Cu	96.6	<LOD	60.68	106.10	162.26	528.39
Zn	74.6	<LOD	0.36	317.10	821.13	6107.56

Table 2. (Continued)

Chemicals	DF (%)	Min	25 th	Median	75 th	Max
Ga	40.7	<LOD	<LOD	<LOD	0.84	4.09
As	97.5	<LOD	0.86	2.19	4.77	12.79
Se	97.5	<LOD	19.01	29.80	47.62	100.18
Rb	96.6	<LOD	118.90	210.66	444.46	1442.59
Sr	95.8	<LOD	6.68	16.75	37.17	190.92
Ag	29.7	<LOD	<LOD	<LOD	0.22	8.73
In	23.7	<LOD	<LOD	<LOD	<LOD	4.37
Cs	97.5	<LOD	3.28	6.05	9.04	16.86
Ba	46.6	<LOD	<LOD	<LOD	3.71	99.28
Tl	70.3	<LOD	<LOD	0.04	0.08	6.69
Pb	97.5	<LOD	0.02	0.02	0.02	354.67
Bi	20.3	<LOD	<LOD	<LOD	<LOD	4.72
U	0.8	<LOD	<LOD	<LOD	<LOD	0.06
<i>Bisphenols</i>						
BPA	86.4	<LOD	1.18	2.31	4.27	14.84
BPF	78.0	<LOD	0.07	0.24	0.50	3.18
BPE	85.6	<LOD	0.10	0.23	0.55	14.55
BPB	72.0	<LOD	<LOD	0.24	0.70	16.39
BPS	86.4	<LOD	0.07	0.25	0.66	24.00
BPC	79.7	<LOD	0.07	0.19	0.66	4.64
BPZ	38.1	<LOD	<LOD	<LOD	0.10	0.39
BPAP	82.2	<LOD	0.07	0.26	0.70	6.76
BPG	59.3	<LOD	<LOD	0.37	1.31	31.26
BPAF	69.5	<LOD	<LOD	0.02	0.06	1.83
BPM	65.3	<LOD	<LOD	0.10	0.22	1.67
BPP	77.1	<LOD	0.05	0.20	0.36	6.68
BPBP	55.1	<LOD	<LOD	0.03	0.09	0.43
<i>Parabens</i>						
PrP	78.8	<LOD	0.09	0.50	3.18	181.61
BuP	86.4	<LOD	0.03	0.18	0.81	16.26
<i>Triclosan</i>						
TCS	95.8	<LOD	0.05	0.41	1.37	24.92
<i>PAEs</i>						
MMP	85.6	<LOD	2.20	6.56	20.59	148.69
MEP	85.6	<LOD	0.71	3.45	9.91	186.38
MiPrP	83.1	<LOD	0.35	1.33	3.22	136.96
MBP	85.6	<LOD	1.29	4.04	10.75	85.26
MiBP	85.6	<LOD	1.43	4.12	9.73	66.99
MCHP	84.7	<LOD	2.44	10.95	21.99	118.37
MHXP	79.7	<LOD	0.04	0.21	0.70	6.65
MBzP	83.1	<LOD	0.53	1.57	3.46	31.49
MiHeP	83.1	<LOD	0.48	1.81	3.72	111.16
MEHP	89.8	<LOD	1.24	4.08	11.70	148.34

Table 2. (Continued)

Chemicals	DF (%)	Min	25 th	Median	75 th	Max
mEOHP	73.7	<LOD	<LOD	4.11	11.25	50.91
mEHHP	72.9	<LOD	<LOD	3.10	7.39	27.52
mECP	85.6	<LOD	7.93	29.04	54.68	190.61
MiNP	83.9	<LOD	0.27	0.86	3.62	34.92
MnOP	78.8	<LOD	0.56	2.43	5.92	62.82
MHiNP	85.6	<LOD	3.16	12.82	30.19	185.21
MCIOP	64.4	<LOD	<LOD	0.76	3.69	172.46
<i>OPEs</i>						
DMP	98.3	<LOD	1.16	2.24	5.07	94.12
DEP	91.5	<LOD	0.02	0.02	0.06	0.96
BCEP	95.8	<LOD	2.63	5.94	10.97	100.44
BCIPP	94.1	<LOD	0.29	0.65	1.38	8.51
BMPP	87.3	<LOD	0.17	0.62	2.75	30.48
BBOEP	82.2	<LOD	0.22	1.54	6.55	59.69
DPHP	99.2	<LOD	0.04	0.09	0.18	1.04
BDBPP	57.6	<LOD	<LOD	0.37	0.56	3.95
BDCPP	84.7	<LOD	0.25	0.71	1.25	3.29
BEHP	33.1	<LOD	<LOD	<LOD	<LOD	0.45
DnBP	47.5	<LOD	<LOD	<LOD	0.02	3.17
<i>Pyrethroid</i>						
3-PBA	67.8	<LOD	<LOD	0.06	0.10	1.28

Notes: Concentrations of PFAS refer to the levels detected in the serum, while the concentrations of metals, bisphenols, parabens, triclosan, PAEs, OPEs and pyrethroid are the levels in urine after adjustment for specific gravity. Full names of chemicals refer to Table S1. Abbreviations: DF, detection frequency. LOD, limit of detection.

showed that the DF of chemicals varied. The DF for PFASs and metals ranged from 11% to 95% and 1% to 97%, respectively, while the DF for BPs, parabens, PAEs, OPEs and pyrethroid were above 50% (Figure 2A). Among all measured PFASs from maternal blood, the concentrations of perfluorooctanoic acid (PFOA, median 5.34 vs 3.48 ng/mL, $P<0.001$) and perfluorooctanesulfonic acid (PFOS median 6.16 vs 5.00 ng/mL, $P=0.038$) were higher in mothers diagnosed with PE than that of those healthy mothers (Table S2). For metals, only arsenic (As, median 7.61 vs 1.56 ng/mL, $P<0.001$) was found with higher concentration in urine of PE women compared to healthy ones. In other groups of chemicals, BPA (median 9.29 vs 1.93 ng/mL, $P<0.001$), butyl paraben (BuP, median 0.50 vs 0.11 ng/mL, $P=0.016$) and mono(2-ethylhexyl) phthalate (MEHP, median: 19.33vs 3.56 ng/mL, $P<0.001$) also demonstrated significantly higher concentrations in the PE group than in the healthy controls. The results above suggest widespread exposure to chemical substances in pregnant women, which is a mixture of low-dose and various types of exposure, requiring further analysis of the exposure risks.

Epidemiological assessment of chemical exposure and PE risk

To explore the associations between chemicals with a DF above 50% and PE in a case-control study design from an ongoing birth cohort. Conditional logistic regression and mixed effect modelling were used to determine single and mixture effect, respectively. The models were either unadjusted or adjusted for maternal BMI (<18.5, 18.5-23.9, ≥24, kg/m²), educational level (high school or below, high school and beyond), occupation (heavy physical work, light physical work and others), alcohol consumption (never, ever), and annual household income (<30k, 30k-90k, ≥90k CNY). Figure 2A showed the DF of 99 substances, of which 76 are greater than 50%. The results showed (Figures 2B-C) that in the conditional logistic regression, PFOA (OR: 1.44,

95%CI: 1.00-2.08), As (OR: 1.32, 95%CI: 1.03-1.69), BPA (OR: 1.21, 95%CI: 1.01-1.45), BuP (OR: 1.23, 95%CI: 1.04-1.45), TCS (OR: 1.23, 95%CI: 1.04-1.46), MEHP (OR: 1.19, 95%CI: 1.03-1.37), diphenyl phosphate (DEP) (OR: 1.34, 95%CI: 1.06-1.70), DPHP (OR: 1.55, 95%CI: 1.12-2.16) and 3-phenoxybenzoic acid (3-PBA, OR: 1.42, 95%CI: 1.10-1.83) were all significantly associated with PE in crude model. In adjusted model, exposure to As (OR: 1.27, 95%CI: 1.00-1.62), BPA (OR: 1.27, 95%CI: 1.02-1.58), BuP (OR: 1.22, 95%CI: 1-1.49), MEHP (OR: 1.26, 95%CI: 1.03-1.52), DEP (OR: 1.50, 95%CI: 1.07-2.10) and 3-PBA (OR: 1.51, 95%CI: 1.08-2.12) remained significant for PE. Through conditional logistic regression, 19 substances significantly associated with PE were screened out for further mixture analysis.

In the forward gWQS model, where exposure contributes positively to the risk of disease (Figures 2D-E), PFASs and PAEs were among the top-weighted chemicals, with PFOA (0.208), PFBA (0.201), MEHP (0.172) and As (0.148) being the top-ranked individual ones in adjusted model. For further non-additive and non-linear associations between mixed chemical exposure and PE, BKMR modelling was used. Chemicals of TCS, PFOA and BPA exhibited the highest PIP in BKMR model (Figure 2F). The top-ranking substances of exposure included MEHP, PFOA, BPA, As and TCS exposure risk (Figure 2G). The weights of chemicals in each model (OR from logistic regression, weights from gWQS and PIPs from BKMR) were normalized and assessed for human health epidemiological risk (Figure 2H). Finally, the weights of the chemicals in each model (logistic regression, weight from gWQS and PIPs from BKMR) were normalized and added for an exposomic and epidemiological score, for which BPA, PFOA and TCS were highest in risk ranking (Figure 2I). Based on the ranking of all chemicals and the positive or negative nature of their risk relationships, nine top-ranked substances were selected from each category. These nine substances (BPA, TCS, BuP, MEHP, PFOA, DPHP,

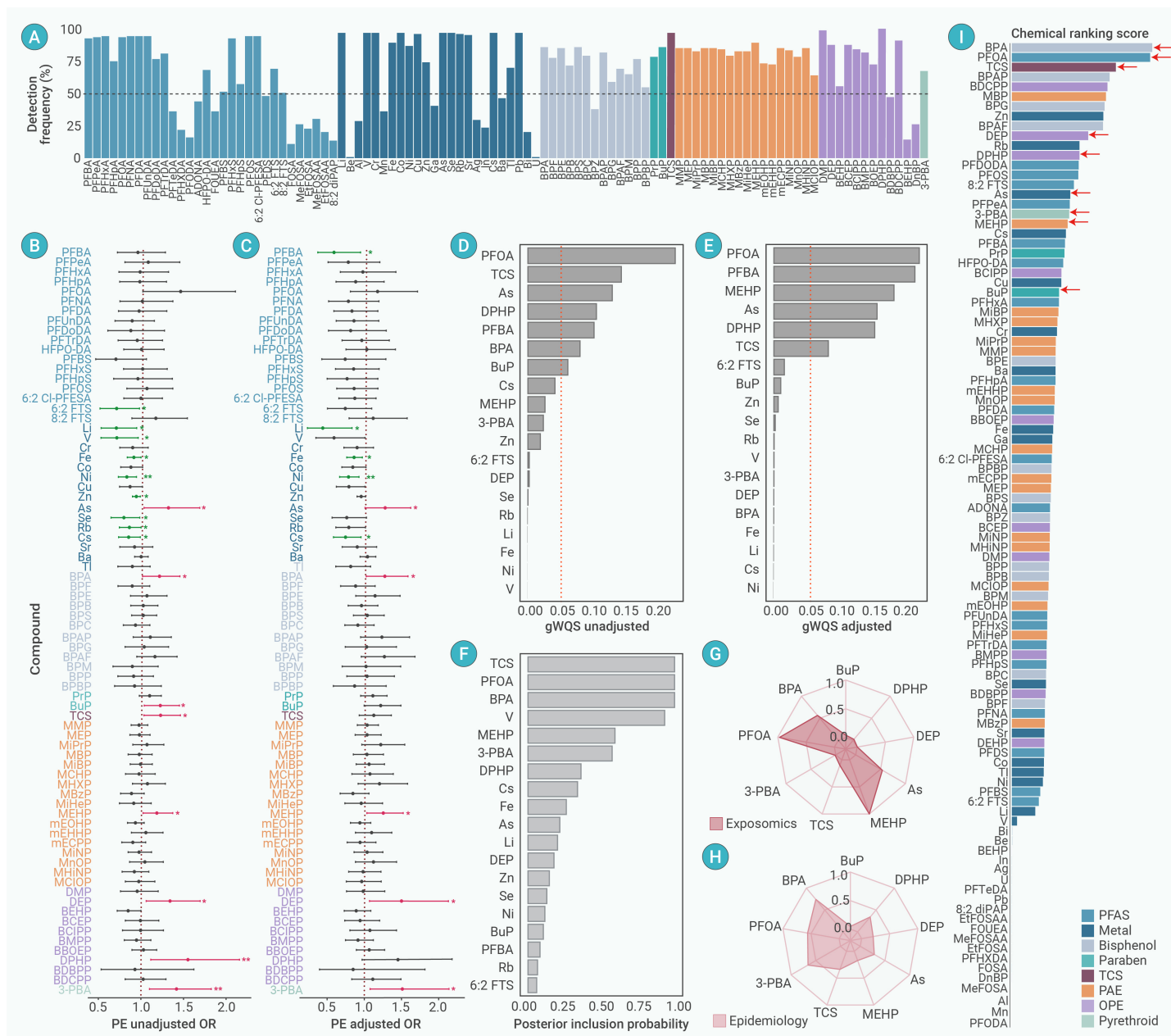


Figure 2. Exposomic profiling and epidemiological screening for chemicals in relation to elevated risks (exposure risk and human health risk) of PE in the pregnant women (A) Detection frequency (DF) of 99 chemicals in maternal urine or blood (B-C) Logistic regression model assessing associations between 76 single chemicals (*ln*-transformed) and risk of PE before and after adjusted for covariates. Adjusted covariates: maternal BMI, education level, occupation, alcohol consumption and annual household income. (D-E) Chemical weight in the correlation between positive generalized weighted quantile sum (gWQS) index and PE before and after adjusted for covariates. (F) Posterior Inclusion Probability values of chemicals from Bayesian kernel machine regression (BKMR). (G) Chemical exposure risk ranking score based on concentrations (H) Epidemiological scores of human health risk based on odds ratio, weights derived from gWQS and BKMR. (I) Score based on the concentrations of all chemicals, conduct conditional logistic regression for chemicals with DF greater than 50%, and perform weighted quantile sum and Bayesian kernel machine regression analyses for weighting. This yields a preliminary ranking of 99 chemicals, followed by further analysis of those with significant risk.

DEP, 3-PBA, As) were undergone further *in vitro* and *in silico* analyses to assess cytotoxicity, metabolomic disruption, and *in silico* toxicity.

In vitro experiment and metabolomic analysis

Next, we further evaluated the effects of key substances, namely, the selected nine chemicals, on the proliferation of placental trophoblast cells for placental function which may be a key event characterizing PE. Cell viability assay and metabolomics testing were divided into separate groups for single chemical exposure and mixed exposures. For single chemical exposure, three concentrations were used (MEDIAN, MAX and 100MAX, corresponding to the median, 95th percentile, and 100 times of the 95th percentile concentration from the studied population). Cell viability assay showed that when treated with single exposure, most chemicals inhibited the proliferation of trophoblast cells at 100MAX concentration compared to the control group, except BPA

(Figure 3A). The effect started to be attenuated in a dose-dependent manner as treatment dosage decreased to the MEDIAN concentration. Specifically, DEP significantly inhibited cell proliferation at all three concentrations, suggesting its adversely affects to placental function even at lower concentrations. For mixed chemical exposures, the toxicity of chemical mixtures to trophoblast cells was greater than the effects of individual chemicals, indicating that mixed exposures might enhance toxicity to placental cells (Figure 3B). Under 100MAX concentration, mixed exposures significantly reduced cell viability. However, in the MAX group, the MIX-BPA group exhibited a partial toxic excitatory effect. In the mixed exposure group, "leave-one-out" method was also employed.²⁴ By systematically removing individual chemicals from the mixture, we observed that the toxicity of the mixture diverged into two distinct pathways compared to mixture group. In the group where TCS and BuP were removed, cell viability increased, whereas in the group where BPA

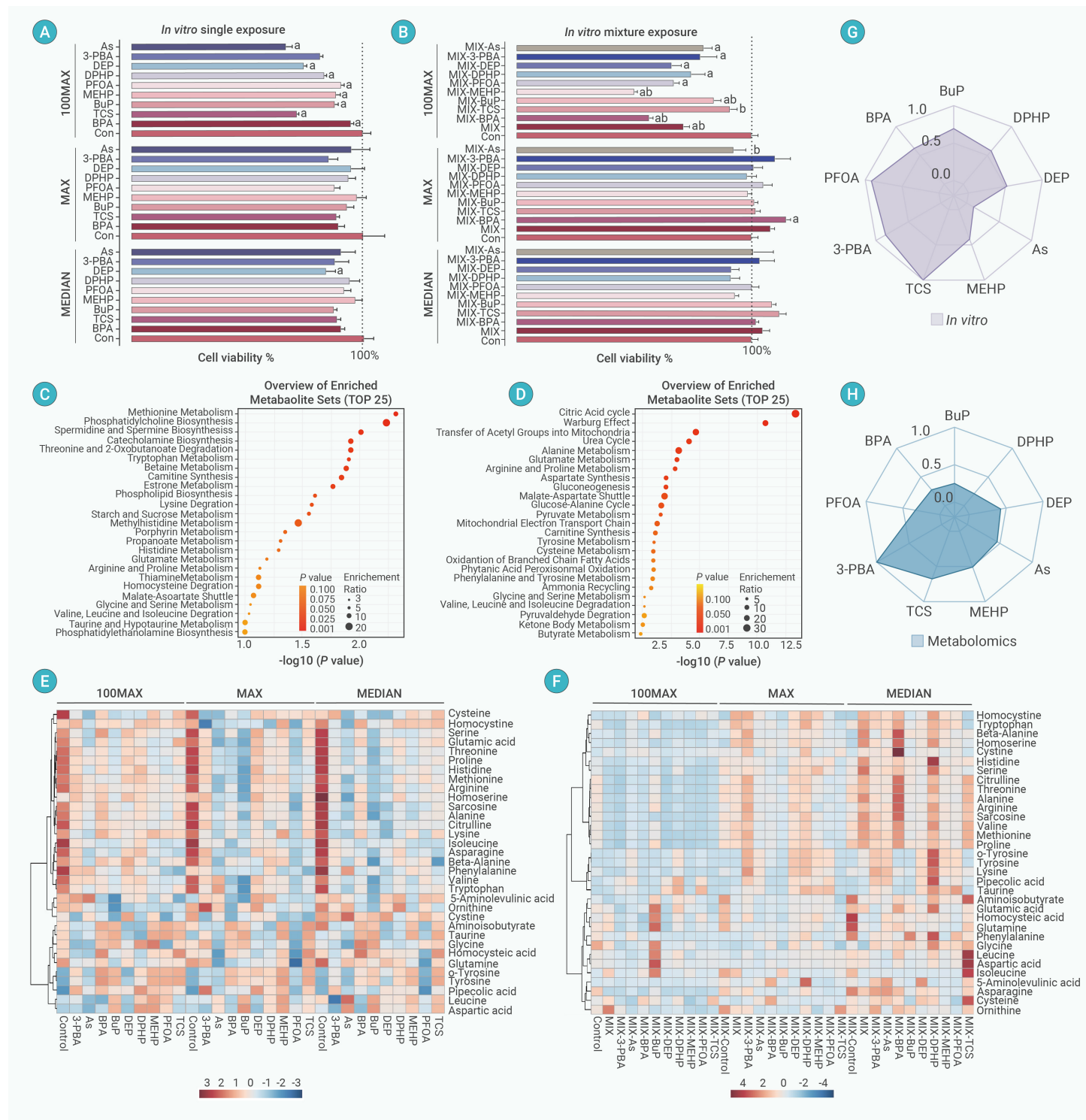


Figure 3. *In vitro* cytotoxicity and metabolism disruption of screened chemicals (A-B) Exposure to BPA, PFOA, 3-PBA, TCS, MEHP, As, DEP, DPHP, BuP on proliferation of Human trophoblast HTR-8/SVneo cells, using single and mixed exposures by leave-one-out method for 24 hours, respectively. Median, MAX and 100MAX corresponds to the median, 95th percentile, 100 times of 95th percentile concentration of the population, respectively. Multiple comparisons were corrected using the Benjamini-Hochberg method to control the false discovery rate (FDR). 'a' indicates that the difference between the other groups and the control group is statistically significant; 'b' indicates a significant difference between the other groups and the nine chemical mixture group. (C-D) Bubble plot of differential metabolite enrichment analysis between single-exposure chemicals and control group, as well as mixed exposure and control group. (E-F) Effects of chemical exposure on key metabolites in the cell amino acids and mitochondrial TCA cycle of HTR-8/SVneo cells. (G-H) Cell cytotoxicity score based on cell viability and metabolomic disturbance score (TCA and amino acid metabolic toxicity).

and As were removed, cell viability decreased. This indicates that TCS and BuP enhance the toxicity of the mixture, while BPA and As reduce it. Similarly, in the MAX group, the removal of As yielded comparable results. However, the removal of BPA in the MAX group led to increased cell viability compared to the mixture group, indicating that different concentration ratios influenced the concentration of BPA within the mixture (Figure 3B).

To further evaluate the metabolism-disrupting features of these chemicals, cells from control and treatment group were collected to determine the changes in the concentrations of several key metabolites, including amino acids, nucleotides and nucleic acids, coenzymes and vitamins, carbohydrates, lipids, organic acids, and other small molecules. The differences between the treatment and control groups were then subjected to KEGG

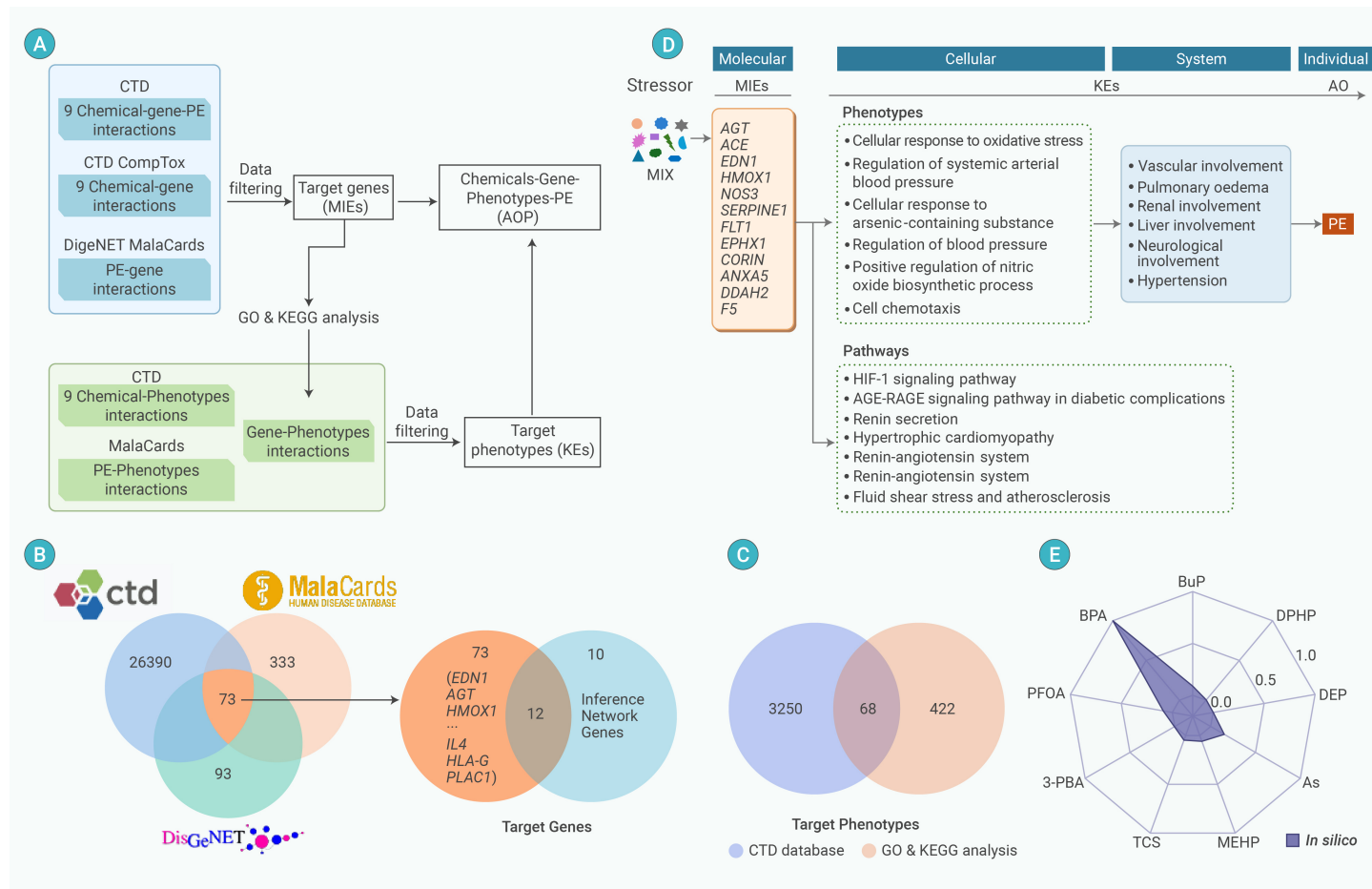


Figure 4. *In silico* toxicity of screened chemicals (A) Workflow of *in silico* analysis constructed. (B-C) Target genes and phenotypes associated with PE for nine chemicals. (D) Overview of the AOP pathway treated with three levels (subcellular, cellular, system) and linked together by their relationships. Specific phenotypes and pathways are shown in Tables S12-S13. (E) *In silico* score based on interaction score, phenotype score and pathway score.

pathway enrichment analysis. Results of all treatment groups using single chemical exposure were combined, and the differential pathway enrichment analyses were performed between the control and treatment group only. Differentially enriched metabolic pathways included phosphatidylcholine biosynthesis, spermidine and spermine biosynthesis, methylhistidine metabolism, and threonine and 2-oxobutanoate degradation. These pathways are related to lipid transport, DNA stability, and amino acid metabolism (Figure 3C). In mixed exposure group, KEGG pathway enrichment analysis compared to the control group revealed significant differences (Figure 3D) in pathways such as the citric acid cycle, transfer of acetyl groups into mitochondria, alanine metabolism, malate-aspartate shuttle, and glucose-alanine cycle. These pathways are related to energy metabolism, glycolysis, fatty acid oxidation, and energy storage and release in cells.

In the treatment groups of all 9 individually exposed chemicals and mixed exposure groups, amino acid metabolism concentrations are shown in Figures 3E-F, including essential amino acids, non-essential amino acids, conditionally essential amino acids, and other amino acids. In the individual exposure groups, the treatment groups showed a downregulation of glutamate, homocysteine, arginine, alanine, isoleucine, valine, and others (Figure 3E). In the mixed exposure groups (Figure 3F), almost all amino acids in the 100MAX group of all mixtures showed a trend of downregulation compared to the control. The simultaneous changes in these various functional amino acids suggest widespread metabolic disturbances in the cells. Another group of amino acids, including taurine, glutamate, homocysteic acid, glutamine, phenylalanine, leucine, aspartate, and isoleucine, were significantly downregulated.

As TCA cycle and amino acid metabolism were the most prevalent changes seen after exposure to these chemicals, two scoring system for cytotoxicity and metabolism-disrupting property were derived from cell

viability and metabolomic profiling tests, respectively, through standardization and normalization (Figures 3G-H). The results showed that the most toxic substances in the cell experiment were 3-PBA, TCS and PFOA. *In vitro* experiments provide a direct and effective means of assessing the toxicity of chemicals. They allow for precise analysis of chemical toxicity in a controlled environment, reducing the complexities of interactions within a living organism, and utilizing human cell lines to enhance the relevance of the results.

***In silico* analyses based on public toxicity databases**

Through these tests and analyses described above, nine chemicals with highest toxicity rating score were considered for *in silico* analyses for studying the complex interactions between chemicals, genes, and phenotypes. Figure 4A shows a schematic diagram of the workflow of this study. We searched CTD, DisGeNET, and MalaCard to collect interactions among the nine chemicals, relevant genes and phenotypes (phenotype refers to a biological outcome that is not inherently a disease), as well as PE.²⁷ Bioinformatics analyses, including GO and KEGG enrichment analyses, were used to obtain gene-phenotype interactions. After confidence assessment according to the Organization for Economic Co-operation and Development's (OECD) AOP handbook, AOPs with higher confidence were used to illustrate the relationship and potential mechanisms between nine chemicals and PE. The target entries of nine chemicals and PE-related genes screened from CTD (Table S4), and filtered genes and phenotypes collected from different databases were pre-curated and summarized in Table S5. Additionally, MalaCards identified 406 genes and DisGeNet identified 166 genes, with a total of 572 genes related to PE. We identified 210 phenotypes related to the nine chemicals and 77 PE-related phenotypes from CTD and MalaCards. From 26889 genes related to nine chemicals and PE screened from CTD, DisGeNET, and MalaCards, 73 overlapping genes were selected as target

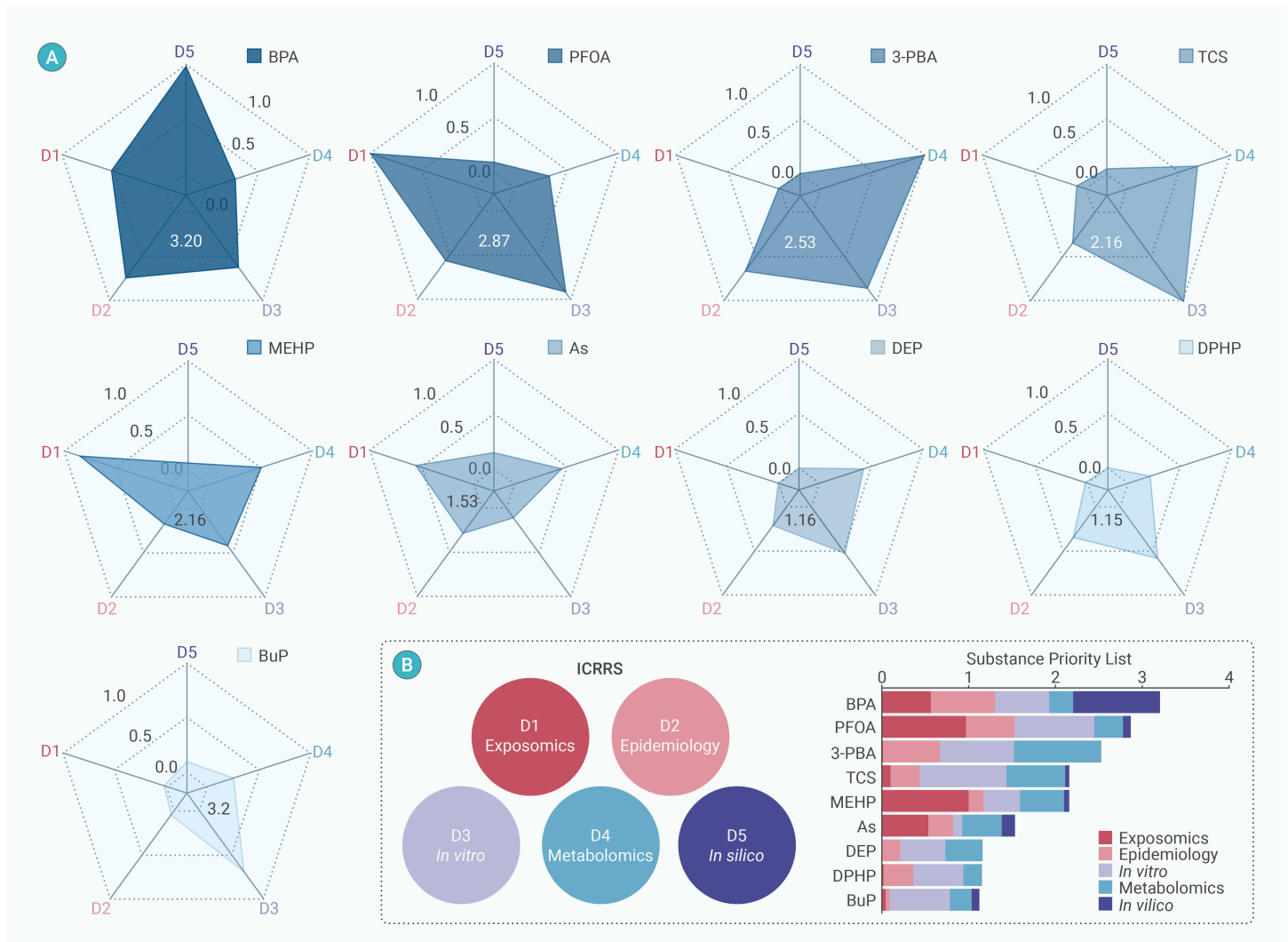


Figure 5. SPL of environmental chemicals for PE using the ICRRS (A) Standardized scores of the nine screened chemicals in relation to the risk of PE corresponding to the five dimensions (exposure risk, human health risk, cytotoxicity, *in vitro* metabolomic disruption, and *in silico* toxicity). (B) Prioritization of screened chemicals based on cumulative scores from the five dimensions described above. SPL stands for substances prioritization list.

genes (Figure 4B). Among these 73 target genes, 12 were directly annotated for nine chemicals and their corresponding PE in CTD. Based on the data, 12 genes related to both chemicals and PE were selected. These target genes are potential MIEs with high confidence between chemicals and PE. Using panther classification (version 16.0),²⁸ these 12 target proteins were classified into the following eight protein classes: cardiovascular regulatory proteins (*AGT*, angiotensinogen; *ACE*, angiotensin-converting enzyme; *EDN1*, endothelin 1; *NOS3*, nitric oxide synthase 3; *SERPINE1*, plasminogen activator inhibitor), metabolic and antioxidant proteins (*HMOX1*, heme oxygenase 1; *CORIN*, atrial natriuretic peptide-converting enzyme), inflammation and immune regulatory proteins (*FLT1*, Fms-like tyrosine kinase 1; *EPHX1*, epoxide hydrolase 1), and lipid and energy metabolic proteins (*ANXA5*, annexin A5).

In addition to the phenotypes retrieved from CTD and MalaCards, 490 GO annotations were obtained for 16 target genes across 13 KEGG pathways. Venn diagram analysis of 3318 phenotypes (from CTD) and 490 phenotypes (from GO and KEGG enrichment analysis) revealed 68 overlapping phenotypes, including 68 GO terms and 3 pathways (Figure 4C). These phenotypes were mapped into three KE levels in the GO hierarchy: subcellular, cellular, and system level. Some GO phenotypes with similar biological themes appeared at one or more KE levels. For example, "amino acid metabolic process" (GO:0006520) at the subcellular level and "cellular response to oxidative stress" (GO:0006979) at the cellular level are both related to oxidative stress response and cell proliferation and migration. Thus, we constructed AOPs based on hierarchical relationships and physiological

mechanisms. These target phenotypes were retained as potential KEs linking chemicals and PE. Figure 4D depicts an inferred pathway of mixture MIX to PE outcome, showing how environmental chemicals interfere with normal biological processes by affecting the expression or activity of specific genes, including subcellular, cellular, and systemic levels, ultimately leading to disease. In this network, several genes are listed, such as *AGT*, *ACE*, *EDN1*, which influence cell functions at the molecular level through different signaling pathways. These genes and related signaling pathways are associated with the development of hypertension, which is a significant risk factor for the onset of PE. Figure 4E describes the *in silico*-based chemical scores and the database-based inference scores (IS). BPA has the highest weight score, followed by As and PFOA. The *in silico* analyses leveraging public toxicity databases illuminated the intricate interplay between nine toxic chemicals, while their associated genes, phenotypes, and pathways supplemented the comprehensive scoring from integrated research databases, providing further evidence and reference for the ranking of these chemicals.

Toxicity scoring and priority ranking of chemicals for PE

To calculate the toxicity scores and priority ranking, we used five evaluation criteria, namely exposure risk, human health risk, cytotoxicity, metabolomic disruption, *in silico* toxicity, which correspond to the five dimension above in Figure 5 (exposomic, epidemiology, *in vitro*, metabolomic, *in silico*). The radar plot in Figure 5A visually represents the score of each chemical across the five dimensions. Finally, the PE-specific SPL for the chemicals is shown in Figure 5B, with the cumulative scoring order from top-

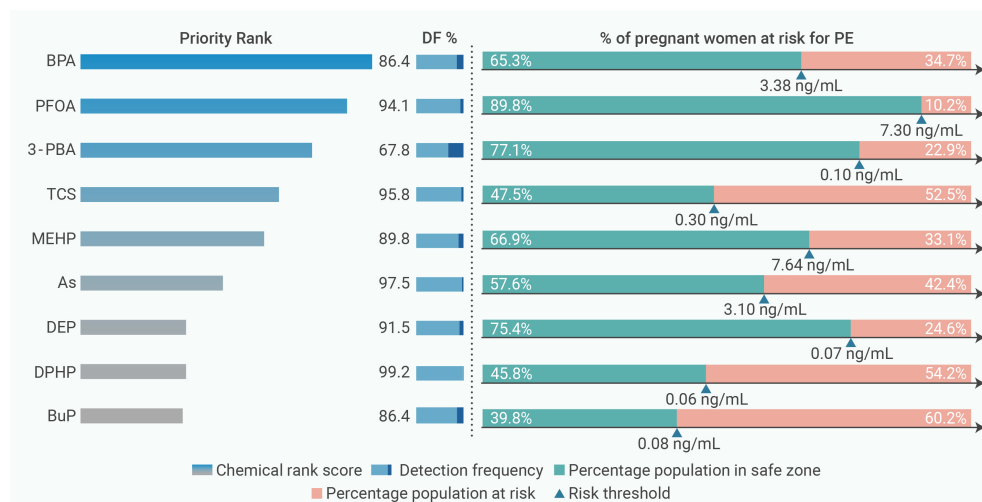


Figure 6. Precision prioritization of chemicals considering both our PE-specific SPL and exposure risk in this study Left: PE-specific SPL derived from the integrated chemical risk ranking score. Middle: the detection frequency (DF) of chemicals is presented. Right: Women with endogenous level of chemicals below the threshold are deemed safe for specific-chemical related risk of preeclampsia (green area), while those of the red section represent those at-risk. The risk threshold was determined by the value where the restricted cubic spline (RCS) intersects $y = 1$.

down being BPA, PFOA, 3-PBA, TCS, MEHP, As, DEP, DPHP and BuP. Among these chemicals, BPA scored highest in epidemiological and *in silico* analyses, while PFOA, 3-PBA and TCS scored highest in exposomics, metabolomics and *in vitro* experiments, respectively, demonstrating that each chemical has unique toxicological characterizations in different aspects. The linear trend consistency analysis of rankings for the 9 chemicals is presented in Table S18. 3-PBA, DEP, and BuP demonstrated good ranking consistency, indicating similar rankings across each dimension, whereas BPA, PFOA, and MEHP exhibited higher inconsistency in rankings across various dimensions (Table S18).

Ultimately, we obtained a framework for precision prioritization of chemicals specifically in relation to PE and identified risk thresholds for PE in pregnant women (Figure 6). The nine screened chemicals were all with a DF of greater than 80%, and the risk threshold for PE was calculated using restricted cubic spline regression. The percentage of pregnant women at elevated risk of PE due to individual chemical exposure and relevant threshold were as follows: BPA (34.7%, 3.38 ng/mL), PFOA (10.2%, 7.30 ng/mL), 3-PBA (22.9%, 0.10 ng/mL), TCS (52.5%, 0.30 ng/mL), MEHP (33.1%, 7.64 ng/mL), As (42.4%, 3.10 ng/mL), DEP (24.6%, 0.07 ng/mL), DPHP (54.2%, 0.06 ng/mL), and BuP (60.2%, 0.08 ng/mL). It should be noted that the priority ranking and risk of chemical exposure need to be considered in combination. For example, although TCS only ranked fourth in place in prioritization list, the DF and proportion of population at increased risk of PE was higher than that of the other top three chemicals. Taken together, such comprehensive disease-specific risk prioritization depiction allows more deep understanding and ready-to-adopt knowledge of SPL for PE. Correlation and sensitivity analysis showed that the rankings of core high-risk substances were highly consistent, supporting the robustness of the ICRRS conclusions (Text S8, Table S19).

DISCUSSION

Identifying exogenous substances that are most relevant to the elevated risk of disease has long been a challenge. In this study, using a detrimental pregnancy complication as example, we constructed an ICRRS system for precision prioritization of chemicals in relation to risk of PE, by incorporating evidence from five dimensions, including exposomic profiling, epidemiological analyses, *in vitro* cytotoxicity, metabolic disruption, and *in silico* data linkage and analyses. Using the ICRRS strategy developed, we screened from 99 chemicals detected in maternal biological samples and shortlisted nine chemicals of concern that are significantly associated with the risk of PE. This approach for disease-specific chemical risk ranking can be amended for other diseases that might root in environmental contaminant exposures, which enables precision prioritization to inform government and authorities for the regulation and control of chemical uses.

The foundation of precision prioritization of chemicals lies in the accurate characterization of exposure burden in targeted population for comprehensive exposomic profiling and establish associations between observed diseases and contaminant exposures through epidemiological analyses. A

limited chemicals being measured.¹⁷ Building on previous research, we measured 99 chemicals of 8 different categories and used single element and mixture analyses for studying the association between (co-)exposure to a range of chemicals and its relationship with PE. Several studies investigated the associations between highly detected PFASs and the risk of PE or gestational hypertensive disorders reported mostly null associations, likely due to lower exposure level.²⁹⁻³¹ We found legacy PFOA, with a DF of 100% and a median of 3.89 ng/mL, was significantly related to elevated risk of PE. In our study, BPA was found to be strongly associated with the risk of PE in both single and mixture effect model. Although the DF of BPA was slightly lower in the studied population as compared to previous reports (<90% versus nearly 100%), the measured concentration (2.31 ng/mL) was higher than that reported in Shanghai (0.95 ng/mL) and Sweden (1.55 ng/mL), respectively,^{32,33} reflecting both regional and temporal differences in the exposure burden. A primary risk assessment taking both DF and absolute concentration of chemicals into consideration is the fundamental step for constructing a SPL.

AT approach is encouraged in modern toxicological research due to its high throughput at lower costs related to labor and experimental expenses, the accuracy of which has been proven to be at least equivalent to that derived from conventional animal testing.³⁴ AT is therefore the preferred method in screening large number of chemicals in identifying the real effect driver towards a certain disease from a mixture of substances. In addition, a distinctive strength of AT is that it is suitable for studying the potential toxicological effect of benign substances that might have low exposure level in the population, for which the toxicity effect might be overlooked. Our study employed a 2D *in vitro* model to determine the cytotoxicity and metabolic disrupting property of screened chemicals, which can be benefited by including more complex AT models, including *ex vivo* assay and organ-on-a-chip that provides toxicity data on tissue and organ level, respectively.³⁵

Computational toxicology using data from publicly available databases in predicting the toxicological effects of substances in relation to any disease or phenotypes has been increasingly recognized as a valued AT approach. Computational toxicology demonstrated its potential in decoding the molecular features related to toxicological effect through the development of chemical descriptors or predicting the cytotoxicity of contaminants using morphological descriptors and its fingerprint.^{36,37} Recent advances in computational toxicology were also prompted by the development of large publicly available databases, which allowed fast screening of chemicals in relation to specific biological effects and/or diseases. Previous research focused only on the metabolomic disturbances caused by exposure in creating a SPL without targeting a specific endpoint. Considering the potential interactions between chemicals and genes in the progression of diseases, in our *in silico* toxicity evaluation, we incorporated three databases to thoroughly deciphering the interactions in between chemical exposures, PE and genes. Computational toxicology has gained recognition as a valuable approach for predicting the toxicological effects of substances related to diseases, leveraging publicly available databases to analyze molecular features, assess cytotoxicity, and

explore chemical-gene interactions in disease progression.³⁸

Each region faces unique health threats posed by environmental contaminants due to differences in the agricultural and industrial applications, thus the United States of America, Republic of South Korea and the European union have all developed their own human biomonitoring programs and SPLs.^{12,14,39} Considering the chemical DF, potential or suspected toxicity and human exposure, the top three substances in the SPL of United States were all metals (As, lead and mercury).⁴⁰ Using effect-based methods quantifying the toxicological effects of given substances on organism and cellular level, in 2023, the European union proposed to update the SPL for the quality control of water to include antibiotics, PFASs, estrogenic hormones in a total of 46 substances.⁴¹ The SPL list of Korean is mainly composed of substances that are carcinogenic, mutagenic and reprotoxic, bioaccumulative or persistent in the environment, or with specific target organ toxicity.⁴² SPL developed by the European Union and Republic of Korea focused more on the chemical property and toxicological effect of the substances, while that of the United States incorporated human exposure risk. An integrated system encompassing all these components would reflect a more thorough SPL. Nevertheless, chemicals on SPL might demonstrate a broad-spectrum toxicity in all monitored populations, in consideration of the health equity, SPL for vulnerable populations is necessary in improving the quality of life of all. The increases in the prevalence of non-communicable diseases and infertility coincided with the ever-increasing production and usage of chemical contaminants, development of disease-specific SPL is also warranted in dealing with the crisis. Our integrated scoring approach synthesizes evidence across exposure science, epidemiological associations, mechanistic toxicology, and computational predictions, aligning with established frameworks for chemical prioritization.⁴³ Exposure risk quantification followed validated exposome-ranking methods, while human health risk integrated Z-score normalized effect estimates from logistic regression, gWQS weights, and BKMR PIPs.⁴⁴ Weight assignment via analytic hierarchy process prioritized placental toxicity pathways based on their established role in preeclampsia pathogenesis, with *in silico* prediction-sand exposure magnitude providing complementary evidence. This multi-criteria decision analysis framework demonstrated robustness in sensitivity testing and consistency with EPA's Chemical Safety for Sustainability principles. While expert-informed weighting introduces subjectivity, our approach advances upon single-dimension prioritization models by transparently reconciling population-level associations with molecular mechanisms. Future iterations could refine weights using toxicogenomic anchors (e.g., ToxCast assay consensus) and incorporate non-targeted exposomics to capture emergent contaminants.

The novelty of this research lies in the development of the ICRRS using evidence from exposome, epidemiology, *in vitro* cytotoxicity, metabolomic disruption, and *in silico* toxicity for a precision prioritization of chemicals associated with the risk of disease-specific health outcomes including PE. Our ICRRS integrated the core of SPL construction strategy used by different countries (or region), considered both human exposure risk (DF and concentration) and potential toxicity (*in vitro* and *in silico* toxicity assessment).⁴⁵ We further incorporated epidemiological associations for identification of chemical threshold related to elevated risk of PE, which can be used specifically for health advisory for individuals. However, there were several limitations that should be acknowledged. Although a panel of nearly 100 chemicals were measured in our study, one might argue that it is far from a real reflection of human exposomic atlas. By using targeted exposomics, we intended to accurately characterize the exposure burden of commonly reported environmental chemicals, which might have compromised the coverage of other substances that may otherwise be (semi)-quantified using non-targeted high resolution mass spectrometry technique. Considering the rapid revolution of industrial and domestic products, future studies should expand the scope to capture more of the emerging and region-specific contaminants that are more likely to explain differences in the prevalence of diseases in different countries and regions. Besides, the relatively small sample size and cross-sectional study design warrants further larger-scale prospective validations, although other studies have reported similar finding of exposure to PAFS, PAEs, OPEs and BPs et al. PFOA, BPA, As, TCS, MEHP was associated with

the risk of PE as reported in our study.^{18,19,46-50}

CONCLUSION

In conclusion, we proposed an ICCRS system for shortlisting chemicals of concern in relation to the risk of PE. This mode of precision prioritization can be applied in developing other disease-specific SPL, the significance of which has three main aspects. For legislation and regulation purposes, the ICCRS strategy informs precision prioritization of chemicals for stringent controls. For clinicians and healthcare professionals, to determine endogenous level of key environmental contaminants for patients with unexplained cause disease might assist the treatment of diseases through health advisories. The risk threshold in relation to diseases derived from our ICCRS system could also be informative for individuals in making lifestyle decisions.

REFERENCES

1. Vermeulen R., Schymanski E. L., Barabási A.-L., et al. (2020). The exposome and health: Where chemistry meets biology. *Science* **367**:392–396. DOI:10.1126/science.aay3164
2. Punt A., Bouwmeester H., Blaauwer B. J., et al. (2020). New approach methodologies (NAMs) for human-relevant biokinetics predictions: Meeting the paradigm shift in toxicology towards an animal-free chemical risk assessment. *Altex* **37**:607–622. DOI:10.14573/altex.2003242
3. Major M. B. and Moon R. T. (2009). "Omic" risk assessment. *Sci. Signal.* **2**:eg7. DOI:10.1126/scisignal.272eg7
4. Drakvik E., Altenburger R., Aoki Y., et al. (2020). Statement on advancing the assessment of chemical mixtures and their risks for human health and the environment. *Environ. Int.* **134**:105267. DOI:10.1016/j.envint.2019.105267
5. Posthuma L., Altenburger R., Backhaus T., et al. (2019). Improved component-based methods for mixture risk assessment are key to characterize complex chemical pollution in surface waters. *Environ. Sci. Eur.* **31**:1–11. DOI:10.1186/s12302-019-0246-5
6. Knäpen D., Angrish M. M., Fortin M. C., et al. (2018). Adverse outcome pathway networks I: Development and applications. *Environ. Toxicol. Chem.* **37**:1723–1733. DOI:10.1002/etc.4125
7. Chen Q., Wei X., Xie R., et al. (2024). Beyond physical particles: A holistic assessment of human health risks from real-world micro-nanoplastic exposure. *Innov. Med.* **2**:100104. DOI:10.59717/j.xinn-med.2024.100104
8. Fang M., Hu L., Chen D., et al. (2021). Exposome in human health: Utopia or wonderland. *The Innovation* **2**:100172. DOI:10.1016/j.xinn.2021.100172
9. Zhao F., Li L., Chen Y., et al. (2021). Risk-based chemical ranking and generating a prioritized human exposome database. *Environ. Health Persp.* **129**:047014. DOI:10.1289/EHP7722
10. Feng X., Li D., Liang W., et al. (2021). Recognition and prioritization of chemical mixtures and transformation products in Chinese estuarine waters by suspect screening analysis. *Environ. Sci. Technol.* **55**:9508–9517. DOI:10.1021/acs.est.0c06773
11. Vryzas Z., Ramwell C. and Sans C. (2020). Pesticide prioritization approaches and limitations in environmental monitoring studies: From Europe to Latin America and the Caribbean. *Environ. Int.* **143**:105917. DOI:10.1016/j.envint.2020.105917
12. Agency for Toxic Substances and Disease Registry Division of Toxicology and Human Health Sciences. (2022). <https://www.atsdr.cdc.gov/index.html>.
13. List of New Pollutants to be Controlled (2023 Edition). https://www.mee.gov.cn/xxgk2018/xxgk/xxgk02/202212/t02221230_1009167.html.
14. Agency E. E. Europe has the Human Biomonitoring for Europe. (2022). <https://www.hbm4eu.eu/>.
15. Wang F., Xiang L., Sze-Yin Leung K., et al. (2024). Emerging contaminants: A One Health perspective. *The Innovation* **5**:100612. DOI:10.1016/j.xinn.2024.100612
16. Burton G. J. and Fowden A. L. (2015). The placenta: A multifaceted, transient organ. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **370**:20140066. DOI:10.1098/rstb.2014.0066
17. Rosen E. M., Muñoz M. I., McElrath T., et al. (2018). Environmental contaminants and preeclampsia: A systematic literature review. *J. Toxicol. Environ. Health B Crit. Rev.* **21**:291–319. DOI:10.1080/10937404.2018.1554515
18. Cantonwine D. E., Meeker J. D., Ferguson K. K., et al. (2016). Urinary concentrations of bisphenol A and phthalate metabolites measured during pregnancy and risk of preeclampsia. *Environ. Health Perspect.* **124**:1651–1655. DOI:10.1289/Ehp188
19. Hirke A., Varghese B., Varade S., et al. (2023). Exposure to endocrine-disrupting chemicals and risk of gestational hypertension and preeclampsia: A systematic review and meta-analysis. *Environ. Pollut.* **317**:120828. DOI:10.1016/j.envpol.2022.120828
20. Tian Y., Zhou Q., Zhang L., et al. (2023). In utero exposure to per-/polyfluoroalkyl substances (PFASs): Preeclampsia in pregnancy and low birth weight for neonates. *Chemosphere* **313**:137490. DOI:10.1016/j.chemosphere.2022.137490
21. Borghese M. M., Walker M., Helewa M. E., et al. (2020). Association of perfluoroalkyl substances with gestational hypertension and preeclampsia in the MIREC study. *Environ. Int.* **141**:105789. DOI:10.1016/j.envint.2020.105789
22. Tao L., Tang W., Xia Z., et al. (2024). Machine learning predicts the serum PFOA and

- PFOS levels in pregnant women: Enhancement of fatty acid status on model performance. *Environ. Int.* **190**:108837. DOI:10.1016/j.envint.2024.108837
23. Deng M., Gao T., Tao L., et al. (2023). Are human exposure assessment the same for non-persistent organic chemicals-from the lens of urinary variability and predictability. *Sci. Total. Environ.* **868**:161542. DOI:10.1016/j.scitotenv.2023.161542
 24. Liu M., Jia S., Dong T., et al. (2020). Metabolomic and transcriptomic analysis of MCF-7 cells exposed to 23 chemicals at human-relevant levels: Estimation of individual chemical contribution to effects. *Environ. Health Perspect.* **128**:127008. DOI:10.1289/EHP6641
 25. Fourth national report on human exposure to environmental chemicals: updated tables, March 2018, Volume two. (2018). https://dtsc.ca.gov/wp-content/uploads/sites/31/2018/10/CDC_Report_2.pdf.
 26. Zhang Y., Lv J., Fan Y. J., et al. (2023). Evaluating the effect of gestational exposure to perfluorohexane sulfonate on placental development in mice combining alternative splicing and gene expression analyses. *Environ. Health Perspect.* **131**:117011. DOI:10.1289/ehp13217
 27. Davis A. P., Wiegiers T. C., Johnson R. J., et al. (2023). Comparative toxicogenomics database (CTD): update 2023. *Nucleic Acids Res.* **51**:D1257–D1262. DOI:10.1093/nar/gkac833
 28. Mi H., Muruganujan A., Casagrande J. T., et al. (2013). Large-scale gene function analysis with the PANTHER classification system. *Nat. Protoc.* **8**:1551–1566. DOI:10.1038/nprot.2013.092
 29. Rylander L., Lindh C. H., Hansson S. R., et al. (2020). Per- and polyfluoroalkyl substances in early pregnancy and risk for preeclampsia: A case-control study in southern Sweden. *Toxics* **8**:43. DOI:10.3390/toxics8020043
 30. Bommarito P. A., Ferguson K. K., Meeker J. D., et al. (2021). Maternal levels of perfluoroalkyl substances (PFAS) during early pregnancy in relation to preeclampsia subtypes and biomarkers of preeclampsia risk. *Environ. Health Perspect.* **129**:107004. DOI:10.1289/EHP9091
 31. Huang R., Chen Q., Zhang L., et al. (2019). Prenatal exposure to perfluoroalkyl and polyfluoroalkyl substances and the risk of hypertensive disorders of pregnancy. *Environ. Health-Glob.* **18**:5. DOI:10.1186/s12940-018-0445-3
 32. Wang Z., Zhou Y., Liang H., et al. (2021). Prenatal exposure to bisphenol analogues and digit ratio in children at ages 4 and 6 years: A birth cohort study. *Environ. Pollut.* **278**:116820. DOI:10.1016/j.envpol.2021.116820
 33. Engdahl E., Svensson K., Lin P.-I. D., et al. (2021). DNA methylation at GRIN2B partially mediates the association between prenatal bisphenol F exposure and cognitive functions in 7-year-old children in the SELMA study. *Environ. Int.* **156**:106617. DOI:10.1016/j.envint.2021.106617
 34. Hartung T. (2019). Predicting toxicity of chemicals: Software beats animal testing. *EFSA J.* **17**:e170710. DOI:10.2903/j.efsa.2019.e170710
 35. Cong Y., Han X., Wang Y., et al. (2020). Drug toxicity evaluation based on organ-on-a-chip technology: A review. *Micromachines (Basel)* **11**:381. DOI:10.3390/mi11040381
 36. Townsend P. A. and Grayson M. N. (2021). Density functional theory in the prediction of mutagenicity: A perspective. *Chem. Res. Toxicol.* **34**:179–188. DOI:10.1021/acs.chemrestox.0c00113
 37. Sedykh A. Y., Shah R. R., Kleinstreuer N. C., et al. (2021). Saagar-A new, extensible set of molecular substructures for QSAR/QSPR and read-across predictions. *Chem. Res. Toxicol.* **34**:634–640. DOI:10.1021/acs.chemrestox.0c00464
 38. Liang C., Ding R., Sun Q., et al. (2024). An overview of adverse outcome pathway links between PM(2.5) exposure and cardiac developmental toxicity. *Environ. Health* **2**:105–113. DOI:10.1021/envhealth.3c00143
 39. Choi W., Kim S., Baek Y.-W., et al. (2017). Exposure to environmental chemicals among Korean adults-updates from the second Korean National Environmental Health Survey (2012–2014). *Int. J. Hyg. Envir. Heal.* **220**:29–35. DOI:10.1016/j.ijheh.2016.10.002
 40. Agency for Toxic Substances and Disease Registry. (2024). <https://www.atsdr.cdc.gov/index.html>.
 41. Vivienne H. (2024). Pollutants in EU waters: Update of chemical substances listed for control. *European Parliamentary Research Service* 749.772. [https://www.europarl.europa.eu/RegData/etudes/BRIE/2023/749772/EPRS_BRI\(2023\)749772_EN.pdf](https://www.europarl.europa.eu/RegData/etudes/BRIE/2023/749772/EPRS_BRI(2023)749772_EN.pdf).
 42. Substances of Priority Control and Product Notification under K-REACH. (2019). https://www.chemsafetypro.com/Topics/Korea/Substances_of_Priority_Control_and_Product_Notification_under_K-REACH.html.
 43. Whaley P., Halsall C., Ågerstrand M., et al. (2016). Implementing systematic review techniques in chemical risk assessment: Challenges, opportunities and recommendations. *Environ. Int.* **92–93**:556–564. DOI:10.1016/j.envint.2015.11.002
 44. Zhao F., Li L., Chen Y., et al. (2021). Risk-based chemical ranking and generating a prioritized human exposome database. *Environ. Health Perspect.* **129**:47014. DOI:10.1289/ehp7722
 45. Han P., Li X., Yang J., et al. (2024). Advancing toxicity predictions: A review on in vitro to in vivo extrapolation in next-generation risk assessment. *Environ. Health* **2**:499–513. DOI:10.1021/envhealth.4c00043
 46. Zhao Y., Zhou J., Xu H., et al. (2023). Prenatal exposure to perfluorooctane sulfonate and perfluorooctanoate and the risk of preeclampsia: A nested case-control study in Shanghai, China. *Chin. Med. J.* **136**:857–859. DOI:10.1097/cm9.0000000000002414
 47. Liu H., Pu Y., Ai S., et al. (2022). The relationship between preeclampsia and arsenic concentration in the peripheral blood. *Biol. Trace. Elem. Res.* **200**:3965–3974. DOI:10.1007/s12011-021-02988-5
 48. Ye Y., Tang Y., Xiong Y., et al. (2019). Bisphenol A exposure alters placentalation and causes preeclampsia - like features in pregnant mice involved in reprogramming of DNA methylation of WNT2. *FASEB J.* **33**:2732. DOI:10.1096/fj.201800934RRR
 49. Wang Y., Wang K. K., Han T. B., et al. (2020). Exposure to multiple metals and prevalence for preeclampsia in Taiyuan, China. *Environ. Int.* **145**:106098. DOI:10.1016/j.envint.2020.106098
 50. Honkisz E., Zieba-Przybylska D. and Wojtowicz A. K. (2012). The effect of triclosan on hormone secretion and viability of human choriocarcinoma JEG-3 cells. *Reprod. Toxicol.* **34**:385–392. DOI:10.1016/j.reprotox.2012.05.094

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AUTHOR CONTRIBUTIONS

Tianrui Gao: Investigation, Data Curation, Formal Analysis, Visualization, Writing-Original Draft, Writing - Review & Editing. Lin Tao and Chang Gao: Methodology, Data Curation, Formal Analysis, Writing - Review & Editing. Lin Luo, Liping Cui and Liyan Guo: Investigation, Data Curation. Jia Lv and Weitang Tang: Investigation, Validation, Data Curation. Jiayue Hu and Yijun Fan: Resources, Project Administration, Writing - Review & Editing. Hua Wang: Resources, Writing - Review & Editing. Quancai Sun: Formal Analysis, Validation, Writing - Review & Editing. De-Xiang Xu: Resources, Writing - Review & Editing, Supervision. Yichao Huang: Conceptualization, Investigation, Methodology Project Administration, Resources, Writing - Review & Editing, Supervision, Funding Acquisition. All authors contributed to and approved the manuscript

DECLARATION OF INTERESTS

The authors declare no competing interests.

ETHICAL STATEMENT AND PATIENT CONSENT

The protocol of TIMFEM study was approved by the Ethic Committee of the Second Affiliated Hospital of Anhui Medical University (YX2021-091F1), all enrolled participants provided written consents.

DATA AND CODE AVAILABILITY

The data and code that support the anticipated results are available from the corresponding author upon reasonable request.

SUPPLEMENTAL INFORMATION

It can be found online at <https://doi.org/10.59717/j.xinn-med.2025.100136>