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Early-life chemical exposome: Comprehensive strategies for wide-scope screening of organic compounds

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ABSTRACT

Exposure to organic compounds during early-life stages can disrupt human development and increase susceptibility to adverse health outcomes later in life. Despite evidence of these impacts, research has primarily focused on targeted analyses, missing the complex nature of early-life exposures. Comprehensive approaches are needed to better characterize these complex mixtures. Recent advances in high-resolution mass spectrometry and computational science have enabled the comprehensive analysis of numerous chemicals through wide-scope screening approaches, yet their application in early-life exposome studies is still limited. This review critically examines studies from the past decade using wide-scope screening to investigate the early-life chemical exposome, emphasizing organic compounds. It evaluates each step of the analytical workflow, including sample collection, preparation, analysis, and data processing, and concludes with recommendations for future research to improve understanding of the early-life chemical exposome and its health implications.

Abbreviation	Definition
ACN	Acetonitrile
CE	Collision energy
CECs	Constituents of Emerging Concern
DCM	Dichloromethane
DLLE	Dispersive liquid-liquid extraction
DOHaD	Developmental Origins of Health and Disease
EOAs	Environmental organic acids
FT/FTs	Feature/s
GC	Gas chromatography
HILIC	Hydrophilic interaction chromatography
HRMS	High-resolution mass spectrometry
ISs	Internal standards
LC	Liquid chromatography
LLE	Liquid-liquid extraction
MS	Mass spectrometry
MTBE	*Tert*-Butyl Methyl Ether
NCDs	Non-communicable diseases
NTA	Non-targeted analysis
PAHs	Polycyclic aromatic hydrocarbons
PFCs	Perfluorinated compounds
PFASs	Per- and polyfluoroalkyl substances
POPs	Persistent organic pollutants
PPRP	Protein and Phospholipid Removal Plates
PPT	Protein precipitation

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QA	Quality assurance
QC	Quality control
QTOF	Quadrupole time-of-flight
RP	Reverse phase chromatography
SLE	Solid-liquid extraction
SPE	Solid phase extraction
SSA	Suspect screening analysis
UPLC	Ultrahigh-performance liquid chromatography
VOCs	Volatile organic compounds
UVFs	Ultra-Violet Filters
WHO	World Health Organisation

1. Introduction

1.1. Early life and chemical exposome

In 2005, chemical and nonchemical external factors, as well as their internal biological responses were enclosed in the concept of exposome by C.P. Wild to elucidate the environment-human health associations [1]. The study of the exposome, also called *exposomics*, is a new 'omics' field focused on evaluating human exposures from conception onwards [2,3]. Lifestyle factors (e.g. daily activities, diet habits, or smoking), social factors (e.g. socioeconomic status and level of education), physical-chemical exposures (e.g. electromagnetic fields, radiation, pharmaceuticals, outdoor and indoor air pollution, pesticides, plasticizers, and persistent organic pollutants), and ecosystem/climate are the primary exposures considered [3,4].

The growing and extensive use of chemicals, commonly referred to as xenobiotics, results in daily exposure for individuals through inhalation, ingestion, and/or dermal absorption. This continuous exposure has been shown to predispose individuals to adverse health effects. Interactions with chemicals can potentially interfere at various biological functional levels, including the genome, proteome, epigenome, transcriptome and metabolome [2,5–8]. Due to organ and tissue development, the early-life period – from in-utero to puberty - represents a critical window of susceptibility and vulnerability to chemical exposures. According to the World Health Organization (WHO) and the Developmental Origins of Health and Disease (DOHaD), environmental exposures during the intrauterine period can profoundly affect the developing fetus. These exposures may predispose individuals to conditions such as premature birth and low birth weight and can contribute to the development of non-communicable diseases (NCDs) later-in-life, such as endocrine and metabolic disorders, respiratory and cardiovascular diseases, and neurological morbidities [3,9–13].

Many early-life exposome studies primarily focus on determining specific chemical families through targeted analysis. However, there is a notable scarcity of studies employing a comprehensive human biomonitoring approach to assess the chemical exposome in early-life. Targeted analysis, mainly based on multiple reaction monitoring with triple quadrupole instruments, offers high accuracy and sensitivity for the unequivocal identification and quantification of specific classes of chemicals at trace levels. However, this approach is limited to a predefined list of xenobiotics, thereby overlooking the potential health effects of the complex mixtures. Given the temporal variability and matrix-dependency of the chemical exposome, there is a critical need for powerful tools that enable comprehensive analysis, enhance coverage of the chemical space, and elucidate the impact of the chemical exposome on human health.

Recent advances in analytical, computational and data processing tools, particularly high-resolution mass spectrometry (HRMS) and computational science, have significantly enhanced the adoption of wide-scope screening approaches. These approaches, such as Suspect Screening Analysis (SSA) and Non-Targeted Analysis (NTA), are designed to analyze hundreds to thousands of chemicals using HRMS, and retrieving information about new analytes. In the context of exposomics, wide-scope screening experiments play a crucial role in comprehensively characterizing chemicals of emerging concern (CECs) and assessing their potential effects on human health [14,15]. These methods enable researchers to explore the complete chemical space in biological and environmental samples, providing insights into exposures that traditional targeted methods might miss.

In recent years, there has been a significant increase in interest in applying wide-scope screening approaches to characterize the chemical exposome. Over the past decade, research focused on wide-scope screening exposomics has seen substantial growth, expanding from seven publications to over fifty, encompassing various biological matrices across different stages of life as well as environmental samples (Fig. 1, blue line). Despite the overall growth in wide-scope screening approaches from 2013 to 2023, only twenty-three out of approximately three hundred studies have specifically focused on the chemical exposome during early-life stages (Fig. 1, orange line). This limited focus can be attributed to the major challenges in routine human biomonitoring in exposomics. These challenges include the lower concentration of xenobiotics compared to endogenous compounds (typically pg/mL or below) [16–18], the use of non-selective sample preparation methods leading to lower sensitivity and higher matrix effect [19–22], the complexity and size of generated data, the lack of extensive experimental spectral databases, and the unavailability of some reference standards [23–25]. Recent reviews on exposomics have tackled these challenges by highlighting the need for standardization. Gu et al. analyzed the advantages and challenges associated with current sample preparation procedures for mass spectrometry in human exposome research. These methods include solid-phase extraction, liquid extraction, QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe), filtering, and dilute and shoot [26]. Other publications have reviewed the application of SSA and NTA approaches in exposomics, comparing the coverage of chemical space in human samples and environmental matrices, as well as discussing the quality assurance and control efforts for NTA [27–29].

However, to the best of our knowledge, there is a gap in the literature regarding the comprehensive investigation - from sampling to data processing - of the chemical exposome during early-life stages using wide-scope screening approaches. Therefore, this review aims to provide an overview of the entire wide-scope screening workflow for assessing the early-life chemical exposome, with a specific focus on exposure to organic compounds. We critically assess the methodologies at each step of the analytical workflow, including sampling, sample preparation and analytical parameters specific to biological matrices. Additionally, we review data acquisition and processing tools. Finally, we offer concluding remarks and recommendations for future studies to contribute to a deeper understanding of the early-life chemical exposome and its impact on human health.

1.2. Selection of the papers

The literature review covers original research work published from 2013 to August 2023 sourced from PubMed, Scopus and Web of Sciences Core Collection. A summary of the data is depicted graphically in Fig. 1. Studies exploring the chemical exposome using wide-scope screening strategies in both human and environmental samples were retrieved using a comprehensive multiple-query search strategy. The queries "(exposome OR exposure OR exposomic OR exposomics) AND high resolution AND (suspect screening OR untargeted OR non-targeted OR wide-scope) AND chromatography yielded a total of 305 non-duplicated publications (Fig. 1, blue line). Detailed records of article titles and corresponding keywords can be found in Supplementary Data (Sheet: General_list).

A secondary research was performed to identify studies focusing on early-life stages. The candidate queries "(exposome OR exposure OR exposomic OR exposomics) AND high resolution AND (suspect screening OR untargeted OR non-targeted OR wide-scope) AND chromatography

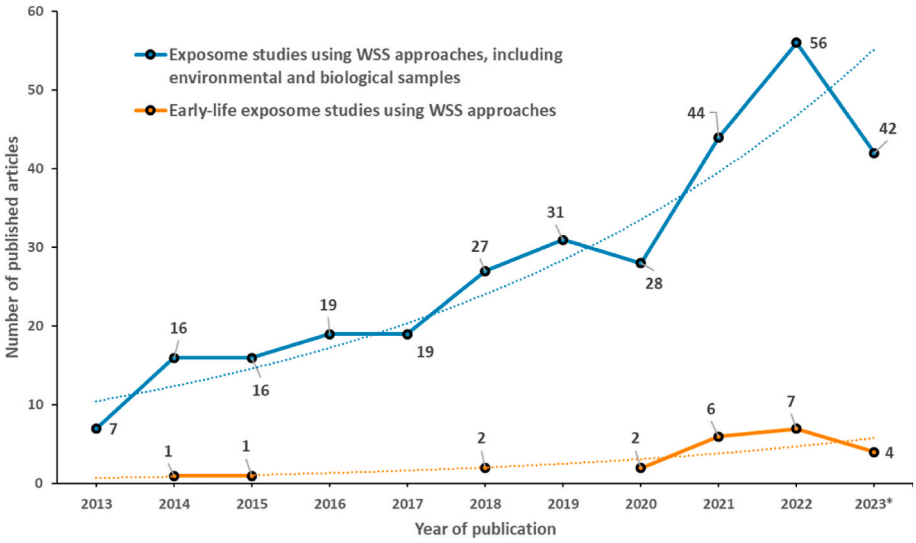

Fig. 1. Trend of studies using wide-scope screening approaches to characterize the chemical exposome (2013–2023). The blue line depicts studies encompassing environmental and biological samples across all stages of life, while the orange line specifically represents studies focusing on early-life stages included in this review. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

AND (early life OR child OR children OR infant OR pregnant OR pregnancy OR maternal)" yielded 179, 244, and 149 publications in PubMed, Scopus and Web of Science, respectively. After removing duplicate papers, the search results were manually filtered according to strict criteria, including: (A) original data from observational and experimental studies involving biological matrices from parents and/or children exposed to xenobiotics; (B) suspect screening or non-targeted analysis of exogenous compounds. Reviews, protocols, studies focused solely on endogenous metabolites, predictive models, simulations, visualization tools, or methodology discussions were excluded. The search process identified 23 studies that met the selection criteria. The titles of these articles and corresponding keywords are documented in Supplementary Data (Sheet: Selected_papers).

Comprehensive experimental and instrumental parameters from the selected studies, encompassing study design, sample preparation, chromatographic separation, high-resolution mass spectrometry settings, and data processing methodologies, are documented in Supplementary Data (Sheet: Selected_papers). Sample preparation methods applied in recent wide-scope screening exposome-type studies that analyzed human samples, as well as data acquisition and processing tools, and the detected families of chemicals are outlined in Table 1. Additionally, Table 2 offers a comprehensive overview of the tools, relevant parameters, and databases utilized within the data processing workflows. The number of identified or annotated features is also reported for each study, categorized based on the identification confidence levels proposed by Schymanski et al. [30].

2. Early-life chemical exposome: sampling & analytical considerations

The chemical properties and analytical workflows significantly influence the chemical coverage in early-life exposome studies. Physicochemical characteristics, particularly lipophilicity, half-lives of organic compounds in the biological matrices, and duration of exposure, play pivotal roles in understanding the toxicity and the human capacity to metabolize, excrete, or accumulate xenobiotics in various biological matrices [54–57]. Organic compounds entering the body can undergo extensive metabolism, partial metabolism, or remain unmetabolized before excretion through urine or stool. Lipophilic chemicals tend to partition into lipid-rich structures such as the liver, adipose tissues, and human milk. The stool is the primary route of elimination for these compounds due to their longer half-life in these matrices compared to others [58,59].

As an example, in blood, persistent organic pollutants (POPs) tend to accumulate in lipid-rich structures, leading to their rapid decline [59,60]. In contrast, polar and hydrophilic compounds are quickly filtered by the kidneys and excreted into urine either in their metabolized or unchanged forms. During urine formation, polar compounds undergo metabolism through Phase-I reactions involving functional group substitution/introduction or Phase-II reactions where additional moieties like glucuronide or sulfate groups are added. These transformations enhance the water solubility of xenobiotics [61–66]. Pesticides, phthalates, and volatile organic compounds (VOCs) are effectively detected in blood and urine [59,67,68]. The distribution of different organic compounds in solid matrices presents an additional challenge that must be considered during the sampling step. For example, recent studies have demonstrated heterogeneous spatial distributions of polybrominated diphenyl ethers (PBDEs) and iodine in the placenta between the maternal and fetal sides [69,70]. Therefore, in biomonitoring, the early-life chemical exposome, the physicochemical characteristics of the xenobiotics of interest play a crucial role in selecting the biological matrix, as they determine the type of exposure assessed and the chemical coverage of the analysis. In this review, the majority of studies on the early-life exposome analyzed maternal and cord blood samples (41 %). Other matrices included urine from parents and children (23 %), as well as placental samples (16 %). A subset of studies (8 %) compared the distribution of xenobiotics between the placenta and paired maternal and umbilical cord blood to track chemicals that can cross the placenta and be transferred to the fetus [35,37]. Wide-scope screening approaches have been also conducted on human milk (8 %), teeth (8 %), and amniotic fluid (4 %).

In addition to selecting the biological matrix, crucial aspects during study design include the number and frequency of samples, as well as the size of the cohort, to comprehensively investigate the early-life exposome and assess epidemiological aspects. Previous studies have demonstrated that relying solely on single-spot samplings may introduce higher errors and only reflect recent exposures due to the temporal variability of the exposome and the compounds' half-life [71]. To accurately assess the temporal variability of analytes and establish exposure profiles, sequential samples collected at regular intervals over extended periods are essential. While most studies have relied on single-spot samples, Huber et al. [42] used composite urine samples

Table 1
Overview of sample preparation, chromatographic and mass spectrometry parameters, data processing software and chemical coverage in selected articles characterizing the early-life exposome via wide-scope screening.

Ref.	Matrix	Vol.	Sample preparation	Chromatography	MS and MS/MS parameters	Data Processing		Chemical coverage
						Open-source tools	Commercial software	
Blood								
Prenatal exposome								
[31]	**maternal & cord serum (n=295)**	250 µL	• Centrifugation • PPT extraction (MeOH)	• LC-QTOF/MS • RP column (C_{18})	**ESI ± MS:** full scan (*m/z* 100–1000) **MS/MS:** DDA at 10, 20, 40 eV	- *in-house algorithm* - MS-Dial	- MassHunter Profinder (Agilent) - Mass Profiler Professional (Agilent)	PFASs and cyclic volatile methylsiloxanes
[32]	**maternal serum (n=75)**	250 µL	• Centrifugation • SPE (Oasis HLB)	• LC-QTOF/MS • RP column (C_{18} at 55 °C)	**ESI-MS**: dynamic mode (*m/z* 80–600) **MS/MS**: DDA	- R software	- MassHunter Qualitative Analysis (Agilent): Find-by-Formula	Phenols, pesticide metabolites, phthalate metabolites, PFASs, phenolic pesticides, acidic pesticides
[33]	**maternal & cord blood (n=30)**	250 µL	• Centrifugation • PPT extraction (MeOH with 0.1 % HCOOH)	• LC-QTOF/MS • RP column (C_{18})	**ESI ± MS:** full scan (*m/z* 100–1000) **MS/MS:** DDA	- MS-Dial	- MassHunter Profinder (Agilent)	Environmental organic acids (EOAs)
[34]	**maternal serum (n=20)**	250 µL	**Method 1** • Centrifugation • SPE (Oasis HLB) **Method 2** • Centrifugation • PPT extraction (ACN:MeOH) • Centrifugation	• LC-QTOF/MS • RP column (C_{18} at 55 °C)	**ESI-MS**: dynamic mode (*m/z* 80–600) **MS/MS**: DDA	–	- MassHunter Qualitative Analysis (Agilent): Find-by-Formula	Pesticides, pesticide metabolites, phenol, PFC, phthalates, phthalate metabolites
[35]	**maternal & cord serum (n=50)**	500 µL	• Vortex with $MgSO_4$ & NaCl (QuEChERS) • UAE (ACN) • Centrifugation	• LC-QTOF/MS • RP column (C_{18} at 35 °C)	**ESI-MS**: full scan (*m/z* 50–1250) **MS/MS**: DIA (*m/z* 50–1000)	–	- UNIFI screening platform (Waters)	PFASs
[36]	**cord blood (n=69)**	500 µL	• Centrifugation • LLE (NaCl in MeOH & MTBE)	• LC-Orbitrap-MS • RP column • (C_{18})	**ESI ± MS**: full scan (*m/z* 67–1000) **MS/MS**: DDA (40 eV) & DIA (ramp of 10–40 eV)	- MS Convert - Norman Digital Sample Freezing Platform	–	UVFs, surfactants, analgesics, plasticizers, emollients, pharmaceuticals, PCPs

Ref.	Matrix	Vol.	Sample treatment	Chromatography	MS and MS/MS parameters	Data Processing		Chemical coverage
						Open-source tools	Commercial software	
[37]	**maternal blood (n=10)**	150 µL	• LLE (ACN) • Centrifugation	• LC-Orbitrap-MS • RP column (C_{18} at 40 °C)	**ESI ±MS**: full scan (*m/z* 67–1000) **MS/MS**: DDA (35 eV) and DIA (25 eV)	- MS Convert - Norman Digital Sample Freezing Platform - Mzmine2 - SIRIUS	–	Flame retardants, food components, herbicides, pesticides, industrial chemicals, PFASs, stimulants, pharmaceuticals, PCPs
[38]	**maternal & cord blood (n=294)**	250 µL	• PPT (MeOH 0.1 % HCOOH) • Centrifugation	• LC-QTOF/MS • RP column (C_{18})	**ESI ± MS:** full scan (*m/z* 100–1000) **MS/MS**: no performed	–	- MassHunter Profinder (Agilent) - Mass Profiler Professional (Agilent)	Pesticides, PFASs, plasticizers, PCPs, pharmaceuticals
[39]	**maternal (n=75) & cord blood (n=64)**	250 µL	• PPT (MeOH 0.1 % HCOOH) • Centrifugation	• LC-QTOF/MS • RP column (C_{18})	**ESI ± MS:** full scan (*m/z* 100–1000) **MS/MS**: no performed	–	- MassHunter Profinder (Agilent) - Mass Profiler Professional (Agilent)	Pesticides, food additives, herbicides, plasticizers, industrial chemicals, PFASs, pharmaceuticals, PCPs
[40]	**maternal serum (n=30)**	250 µL	• PPT (MeOH 0.1 % HCOOH) • Centrifugation • Evaporation N_2 • LLE (MeOH) • Centrifugation	• LC-QTOF/MS • RP column (C_{18})	**ESI ±MS:** full scan (*m/z* 100–1000)	–	- MassHunter Profinder (Agilent) - Mass Profiler Professional (Agilent)	Pesticides, food additives, herbicides, plasticizers, industrial chemicals
[41]	**maternal & cord blood (n=117)**	500 µL	• Vortex with $MgSO_4$ & NaCl (QuEChERS) • Extraction (ACN) in US • Centrifugation	• LC-QTOF/MS • RP column (C_{18} at 35 °C)	**ESI- MS**: full scan (*m/z* 50–1250) **MS/MS**: DIA (*m/z* 30–1000)	–	- PeakView & MarkerView (Sciex) - MATLAB	PFASs

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Table 1 (*continued*)

Ref.	Matrix	Vol.	Sample treatment	Chromatography	MS and MS/MS parameters	Data Processing		Chemical coverage
						Open-source tools	Commercial software	
Urine								
Prenatal exposome								
[42]	**mothers & fathers urine (n=500)**	500 µL	• SPE (Oasis HLB)	• LC-Orbitrap-MS • RP column (C_{18} at 50 °C)	**ESI ± MS**: full scan[a] **MS/MS**: DDA	- MS Convert - MetAlign - R packages (mzR, msPurity, Spectra) - SIRIUS	–	Fungicides, insecticides, acaricides, herbicides
[43]	**maternal urine (n=40)**	N/A	• Dilution 1:1 (H_2O:CH_3OH:CH_3CO_2H, 95:5:0.1)	• LC-Orbitrap-MS • RP column (C_{18} at 35 °C)	**ESI ± MS & MS/MS**: *m/z* 60–800	–	- MetWorks - Xcalibur QualBrowser & QuantBrowser (Thermo)	Pesticides
Postnatal exposome								
[42]	**children's urine 6–11 y.o. (n=500)**	500 µL	• SPE (Oasis HLB)	• LC-Orbitrap-MS • RP column (C_{18} at 50 °C)	**ESI ± MS**: full scan[a] **MS/MS**: DDA	- MS Convert - MetAlign - R packages (mzR, msPurity, Spectra) - SIRIUS	–	Fungicides, insecticides, acaricides, herbicides
[44]	**children's urine <2 y.o.** (n = 11)	250 µL	• Acidification (H_2O 0.1 % HCOOH) • Centrifugation	• LC-Orbitrap-MS • RP column (C_{18} at 45 °C)	**ESI ± MS:** full scan (*m/z* 100–1000) **MS/MS:** DDA	–	- Compound Discoverer (Thermo)	Pesticides, industrial chemicals, plasticizers
[45]	**children's urine 2–4 y.o. (n=5)**	500 µL	• Filtration (0.2 µm) • β-glucuronidase and arylsulfatase enzymes • Direct injection in an online SPE (Hypersil Gold aQ)-LC-HRMS system	• LC-Orbitrap-MS • RP column (C_{18})	**ESI ±**	–	- Compound Discoverer (Thermo)	Natural products, pharmaceuticals, pesticides, PCPs, industrial chemicals
[46]	**children's urine 6–9 y.o. (n=200)**	1000 µL	• β-glucuronidase enzymes treatment • SPE (Oasis HLB)	• LC-Orbitrap-MS • RP column (HSS-T_3 at 35 °C)	**HESI ± MS**: full scan (*m/z* 100–900) **MS/MS**[a]	- MS Convert - MZmine - CFM-ID - Sirius-CSI: Finger-ID (CFM-ID)	–	Pesticides, industrial chemicals, plasticizers, phthalates, surfactants, bisphenols, pharmaceuticals, PCPs, tobacco-related compounds
Placenta								
[35]	**placenta (n=50)**	500 mg	• Vortex with $MgSO_4$ and NaCl • Extraction (ACN) • Centrifugation	• LC-QTOF/MS • RP column (C_{18} at 35 °C)	**ESI-MS**: full scan (*m/z* 50–1250) **MS/MS**: DIA	–	- UNIFI screening platform (Waters)	PFASs
[37]	**placenta** (0.4 × 0.4 mm, full-thickness from two different areas) **(n=9)**	200 mg	• LLE (citrate buffer: ACN) & centrifugation (x3) • SPE (home-made cartridges)	• LC-Orbitrap-MS • RP column (C_{18} at 40 °C)	**ESI ± MS**: full scan (*m/z* 67–1000) **MS/MS:** DDA and DIA	- MS Convert - Mzmine2 - SIRIUS - Norman Digital Sample Freezing Platform	–	Flame retardants, food components, herbicides, pesticides, industrial chemicals, PFASs, stimulants, pharmaceuticals, PCPs
[47]	**placenta** (5 sections of 10 cm^3 from different areas) **(n=40)**	100 mg	• **Method 1:** extraction (MeOH), PPT (MeOH) & centrifugation • **Method 2:** extraction (MeOH), acidification with HCOOH (1 %) & PPRP (Phree 96-Well Plate) • **Method 3:** extraction (MeOH), Strata-X cartridges (MeOH and H_2O), SPE (Polymeric Strata-X) H_2O with 5 % MeOH • **Method 4:** Method 2 & 3 were combined	• LC-QTOF/MS • RP column (HSS-T_3 at 40 °C)	**ESI ± MS**: full scan (*m/z* 50−1100) **MS/MS**: DIA (SWATH) (*m/z* 50–900)	- MS Convert - MS-DIAL - MetFrag	- MarkerView (Sciex) - Reifycs Abf converter	Biocides, pharmaceuticals, PCPs
[48]	**placenta (n=35)**	500 mg	• SLE (H_2O:ACN), vortex & centrifugation • SLE (ACN), vortex & centrifugation	• LC-QTOF/MS • RP column (C_8 at 40 °C)	**ESI ± MS**: full scan (*m/z* 100–1000) **MS/MS**: DDA	- NTA WebApp	- MassHunter Profinder (Agilent)	Drugs, PCPs, industrial chemicals, plasticizers, insecticides, repellents

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Table 1 (*continued*)

Ref.	Matrix	Vol.	Sample treatment	Chromatography	MS and MS/MS parameters	Data Processing		Chemical coverage
						Open-source tools	Commercial software	
			• Filtration (Captiva EMR-lipid) of the two supernatants				- Mass Profiler Professional (Agilent)	
Human milk								
[49]	**human milk (n=4)**	1 mL	• Vortex • Extraction (ACN, Na_2SO_4, NaCl) • Centrifugation at 4 °C • PPT (−20 °C for 12h) • Evaporation • Acidification (ACN 0.1 % HCOOH) • Filtration (Captiva ND-lipid)	• LC-Orbitrap-MS • RP column (C_{18} at 50 °C)	**ESI ± MS:** full scan (*m/z* 70–1050) **MS/MS**: DDA with an automatic intensity threshold and dynamic exclusion (*m/z* 70–1050)	–	- Compound Discoverer (Thermo)	Plasticizers, UV filters, food additives, drugs, phytoestrogens, stimulants, surfactants, alkaloids, artificial sweeteners, industrial chemicals
[50]	**human milk (n=3)**	16 mL	• Centrifugation • Frozen overnight for PPT (−20 °C) • lipid portion: dilution (acetone:hexane & Na_2SO_4), centrifugation, dissolution in cyclohexane & ethylacetate, GPC, silica SPE with hexane: DCM • water portion: centrifugation, SPE (Oasis HLB), centrifugation and reconstitution with DCM	• GCxGC/QTOF-MS • Restek Rtx-5ms & Restek Rtx-17 column	**EI** **MS**: full scan (*m/z* 100–1000)	–	- ChromaTOF (LECO)	POPs and PAHs
Amniotic fluid								
[51]	**amniotic fluid (n=4)**	5 mL	• SPE (Oasis HLB) • Evaporation N_2	• GC-Orbitrap-MS • Restek Rtx-5Sil column	**EI** full scan (*m/z* 50–750)	–	- TraceFinder with Deconvolution Plugin (Thermo)	Endocrine disruptors and xenobiotics
Teeth								
[52]	**teeth (n=5)**	5–25 mg	• solid phase extraction with polymeric sorbent: acidic analytes (CH_3COOH in ACN), neutral analytes (ACN) and basic analytes (NaOH) • C_{18} column extraction: acidic and neutrals analytes (HCl in ACN), and basic analytes (NH_4OH in ACN) • evaporation & reconstitution with ACN	• LC-QTOF/MS • RP column (C_{18} at 35 °C)	**ESI ± MS**: dynamic range (*m/z* 50–1700) **MS/MS**: no performed	–	- MassHunter Profinder (Agilent): Find-by-Formula, Molecular Feature Extraction & Batch Recursive Feature Extraction	Bisphenols, parabens, UV filters, pesticides, polyfluorinated chemicals, tobacco-related compounds
[53]	**teeth (n=31)**	5–25 mg	• solid phase extraction with polymeric sorbent: acidic analytes (CH_3COOH in ACN), neutral analytes (ACN) and basic analytes (NaOH) • C_{18} column extraction: acidic and neutrals analytes (HCl in ACN), and basic analytes (NH_4OH in ACN) • evaporation & reconstitution with ACN	• LC-QTOF/MS • HILIC column (zwitterionic) at 25 °C & RP column (C_{18} at 50 °C)	**ESI ± MS**: full scan[a] **MS/MS**: DDA	- MS Convert - XCMS R package - GNPS	- MassHunter Profinder (Agilent) - Mass Profiler Professional (Agilent)	Phthalates, parabens, perfluoroalkyl compounds, cotinine

Note: **LC**: Liquid chromatography; **GC**: Gas chromatography; **ESI+**: Electrospray ionization in positive mode; **ESI-**: Electrospray ionization in negative mode; **EI**: Electronic impact; **PPT**: Protein precipitation; **PPRP**: Protein and phospholipid removal plates; **SPE**: Solid phase extraction; **LLE**: Liquid/liquid extraction; **DLLE**: Dispersive liquid/liquid extraction; **UAE**: ultrasound-assisted extraction; PFASs: Perfluoroalkyl and Polyfluoroalkyl Substances; PCPs: personal care products.

[a] no further information reported in the article.

Table 2
Criteria followed for feature generation, MS1 and MS2 mining, and *in-silico* tools for early-life *exposomics*. The level of confidence and chemical families of the annotations are reported for each study based on E. Schymanski's definitions [30].

Ref.	Feature generation	MS1 mining	MS2 mining	In-silico prediction	Databases	Annotations with level of confidence
[31]	- **Agilent MassHunter Profinder** to generate FTs - **MPP** to align FTs and subtract the blank Parameters: mass error <5 ppm, peak intensity >1500 a.u., alignment range: 30 s - **MS-Dial.** Same parameters	- Matching FTs with ***in-house* database** (65535 compounds) Parameters: mass accuracy and isotopic pattern score >70	- Screening MS1 FT list with MS/MS databases: **MassBank of Europe and North America, HMDB, mzCloud,** and **MS-Dial** ("All public MS/MS database") Parameters: mass error <10 ppm, at least one fragment aside from the molecular ion	- Screening MS1 FT list with *in-silico* databases using **CFM-ID** Parameters: mass error <10 ppm, at least one fragment aside from the molecular ion	- **MS database**: 65535 entries (2420 unique chemical formulas) from the U.S. EPA Chemical Data Reporting list (CDR) 2016 and *in-house* database - **MS/MS databases**: MassBank of Europe and North America, HMDB, mzCloud, EPA's Chemistry Dashboard databases - ***In-silico* tools**: CFM-ID	**level 1:** 19 compounds **level 2:** 73 compounds **level 3:** 98 putative annotations (38 exogenous & 60 endogenous) **level 4:** 288 putative (273 exogenous & 15 endogenous)
[32]	- **Agilent MassHunter** (Find-by-Formula) to generate FTs Parameters: mass error <10 ppm, isotopic pattern - **MassHunter Qualitative Analysis** Parameters: peak area >2 times the blank, S/N > 3	- Matching FTs with the **database of EOAs** and **EPA's CompTox Chemistry Dashboard database** Parameters: target score >70	- Screening MS1 FT list with MS/MS ***in-house* EOAs database** Parameters: target score >70 and present in >80 % participants	–	- **MS & MS/MS database:** 696 entries (516 unique molecular formulas)	**level 5**: 455 putative annotations
[33]	- **Agilent MassHunter Profinder** (MFE & MPP) to generate and align FTs Parameters: S/N > 2, 100 % in triplicates - **MS-Dial** Same parameters	- Matching FTs with **MS-Dial, MassBank of Europe and North America, HMDB** and **mzCloud databases** Parameters: molecular ion, isotopic pattern >70, 100 % matches with frequency in paired samples and demographic differences	- Screening MS1 FT list with MS/MS databases: **MS-Dial, MassBank of Europe and North America, HMDB** and **mzCloud**	- Screening MS1 FT list with *in-silico* spectral data (**CFM-ID and MetFrag**)	- **MS database:** 65535 entries (2420 unique chemical formulas) from the U.S. EPA Chemical Data Reporting list (CDR) 2016 and *in-house* database - **MS/MS database:** MS-DIAL, MassBank of Europe and North America, mzCloud - ***In-silico* tools**: CFM-ID & MetFrag	**level 1:** 15 EOAs **level 2:** 73 EOAs
[34]	- **Agilent MassHunter** to generate FTs	- Matching FTs with the ***in-house* database of EOAs** & **EPA's CompTox Chemistry Dashboard database** by **Find by Formula** (Agilent) Parameters: mass error <10 ppm, peak area >500 a.u., S/N > 3, target score >70, and participants filtering >80 % - **Retention time plausibility** Parameters: ΔRT ± 0.5 min across the samples, predicted polarity	- Confirmation of 3 of MS1 matched compounds with standards	–	- **MS & MS/MS database:** 693 environmental organic acids	**level 1:** 2 EOAs **level 4:** 282 putative EOAs annotated
[35]	- **UNIFI** (Waters) to generate FTs	- Matching FTs with the ***in-house* list** of PFAS from **NORMAN Suspect List Exchange, the U.S. EPA CompTox Chemistry Dashboard** and **an *in-house* database** Parameters: mass error <5 ppm, fragment error <12.5 ppm, isotopic pattern fit <10 %, peak intensity >25000 a.u.	- Screening MS1 FT list with MS/MS databases: **NORMAN SLE, the U.S. EPA CompTox Chemistry Dashboard** and ***in-house* database** Parameters: at least three fragments aside from the molecular ion	–	- **MS & MS/MS database:** 3000 PFASs from the NORMAN Suspect List Exchange, the U.S. EPA CompTox Chemistry Dashboard and *in-house* database	**level 2:** 49 PFAS
[36]	- **ProteoWizard** (to mzML) - **NORMAN DSFP** to generate FTs Parameters: mass error <3 ppm, peak intensity >500 a.u.	- Matching FTs with **EUCOSMETICS list (NORMAN-SLE)** Parameters: mass accuracy, isotopic pattern, predicted RT, blank subtraction, removing endogenous compounds	- Screening MS1 FT list with MS/MS databases: **MassBank, mzCloud,** and **PubChem databases**	–	- **MS & MS/MS database:** EUCOSMETICS list (NORMAN-SLE), MassBank of Europe and North America, mzCloud and PubChem	**level 1:** 10 compounds **level 2:** 3 compounds
[37]	**Method 1:** - **ProteoWizard** (to mzML)	- Matching FTs with **EXPHRMSMSAVAL (NORMAN SLE)**	- Screening MS1 FT list was checked with **MassBank, MoNA, Metlin, mzCloud** and **PubChem databases**	- **SIRIUS (ZODIAC, CSI: FingerID, CANOPUS)** to	- **MS & MS/MS database:** EXPHRMSMSAVAL list (NORMAN-SLE), MassBank of Europe and North	**level 1:** 27 compounds **level 2:** 12 compounds

(*continued on next page*)

Table 2 (*continued*)

Ref.	Feature generation	MS1 mining	MS2 mining	In-silico prediction	Databases	Annotations with level of confidence
	- **NORMAN DSFP** to generate FTs Parameters: mass error <3 ppm, peak intensity >500, peak points >3 **Method 2:** - **ProteoWizard** (to mzML) - **MzMine** to generate FTs Parameters: mass error <3 ppm, peak intensity $< 1 \times 10^5$, S/N > 10	Parameters: mass accuracy, isotopic pattern, predicted RT, and blank subtraction, removing endogenous compounds		assign molecular formula and annotate FTs Parameters: mass error <3 ppm, isotopic pattern, predicted RT, and adducts	America, Metlin, HMDB and mzCloud - ***In-silico* tools**: Sirius-CSI: FingerID	**level 3:** 2 putative annotations
[38]	- **MFE** to generate FTs Parameters: mass error <50 ppm, RT window ± 30 s, FTs intensity ≥2 times procedural blank - **MPP** to align the FTs Parameters: mass error <10 ppm, RT correction window 5 % ± 30 s, RT alignment window 20 s	- Matching FTs with **in-house database for human exposures** to assign the molecular formulas Parameters: mass error <5 ppm, isotopic abundance and pattern >70 - Matching FTs with **HMDB database** to exclude endogenous compound Parameters: mass error <5 ppm, isotopic pattern score >70 - Matching chemical structures with isomers in **EPA's Chemistry Dashboard** using a score from *in-house algorithm*	–	–	- **MS database**: 65535 entries (2420 unique chemical formulas) from the U.S. EPA Chemical Data Reporting list (CDR) 2016 and *in-house* database	**level 4:** 685 putative annotations
[39]	- **MFE** to generate FTs Parameters: mass error <50 ppm, RT window ± 30 s, FTs intensity ≥2 times procedural blank - **MPP** to align the FTs Parameters: mass error <10 ppm, RT correction window 5 % ± 30 s, RT alignment window 20 s	- Matching FTs with ***in-house* database for human exposures** to assign the molecular formulas Parameters: mass error <5 ppm, isotopic abundance and pattern >70 - Matching FTs with **HMDB database** to exclude endogenous compound Parameters: mass error <5 ppm, isotopic pattern score >70 - Matching chemical structures with isomers in **EPA's Chemistry Dashboard** using a score from *in-house algorithm*	–	–	- **MS database**: 65535 entries (2420 unique chemical formulas) from the U.S. EPA Chemical Data Reporting list (CDR) 2016 and *in-house* database	**level 4:** 2167 putative annotations
[40]	- **MFE** to generate FTs Parameters: mass error <50 ppm, RT window ± 30 s, FTs intensity ≥2 times procedural blank - **MPP** to align FTs Parameters: mass error <10 ppm, RT correction window 5 % ± 30 s, RT alignment window 20 s	- Matching FTs with ***in-house* database for human exposures** to assign the molecular formulas Parameters: mass error <5 ppm, isotopic abundance and pattern >70, ΔRT <30 s - Matching FTs with **HMDB database** to exclude endogenous compound Parameters: mass error <5 ppm, isotopic pattern score >70	- Screening MS1 FT list with MS/MS databases: **MassBank of Europe and North America, HMDB** and **mzCloud** Parameters: at least two fragmentation peaks, mass error <10 ppm	- **CFM-ID and MetFrag** to generate *in-silico* spectral data and screen MS1 FT list Parameters: at least two fragmentation peaks, mass error <10 ppm	- **MS/MS database:** 3518 compounds from U.S. EPA Chemical Data Reporting (CDR) 2016 database - **MS/MS database:** MassBank of Europe and North America, HMDB and mzCloud - ***In-silico* tools**: CFM-ID & MetFrag	**level 1:** 17 compounds **level 2:** 7 compounds **level 3:** 9 compounds **level 4:** 207 putative annotations
[41]	- **PeakView** to generate FTs Parameters: S/N > 3, intensity >100 - **MarkerView** to align FTs Parameters: mass error <10 ppm, RT window ± 2 min	- Matching FTs with ***in-house* PFASs database** from **U.S. EPA CompTox Chemistry Dash-board, NORMAN SLE** Parameters: mass and isotopic pattern - MATLAB script to flag FTs Parameters: mass defect <0.15 or >0.85	- **MATLAB** script to flag FTs with characteristic neutral losses (-CF2-) - Screening MS1 FT list and FTs flagged by **MATLAB** with MS/MS databases: **MassBank, mzCloud,** and **an *in-house* database**	–	- **MS database:** 2893 PFASs compiled from U.S. EPA CompTox Chemistry Dash-board, NORMAN SLE and *in-house* library - **MS/MS database**: MassBank, mzCloud, and an *in-house* library	**level 1:** 2 PFASs **level 2:** 2 PFASs **level 3:** 25 PFASs

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Table 2 (*continued*)

Ref.	Feature generation	MS1 mining	MS2 mining	In-silico prediction	Databases	Annotations with level of confidence
[42]	- **MetAlign** Parameters: mass error <1.5 ppm, intensity $>10^3$-10^4 a.u, isotopic pattern	- Matching FTs with ***in-house* pesticide metabolites database**	- Screening MS1 FT list with MS/MS ***in-house* pesticide metabolites database** Parameters: mass error <10 ppm, at least three fragments, manual review	*In-silico* deconjugation procedure to generate a pesticide metabolites list for MS2 matching - **SIRIUS** to assign molecular formulas	- **MS and MS/MS *in-house* database**: 4600 entries of pesticide metabolites from *in-house* library	**level 1:** 18 pesticides **level 2:** 21 pesticides **level 3:** 46 pesticides **level 4:** 14 parent pesticides and 71 metabolites (including 16 glucuronide and 11 sulfate conjugates)
[43]	- **MetWorks** to generate FTs Parameters: mass error <5 ppm, peak intensity $> 5 \times 10^3$	- Matching FTs with ***in-house* database of 47 pesticides** using **XCalibur QualBrowser** and **QuantBrowser** Parameters: mass error <5 ppm, isotopic pattern	- MS1 FT list was filtered using **XCalibur QualBrowser** and **QuantBrowser** Parameters: isotopic pattern, MS2 spectral matches with metabolites from rat urine, confirmation with standard if available	–	- **MS and MS/MS *in-house* database**: 47 pesticides from the International Program on Chemical Safety (IPCS) database, the European Food Safety Authority, the Pesticide Properties Database, and Metabolic Pathways of Agrochemicals	**level 1:** 23 pesticides **level 3:** 5 pesticides
[44]	- **Compound Discoverer** to generate FTs Parameters: peak intensity $> 2 \times 10^5$, mass error <5 ppm, S/N > 3, intensity error 30 %	- Matching FTs with **mzCloud** Parameters: database match score >80	- Screening MS1 FT list with MS/MS database: **mzCloud** Parameters: mass error fragments <5 ppm, spectral fitting >80 %	–	- **MS/MS databases**: MzCloud	**level 2:** 7 compounds
[45]	- **Compound Discoverer** to deconvolute peaks, subtract background, group FTs, evaluate isotopic pattern and fragment matching Parameters: mass error <5 ppm, intensity tolerance: 30 %, peak intensity >500000 a.u., S/N ratio> 3	- Matching FTs with **Chemspider, MzCloud, MzVault, DrugBank, EAWAG (Biocatalysis & Biodegradation), USEPA Toxcast** and **DSSTox databases** Parameters: mass error <5 ppm, FTs intensity ≥3 times procedural blank, isotopic pattern, RT ± 2 min $RT_{predicted}$	- Screening MS1 FT list with MS/MS databases: **Chemspider, MzCloud, MzVault, DrugBank, EAWAG Biocatalysis/ Biodegradation, USEPA Toxcast** and **DSSTox** Parameters: mass error <5 ppm, isotopic pattern	–	- **MS/MS databases**: Chemspider, MzCloud, MzVault, DrugBank, EAWAG Biocatalysis/Biodegradation, USEPA Toxcast and DSSTox	**level 2:** 265 compounds
[46]	- **ProteoWizard** (to mzXML) - **MzMine** to generate FTs Parameters: mass error <5 ppm, peak intensity $> 1 \times 10^4$, S/N > 10, blank subtraction	- Matching FTs with ***in-house* database** of **801 xenobiotics** from **Exposome Explorer, T3DB** and **HBM4EU databases**	- Screening MS1 FT list with **MoNA database** Parameters: mass error precursor <6 ppm, mass error fragments <7 ppm, library matches (KEGG, ChEBI, PubChem) > 60 %, biomarkers of exposure (BoE), cosine similarity >0.8, at least 5 fragment ions matching	- **CFM-ID** to generate in silico spectral data	- **MS database**: Exposome Explorer, T3DB and HBM4EU databases (801 entries) - **MS/MS database**: MassBank of North America - ***In-silico* tools**: Sirius-CSI: FingerID	**level 2:** 36 compounds **level 3:**38 compounds
[47]	- **MakerView** to generate FTs Parameters: peak intensity >100, mass error <10 ppm, alignment range 30 s - **MS-Dial** Parameters: peak intensity >100, mass error <10 ppm	- Matching FTs with public databases: **MS-Dial, Metlin, MassBank of Europe and North America and HMDB**	- Screening MS1 feature list with MS/MS databases: **MS-Dial, Metlin, MassBank of Europe** and **North America, and HMDB**	- **MetFrag**[a]	- **MS/MS databases:** MS-DIAL, Metlin, MassBank of Europe and North America, HMDB - ***In-silico* tools:** MetFrag	**level 1:** 12 compounds **level 2a:** 78 compounds **level 2b:** 50 compounds **level 3:** 26 putative compounds **level 5:** 31 putative compounds
[48]	- **Agilent Profinder** to generate and align FTs Parameters: mass error <15 ppm, peak intensity >1000, RT ± 30 s,100 % replicates	- **Agilent Mass Profiler** to assign molecular formulas using **DSSTox-MS-Ready Formulae** Parameters: mass error <10 ppm, RT ± 10 s, blank subtraction	- Screening MS1 FT list with MS/MS databases: **Agilent Personal Compound Database and Library (PCDL)**	- *in-silico* predicted MS2 spectra for all **DSSTox database** compounds	- **MS database:** DSSTox database from EPA's Distributed Structure-Searchable Toxicity and Product Categories (CPCat) - **MS/MS databases:** 11324 unique compounds from six Agilent Personal Compound Database and Library (PCDL)	**level 1:** 27 compounds **level 2:** 12 compounds **level 3:** 2 compounds

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Table 2 (*continued*)

Ref.	Feature generation	MS1 mining	MS2 mining	In-silico prediction	Databases	Annotations with level of confidence
					databases (Environmental water screening, Pesticides, Forensic toxicology, Veterinary drugs, Metlin, and Extractables and leachables) - ***In-silico* tools:** CFM-ID	
[49]	- **Compound Discoverer** to generate FTs Parameters: peak area $> 10^7$, 100 % in triplicates, group variance <30 %, S/N > 10	- Matching FTs with **mzCloud** using **Compound Discoverer** Parameters: mass and isotopic pattern - Matching FTs with **HMDB database** to exclude endogenous compound	- Screening MS1 FT list with MS/MS database: **mzCloud** Parameters: spectral fitting >70 %, ΔRT ± 0.1 min	–	- **MS & MS/MS databases:** mzCloud (17800 compounds)	**level 1:** 13 compounds **level 2a:** 12 compounds **level 2b:** 12 compounds **level 3:** 5 compounds **level 4:** 8 putative annotation
[50]	- **LECO Chroma TOF** to generate FTs Parameters: S/N > 50, RT > 806 s	- Matching FTs with **2011 NIST Mass Spectral Library** - Filtering halogenated, aromatic FTs	–	–	- **EI databases:** 2011 NIST Mass Spectral Library (212961 spectra)	**level 1: 4**0 compounds **level 2:** 132 compounds
[51]	- **TraceFinder** to generate FTs Parameters: S/N ratio >10, peak intensity >1000 a.u., ion overlap window >99 %	- Matching formula and retention index with **2017 NIST Mass Spectra Library** & ***in-house* library** - Comparison between library matches and 350 standards using MATLAB Parameters: RSI/Rev score >700, FTs intensity ≥5 times blanks	–	QSARs *in-silico* prediction models to prioritize compounds with endocrine activity	- **EI databases:** 267000 compounds from 2017 NIST Mass Spectra Library (NIST/EPA/NIH EI and NIST Tandem Mass Spectral Library) and 300 compounds from an *in-house* database	**level 1:** 9 endocrine disruptors **level 2:** 110 exogenous compounds
[52]	- **Agilent MassHunter** (Find-by-Formula) to generate the FTs[a]	- Matching FTs with **Personal Compound Database (PCD)** using **MFE** and **BRFE** Parameters: peak intensity >1500 a.u., ion abundance >70 %	–	–	- **MS/MS MS database:** Personal Compound Database (PCD)	**level 4:** 26 organic compounds
[53]	- **ProteoWizard** (to mzXML) - **XCMS** to generate FTs Parameters: mass error <3 ppm, peak intensity >500 a.u., 3 minimum peak points, 100 % in triplicates, S/N > 3	- Matching with ***in-house* Personal Chemical Database Library (PCDL)** Parameters: mass error <20 ppm, ΔRT ± 0.3 min	- Screening MS1 FT list with **GNPS** Parameters: mass error precursor <2 ppm, cosine similarity >0.7, MS/MS tolerance 0.5 Da - Screening MS1 FT list with **Metlin** Parameters: mass error precursor <20 ppm, MS/MS tolerance 0.03 Da	–	- **MS database:** *in-house* Personal Compound Database Library (PCDL) - **MS/MS database:** Metlin and GNPS Personal Compound Database (PCD)	**level 2:** 250 compounds (including endogenous)

BRFE: Batch Recursive Feature Extraction; **MFE**: Molecular Feature Extraction (Agilent); **MPP**: Mass Profile Professional (Agilent).
[a] no further information reported in the article.

from five different mornings, and Tkalec et al. [46] collected two urine samples to assess seasonal variations. Previous research has highlighted significant spatial variability of xenobiotics within the same biological samples, particularly in large and solid matrices like the placenta, where composition varies by location and orientation due to heterogeneous enzyme distribution [72–74]. Despite the increased costs and logistical challenges, employing multiple tissues/biofluids and repeated samplings provide complementary insights and a more comprehensive understanding of temporal exposures.

Large cohorts provide robust statistical power, enhancing the understanding of chemical exposures and determining environment-health associations [75]. Considerable investments in exposome research have been made in the USA and Europe [76], with large projects and cohorts such as HEALS (Health and Environment-wide Associations based on Large Population Surveys) [77], EXPOsOMICS (Enhanced exposure assessment and omic profiling for high-priority environmental exposures in Europe) [78], HBM4EU (European Human Biomonitoring Initiative) [79], CHEAR (Children's Health Exposure Analysis Resource) [80], or HERCULES (Human Exposome Research Center: Understanding Lifetime Exposures) [81]. Research projects evaluating the early-life chemical exposome are emerging, with the implementation of larger and more representative cohorts, such as HELIX (Human Early Life Exposome) [82], ATHLETE (The Advancing Tools for Human Early Lifecourse Exposome Research and Translation) [83], The LifeCycle Project – EU Child Cohort Network [84], and the INMA-Sabadell Birth Cohort [85]. Among the studies reviewed, participant numbers ranged from 3 to 500. Small cohorts are employed in pilot studies to evaluate the feasibility of using a particular biological matrix or workflow for early-life exposomics. Human biomonitoring cohorts ideally require a significant sample size to establish robust statistical evidence of correlations, typically aiming for 200–300 participants for studies aiming to elucidate adverse health outcomes linked to early-life chemical exposures. However, only 9 studies utilized cohorts with 50 or more participants, and just 4 studies included over 200 individuals.

The storage and sample preparation play a central role in the analytical workflow, impacting the comprehensiveness, accuracy, and

reliability of the results. The storage temperature is a critical parameter in sample handling. Traditionally, a storage temperature of −80 °C is considered optimal. While approximately 83 % of studies have adhered to this standard, 17 % have utilized −20 °C. However, it is essential to acknowledge that prolonged storage at −20 °C may potentially compromise the accuracy of findings, particularly for contaminants in human samples. Further investigation into the effects of storage temperature on sample integrity is warranted. In wide-scope screening approaches, the choice of sample preparation strategy depends on the matrix type and the availability of sample volume or weight. For solid matrices, common extraction methods include solid-phase extraction (SPE), solid-liquid extraction (SLE), and ultrasound-assisted extraction (UAE). In contrast, for liquid samples, protein precipitation (PPT) using organic solvents such as methanol (MeOH) or acetonitrile (ACN), followed by centrifugation, liquid-liquid extraction (LLE), dispersive liquid-liquid extraction (DLLE), or their combinations, are typically employed. A detailed comparison of these methods will be provided in Section 3, "Biological Matrices," below.

In instrumental analysis, liquid chromatography (LC) coupled with high-resolution mass spectrometry (HRMS) emerges as the predominant approach (91 %). Conventional reverse-phase columns (C_{18}, C_8, HSS-T_3) are used in all studies. Electrospray ionization (ESI) stands as the predominant ionization source in LC-based studies. However, there is no reported use of alternative ionization sources for LC, such as atmospheric pressure photoionization (APPI) or atmospheric pressure chemical ionization (APCI), despite their potential to broaden chemical space coverage. A gradient of aqueous and organic phases, typically employing solvents like MeOH or ACN, constitutes the mobile phase in 87 % of the studies, facilitating the separation and detection of polar and semi-polar compounds.

Conversely, for less polar, hydrophobic and volatile compounds, gas chromatography (GC) is a widely used technique [86]. However, in early-life exposomics, GC has only been applied in only 9 % of the studies. Non-polar stationary phases, such as diphenyl dimethyl polysiloxane or diphenyl and dimethyl polysiloxane or those functionalized with cyano and phenyl groups, along with electron ionization (EI), are commonly employed conditions. Due to the limited availability of mass spectral databases, chemical ionization (CI) and atmospheric-pressure chemical ionization (APCI) are not commonly utilized [50].

In metabolomics, different methodologies such as complementary analytical techniques or columns with different polarity are usually combined in the same study to enhance the coverage of the chemical space and gain deeper insights into phenotypical changes [87,88]. This approach is crucial because the diversity of metabolite groups in terms of their physicochemical properties limits the detection capability of single analytical approaches to capture the entire metabolome. Contrarily, in exposomics, there are relatively few studies that utilize orthogonal approaches to broaden the chemical coverage as reported in the recent review of Manz et al. [89]. LC stands as a prevalent choice for wide-scope screening exposomics, whereas GC remains underutilized in the field, potentially leading to the incomplete characterization of a subset of the chemical exposome [90]. The complementary use of both chromatographic techniques, as well as different stationary phases, not only expands the coverage of the chemical exposome but also enhances confidence in annotations and identifications. In early-life exposomics, only two studies applied orthogonal methods to the same cohort to broaden the characterization of the environmental contaminants. In particular, Tran et al. [50] used two-dimensional gas chromatographic separation to determine unexpected contaminants in human breast milk [50]. The combination of polar and non-polar stationary phases provides improved chromatographic separation compared to one-dimensional GC, which is advantageous for the analysis of complex matrices [91]. Yu et al. conducted an orthogonal analysis using zwitterionic-HILIC and reverse-phase column to better profile small molecules from prenatal and postnatal tooth fractions, a direct window into fetal exposure [53]. The employment of chromatographic columns with orthogonal phases of different polarities [31,53] as well as alternative ionization techniques to ESI, such as atmospheric pressure photoionization or atmospheric pressure chemical ionization, have demonstrated potential in environmental sample analysis for detecting organic compounds not efficiently ionized by ESI [29]. Fig. 2 summarizes these protocols and techniques separated by biological matrix.

3. Biological matrices

3.1. Blood

Blood is a complex biofluid that comprises red and white blood cells, platelets, proteins, hormones, carbohydrates, minerals, lipids, and other small organic molecules [92,71]. Due to its close interaction with almost all body tissues, blood contains a diverse range of compounds and their metabolites, offering a snapshot of the current exposome status. However, it has limitations in fully representing certain exposure routes and long-term exposures.

Blood collection, despite its invasive nature, is widely adopted in biomonitoring protocols due to standardized and straightforward protocols. In these studies, plasma and serum are typically chosen for screening multiple environmental pollutants, while whole blood is preferred for evaluating metals due to their distribution between extracellular and intracellular compartments. In early-life exposomics, 48 % of the selected studies have investigated the chemical exposome using maternal and/or cord blood samples. Specifically, 36 % of the studies focused on maternal blood, 9 % exclusively on cord blood, and 55 % of them analyzed both types of samples. This approach allows researchers to assess both prenatal exposure through maternal blood and fetal exposure through cord blood, providing insights into the transfer of environmental contaminants across the placenta and potential impacts on fetal development. For example, analysis of matched maternal and cord samples has revealed similar profiles for exposures to PFASs and EOAs, denoting the permeability of the placenta to these chemicals [32,35].

In blood exposomics, the first step of sample treatment involves centrifuging or vortexing a variable volume of blood (50–500 μL) to separate cellular components such as red and white blood cells. Subsequently, solid-phase extraction with non-polar solvents [32,34,37], or protein precipitation [31,38,39] combined with centrifugation are commonly employed. Less frequently used methods include ultrasound-assisted extraction [35], liquid-liquid extraction [36], or a QuEChERS extraction [41]. MeOH and ACN are frequently used as clean-up solvents thanks to their effectiveness in removing proteins.

Regarding the chromatographic conditions, all the studies used reversed-phase columns (C_{18}), electrospray ionization in negative or both modes and HRMS (80 % QTOF and 20 % Orbitrap). The number of features identified with a level 1 confidence ranged from 10 to 30, with most annotations having a lower confidence level (level 4 or 5). Out of the total annotations, only 4 % were categorized as level 1, 9 % as level 2, and 5 % as level 3, highlighting the critical need for expanding spectral databases and developing standards. The principal chemical families identified or annotated by wide-scope screening approaches include personal care products (such as surfactants, ultraviolet filters, and fragrances), food-derived compounds, pesticides (including herbicides and insect repellents), pharmaceuticals, and plasticizers.

3.2. Urine

Urine, a liquid waste product excreted through the urinary system, serves as a valuable biological sample in exposomics research. It provides insights into recent exposures, particularly for substances with low bioaccumulation due to their short half-lives. Urine is favored in exposomics for its non-invasive collection method, facilitating large cohort studies [93]. Its ease of sampling allows for collection at multiple time points, enabling assessment for both short-term and long-term

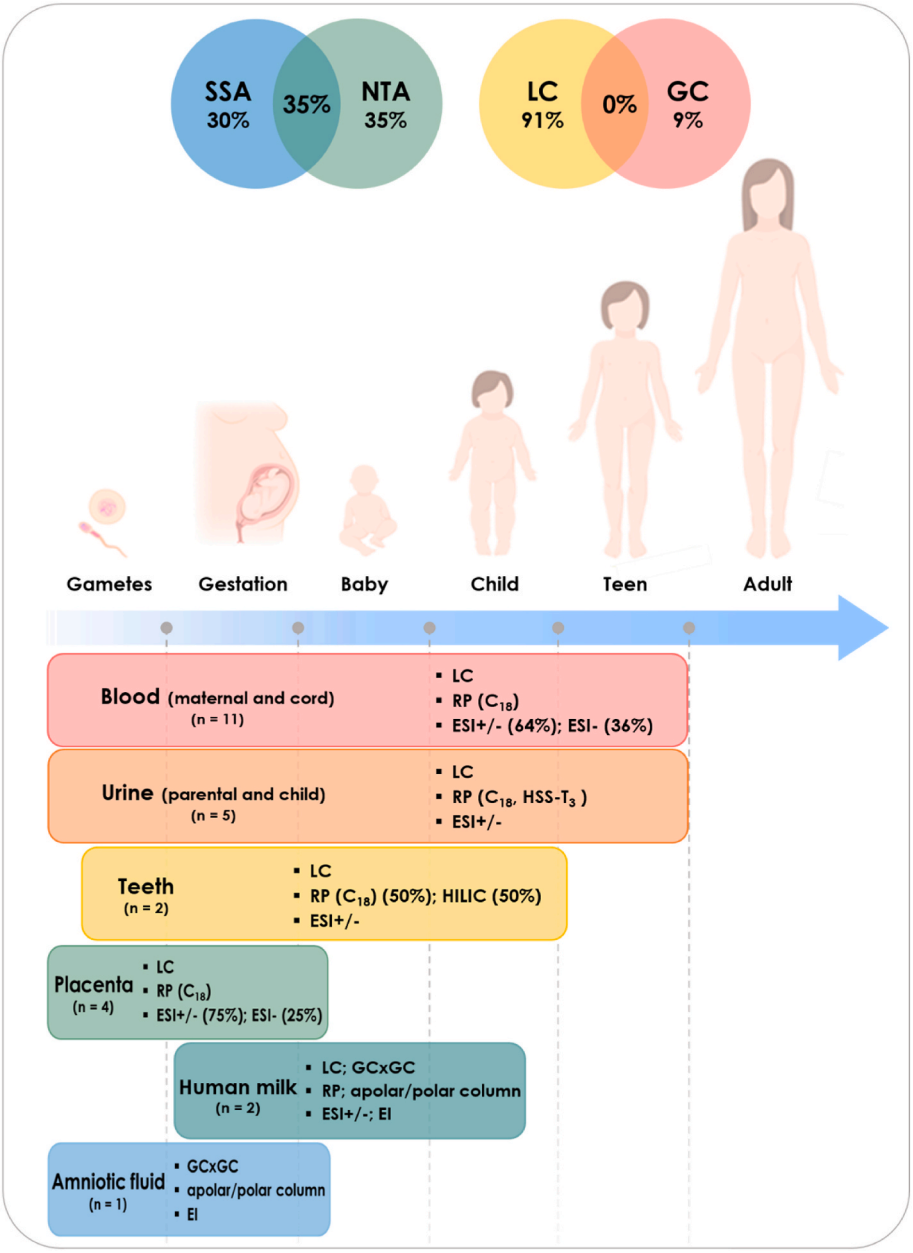

Fig. 2. Summary of the screening strategies and chromatographic techniques employed in early-life exposomics. Analytical information, including chromatographic technique, column type, and ionization modes are detailed for each biological matrix.

exposures. However, to mitigate matrix effects and ion suppression, robust sample preparation steps are needed to remove impurities and enhance the sensitivity and reproducibility of the analytical results.

3.2.1. *Prenatal chemical exposome: maternal urine*

Exploring the prenatal chemical exposome via maternal urine analysis offers valuable insights into the exposures that may influence fetal development during pregnancy. Two studies in the literature analyzed maternal urine by reversed-phase columns, ESI in both modes and Orbitrap/MS. Huber et al. studied the largest cohort (n = 500) using a 96-well plate SPE (Strata-X) resulting in a fivefold preconcentration. They tentatively annotated 14 pesticides and 71 of their metabolites, with 18 identifications (level 1) and 21 annotations (level 2) [42]. Additionally, Jamin et al. confirmed 20 pesticide metabolites using commercial standards (level 1) and annotated an additional 3 at level 3 using a dilute-and-shoot approach [43].

3.2.2. *Postnatal exposome: children's/infants' urine*

Infants and children are particularly vulnerable to environmental exposures due to their ongoing growth and organ development. Analyzing postnatal urine can help identify critical developmental stages during which certain exposures may exert a more significant impact on health. Four studies have been conducted with children's urine. One study included urine samples from both mothers and children, while the other three studies focused exclusively on children's urine (ages 1–11 years old). All studies employed reversed-phase columns, ESI in both modes and Orbitrap/MS. Two studies utilized SPE (Strata-X and HLB), while the other two used dilute-and-shoot approach. The differences in urine treatment methods significantly influenced the number of annotated and identified exposome metabolites. The tentative annotations of Huber et al. were those above described. Tkalec et al. tentatively annotated 38 compounds with level 3, and 36 with level 2 via SSA and NTA [46]. Cui et al. annotated 5121 features (level 5), with 265 features detected in more than 60 % of the urine samples and confirmed

9 compounds with commercial standards at level 1 confidence [45]. The majority of the features annotated by Cui et al. were natural products, and 3 pharmaceuticals: dobutamine, pactamycin, and phenacetin. Lupolt et al. annotated 7 compounds with level 2 confidence, which included plasticizers, pharmaceuticals, and natural products [44]. The difference in the total number of identified or annotated organic compounds in urine from both mother and children, comparing SPE and dilute-and-shot strategies can be likely attributed to matrix effects. SPE typically allows for the determination of more features because it effectively removes interfering substances from complex urine matrices, thus enhancing analyte detection. However, despite the increased number of features observed with SPE, the sensitivity of detection may be compromised compared to dilute-and-shot strategies. A recent study compared various sample preparation methods for urine, including dilute-and-shot, different SPE sorbent materials (Sepra ZT,30 μm, 85 Å; Sepra ZTL-WCX, 100 μm, 300 Å; Sepra ZTL-WAX, 115 μm, 330 Å and Isolute ENV+), and clean-up using CAPTIVA cartridges. The findings revealed that clean-up cartridges provided a larger chemical coverage and higher sensitivity compared to other methods [94]. This indicates that while SPE can improve the detection of analytes by reducing matrix effects, newer clean-up techniques might offer even better performance in terms of both comprehensiveness and sensitivity in exposome studies.

3.3. Placenta

Placenta is a complex and dynamic organ responsible for exchanging nutrients and waste through passive diffusion and active transport between the mother and the fetus [72,95]. It also serves as a barrier to protect the fetus from toxic substances [41]. Despite its protective function, various chemicals including drugs, pesticides, and metals can cross the placenta and potentially impact fetal development [96–98]. The placenta is an accessible and non-invasive biospecimen to investigate the prenatal chemical exposome, that can be easily obtained during delivery. However, due to its diverse cellular diversity and heterogeneous distribution of xenobiotics, careful monitoring of chemical accumulation across different placental areas is essential. Standardized sampling and pre-treatment methods are crucial to ensure the accuracy and reliability of exposome data derived from placental samples [95]. Among the reported studies, methodologies vary. Al-Salhi et al. [47] analyzed five sections from different placental positions, Bao et al. [35], homogenized the entire placenta, Chao et al. [48], employed a randomized sampling approach, while Gil-Solsona et al. [37], analyzed a single-point sample. Each method has its strengths and limitations, impacting the interpretation and comprehensiveness of chemical exposure assessments in placental tissues.

The four studies on placental analysis have employed different solvent extractions and sample preparations to detect low-abundant xenobiotics, highlighting the need for harmonization in methodology. Al-Salhi et al. evaluated several solvent extraction methods (ACN, MeOH, MeOH/H_2O (50/50, v/v), MTBE/methanol/H_2O), and four sample preparation techniques (PPT, SPE (Polymeric Strata-X), PPRP and a combination of SPE with PPRP) for SSA. They found that methanolic extraction followed by either protein precipitation or protein and phospholipid removal plates yielded the best coverage of chemical space and repeatability for detecting low-abundant xenobiotics. Chao et al. used a double SLE with ACN and filtration using Captiva filters. In contrast, Gil-Solsona et al. opted for SPE for pre-concentration instead of Captiva filters during the clean-up process. Instrumental analysis across all the studies used reversed-phase columns (HSS-T_3, C_{18} and C_8). HRMS was predominantly performed with a QTOF mass analyzer in positive and negative modes, except for Gil-Solsona et al., who used an Orbitrap mass analyzer.

The main xenobiotics annotated and identified in the placenta have been pharmaceuticals, plastic additives, pesticides, food-derived compounds, personal care products and industrial chemicals. Gil-Solsona et al. annotated a total of 37 compounds, with 28 of these compounds confirmed using standards. Their study, along with that of Bao et al., expanded the analysis to include maternal serum and cord blood to compare the distribution of xenobiotics in different matrices. Gil-Solsona et al. reported that the placenta tends to bioaccumulate compounds compared to serum samples, noting that 40 % of the detected compounds were found exclusively in the placental tissue. However, Bao et al. demonstrated that PFASs can cross the placenta barrier and affect newborn health by increasing serum levels of thyroid hormones and glucocorticoids [35]. In Chao et al. study, 44 features were attributed to exogenous compounds, with 26 of them identified at level 1 of confidence.

3.4. Human milk

Human milk is a highly nutrient-rich source for newborns and infants, providing essential proteins, fats, carbohydrates, vitamins and minerals. Due to its non-invasive collection method and minimal risks to both mother and baby, human milk is considered a valuable biological matrix for exposomics [99]. Despite its usefulness and excellence as a biological record of maternal exposure to various environmental factors such as dietary components, contaminants, pollutants, and toxins during pregnancy and breastfeeding, only two studies involving small cohorts ($n < 5$ participants) have used human milk for biomonitoring.

Because of its high protein and fat content, a multi-step process and more complex extraction protocols are required compared to blood or urine. Musatadi et al. employed multiple extractions in their study [49]. Initially, samples were extracted with ACN, centrifuged, and frozen to precipitate proteins. The extracts were then filtered with Captiva filters and extracted with SPE with Oasis HLB cartridges. Similarly, Tran et al. used centrifugation and freezing protocols to separate water from the lipidic content, followed by gel permeation chromatography and extraction by SPE with ENVIRO CLEAN cartridges. The water fraction was subsequently centrifuged and extracted with an Oasis SPE cartridge [50].

In their study, Musatadi et al., used a C_{18} reversed-phase column, ESI in both modes and LC-Orbitrap/MS. Among the 34 xenobiotics (10 with level 1 confidence, 14 with level 2, and 10 with level 3 or lower), the principal compounds annotated included stimulants, plasticizers, UV filters, alkaloids, pharmaceuticals and food components [49]. Tran et al., using two-dimensional gas chromatography (GCxGC), identified 30 compounds and annotated 40, including PAHs, POPs, pesticides, organophosphorus compounds and plasticizers [50].

3.5. Teeth

Teeth, the hardest part of the human body, begin to develop in the second trimester of intrauterine development and are naturally shed during childhood or adolescence [100]. Unlike other biological matrices, teeth are a time-capsule and non-invasive biomatrix capable of determining early-life environmental chemical exposures from intrauterine to progressive life stages. The dentine layer can capture and store a broad range of compounds circulating in the blood such as pesticides, plastic additives, pharmaceuticals, illegal drugs, and metabolites of alcohol and tobacco [101]. However, only two studies, conducted by the same research group, have used wide-scope screening approaches to investigate biomarkers related to chemical exposures in dentine layers of deciduous teeth. These studies employed light microscopy and an advanced laser robotic platform to micro-section stage-specific dentine layers from prenatal (2nd and 3rd trimesters) and postnatal (up to 6 months from birth) phases [52,102]. In both studies, the sample treatment consisted of extraction with solvents (CH_3COOH in ACN, ACN and NaOH, for acidic, neutral and basic analytes, respectively) followed by pre-concentration with a polymeric sorbent. The eluents used were HCl in ACN for neutral and acidic fractions and NH_4OH in ACN for the basic fraction. Yu et al. compared the performance of two chromatographic columns and different LC-HRMS analytical modes (ZIC-HILIC in ESI

±and C_{18} RP in ESI+/−) to optimize analysis conditions for determining xenobiotics in dentine [53]. Based on the total number of features, the molecular mass, and signal intensity distribution of the tooth exposome, reverse-phase chromatography in positive mode provided the best results. This suggests a broad range of small and larger molecules and diverse lipophilicity in the tooth exposome.

Both studies revealed changes in the prenatal and postnatal levels of various compounds, including bisphenols, parabens, UV filters, pesticides, polyfluorinated chemicals, and tobacco-related compounds.

3.6. Amniotic fluid

Amniotic fluid is the liquid that surrounds the developing fetus during pregnancy, playing a crucial role in fetal development and protection. As directly contacts the fetus during gestation, it offers a comprehensive assessment of fetal exposure. However, collecting amniotic fluid involves an invasive procedure known as amniocentesis, which carries a small risk of complications, including miscarriage.

Dusza et al. screened for endocrine disruptors in amniotic fluid via GC-HRMS. The extraction protocol involved SPE (Oasis HLB), initially with deionized water followed by MeOH. DLLE was performed on the aqueous eluents (DCM as the extraction solvent and acetone as the dispersive solvent), followed by acidification with acetate/acetic acid buffer and centrifugation. This multi-step sample preparation facilitated the annotation of 119 exogenous compounds (level 2 of confidence), with 9 identified using standards, including plasticizers, flame retardants, insecticides, and industrial chemicals [51].

4. Quality assurance/quality control

Quality assurance and quality control (QA/QC) measures are essential in wide-scope screening workflows to ensure the reliability and consistency of results and to mitigate the risk of overlooking potential substances of interest. A previous review on human monitoring via SSA and NTA [5] proposed a standardized set of QA/QC protocols, including the use of solvent, procedural and field blanks. Obtaining field blanks can be challenging as it requires the involvement of healthcare professionals. Furthermore, biobank collections often lack field blanks, leading researchers to commonly use synthetic matrices or solvents instead. While solvent blanks are reported in all the studies, the use of procedural or field blanks is not consistently mentioned across all studies [42,43]. Most studies only used procedural blanks, and only six employed field blanks in their analysis, replicating the same steps with synthetic matrix samples [32–34,38,39,47].

The number of each type of blank sample is also a relevant parameter. It should be selected in advance and ensure representativeness, typically covering at least 10 % of the total number of samples [5]. Among the selected 23 studies, only 67 % used a number of blanks equal to or exceeding 10 % of the total number of samples.

Surrogates or isotopically labeled internal standards are employed to assess analyte recovery, correct for potential losses during extraction/analysis, and monitor instrumental variations. The selection of internal standards should cover the complete chemical space and chromatographic run, ideally including at least one internal standard per anticipated chemical family [5]. All studies reviewed here employed a varying number of internal standards.

Quality control samples (QC) are commonly pooled samples comprising small aliquots from all samples within an experiment. Injection of QCs during the baches is recommended to control the performance of wide-scope screening analysis, correct retention time and m/z shifts, and assess the instrument stability and daily instrument performance. Despite its potential benefits, 30 % of the studies did not incorporate QCs in their methodologies [35,36,44,48,52,53].

Authentic standards, when available, are also essential during the data processing to validate the tentative annotations in suspect/non-targeted workflows. This is a well-established practice for annotation confirmation, yet only 15 out of the 23 studies (65 %) procured available standards to verify their findings.

Regarding GC analysis, a well-established QA/QC practice involves calculating the retention time indexes (RTI) [50,51]. This practice facilitates the correction of retention time shifts between samples and batches, thereby enhancing annotation confidence. Finally, other QA/QC practices, such as interlaboratory comparison of the methodology, were not employed in any of the included studies.

5. Data acquisition & processing

Data acquisition represents a critical step in the analysis workflow, profoundly impacting the final study outcomes. Strategies vary based on available instrumentation, experimental design, and study objectives. Table 2 summarizes the acquisition strategies and parameters employed in wide-scope screening studies of early-life exposome, alongside the number of identified compounds categorized according to Schymanski's level of identification confidence. Across all reviewed studies, full scan mode was consistently employed, typically within the 100–1000 m/z range (65 %), with mass accuracy errors <5 ppm (or lower) and resolving power ranging between 40000 and 100000 FWHM (73 %). However, there was a notable diversity in MS2 acquisition approaches. Among the 21 works included in this review (excluding the two GC papers), 14 % did not conduct MS2 acquisition. Among those that did acquire MS2 data, 77 % utilized data-dependent acquisition (DDA), 6 % employed data-independent acquisition (DIA), 11 % employed both DDA and DIA, and 6 % did not specify the MS2 acquisition method. In studies using DDA, the most common approach was stepped collision energy (CE), ranging from 10 eV to 40 eV. Typically) between 3 and 20 of the most intense ions (TopN) were selected for MS2, with resolution values varying widely (15000–60000 FWHM). Similar variability was observed in studies using DIA. One study [47] employed different acquisition windows (SWATH), while others utilized high-energy acquisition across the entire mass range. Thirteen percent of the studies employed an acquisition strategy based on feature prioritization for MS2 analysis. This approach initially acquires data at the MS1 level, prioritizes and selects features of interest for subsequent MS2 acquisition. Given that most of the exposome-related analytes exist at trace levels, a strategy that prioritazes sample-specific MS1 precursor ions, filters in-source fragment ions, and subtracts blanks is crucial for obtaining high-quality MS2 data. High-quality MS2 data enhances the confident identification of analytes at trace level in the exposome context. For instance, Tkalec et al. used calculated m/z values from a list of 801 xenobiotics complied from Exposome Explorer, T3DB and HBM4EU databases to create an inclusion list [46]. Jiang et al. based the feature prioritization on (i) detection frequency in the cohort, (ii) features highlighting demographic differences, and (iii) features correlating in maternal and cord blood samples [40].

Beyond data prioritization strategies, certain early-life studies have leveraged sample characteristics to prioritize features of interest, facilitating comparisons across different matrices, populations, or disease control/case groups. For instance, in the two studies analyzing teeth [52,53], only features that showed significant differences between prenatal and postnatal samples were selected to investigate potential exposures during these periods. Abrahamsson et al. and Wang et al. employed this approach to prioritize features showing a high correlation between fetal and maternal serum, thereby highlighting placental transfer dynamics [31,33]. Additionally, Chao et al. prioritized features associated with preeclampsia [48]. Furthermore, some studies filtered features based on socioeconomic [38], geographic [39], or demographic differences [32,38] within the studied population.

For MS data analysis, vendor software is commonly employed for peak picking, spectral deconvolution, and feature alignment. In the selected studies, vendor software included MassHunter Profinder (Agilent) [31–34,38–40,48,52], Compound Discoverer [44,45,49],

TraceFinder [51], or MetWorks (Thermo Scientific) [43], MarkerView (Sciex) [41,47], Chroma TOF (LECO) [50], and UNIFI (Waters) [35]. However, there has been a recent increase in using open-source software applications, which provide enhanced flexibility in data processing compared to vendor software, where some parameters cannot be modified. Proteowizard [36,37,46,53] is commonly used for the transformation of vendor raw files into open formats, while MS-Dial [31,33,47], MetAlign [42], R software [32], XCMS [53], MZmine [37,46], and the Digital Sample Freezing Platform (Norman Network) [35–37] have been applied for feature extraction in biomonitoring studies. Data processing parameters used in the studies are often quite similar: the signal-to-noise ratio is typically set at 3, mass tolerance is generally kept under 5 ppm, and the time range for alignment is commonly set at 30 s. Minimum peak height, intensity, or area is sometimes included as a threshold (61 %), but specific values can vary widely depending on the software or peak parameter employed. Additionally, the removal of features not detected in all replicates (17 %) or those detected in blank samples (with studies using thresholds from 2 to 10-fold difference) was also applied.

5.1. MS1 data mining

At the MS1 level, all studies included in this review utilized databases for initial screening of features by exact mass matching, typically with a mass tolerance ranging between 5 and 20 ppm (Table 2). The standard approach at the MS1 level involves screening MS1 raw data with an *in-house* database [31–35,38,39,42,43,49,51,52], which includes US EPA databases (CompTox, ToxCast, DSSTox, etc) [32–35,41,45,48] and the Norman Suspect List Exchange [35–37,41], to identify MS1 feature matches for subsequent MS/MS acquisition. Other databases used for MS1 matching included the Human Metabolome Database (HDMB) [31,40,46,47], mzCloud [31,40,44,45], METLIN [47,48,53], and MassBank Europe or North America databases [31,40,47]. The HDMB and the HUMANBLOOD database were specifically employed to exclude endogenous compounds [31,38–40,46,47]. The Digital Sample Freezing Platform (DSFP) [43] enables the upload of open-access raw files and utilizes the Norman Suspect List Exchange (>110,000 structures) for screening at both MS1 and MS2 levels, thereby expanding the chemical coverage. However, these databases often lead to numerous false positive identifications, necessitating manual refinement efforts. Additionally, some studies employed selective screening processes for specific chemical families, which may limit the comprehensive evaluation of the early-life chemical exposome, albeit with reduced identification confidence. Common chemical families screened using these processes included EOAs [32], PFASs [35,41], and pesticides [42,43]. A common method for feature prioritization at the MS1 level involved using characteristic isotopic patterns. Further parameters are retention time consistency, occurrence in a certain percentage of participants, blank subtraction, and mass defect were employed to enhance annotation accuracy and confidence.

Huber et al. prioritized features containing halogenated elements using a pesticide database. They then filtered the feature list by excluding (i) pharmaceutical annotations, (ii) small molecules such as phenols, cresols, or benzoates, (iii) and features not fitting the isotopic pattern [42]. Tran et al. [50], filtered the features presenting halogenated isotopic patterns and fragments due to halogen loss, while Jamin et al. [43], used isotopic patterns to identify sulfated, chlorinated, or brominated compounds.

Mass defect, defined as the difference between the nominal mass and the monoisotopic mass, was used to filter potential candidates in wide-scope screening. Molecules with a higher proportion of heteroatoms (e.g., oxygen, nitrogen, fluorine) relative to hydrogen exhibit low or negative mass defect values, which facilitates their prioritization. For example, Gil-Solsona et al. used negative mass defect to screen for halogenated compounds, Li et al. [41] filtered for mass defects <0.15 or >0.85 to identify PFAS homologs, and Cui et al. [45], utilized Kendrick mass defect plots to depict unique patterns of anthropogenic compounds. Fourteen percent of the studies [38,39,52] conducted data mining at MS1 level, reporting annotations with identification confidence at level 4 or lower. However, the prevailing strategy was to leverage MS2 data to enhance the confidence of the reported annotations.

5.2. MS2 data mining & compound annotation

At the MS2 level, a variety of strategies are employed (Table 2). The most commonly used parameter for matching is a spectral similarity score (typically set >70–80). Additionally, criteria such as mass error (<5–10 ppm) and a minimum number of fragments (1–5 fragments) are used to refine MS2 matching conditions. The predominant approach involves using databases at the MS2 level. Among these databases, MzCloud [31,33,36,37,40,41,44,49] and Mass Bank Europe and North America [31,33,36,37,40,41,46,47] were the most commonly used, followed by HMDB [31,33,40,47], MS-Dial [31,33,47], Metlin [37,47,53], and the NORMAN Suspect List Exchange [35–37]. The majority of studies performed MS2 screening based on MS1 matches or *in-silico* fragmentation tools, but 2 of them initially screened features at the MS2 level and subsequently validated them at the MS1 level. Li et al. [41] searched for diagnostic neutral losses and fragments of novel PFAS at the MS2 level. Flagged features were subsequently screened using MS1 and MS2 libraries for potential matching [41]. Tkalec et al. [46] submitted all features with acquired MS2 data for molecular formula *in-silico* prediction. Only features meeting specific criteria (Table 2) were screened against MS2 databases (T3DB, Exposome Explorer and Chemistry Dashboard) [46].

Recently, *in vitro* or *in vivo* metabolization experiments have emerged as a cost- and time-effective approach used at the MS2 level to generate spectral information when standards are unavailable. This method has found application in various fields, including drug discovery, detection of psychoactive substances, and human biomonitoring studies of contaminants [103–107]. For instance, Huber et al. [42] conducted human liver S9 *in vitro* incubation experiments with 69 pesticides to generate MS2 spectra of their metabolites and used this spectral information to screen the adult and child urine samples [42]. Similarly, Jamin et al. [43] administrated pesticide parent compounds to a murine model to produce their metabolites *in vivo*, generating MS2 spectral information to detect their presence in urine samples from pregnant women [43].

Despite efforts to select or query features against databases, a significant proportion of the extracted features from raw data remain unannotated and categorized as unknown. *In-silico* fragmentation tools such as Competitive Fragmentation Modelling for Metabolite Identification (CFM-ID) [31,40,48], SIRIUS-CSI [37,46], or MetFrag [40,47] have been employed in early-life chemical exposome studies to provide candidate lists based on the MS/MS spectral information (Table 2) [31,40,48] [37,40,46,47]. These tools are used for (i) molecular formula assignment, (ii) generation of in-silico MS2 spectra based on structure, or (iii) annotation of unknown features based on MS1 and MS2 data. However, regardless of the selected approach, manual curation remains essential to significantly enhance identification confidence.

5.3. Other strategies for feature prioritization

Shifting from targeted analysis to more holistic approaches in the investigation of environment-health associations has led to a new structure of data. Wide-scope screening approaches produce information-rich, high-dimensional, and complex datasets that are challenging to handle and fully interpret. Computational tools and algorithms for data reduction and data visualization are used to analyze these large and complex datasets, discern patterns, correlations, or relationships between features and samples, and determine adverse effects. Multiple network strategies can be applied for data mining including association networks based on quantitative information, mass

spectra similarity networks to assist chemical annotation and biochemical networks for systematic data interpretation. Grouping spectra into clusters based on the cosine similarity, Feature-Based Molecular Networking (FBMN) and Feature Clustering are valuable bioinformatic tools for identifying and prioritizing chemicals of interest. FBMN was used by Abrahamsson et al. [31], to identify correlations between exogenous chemicals and endogenous metabolites in maternal and cord blood: Chao et al. [48] used it to find clustering features related to preeclampsia; Wang et al. to differentiate clusters between maternal and cord blood [33]; and Yu et al. to describe the relationship of features between prenatal and postnatal profiles in teeth [53]. Other computational tools used in early-life exposomics for the identification and separation of certain phenotypes included principal component analysis (PCA) [31,43,44], partial least squares-discriminant analysis (PLS-DA) [43], heatmaps [31,48,51], volcano plots [48,49], and Variable Importance in Projection (VIP) scores [43,46].

6. Concluding remarks & future perspectives

The use of wide-scope screening strategies play a crucial role in enhancing chemical coverage in human biomonitoring, particularly in characterizing the early-life chemical exposome. This approach enables a comprehensive understanding of how early exposure to organic compounds impacts not only early development but also the risk of non-communicable diseases later in life. However, its application in early-life exposomics is still limited, likely due to the lack of standardization in the field. While standardized protocols, including sample collection, preparation, analysis, and data processing, have been proposed and implemented in metabolomics, adapting them to wide-scope screening experiments for exposome studies remains challenging, despite some efforts made in large international initiatives.

For effective future research, it is essential to prioritize the inclusion of large and diverse early-life cohorts representing varied geographical, socioeconomic, and ethnic backgrounds. This approach enables the capture of a comprehensive range of exposures and facilitates the identification of statistically significant correlations with potential health outcomes. Numerous studies have demonstrated significant variations in contaminant biomarkers across various matrices, highlighting the need for longitudinal over cross-sectional studies. Longitudinal designs are essential for capturing temporal dynamics and providing a more accurate representation of exposure patterns, which is crucial for understanding the impact of exposures on individual health. To achieve robust results, it is important to balance the cohort size with the frequency of sampling for each individual, utilizing recurrent sampling to accommodate the high dynamism and spatiotemporal variability of chemical exposures [108].

Additionally, it is recommended to utilize diverse and complementary matrices to gain a comprehensive understanding of exposures to organic compounds, even beyond early-life contexts. Wide-scope screening sample preparation and analysis should aim for non-specificity to capture a broad range of organic chemicals. However, it is important to acknowledge that different extraction and analysis techniques can affect selectivity and efficiency. Therefore, employing orthogonal techniques such as various chromatographic methods, columns with different polarities, and alternative ionization methods like APPI and APCI is encouraged to enhance the chemical space coverage. Furthermore, integrating both targeted and wide-scope screening methodologies allows for the comprehensive detection of both known and unknown xenobiotics and their metabolites.

The final step of the workflow, the data processing strategy, varies depending on the sensitivity and selectivity characteristics of the acquisition instrument, as well as the data acquisition methodology and settings. To ensure transparency and flexibility in data processing, open-source processing workflows that empower analysts to control all steps of the process are highly encouraged. By leveraging open-source processing workflows, analysts can effectively process and analyze complex data generated from wide-scope screening experiments, enabling comprehensive characterization of the chemical exposome and identification of potential biomarkers of exposure. Additionally, the poor quality or absence of spectral data for certain organic compounds poses a bottleneck in their identification. The scientific community is actively tackling this issue by expanding the quantity and quality of spectral data available, mainly through open-source databases [109–111].

As a final remark, integrating multiple omics approaches within the same samples presents a compelling opportunity. Combining early-life exposomics with various omics disciplines provides complementary insights into the mechanisms and physiological alterations induced by detected chemicals. This interdisciplinary approach offers a more comprehensive understanding of the complex interactions between early-life chemical exposure and human health, advancing our ability to assess and mitigate the impacts of the chemical exposome on public health. For instance, integrating early-life exposomics with metabolomics has successfully revealed changes in the endogenous metabolic profile induced by identified and quantified exogenous compounds in several studies [31,47,48,52,53]. Furthermore, integrating transcriptomics and epigenomics with early-life exposomics allows the assessment of modifications and alterations in gene expression and regulation levels [48].

Despite the limited available works, the field of early-life exposomics and the application of multi-omics studies applied to early-life chemical exposome via wide-scope analysis is rapidly evolving. This evolution extends to the analytical techniques and tools employed in every step of the workflow. The data generated from these studies provide substantial and comprehensive insights into environmental exposures and their impact on early development. Notably, the increasing number of studies in this direction reflects a growing recognition of the importance of understanding early-life chemical exposures and their implications for human health.

CRediT authorship contribution statement

Camilla Guerrini: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Adrià Sunyer-Caldú:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Ruben Gil-Solsona:** Writing – review & editing, Validation, Supervision, Conceptualization. **Joaquín Escribano:** Writing – review & editing, Funding acquisition. **Maria Vinaixa:** Writing – review & editing, Validation, Supervision, Conceptualization. **Pablo Gago-Ferrero:** Writing – review & editing, Validation, Supervision, Conceptualization. **Noelia Ramírez:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data is shared in the Supplemental Information excel file

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Fig. 2 and the graphical abstract include icons from BioRender (https://www.biorender.com/).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.trac.2024.117903.

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