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Review

Toxicant early-life exposure: A mini review of the comprehensive LC-MS/MS analysis of xenobiotics in breast milk samples

Si-Hung Le*

Application Department, Thang Long Instruments

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Abstract

The analysis of xenobiotics in breast milk is crucial for understanding the impact of early-life exposure on infant health. This mini-review highlights some recent advancements in mass spectrometry, focusing on SCIEX MS systems, to detect and quantify a wide range of xenobiotics in breast milk, an important nutrient source for infants. Advanced LC-MS/MS methods have been developed to achieve high sensitivity, with detection limits reaching picogram to nanogram per milliliter levels. These methods allow for the identification of xenobiotics like plasticizers and perfluorinated substances, as well as novel detections of compounds such as pyrrolizidine and tropane alkaloids in breast milk. The review also showcases and discusses the use of LC-HRMS/MS-based non-targeted analysis for profiling xenobiotics in breast milk and stool samples from mother-infant pairs. The approach was able to identify thousands of chemical features, revealing the overlap and distinct chemical patterns across matrices. The LC-HRMS/MS non-targeted analysis has proven to provide new insights into how the chemical exposome interacts with the gut microbiome, highlighting potential health risks associated with dietary and environmental exposures during early development.

Keywords*: xenobiotics, suspect screening, breast milk, infants, LC-MS/MS*

1. INTRODUCTION

Humans are exposed daily to various xenobiotics from the consumed food and surrounding environment, along with other factors like stress, lifestyle, and diet [1, 2]. The term "xenobiotic", which originated from the Greek words xenos (strange) and bios (life), refers to the unexpected/strange chemical substances (e.g., carcinogens, drugs, environmental pollutants, food additives, hydrocarbons, and pesticides) found within an organism. Moreover, the xenobiotics introduced to the body during the ADME process (Absorption, Distribution, Metabolism, and Excretion) can undergo biotransformation via several metabolisms (e.g., oxidation, glucuronidation…), thus resulting in related metabolites. Both the original xenobiotic and its metabolites could be considered xenobiotics, as they are not naturally present in the body. There are several xenobiotics classifications, including a wide range of chemical groups such as natural toxins, trace metals, pesticides and veterinary drugs, environmental pollutants, processing-related contaminants, and their related metabolites. Common xenobiotics in early-life exposure include mycotoxins, which are toxic fungal metabolites found in contaminated foods like maize, nuts, and cereals. These include aflatoxins, fumonisins, and sterigmatocystin, which are frequently detected in breast milk, complementary foods, and urine, indicating widespread co-exposure among infants [3]. Exposure to these mycotoxins, particularly aflatoxins and fumonisins, poses significant health risks, including liver cancer, impaired child growth, and nephrotoxicity. Another group of concern is pyrrolizidine alkaloids, naturally occurring plant toxins found in herbal teas and food supplements, which are genotoxic and carcinogenic [4]. Additionally, veterinary drugs like antibiotics and pesticides can contaminate animal-derived foods such as meat, milk, and eggs, leading to potential

*Corresponding author: Si-Hung Le (E-mail: hung.le@thanglonginst.com)

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antimicrobial resistance and long-term health risks like cancer [2]. Bisphenol A, used in food packaging, is another prevalent xenobiotic classified as an endocrine-disrupting chemical linked to cancer, diabetes, and developmental disorders [5]. A study indicated that women who used lotions, shampoos, conditioners, and cosmetics within the previous 24 hours had significantly higher concentrations of parabens in their urine, indicating the potential transfer of these chemicals to breast milk [6]. Additionally, the combination of residual chemicals presenting in a meal may produce either additive or synergistic effects, with the potential health impacts greatly influenced by the timing, duration, intensity of exposure, interactions between chemicals, and individual genetic differences [7]. The potential risks from such xenobiotics are particularly concerning during early human development when vulnerabilities to chemical exposures can be intensified.

This mini-review provides an overview of early-life toxic exposures in infants while discussing methods for studying and quantifying xenobiotics with a focus on the role of SCIEX mass spectrometry instruments for sensitive and comprehensive analysis. The review also addresses limitations in early-life biomonitoring and suggests improvements through advances in MS technology.

2. XENOBIOTICS AND INFANT'S HEALTH IMPACT

2.1. Xenobiotics in infants' food

Breast milk, which is the ideal food for infants, is not free of xenobiotics coming from either the mother's diet or environmental exposure. The results of xenobiotic concentrations in breast milk from various countries indicate distinctive patterns based on the categories and regions. It also reveals the relationship of these chemicals with their historical and industrial uses. For instance, results from a study in Norway highlighted the contamination of polybrominated diphenyl ethers (PBDEs), organochlorine pesticides (e.g., DDT), and their metabolites, while polycyclic aromatic hydrocarbons (PAHs) and perfluoroalkyl substances (PFAS) were notably detected in Portugal and Korean samples, respectively (**Figure 1**) [1]. Moreover, when switching the dietary to complementary foods, infants are exposed to more contaminants (e.g., mycotoxins), highlighting the need for breastfeeding and careful selection of these foods. Breast milk contains a mix of bioactive compounds like polyphenols and carotenoids that may impact infant health, with ongoing research into their benefits and risks. Ezekiel *et al.* reported the widespread contamination of mycotoxin in breast milk, complementary foods, and infant urine, highlighting the increasing exposure to these toxins when consuming complementary foods [3]. Mycotoxins, which are produced by molds, contaminate staple foods (e.g., maize, rice, and wheat) globally, while pyrrolizidine alkaloids (PAs) found in herbal teas are genotoxic carcinogens, posing risks even in small quantities [4, 8]. PA contamination in teas marketed to infants and pregnant/lactating women has been reported as a growing concern since 2015 [9]. Other xenobiotics like veterinary drugs, pesticides, and bisphenol A (BPA) in food packaging pose additional risks [2, 5]. Despite suboptimal maternal diets (e.g., maternal fruit and vegetable intake), breast milk still contains polyphenols, which have health benefits but can also affect iron absorption and hormone balance [10, 11]. Moreover, the intake of legumes and tea significantly influences breast milk's phenolic composition [10]. A study from Song *et al.* indicated that phytochemicals (i.e., carotenoids and flavonoids) in breast milk decrease over lactation, offering potential benefits to breastfed infants [12]. Research continues to explore how maternal diet and other factors affect breast milk composition, and while formula milk is an alternative, it leads to different health outcomes [3, 13].

2.2. Health impact of xenobiotics

Early human life is a critical period of high susceptibility to external exposures because the immune system and enzymes for processing xenobiotics are not fully developed; this vulnerability is especially pronounced during developmental stages such as the embryonic and fetal periods and extends through early childhood up to three years old [1, 14, 15]. Fetuses and infants can be exposed to toxicants through several pathways, including prenatal exposure via the placenta and postnatal exposure through oral, injectable, inhalation, and skin contact routes [1]. Prenatal exposure occurs either through direct placental transfer or by disrupting placental functions. Nanoparticles can also cross the placenta, potentially enhancing toxicity by carrying other contaminants. After birth, the exposure can happen through breast milk, infant formula milk, food, drinking water, and medical products. Additionally, the higher food intake per kilogram of body weight in infants compared to adults and the limited nutritional sources during early development increase the chance of toxic compounds exposure from food, potentially leading to significant health impacts in infants [14 - 16]. For

instance, exposure to endocrine-disrupting chemicals during the first thousand days of life has been linked to the development of chronic diseases later in life, such as promoting neurodevelopmental disorders [17 - 20]. The relationship between early-life exposure to xenobiotics (e.g., bisphenol A, phthalates, triclosan, and perfluoroalkyl substances) and childhood neurobehavioural disorders and obesity is also discussed in several epidemiological studies [21]. However, not all xenobiotics exhibit harmful health effects. For instance, exposure to polyphenols can offer several health advantages, such as aiding in the prevention of chronic diseases and supporting microbiome development in humans, including infants [10, 11, 18, 22]. Ellagitannins contained in pomegranate can be converted to ellagic acid, which is then absorbed into the bloodstream of pregnant women and has shown neuroprotective effects for their infants [23]. Moreover, the development of intestinal microbiota during early life, which is influenced by these exposures, plays an essential role in long-term health impacts, thus emphasizing the importance of understanding early-life exposure to xenobiotics.

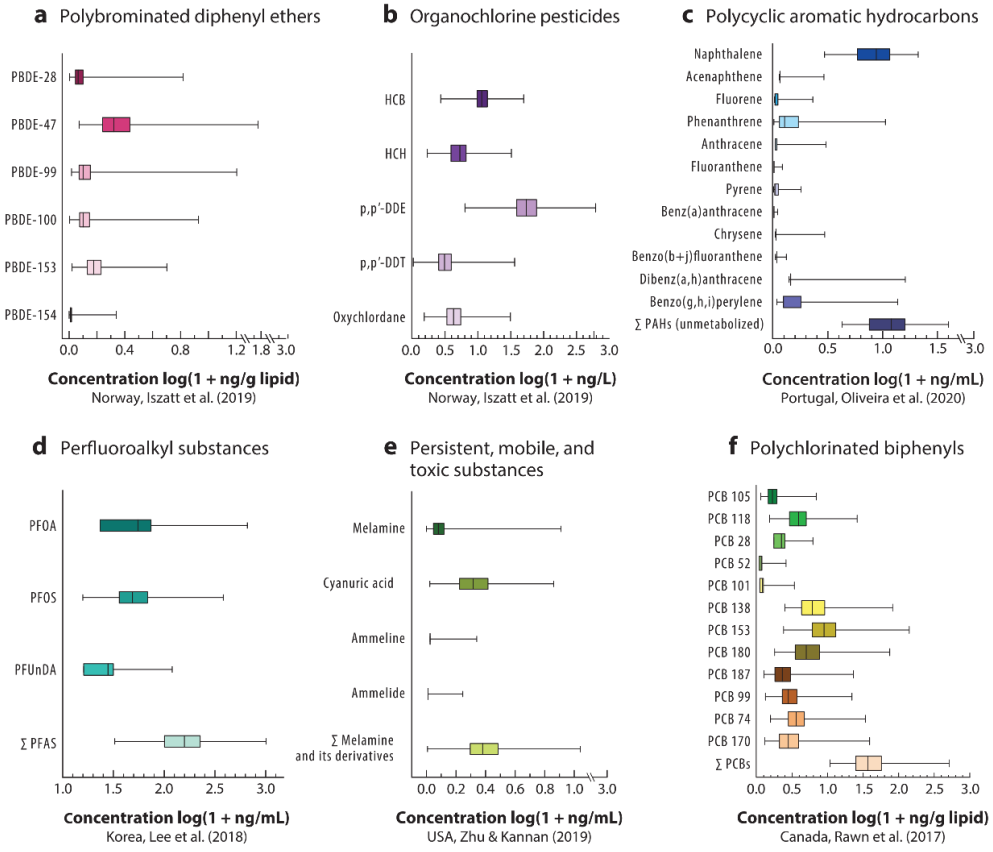

Figure 1. *Synthetic chemical concentrations in breast milk from various countries reveal distinct contamination patterns based on xenobiotic classes and regions [1]*

The early development of the intestinal microbiota, which is affected mainly by nutrition and initial colonization by pioneer bacteria, is crucial for establishing host-microbe interactions that promote long-term health through a stable symbiotic relationship (the mutually beneficial interaction between the host and the microbes in the gut) [22, 24]. During the first months of life, this microbial assembly forms specialized ecosystems in different gut compartments, significantly influenced by environmental factors and nutrition, particularly breastfeeding [22, 25, 26]. Since breastfeeding can support healthy microbiota, as human milk contains components that influence microbial growth, and maternal diet may further enhance/limit infant gut microbiota through its impact on milk composition [24]. The newborn's gut microbiota assembly during this particular period of life has been shown to have lasting effects on digestive, immune, metabolic, and behavioral health. Moreover, the gut microbiome plays a significant role in xenobiotic metabolism by influencing the pharmacokinetics of pharmaceuticals, toxicants, and heavy metals [27]. In which the microbiome can directly modify xenobiotics either in the intestines or through enterohepatic circulation, leading to altered metabolism

or bioactivation, depending on microbial enzymes. Some of the enzymes can reverse detoxification processes initiated by the host, while others reduce xenobiotic absorption by enhancing the gut's protective barriers. Moreover, the microbiome regulates host gene expression related to detoxification pathways, such as CYP450 enzymes [27]. Xenobiotics, in turn, can affect the microbiome's composition and metabolism. The gut microbiome in neonates, infants, and toddlers is highly dynamic and only starts to stabilize around age three; therefore, exposure during this developmental period can disrupt the gut environment, potentially leading to microbial dysbiosis and negatively affecting long-term health [25]. In recent years, many studies have been conducted in Vietnam to determine and evaluate food hazards. While most studies have focused on determining the presence or content of hazards, there has been insufficient emphasis on assessing the risks these hazards pose to human health through diet. However, in recent years, a number of studies have been systematically conducted following the 4-step risk assessment for identifying the risks of chemical and microbial agents in food, in accordance with Codex's guidelines.

2.3. Importance of xenobiotic analysis

Xenobiotics analysis is vital for evaluating exposure to potentially harmful substances, particularly during critical developmental periods such as infancy. Infants can be exposed to xenobiotics before birth through the placenta and after birth via breast milk, infant formula, food, water, and medical products. These exposures often involve mixtures of chemicals, highlighting the need for a comprehensive assessment of the chemical exposome, which is the totality of chemical exposures throughout a person's life [1]. The interaction between xenobiotics and the gut microbiome is another crucial aspect. Early-life exposures can influence the gut environment, potentially leading to microbial dysbiosis and impacting long-term health outcomes. Understanding these interactions requires detailed research into how xenobiotics affect both the microbiome and health and how dietary factors may mitigate or exacerbate these effects. Environmental factors and diet can impact the gut microbiome, which in turn can affect health and influence how xenobiotics are processed. Especially the early period is a potential time frame for shaping health through microbiota modulation, although fully utilizing this time frame requires a deeper understanding of the underlying mechanisms [24]. Despite significant exposure to various xenobiotics, such as mycotoxins, pesticides, and heavy metals, there is limited research on their impact on the early-life microbiome and subsequent health effects. Additionally, factors like fermented foods, herbal mixtures, and delivery environments, which may affect the microbiome, are often overlooked. Our understanding of exposure, effects, and coexposure of many xenobiotics in this vulnerable population is limited, highlighting the need for more comprehensive research. Infant nutrition is a major source of exposure to toxins during early life. Breastfeeding is linked to lower risks of infection, atopy, and obesity and provides bioactive components, such as proteins and oligosaccharides, that influence the development of the gut microbiome. Breastfed infants tend to have lower gut microbiome diversity compared to formula-fed infants. The gut microbiota, enriched by breastfeeding, produces short-chain fatty acids that support gut health and immune function. Understanding how early nutrition affects the microbiota and its metabolic products is crucial for promoting healthy growth and development [16]. Overall, comprehensive xenobiotic analysis is essential for protecting health by monitoring the exposure, understanding its effects, and guiding public health interventions. Our understanding of the complex relationship between the host, microbiome, and metabolism will advance with new modeling systems, technology development and refinement, and mechanistic studies focused on the contribution of human and microbial metabolism [27].

3. XENOBIOTIC ANALYSIS IN BREAST MILK

3.1. Sample preparation for breast milk samples

The analysis of human breast milk, particularly for xenobiotics, involves several key methodological challenges and preparation steps due to the complex nature of milk, which is rich in fats, proteins, and carbohydrates. This biological matrix often interferes with the extraction and detection of trace-level analytes, requiring highly sensitive and selective methods to ensure accurate quantification. Moreover, xenobiotics vary widely in chemical properties, requiring diverse extraction techniques for optimal recovery. The sample preparation typically begins with the thawing of samples, followed by the addition of internal standards, mostly involving the fully labeled xenobiotics to correct any variations during processing. Moreover, sample preparation is conducted on ice in high-quality plastic tubes (e.g., Eppendorf tubes) to minimize potential

compound degradation and contamination from the container. Extra care needs to be taken to minimize the xenobiotics contamination originating from sampling and analytical tools/instruments. Protein precipitation using acidified acetonitrile, freeze-out techniques, and high-speed centrifugation at low temperatures are commonly employed to separate the analytes from the protein-rich matrix due to its simplicity [11, 14, 15, 25, 28, 29]. Liquid-liquid extraction (LLE) with salting-out is a common approach, where breast milk is mixed with an organic solvent like acetonitrile (ACN), and salts such as magnesium sulfate ($MgSO_4$) and sodium chloride (NaCl) are added to enhance analyte partitioning into the organic phase [30]. After vigorous mixing and centrifugation at high speed (up to 18.000 x g), the ACN layer containing the analytes is collected. Freeze-out methods are also applied to precipitate proteins by chilling samples at -20°C, which helps to reduce the solubility of proteins in the solvent, followed by high-speed centrifugation and supernatant collection (**Figure 2**). Solid phase extraction (SPE) is another technique to use, involving SPE cartridges (e.g., Oasis PRiME HLB cartridges), which selectively retain analytes while washing away interfering compounds [3]. To enhance detection sensitivity, samples are often concentrated through vacuum evaporation or lyophilization, followed by reconstitution in a suitable solvent. Extra vortexing and high-speed centrifugation can be performed after the concentration to ensure cleanup performance, followed by transferring the supernatant to a glass vial and storing at -20°C until the analysis [31]. Additionally, enzymatic deconjugation can be utilized to break down conjugated metabolites, providing more comprehensive insights into the metabolism of xenobiotics; however, the deconjugation can show unwanted interference with some xenobiotics (e.g., polyphenols) [11, 31]. The choice of sample preparation also depends on the analytical purposes. For instance, using SPE strategies can provide better cleanup by removing many interfering compounds, but it tends to focus on specific compound groups, thus introducing bias. In contrast, more global approaches like protein precipitation, which aim to preserve as much unbiasedness as possible for non-targeted analysis, may be more susceptible to matrix effects.

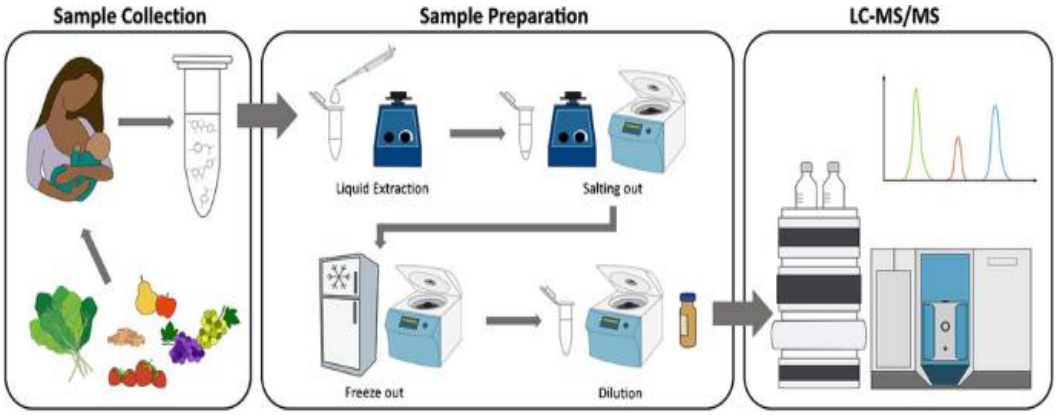

***Figure 2**. Breast milk sample preparation workflow for sensitive polyphenols analysis utilizing SCIEX LC-MS/MS 7500 system [11]*

3.2. Sensitive and selective analytical method for xenobiotics

Several analytical techniques are used to detect and quantify xenobiotics in various matrices, including breast milk, formula, and infant food, with the choice of method depending on the analyte, detection limit requirements, and available resources. Advancements in analytical technologies are continually evolving to enhance sensitivity, selectivity, and throughput for detecting xenobiotics.

The primary analytical techniques utilized to evaluate the chemical exposome are mass spectrometry (MS)-based, often integrated with separation techniques such as liquid chromatography (LC) or gas chromatography (GC). In addition to that, biosensors are emerging as valuable tools that enable rapid, on-site detection of xenobiotics in food samples, presenting a promising approach for real-time monitoring and risk assessment [32]. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is widely preferred for its high sensitivity and selectivity, allowing the detection of trace xenobiotics in complex biological samples, while gas chromatography-mass spectrometry (GC-MS) is ideal for analyzing volatile or semi-volatile compounds like pesticides and organic pollutants [31, 33]. A review from Krausová et al. on advancements in human biomonitoring showed the trend to shift from GC-MS to LC-MS due to the broader analyte range and simpler

sample preparation, while Jamnik et al. emphasized the utility of LC-MS in assessing organic xenobiotics at ultra-trace levels, pointing out the enhanced sensitivity of targeted methods [8, 31]. Additionally, inductively coupled plasma-mass spectrometry (ICP-MS) is suited for detecting heavy metals, such as lead and mercury, in food samples, offering critical insights into the risks associated with heavy metal exposure in infants [33]. These methods are vital for human biomonitoring (HBM), which involves analyzing biological samples to detect xenobiotics. Two main approaches in MS-based analysis are targeted analysis, which focuses on detecting and quantifying a specific set of known analytes, typically employing triple quadrupole (QqQ) mass spectrometry, and non-targeted analysis, which focuses on global profiling unbiased analysis [34]. The main findings of some studies utilizing SCIEX MS instruments for analysis of xenobiotics in biological fluids were summarized in Table 1, thus indicating a trend in xenobiotics analysis.

Table 1. *Main findings of some xenobiotics studies utilizing SCIEX MS for analysis of breast milk samples, including sensitivity targeted analysis and non-targeted analysis studies*

#	Studies	Chemical groups	Main results
1	[3]	14 mycotoxins	- Aflatoxin M1 was found in 18% (4/22) of breast milk samples. Additional mycotoxins like dihydrocitrinone (27%, range: 14.0 - 59.7 ng/L) and sterigmatocystin (5%, 1.2 ng/L) were detected for the first time in breast milk.
2	[31]	80 xenobiotics: Plasticizer/plastic components, PFAS, industrial by-products, and pesticides, as well as endogenous estrogens, phytoestrogens and their metabolites, mycoestrogens and their metabolites, personal care product ingredients, pharmaceuticals and their metabolites, phytotoxins, disinfection by-products, food processing by-products, and air pollutants.	- A highly sensitive LC-MS/MS method was developed and validated, capable of analyzing over 80 diverse xenobiotics across multiple biological matrices, including urine, serum/plasma, and breast milk, with detection limits in the pg - ng/mL range. - Over a 211-day longitudinal collection period post-partum, 29 different analytes were detected in breast milk. Notably, pyrrolizidine and tropane alkaloids were identified for the first time in this matrix.
3	[11]	85 xenobiotics: Dihydrochalcones, hydroxybenzoic acids, hydroxycinnamic acids, hydroxyphenylacetic acids, lignans, stilbenes, anthocyanins, catechins, flavanones, flavones, flavonols, isoflavones, and proanthocyanidins	- A targeted LC-MS/MS assay was developed to analyze 86 polyphenols and applied to breast milk samples from 12 Nigerian mothers at three post-delivery time points. - 50 polyphenols were detected, with phenolic acids making up nearly half. Phase II metabolites like genistein-7-β-D-glucuronide, genistein-7-sulfate, and daidzein-7-β-D-glucuronide were also identified.
4	[15]	242 xenobiotics: Polyphenols, endogenous estrogens, air pollutants, pesticides, food processing by-products, personal care products and pharmaceuticals, industrial side products, mycotoxins, antibiotics, phytotoxins, disinfection by-products, and plasticizers/plastic components.	- Non-targeted analysis of breast milk and stool samples from Nigerian mother-infant pairs revealed various xenobiotics, with stool showing greater diversity. - Exposure increased after introducing complementary foods, and certain xenobiotics were linked to changes in the gut microbiome, indicating interactions between chemicals and microbial development.

The use of innovative LC-MS/MS instruments allowed the changes in analysis from initially focusing on specific groups of xenobiotics, such as mycotoxins or polyphenols, to increasingly comprehensive approaches that target a wider range of substances, including xenobiotic metabolites. Moreover, several highly sensitive instruments (e.g., SCIEX Triple Quad 6500+, SCIEX Triple Quad 7500, Thermo Scientific Stellar MS…) enabled the ability to obtain comprehensive data on challenging compounds that were previously difficult to analyze due to their low levels and the complexity of biological matrices like breast milk, which is rich in proteins, lipids, and other interfering compounds. Recently, there has been a shift towards non-targeted analysis employing high-resolution mass spectrometry (HRMS) instruments (e.g., ZenoTOF 7600, Q Exactive Orbitrap…), thus allowing for the detection of an even wider variety of xenobiotics, including those not previously considered. This progression reflects a growing understanding of the complex chemical exposures in early life, emphasizing the need for more complete assessments of potential health impacts.

3.2.1. Wide targeted xenobiotics analysis in breast milk

Targeted analysis utilizing LC-QqQ boosts high sensitivity and specificity, allowing for the detection of trace levels of specific xenobiotics, which is essential for assessing low-dose exposures. Additionally, these methods have established protocols and readily available databases that facilitate data interpretation. A multi-specimen, multi-mycotoxin LC-MS/MS analysis was conducted by Ezekiel *et al.* on SCIEX QTRAP 6500+ for ultra-trace analysis of 14 mycotoxin co-exposure in 65 infants (aged 1 - 18 months) in Ogun state, Nigeria. The study aimed to investigate the relationship between diet and xenobiotics exposure in exclusively breastfed versus non-exclusively breastfed infants [3]. The method used for highly sensitive analysis of xenobiotics in the breast milk samples was validated according to European Commission Decision 2002/657/EC and the EuraChem Laboratory Guide. The values were reported with the lowest obtained LOD and LOQ values being 0.1 ng/L and 0.3 ng/L (for beauvericin), respectively. Results from the study indicated that aflatoxin M1 was detected in 18% of breast milk samples (4/22), along with six other mycotoxin classes, including dihydrocitrinone in 27% of samples (6/22; 14.0 - 59.7 ng/L) and sterigmatocystin in 5% (1/22; 1.2 ng/L), both reported for the first time in breast milk. Moreover, beauvericin, a mycotoxin mainly produced by the Fusarium genus, was found in 100% (22/22) of breast milk samples. Notably, non-exclusively breastfed infants showed higher frequencies and concentrations of mycotoxins in urine compared to exclusively breastfed infants, highlighting increased exposure as complementary foods are introduced and the importance of breastfeeding to reduce early-life mycotoxin exposure. Several studies now focus on widely targeted analysis of xenobiotics; however, complex biological matrices introduce significant challenges due to various interferences and complicated ion suppression/enhancement effects. Routinely, the quantification using only two specific MRM transitions (i.e., quantitative and qualitative MRM transitions) is sometimes not enough to ensure confident quantification results; however, increasing the number of MRM transitions acquired for each compound may increase the duty cycle of the measurement, hence reducing the total number of xenobiotics can be simultaneously analyzed in a single analysis or reducing the sensitivity of the method.

To overcome the challenges of achieving highly sensitive, widely targeted analysis and confident identification of 80 xenobiotics across various chemical classes in breast milk samples, Jamnik *et al.* utilized a SCIEX QTRAP 6500+ with Enhanced Product Ion (EPI) scanning [31]. This approach employed a linear ion trap to automatically accumulate and scan all product ions of the targeted xenobiotic when detecting the compound in the sample without compromising duty cycle or sensitivity. Due to its ability to accumulate all fragment ions of a compound at various collision energies in the ion trap and its high scan speed (up to 20,000 Da/s), the resulting product ion spectra show significantly improved quality, with more fragments and enhanced sensitivity compared to product ion scanning on a standard triple quadrupole instrument, thus allowing confident compound confirmation via library searching in complex biological matrices. The EPI scan on the QTRAP system allowed the authors to simultaneously achieve sensitive quantification and confident identification of xenobiotics for 86 breast milk samples collected from a mother during the first 211 days postpartum. Results from the study indicated that daidzein, a phytoestrogen, exhibited a wide range of concentrations ranging from < 0.0083 ng/mL to 0.14 ng/mL and had a link to the mother's diet. Synthetic chemicals like parabens and phthalates had quite stable concentrations, hence indicating the exposure from regular use of personal care products and contact with plastics. The PFAS concentrations in the milk sample indicated a constant bioaccumulative nature of PFOS. Compounds like pyrrolizidine alkaloids, tropane

alkaloids, and heterocyclic aromatic amine PhIP were reported for the first time in the breast milk matrix. The results highlighted potential contamination from the mother's consumption of herbal teas and heavily grilled foods, which could expose infants to plant alkaloids and other toxicants through breast milk. Additionally, the authors successfully estimated the hypothetical upper bound intake (hUBI) under a high-exposure scenario and compared it to the recommended tolerable daily intake (TDI) or acceptable daily intake (ADI) values (**Table 2**). This comparison highlighted that PA levels could pose a potential health risk for infants whose mothers consume large amounts of PA through honey or herbal remedies.

***Table 2**. Preliminary exposure/risk assessment using a high-exposure scenario, where hUBI is the calculated hypothetical upper bound intake; TDI is the recommended tolerable daily intake; ADI is the acceptable daily intake for adults and corrected for infants below 16 weeks of age; MoE is the margin of exposure, describing the ratio of a calculated benchmark dose lower confidence limit for a 10% excess cancer risk (BMDL10) to the hUBI for pyrrolizidine alkaloids and PhIP [31]*

Compound	Assumed concentration in breast milk [ng mL^{-1}]	TDI or ADI adults [µg kg^{-1} bw day^{-1}]	TDI or ADI infant corrected [µg kg^{-1} bw day^{-1}]	hUBI [µg kg^{-1} bw day^{-1}]	Ratio TDI or ADI/hUBI or MoE
Bisphenol A (BPA)	0.4	4	1.3	0.067	20
Bisphenol F (BPF)	0.028			0.0047	
Bisphenol S (BPS)	0.051			0.0085	
Mono-n-butyl phthalate	6.4	1.3	1.1	1.1	
Perfluorooctanoic acid	0.092	0.63	0.21	0.015	14
Perfluorooctanesulfonic acid	0.048	0.63	0.21	0.008	26
Prochloraz	0.082	100	33	0.014	2439
8-Prenylnaringenin	0.23			0.038	
Daidzein	0.14			0.023	
Enterodiol	0.025			0.042	
Enterolactone	0.17			0.028	
Glycitein	0.011			0.0018	
Isoxanthohumol	0.078			0.013	
Resveratrol	0.3			0.05	
Xanthohumol	0.22			0.037	
Alternariol	0.51			0.085	
Benzophenone 1	0.039	30	10	0.0065	1538
Benzophenone 2	0.02			0.0033	
Butylparaben (BP)	0.036	2000	667	0.006	111111
Ethylparaben (EP)	0.13	10000	3333	0.022	153846
Methylparaben (MP)	23	10000	3333	3.8	870
Propylparaben (PP)	16	2000	667	2.7	250
Anisodamine	0.031			0.0052	0.0052
Jacobine-N-oxide	0.011			0.018	3889
Riddelliine-N-oxide	0.021			0.0035	20000
Scopolamine	0.0004	0.016	0.0053	0.000067	79
PhIP	0.042			0.007	68571

Taking advantage of the SCIEX QTRAP 7500 system, one of the most sensitive LC-MS/MS systems available to date, along with the linear ion trap, Berger *et al.* developed a targeted LC-MS/MS method using scheduled multiple reaction monitoring with fast polarity switching (i.e., 5 msec). This method was employed to analyze 86 polyphenols in human breast milk samples of 12 Nigerian mothers at three postpartum time points [11]. The xenobiotics were chromatographically separated on a reversed-phase column with a column temperature of 30°C employing a 6 minutes of LC gradient with mobile phases of 0.1% formic acid (FA) in H_2O and 0.1% FA in acetonitrile (ACN). The method was validated in-house following the guidelines set by Eurachem and the EU Commission decision 2002/657/EC. Due to the lack of matrix-matched reference material, blank breast milk samples were prepared by pooling several samples for the matrix-matched calibration curve. The results from method validation showed that 70% of the analytes had mean recoveries within the 80 - 120% range, showing very good recovery performance. Intermediate precision ranged from 5-61% at low, 4 - 56% at medium, and 7 - 62% at high spiking levels, while repeatability was between 4 - 87%, 3 - 59%, and 2 - 71% for these same levels, indicating stable workflow performance. LODs ranged from 0.0041 to 87 ng/mL, with polyphenol classes like flavanones, flavonols, and hydroxycinnamic acids showing particularly low detection limits. The signal suppression/enhancement ranged from 99 - 250%, with 91% of analytes falling between 99 - 130%. Despite the complexity of the breast milk matrix, the optimized sample preparation resulted in high sensitivity, minimal matrix effects, and reliable recoveries for most analytes. The authors successfully quantified nearly 70% of the targeted polyphenols, with 50 identified in breast milk, predominantly phenolic acids, and noted the presence of phase II metabolites of polyphenols, suggesting maternal metabolism and potential infant transfer.

SCIEX triple quadrupole instruments, especially the QTRAP system, have shown great enhancement for xenobiotic analysis in complex matrices like breast milk. These systems are able to provide high sensitivity and specificity, which is very important for analyzing trace levels of xenobiotics in biological samples. Several advanced scan modes offered by the linear ion trap, such as EPI scanning, improve the quality of product ion spectra, enabling confident identification at the same time, highly sensitive quantification even in challenging samples. Studies using these instruments have successfully profiled a wide range of xenobiotics, including mycotoxins and polyphenols, revealing important insights into maternal and infant exposure. Overall, the SCIEX QTRAP systems proved their robustness and versatility for comprehensive xenobiotic profiling, aiding in accurate risk assessments of dietary and environmental exposures in early life.

3.2.2. Non-targeted analysis of xenobiotics in breast milk

While targeted methods offer high sensitivity and specificity for chosen compounds, they have limitations in scope and may overlook unknown or unexpected substances. The targeted strategy may exclude unknown or emerging contaminants and may not accurately capture the complexity of real-world exposures, which often involve mixtures of chemicals that can have synergistic or antagonistic effects. Non-targeted analysis, such as LC in combination with HRMS, offers broader coverage of the chemical space, thus enabling the detection of a wider range of chemicals, including unknowns and emerging contaminants. This approach has shown the potential for discovering novel biomarkers of exposure and disease risk, thus enhancing our understanding of the health impacts of the exposome. HRMS instrumentation continues to push the boundaries of chemical exposomics, facilitating more efficient and comprehensive workflows. Warth *et al.* showcased a workflow utilizing mass spectrometry-based global exposure metabolomics in conjunction with the METLIN Exposome Database, enabling comprehensive exposure assessments [35]. This platform facilitated the identification of various environmental toxicants, food contaminants, supplements, drugs, and antibiotics along with their metabolites while also illustrating how metabolomic-derived pathway analysis could elucidate the biological effects of toxicants, proposing artificial intelligence as a tool for evaluating potential toxicant roles.

Among the latest developments in this field are the Orbitrap Tribrid and Q-Orbitrap Astral from Thermo Fisher Scientific, timsTOF from Bruker, and the innovative ZenoTOF from SCIEX [36]. The ZenoTOF represents a major advancement in HRMS, significantly enhancing sensitivity and versatility in chemical exposomics. Utilizing Zeno trap pulsing, the duty cycle can go up to 90%, hence achieving 5–20 times greater sensitivity than older TripleTOF and X-series models. In addition to that, Zeno Trap releases ions based on their m/z values, ensuring that higher m/z ions are released first, followed by lower m/z ions. This prevents the faster flight of lower m/z ions, which could saturate the extract region, hence affecting higher m/z ions.

Moreover, the instrument offers multiple fragmentation options, including Electron Activated Dissociation (EAD) and Collision Induced Dissociation (CID), and supports "Sequential Window Acquisition of All Theoretical fragment ion spectra" referred to as SWATH-DIA by SCIEX for comprehensive analysis as well as high-resolution MRM. With an ultrafast ToF scanner, ZenoTOF combines high sensitivity, rapid scanning (i.e., up to 133 Hz), and flexible acquisition techniques, positioning it as a key tool for future exposomics research. The emerging technique, SWATH-DIA, a data-independent acquisition method that systematically fragments all ions across pre-defined mass-to-charge (m/z) windows, further improves non-targeted analysis by comprehensively acquiring structural information for all possible compounds present in the samples. Unlike Data-Dependent Acquisition (DDA), which focuses only on a subset of compounds meeting specific criteria (e.g., high-abundance compounds), SWATH-DIA captures data from all detectable ions, reducing the chance of missing low-abundance or less common analytes.

Although non-targeted analysis provides a more comprehensive view of the exposome, it faces challenges in sensitivity for low-abundance compounds and requires complicated data analysis for compound identification [37]. To address the challenges for low-abundance compounds in biological matrices, Oesterle *et al.* utilized SCIEX ZenoTOF 7600, taking advantage of high-resolving power for high selectivity and Zeno Trap for high sensitivity to identify and quantify xenobiotics in breast milk samples (**Figure 3**) [15]. The ZenoTOF was operated in SWATH-DIA mode, enabling comprehensive and retrospective analysis of all detectable analytes. The data were processed using MS-Dial software for peak picking, alignment, and annotation, while MS-Finder provided in silico fragmentation to aid in identifying unknown compounds by comparing experimental spectra with predicted patterns. Compound identification followed a strict confidence system based on established guidelines, ranging from confirmed identifications with reference standards (Level 1) to lower confidence levels derived from spectral matching. The quality control strategy involved preparing pooled QC breast milk samples by combining 10 µL of each sample. The QC samples were measured in triplicates throughout the batch to monitor and correct for any possible signal drift. The procedure blanks were included for identifying background noise and compounds not originating from breast milk samples. Features were filtered based on several criteria. First, those with peak areas in blanks exceeding one-third of the sample average were removed. Next, features were retained only if they were detected in at least two out of three QC replicates with an RSD value below 30% and showed S/N values ≥ 3. Finally, features failing Spearman rank correlation in the QC dilution series were removed. The statistical analysis involved the data normalization with median normalization and log10 transformation using MetaboAnalyst. The non-targeted workflow used by the authors allowed the detection of unexpected or previously uncharacterized substances, with initial analysis identifying 12,192 features in breast milk and 16,461 in stool [15]. Following quality control to remove noise and artifacts, the number of features was reduced to 4,347 in breast milk and 6,905 in stool. A final suspect screening workflow optimized for polyphenols but applicable to other xenobiotics narrowed down these features to 542 in breast milk and 864 in the stool. Spearman rank correlation was used to investigate potential associations between features identified by ANOVA and the stool microbiome. A total of 58 positive and 12 negative microbe-feature correlations were identified, primarily involving phytochemicals. For example, the flavone tricetin showed a strong positive correlation with blautia, which is known to metabolize flavonoids. Kaempferol and biochanin - A were strongly linked to romboutsia, while vulgaxanthin - I had a significant negative correlation with Escherichia-Shigella. Additionally, the mycotoxin fumonisin B1 showed a strong correlation with Streptococcus. These results underscore the need for further investigation into xenobiotic-microbiome interactions using simpler *in vitro* models. Although non-targeted analysis for xenobiotics is a promising tool to get a deep insight into the impact of xenobiotics on health in early life, the requirement of innovative HRMS instruments, sophisticated software (e.g., MZMINE, MS-DIAL…), extensive databases (e.g., MONA, HMDB…) for effective compound identification, lack of authentic standards, and careful quality control process are bottlenecks of the approach.

The SCIEX ZenoTOF 7600 demonstrates significant advantages in non-targeted analysis, particularly through its enhanced sensitivity and comprehensive data acquisition capabilities. Utilizing innovative features like the Zeno Trap and SWATH-DIA, the instrument enables broad coverage of the chemical space, detecting a wide range of xenobiotics, including unknown and emerging contaminants. The high-resolution acquisition paired with advanced fragmentation techniques such as EAD provides detailed structural information,

facilitating the identification of novel biomarkers and unexpected substances. The ZenoTOF has proven to be an essential tool in exposomics research, providing deep, unbiased analysis of complex biological samples and advancing the understanding of the chemical exposome and its potential health impacts in early life.

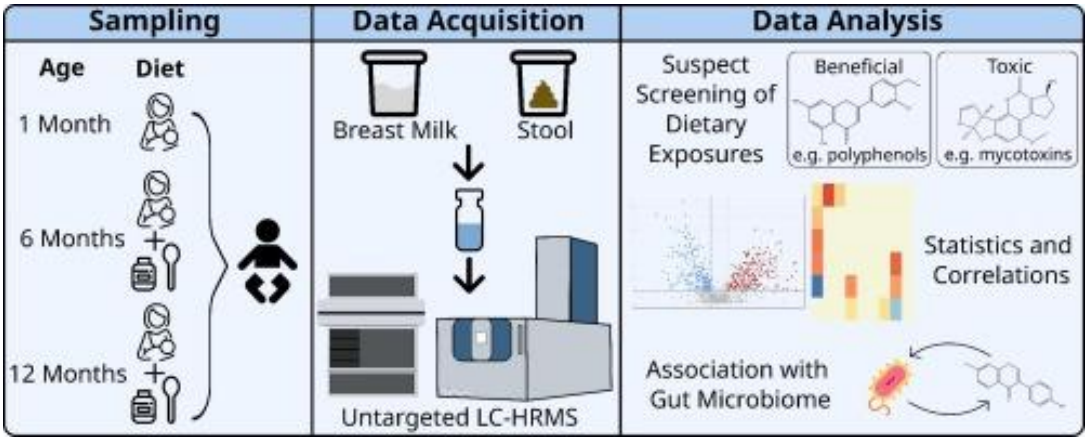

***Figure 3**. Workflow employing ZenoTOF 7600 combined targeted and non-targeted analysis for xenobiotics in complex biological samples [15]*

4. CONCLUSION

There is currently an urgent need for an integrated approach to comprehensively study infant exposure to harmful xenobiotics. Additionally, encouraging breastfeeding remains extremely important, alongside advancing research on the bioavailability and potential health impacts of bioactive compounds in breast milk. Efforts to reduce mycotoxin contamination, particularly in food crops and complementary infant foods, are essential. Furthermore, stringent regulatory frameworks and enhanced monitoring of xenobiotics in food and consumer products must be prioritized. Implementing these strategies will significantly reduce early-life chemical exposure and improve health outcomes from biochemical and toxicological perspectives. The existing regulatory standards governing xenobiotics in neonatal food highlight the necessity for consistent safety levels globally; however, variations across regions and food sources pose significant challenges. Analytical limitations, such as the difficulty in detecting low-level contaminants and the complexity of food matrices, hinder comprehensive exposure monitoring. Additionally, the lack of long-term studies on low-dose exposures underscores the need for improved biomonitoring and more rigorous testing practices. Future efforts should focus on advancing nanotechnology, multimodal detection systems, and portable/fast methods, promoting cleaner agricultural practices, and encouraging global harmonization of food safety standards. Enhanced biomonitoring studies and targeted policy initiatives will be crucial for better protecting early childhood health from xenobiotic exposure.

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