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Meconium in the investigation of prenatal drug exposure

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ABSTRACT

Fetal drug exposure remains a critical public health concern with both maternal and neonatal health implications. In clinical and forensic toxicology, meconium has emerged as a valuable alternative biological matrix to detect prenatal drug exposure. Use of meconium offers a longer detection window than maternal blood or neonatal urine, while enabling non-invasive postnatal collection. Furthermore, advances in analytical techniques, particularly in chromatography and mass spectrometry, have significantly enhanced the sensitivity and specificity of drugs and metabolites detection in meconium. These improvements have expanded its utility in neonatal screening, interventions in maternal care, and legal proceedings related to child protection. Emerging research into the physicochemical and biological mechanisms of xenobiotic incorporation into meconium has improved the interpretation of quantitative results and facilitated the identification of novel biomarkers. The growing application of meconium testing presents an opportunity to refine early intervention strategies, optimize prenatal care, and better characterize long-term neurodevelopmental and behavioral risks associated with in utero substance exposure. This review provides an integrative overview of meconium as an alternative biological matrix for diagnostics and monitoring of the exposure to licit and illicit substances by examining its formation and composition, followed by analytical approaches. It also discusses the implications of meconium toxicology for optimizing prenatal care, guiding neonatal interventions, and informing long-term risk assessment of neurodevelopmental outcomes following prenatal substance exposure.

1. Introduction

Traditional biological matrices such as maternal blood and neonatal urine are routinely used in toxicological screening however, their short detection windows and invasive collection methods limit their diagnostic utility [1]. In contrast, meconium offers a unique opportunity to retrospectively capture cumulative exposure throughout the second and third trimesters of pregnancy [2], while being collected non-invasively after birth [3]. Over the past few decades, advances in analytical chemistry, particularly mass spectrometry and untargeted multi-omics approaches, have expanded the sensitivity and scope of meconium analysis [2]. These features make it particularly useful for identifying in utero exposure to illicit substances [4], prescription medications, and environmental toxins [5].

In a clinical setting, meconium analysis aids in early diagnosis and management of conditions such as neonatal abstinence syndrome (NAS) [6], which allows timely intervention and improved neonatal outcomes [7]. From a public health and child protection perspective, positive meconium findings can support maternal intervention programs, guide postnatal care plans, and inform child protection services in cases of suspected prenatal substance abuse [8].

Despite its advantages, meconium testing presents several analytical and practical challenges. For instance, the composition of meconium can vary significantly between neonates, particularly in preterm births or cases of in utero passage. Furthermore, sample contamination with amniotic fluid or transitional stool may also complicate analysis [9]. To ensure reproducibility and reliability, standardization of collection protocols and analytical validation remains essential [10].

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Although technological and analytical advancements have significantly improved the sensitivity and specificity of toxicological analyses in meconium, liquid chromatography–tandem mass spectrometry (LC-MS/MS) has become the gold standard for confirmatory testing, capable of detecting a wide range of drugs and metabolites accurately. While immunoassays do remain useful for preliminary screening [11], they are prone to cross-reactivity and false positives, necessitating confirmatory testing. Recent integration of high-resolution mass spectrometry (HRMS) and untargeted screening approaches has enabled the detection of emerging psychoactive substances and expanded toxicological profiling [12].

Understanding the mechanisms of xenobiotic incorporation into meconium is also advancing studies and suggests that compound physicochemical properties, placental transport, and fetal metabolism influence deposition patterns [13]. Collectively, these insights may improve interpretability and support the development of more accurate exposure assessment models.

In addition, given the ethical sensitivities and medico-legal consequences associated with perinatal toxicology, including informed consent, maternal privacy [14], and potential legal consequences, testing protocols must be carefully implemented within supportive, non-punitive frameworks [15].

Thus, this review provides a comprehensive overview of meconium as a matrix for monitoring exposure to licit and illicit substances, beginning with its biological formation and composition, followed by analytical methodologies, biomarker discovery, and concluding with clinical, public health, and forensic applications.

2. Fetal drug exposure and the role of meconium in clinical and forensic toxicology

Fetal drug exposure is a critical and multifactorial public health challenge with clinical, developmental, and societal consequences. The global prevalence of substance use among women of reproductive age is rising, driven by both licit (e.g., alcohol, nicotine, prescribed opioids) and illicit substances (e.g., cocaine, methamphetamine) [16]. Epidemiological studies estimate that approximately five million women of childbearing age use psychoactive substances annually in regions including Brazil, the United States, and Europe, resulting in in-utero exposure for over four million neonates each year [16].

The consequences of fetal drug exposure include spontaneous miscarriage, intrauterine growth restriction, congenital anomalies, and long-term neurodevelopmental impairments. Its impact varies depending on the type of substance, frequency, timing, and dosage of use. Prenatal exposure to cocaine, for example, has been associated with uteroplacental vasoconstriction, reduced fetal oxygenation [12], and an increased risk of placental abruption, hypertensive disorders, and spontaneous abortion [13]. Meta-analyses have also linked cocaine exposure to preterm birth, low birth weight, neonatal intensive care unit (NICU) admission [12], and structural abnormalities, including limb defects and neurobehavioral dysregulation [13].

Similarly, opioids, including heroin, methadone, and prescription pain relievers, are associated with the development of neonatal abstinence syndrome (NAS) [8], which affects up to 60–80 % of neonates exposed to opioids in utero [6]. Characterized by symptoms such as hypertonia, tremors, excessive crying, gastrointestinal disturbances, and autonomic instability NAS often necessitates pharmacological intervention and prolonged hospitalization [17]. In the United States, the incidence of NAS rose from 1.5 per 1000 hospital births in 2004 to 8.8 per 1000 by 2016 [17], highlighting the escalating public health burden [6,8] .

Currently, longitudinal studies have consistently demonstrated that prenatal exposure to substances such as alcohol, cannabis, methamphetamines, and benzodiazepines is associated with enduring cognitive, behavioral, and emotional challenges [17]. These challenges may include attention-deficit/hyperactivity disorder (ADHD), executive function deficits, learning disabilities, and emotional dysregulation extending into adolescence and early adulthood. For example, children prenatally exposed to methamphetamine-type substances scored lower on working memory and visual motor integration assessments compared to non-exposed peers at age seven [17].

In this context, meconium toxicological analysis offers a sensitive and clinically valuable method to identify prenatal drug exposure [18]. It also provides a retrospective evidence of chronic or repeated substance use during pregnancy [19–21]. Early detection through meconium testing enables timely and individualized medical interventions, including the initiation of NAS-specific pharmacological protocols [6,22]. Supportive care plans can further improve neonatal outcomes [20]. It also facilitates referrals to evidence-based maternal treatments, such as medication-assisted treatment (MAT) [22]. Behavioral counseling, and social support services provide additional benefits [23] that improve maternal-infant outcomes and reduce recurrence in future pregnancies [16,23–25].

To an extent, hospitals frequently use meconium toxicology testing as part of neonatal screening programs, particularly in cases where there is suspicion of maternal drug use [26]. A positive toxicology result in meconium significantly impacts child protection services [27]. In Australia, for example, maternal substance use during pregnancy may constitute grounds for child welfare intervention [28]. Therefore, the results of toxicological analyses can be used as evidence in legal proceedings, influencing decisions on parental custody, supervised care, and social service interventions [29]. These cases are often complex and require a multidisciplinary approach involving healthcare providers, social workers, and legal professionals to ensure the safety and well-being of the child [30].

Identifying maternal drug use provides an opportunity for healthcare providers to refer mothers to rehabilitation programs, parenting support services, and harm-reduction initiatives [31]. Public health strategies that address perinatal substance use and incorporate meconium testing into broader maternal and child health policies ensure that affected families receive appropriate support [32]. These strategies also facilitate timely intervention when necessary [33].

From a maternal health perspective, meconium toxicology testing can help identify women in need of substance abuse treatment, thereby improving long-term health outcomes for both mother and child [29]. In addition, long-term child development outcomes linked to prenatal drug exposure include cognitive impairments, behavioral disorders, attention deficits, and an increased risk of learning disabilities [30]. Studies have shown that children exposed to substances such as cocaine, methamphetamines, and opioids in utero may experience challenges in emotional regulation and social interaction later in life [31].

3. Formation and composition of meconium

Meconium is a complex biological matrix commonly used to confirm in-utero exposure to drugs, environmental toxins, and other xenobiotics [34]. As the first excretion of a newborn, it is characterized by a viscous, tar-like consistency, dark green to black coloration, and a lack of the typical odor associated with postnatal feces. The composition of meconium reflects its prenatal origin, consisting predominantly of water, desquamated epithelial cells from the gastrointestinal tract and skin [34]. Additional constituents include bile acids and bile salts, cholesterol and sterol precursors, digestive enzymes, mucopolysaccharides, sugars, proteins, trace metals, and various pancreatic and intestinal secretions [34]. Residues of swallowed amniotic fluid also contribute to its composition [34]. Unlike feces, meconium is sterile and non-putrefactive, making it an ideal matrix for retrospective analysis of prenatal exposures [35].

Meconium formation begins in the second trimester of gestation, around the 12th week, and progressively accumulates in the fetal intestine until birth [35], with the majority accumulating in the final eight weeks of gestation. Therefore, the presence of drugs or metabolites in

meconium typically reflects repeated or chronic exposure during the later stages of pregnancy. Isolated or short-term exposures, such as those occurring during the first trimester, may not be detected [40]. Following birth, meconium is typically excreted within the first 24 to 48 h and may continue for up to five days after delivery [36]. Studies employing zinc coproporphyrin as a biomarker indicate that bile pigments specific to meconium can be excreted for up to 125 h (approximately five days) postpartum, after which the formation of transitional and then mature feces predominates [36].

4. Mechanisms and factors influencing xenobiotics incorporation into meconium

During pregnancy, substances consumed by the mother, such as therapeutic drugs, environmental xenobiotics, or drugs of abuse, can cross the placenta and enter the fetal circulation. This occurs primarily through passive diffusion via placental blood vessels [37]. While the efficiency of this transplacental transfer depends on the substance's physicochemical and pharmacokinetic properties, low molecular weight (<500 Da), lipophilic, non-ionized, and unbound (free) compounds diffuse more readily across lipidic membranes [26]. Facilitated diffusion and active transport may also occur, particularly for substances that structurally resemble endogenous compounds, such as glucocorticoids [38]. Placental blood flow and maternal plasma protein binding further influences the rate and extent of fetal exposure [37].

Once in fetal circulation, these compounds undergo partial metabolism by the fetus and are excreted into the amniotic fluid via urine or bile. The fetus then swallows this fluid, allowing the compounds to enter the gastrointestinal tract and incorporate into meconium [2]. This matrix, therefore, captures a cumulative record of maternal metabolism, placental transfer, and fetal metabolism and excretion [39].

Several additional factors influence the extent and interpretation of drug incorporation into meconium. One such factor is ion trapping, a phenomenon driven by fetal blood's slightly more acidic pH than maternal blood. This can cause weak bases to become ionized and thus retained in fetal tissues, limiting their transfer back to the maternal circulation and potentially increasing their accumulation in the fetus' blood and meconium [2]. However, these fetal-specific physiological differences can introduce variability, weakening the correlation between actual fetal exposure and detected concentrations. Even among neonates with similar exposures, such as dizygotic twins, drug levels in meconium can vary significantly due to individual differences in metabolism and placental function [40].

Technical and biological limitations must also be considered. If meconium is excreted prematurely or collected late, the sample may be insufficient or contaminated [41]. A common issue relates to contamination with neonatal urine in the diaper, which can artificially elevate specific analyte concentrations and alter parent-to-metabolite ratios, especially for late gestational exposures [42]. Furthermore, delayed collection may lead to the detection of iatrogenic drugs administered

Fig. 1. Stepwise procedure for neonatal meconium collection. (1) Standard collection kit including a sterile container and spatula; (2) Freshly excreted meconium present in a diaper; (3) Manual extraction of meconium using a sterile spatula; (4) Transfer of meconium into a labeled collection container for subsequent toxicological analysis.

postnatally, complicating the interpretation of results and increasing the risk of false positives not related to in utero exposure [41].

5. Meconium collection and transportation

Meconium collection is a non-invasive process [34]. It is easily collected from diapers by scraping it directly with a spatula and storing it in a collection container (Fig. 1). Care must be taken to avoid collecting urine, which can lead to contamination. The typical sample amount ranges from 0.5 to 2.0 g. Since collection occurs after excretion and without direct contact with the newborn, the procedure itself causes no discomfort [39]. However, it is crucial to consider that the amount of sample available is limited, which could impact the analysis and interpretation [34].

The stability of meconium samples relies on storage conditions. At room temperature, samples remain stable for approximately 24 h [43]. For instance, cocaine maintains its stability in meconium for 24 h at room temperature, but beyond this period, a 25 % reduction in concentration has been reported. When stored at −20 °C, cocaine stays stable for at least nine months [43]. However, prolonged storage can lead to the degradation of certain analytes, such as the heroin metabolite, 6-acetylmorphine (6-AM) [19], which is a key biomarker of heroin intake [19].In the study by Wu, Marin and McMillin (2016) [43], meconium samples stored for two weeks under various conditions (4 °C, room temperature, and 37 °C) showed significant degradation of specific compounds. Concentrations of 7-aminoclonazepam declined by 48.4 % at 4 °C and 71.5 % at room temperature, while chlordiazepoxide decreased by 59.5 % at room temperature. Milder losses were observed in alprazolam (23.5 % at 4 °C), midazolam (20.8 % at 4 °C), nordiazepam (22.8 % at room temperature), and α-hydroxyalprazolam (20.7 % at 4 °C). The 6-AM metabolite was unstable under all temperature conditions. Interestingly, morphine concentrations increased by 33.3 % at 4 °C and 23.4 % at room temperature, likely due to the breakdown of 6-AM. At 37 °C, no significant changes (≥20 %) in morphine levels were observed. Conversely, cocaine and its metabolites, including cocaethylene, benzoylecgonine, and m-hydroxybenzoylecgonine remained stable across all tested temperatures [43]. Overall, it is important to consider that the integrity of the sample and consequently the stability of the analytes of interest may be affected by the storage conditions. Similarly to other biological specimens, stability studies are recommended to assess the impact of storage conditions on the analyte's stability, especially when specimens are expected to be exposed to higher temperatures for prolonged periods and information on the analyte's stability at those temperatures is unavailable.

6. Analytical methods and sample preparation for meconium analysis

Meconium is considered a gold standard for investigating neonatal drug exposure [44], and as such several validated methods have been reported in literature. Given its complex composition, meconium requires extensive sample preparation and clean-up to isolate target analytes, eliminate interfering substances, reduce matrix effects, and enhance analytical sensitivity. Numerous analytical methods have been employed to ensure accurate and reliable detection [45].

Once samples have been collected, aliquoting the required amount for preparation and analysis can be challenging. It is recommended that specimens be stored at low temperatures (−80 °C or − 20 °C) [42]. Therefore, samples will be frozen and further thawed when handled. Given that meconium is a solid, viscous matrix, transferring the required amount to test tubes may be more time-consuming compared to liquid specimens. Nevertheless, overall, sampling and weighing are straightforward processes. The preparation of specimens for analysis may involve multiple steps, including homogenization, liquid or solid-phase extraction, centrifugation, and solvent evaporation. Homogenization can be performed in buffers or solvents and is promoted by mechanical agitation [45–47] or sonication [21], typically followed by centrifugation to isolate the solid content, as described in multiple studies. Hydrolysis is also often required for the analysis of glucuronide conjugates of some compounds, such as 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) [21,34] mainly if a GC–MS-based method is used or if an LC-MS(MS) [48] or LC-HRMS is used but has no glucuronide conjugates in the method's scope [49]. Additionally, for GC–MS-based methods, extractions may be followed by derivatization, depending on the target analytes, which needs to be considered.

Liquid-liquid extraction (LLE) is a well-established technique widely utilized in forensic laboratories. For example, salting-out assisted LLE, which promotes the separation of aqueous and organic layers [47], has been recently reported in the extraction of meconium specimens. Overall, the advantages of using LLE include its ease of performance, the availability of a wide variety of extraction solvents [50] and the possibility of extracting acidic, basic or neutral compounds. LLE is not as selective as other sample preparation techniques, which can be an analytical challenge; nevertheless, LLE can be useful in comprehensively isolating as many compounds as possible, beneficial for screening purposes [51].

Solid phase extraction (SPE) has also frequently been used to determine drugs and metabolites of forensic interest in meconium samples, either alone or in combination with other techniques [35,48]. This is mostly in view of the fact that SPE provides efficient removal of interferences and the extraction can be optimized by selecting different sorbents and elution solvents, as demonstrated by Hernandez et al. (2022) [34] and Jensen et al. (2019) [48]. SPE can also be easily automated, allowing for the extraction of multiple samples at once. However, the application of SPE comes with a few caveats. An initial homogenization/extraction into a solvent is typically required before SPE [47], considering meconium is a solid matrix. Moreover, depending on the particle size of the sorbent, cartridges may clog due to the presence of solid residues, which can extend extraction times. Lastly, SPE methods typically require a considerable amount of solvents for conditioning, washing and elution [51] and some methods may not be considered environmentally friendly.

Over the last few decades, other techniques have been explored and successfully implemented in the extraction of drugs of forensic interest from meconium. Miniaturized extraction techniques typically provide analytical gains by reducing solvent volumes needed for extraction. Formerly known as disposable pipette tip extraction, dispersive pipette XTRaction (DPX® Technologies) is a miniaturized, dispersive SPE technique that employs a solid phase loosely dispersed inside the tip and requires reduced volume of sample and solvents [52], thus making it more environmentally friendly than conventional SPE. This technique has also been demonstrated to effectively extract drugs and metabolites from meconium specimens. For example, Bordin et al. reported this technique in the extraction of cocaine, nicotine, and its metabolites from meconium samples, achieving recoveries between 50 % and 98 % while requiring reduced volumes of solvents for extraction [45].

Reporting also exists for other techniques, such as supported-liquid extraction (SLE) [53] and accelerated solvent extraction (ASE) [54,55]. Labardee et al. (2017) utilized a homogenization in methanol followed by centrifugation, with the supernatant extracted using SLE, and eluted with a mixture of ethyl acetate/isopropanol (90:10) [53]. Unlike SPE, the SLE-based procedure involves fewer steps, avoiding additional steps such as cartridge washes. Mantovani et al. (2014) described a combination of ASE and SPE [55]. Initially, meconium samples were mixed with diatomaceous earth and an internal standard solution, then extracted at 120 °C and 1500 psi using phosphate buffer pH 6; the extract was further subjected to SPE and subsequent derivatization for GC–MS analysis [55]. Similarly, Mantovani et al. (2018) described a method for the extraction of THC-COOH from meconium specimens using a combined ASE-SPE approach; briefly, the extraction was performed at 120 °C and 1500 psi using a 0.4 M NaOH solution, and the extract was further neutralized with 6 M HCl before being processed

using SPE [54].

The analysis of meconium samples has been usually performed via gas or liquid chromatography coupled with mass spectrometry. Although immunoassays have been reported, GC–MS and LC-MS/MS provide chromatographic separation and mass spectral data, which can be used for detection and quantification. Multiple GC–MS-based methods have been published recently [45,56]. Although gas chromatography-tandem mass spectrometry (GC–MS/MS) currently remains marginally explored in meconium testing, Lamy et al. (2017) reported ethyl-glucuronide analysis in meconium [21]. The major drawback associated with GC–MS, however, is that some compounds require derivatization, such as cocaine metabolites [45,55] and Δ^9-tetrahydrocannabinol (Δ^9-THC) metabolites [54,56], which results in longer analytical workflows.

LC-MS-based methods circumvent the need for derivatization and provide acceptable sensitivity and selectivity. Furthermore, it is known that glucuronide conjugates can be targeted in LC-MS-based methods, avoiding the hydrolysis step. For example, Prego-Meleiro et al. (2017) included Δ^9-THC and THC-COOH glucuronides in the scope of their method, and THC-COOH was detected in three authentic specimens using liquid chromatography-triple quadrupole mass spectrometry (LC-QQQ-MS) [51]. Recently, LC-QQQ-MS has been commonly used in the quantitative analysis of different types of drugs, including benzodiazepines and antidepressants [50], cannabinoids [48,51], cotinine and cannabinoids [21], synthetic cathinones [57], opioids, phencyclidine and THC-COOH [44] in meconium. LC-QTrap-MS has also been reported [47,53].

In contrast, in recent literature, applications of liquid chromatography-single quadrupole mass spectrometry (LC-MS) are hardly reported. D'Avila et al. (2016) reported a method for the detection of cocaine and metabolites in meconium using LC-MS [46]. Although LC-MS may be more accessible to many laboratories, the limited applications likely stem from limited sensitivity and selectivity combined to an increasing availability of tandem mass spectrometry instruments in clinical and forensic laboratories. Tandem mass spectrometry-based methods typically provide higher sensitivity and selectivity than single quadrupole MS and are preferred especially in the analysis of concentrations in the low and sub-ng/mL range.

High-resolution mass spectrometry coupled with liquid chromatography may likewise be used for qualitative or quantitative analysis. LC-HRMS provides several analytical advantages, such as untargeted or targeted approaches, utilization of spectral libraries and determination of accurate mass. In the context of novel psychoactive substances (NPS), LC-HRMS can assist with structural elucidation of parent NPS as well as their metabolites. The high sensitivity of LC-HRMS is also remarkable. In the literature, applications of LC-HRMS in meconium testing have been demonstrated using different mass analyzers. López-Rabuñal et al. (2021) developed and validated a semi-quantitative method for 137 drugs, including NPS, using LC-QTOF-MS, achieving significantly low limits of detection (0.04 to 2.4 ng/g) [58]. Likewise, high sensitivity demonstrated by limits of detection in the range of 0.5 to 5 pg/mg were achieved using LC-Orbitrap-MS in a method for 15 substances by Hernandez et al. (2022) [34]. Nevertheless, more applications are yet to be reported likely due to the high instrumentation costs and consequently limited availability compared to low-resolution mass spectrometers.

7. Meconium biomarkers of prenatal drug exposure

As previously described, meconium composition reflects the intrauterine environment and the substances the fetus has been exposed to [59], which may include drug metabolites, environmental contaminants, hormones, and other compounds that cross the placental barrier and then are metabolized by the fetus [60]. The detection and identification of these substances indicate the prenatal exposure to drugs of abuse, prescription medications, alcohol, tobacco, and environmental pollutants [34].

Fetal alcohol exposure is commonly assessed through the detection of fatty acid ethyl esters (FAEEs) in neonatal matrices. These compounds are not transferred from the mother, as they are extensively metabolized and degraded by the placenta. Instead, FAEEs are synthesized by the fetus following transplacental ethanol transfer and subsequent fetal metabolism, thus making them a specific and reliable biomarker of in utero ethanol exposure [61]. Additional alcohol metabolites, including ethyl glucuronide (EtG) and ethyl sulfate (EtS), have been identified as valid biomarkers of prenatal ethanol exposure. However, these compounds have been less extensively studied than FAEEs. According to Keating et al. (2024), while EtS is less established than EtG as a marker in meconium, its inclusion alongside EtG enhances the reliability of detecting fetal alcohol exposure [62].

Moreover, meconium helps in identifying exposure to drugs of abuse. In the context of stimulant drugs, the detection of cocaine can be performed by identifying the parent drug or its primary metabolite, benzoylecgonine, as well as other metabolites such as methyl ecgonine ester (EME), ecgonine, and norcocaine [46]. Exposure to smoked cocaine (crack cocaine) can be revealed by the detection of its pyrolytic products, which are formed during heating, such as methyl ester of anhydroecgonine (AEME) and anhydroecgonine [46]. Amphetamine-type stimulants are detected via their parent compounds amphetamine and 3,4-methylenedioxymethamphetamine (MDMA), for example, as well as metabolites such as p-hydroxymethamphetamine, p-hydroxyamphetamine, and norephedrine [64].

Exposure to cannabis can be investigated through the identification of Δ^9-THC and its metabolites, including THC-COOH, 11-hydroxy-THC, and 8,11-dihydroxy-THC, as well as THC-glucuronide and THC-COOH-glucuronide, along with cannabinol (CBN) and cannabidiol (CBD). THC-COOH is the analyte most frequently detected in meconium [63]. Opioid and its metabolites can be detected in meconium, with morphine being the most prevalent analyte, followed by 6-AM, methadone, codeine, hydromorphone, hydrocodone, and oxycodone [16]. For tobacco exposure, relevant biomarkers include nicotine, cotinine, and trans-3′-hydroxycotinine (OH-COT). These compounds reflect both active and passive maternal smoking during pregnancy [43].

Exposure to prescribed medications, including antidepressants and benzodiazepines, can also be assessed via meconium testing. Antidepressant biomarkers include both parent drugs and metabolites, such as amitriptyline, nortriptyline, imipramine, desipramine, fluoxetine, norfluoxetine, sertraline, norsertraline, paroxetine, venlafaxine, desmethylvenlafaxine, citalopram, and desmethylcitalopram. Benzodiazepines detected include alprazolam, α-hydroxyalprazolam, clonazepam, bromazepam, diazepam, nordiazepam, lorazepam, lormetazepam, oxazepam, flunitrazepam, 7-aminoflunitrazepam, triazolam, midazolam, and zolpidem [58].

The emergence of NPS poses additional challenges for the investigation of prenatal drug exposure. Given that the pharmacology of many NPS remains poorly understood, intentional or unintentional exposure to NPS may lead to adverse effects to the fetus/newborn. Currently, only a few methods targeting NPS in meconium have been published [58]. Nevertheless, the detection of NPS in authentic meconium specimens is still scarce. For example, a study by López-Rabuñal et al. (2021) revealed the detection of acetyl fentanyl, a fentanyl analog, in meconium [58]. This finding highlights the importance of considering the occurrence and potential role of NPS in prenatal exposure, whether used with or separately from traditional illicit or prescribed substances, and the inclusion of NPS in the scope of meconium testing, especially based on national and regional statistics. Although the focus of this review is licit and illicit substances, it is noteworthy that meconium also serves as an effective matrix for assessing prenatal exposure to unintentional xenobiotics, such as environmental pollutants [65].

8. Comparative analysis with other matrices

In toxicology, various biological matrices are utilized to detect drug

use, each with unique characteristics that pose advantages and disadvantages (Table 1). Meconium is beneficial for indicating chronic exposure rather than just recent use. However, as previously mentioned, meconium is limited to newborns and can exhibit variability in drug incorporation based on factors such as placental differences and fetal metabolism [2].

Blood and plasma are widely accepted in clinical and forensic settings [45]. However, they require invasive collection methods (conventional venipuncture method) and primarily reflect recent drug use, limiting their ability to provide information on chronic exposure [66]. Dried blood spots (DBS) could be an alternative to liquid blood for toxicological testing, reducing the discomfort associated with the collection. However, the collection of blood or DBS from newborns will only reveal recent exposure to a given substance, whereas meconium will reflect the continuous exposure throughout pregnancy.

Urine analysis can provide information on recent drug use, however, it has a wider detection window, from hours to a few days, making it a common choice for routine testing [67]. Urine testing is well-established in many laboratories and results from a drug screening in urine can be available in a short period [68]. However, in newborns, urine collection and analysis present some limitations. Collection can be challenging, especially considering the appropriate time for collection, as well as the volume eliminated by the baby might not be sufficient for testing [69].

Specimens collected from newborns are typically diluted, requiring more sensitive analytical methods, with mass spectrometry-based methods being preferred over traditional immunoassay methods [68]. On the other hand, mass spectrometry-based screening methods might not be widely available in hospital laboratories, in contrast to immunoassay instrumentations [68]. Therefore, the analysis of meconium specimens will provide a longer detection window than urine, with the advantage of facilitated collection. Nevertheless, while urine may show the presence of metabolites, it may not accurately reflect immediate drug use [66]. In newborns, the detection of a substance in urine only reflects an exposure that occurred very recently, close to or at the time of the birth [68]. For example, Novikov et al. (2020) reported the detection of fentanyl in urine specimens collected from newborns when the mother was administered fentanyl during birth for analgesia [70]. As stated by the authors of this study, interpreting such findings require caution. A positive result for fentanyl in urine collected from a newborn does not necessarily reflect fentanyl misuse during pregnancy, when the mother was administered fentanyl when giving birth [70]. In this case, additional matrices that provide more expansive detection windows, such as meconium and umbilical cord, would need to be tested [70]. Additionally, during gestation, the fetus can eliminate urine into the amniotic fluid, which can be further absorbed by the fetus and eliminated into meconium [68].

Umbilical cord tissue is another biological matrix explored to assess drug exposure to neonates. This matrix is available at the time of birth and can be readily collected with no discomfort or risk to the neonate [44]. Like meconium [71], umbilical cord tissue reflects drug accumulation during the final weeks of pregnancy [72]. However, the concentration of analytes in the cord tissue is often lower than in meconium, likely due to differences in tissue partitioning and overall analyte concentration [44]. Additionally, the lack of standardized cut-off thresholds and processing protocols limits its standalone diagnostic utility. Nonetheless, it remains a valuable alternative or adjunct to meconium, especially in cases where meconium is unavailable. In contrast, umbilical cord blood directly measures fetal circulating drug levels. Moreover, in pharmacokinetic studies, the umbilical cord can be used to evaluate maternal–fetal drug transfer. Its clinical utility is constrained by a narrow detection window and variability in drug concentrations influenced by metabolism, timing of sampling, and potential contamination with

Table 1
Comparative Evaluation of Biological Matrices for Investigating Prenatal Drug Exposure.

Matrix	Clinical and forensic relevance	Timing of collection	Exposure window	Advantages	Limitations	References
Meconium	Gold standard or comprehensive prenatal drug exposure	1st stool (24–72 h after birth)	2nd trimester to birth	Non-invasive; long detection window; sensitive to repeated/chronic exposure	Cannot detect early 1st trimester exposure; affected by in utero passage or delayed excretion	[3,6,42]
Umbilical Cord Tissue	Practical alternative when meconium is unavailable	At delivery	Late 2nd trimester to birth	Immediate availability, non-invasive, compatible with high-throughput testing	Lower drug concentrations than meconium; may miss early pregnancy exposure	[44,53,71,72]
Umbilical Cord Blood	Detection of acute or peripartum exposure; not cumulative	At delivery	Immediate peripartum exposure	Reflects circulating fetal drug levels; easily obtained at birth	Influenced by time since maternal dosing, only captures acute/last dose exposure	[19,68]
Amniotic Fluid	Valuable in high-risk pregnancies undergoing amniocentesis; mid-gestation exposure data	In utero (via amniocentesis)	Mid-gestation onwards	Reflects renal/fetal swallowing activity; possible during ongoing pregnancy	Invasive; sampling risk; limited availability; varies with gestational age	[76,80,81,84]
Placental Tissue	Exploratory matrix; useful in pharmacology studies	At delivery	Cumulative (local metabolism)	Available at birth; insight into placental drug metabolism	Variability in drug retention; lacks standardization; sampling invasive if in utero	[37,38]
Neonatal Hair	Adjunct to meconium; less commonly used; detection of chronic drug use	After birth (0–3 months)	3rd trimester	Long-term exposure data; suitable for chronic use detection	Affected by melanin content; not all newborns have sufficient hair	[67,74,75]
Human Breast Milk	Assesses postnatal maternal drug use. Useful in breastfeeding safety assessments	During lactation	Postnatal (hours to days)	Non-invasive; monitors postnatal maternal use and infant exposure	Does not assess prenatal exposure; limited drug coverage	[2]
Urine (Neonate)	Routine use for immediate postnatal testing, but limited prenatal relevance	Shortly after birth	A few hours to few days before birth	Rapid screening is commonly used in hospitals	Difficult collection; may not reflect in utero cumulative exposure; requires sensitive methods	[68–70]
Dried Blood Spots (DBS)	Useful for recent exposure screening	At birth (heel prick)	Recent exposure	Minimally invasive; small sample volume	Only reflects very recent use; not suited for chronic exposure detection	[68]
Newborn's Nails	Research use only; potential for long-term exposure data	At birth	Late pregnancy	Possible alternative for long-term exposure	Rarely used; insufficient validation and reference ranges	[37]

maternal blood. Delays in cord clamping may also affect drug levels, complicating interpretation. Therefore, umbilical cord blood is best considered complementary to other matrices, such as meconium and neonatal hair, which better reflect long-term or earlier prenatal drug exposures [37].

Neonatal hair testing is a non-invasive approach to assess cumulative in utero drug exposure, typically from the second to third trimester. Drugs and metabolites are incorporated into the growing hair shaft primarily through passive diffusion from the fetal bloodstream, providing a retrospective timeline of repeated maternal drug use. Strong correlations between maternal and neonatal hair concentrations have been reported, reinforcing its utility in detecting chronic exposure [73]. Furthermore, depending on the length of hair sampled, hair analysis can offer a historical record of drug intake spanning several months. The analysis of newborn hair may provide information about exposures that happened after the 28th week of gestation, and hair samples could still be collected a few months after birth [74], in contrast to meconium, which is only eliminated once and no more than a few days after birth. However, factors including hair pigmentation, the physicochemical properties of the drug, and the potential for external contamination can complicate the analytical interpretation [2,45]. In addition, practical limitations such as insufficient hair volume and cultural objections to cutting hair may restrict its routine application [73]. Although the collection process is relatively non-invasive, it may still be considered uncomfortable or unacceptable in certain contexts [37,75]. Similarly, neonatal nail analysis provides long-term exposure information over several months, offering insights comparable to those obtained from hair. Like hair, nails incorporate drugs during their formation and growth, thereby reflecting chronic exposure. However, nail testing is less commonly employed in clinical and forensic practices, potentially due to slower growth rates, limited sample availability at birth, and a lack of standardized protocols [2].

Placental tissue, as the primary interface for maternal-fetal exchange, can be used as a matrix for mechanistic insight into drug transfer and metabolism during pregnancy. It expresses metabolic enzymes and transporters that can influence the extent and nature of fetal drug exposure. However, drug concentrations measured in placental tissue often reflect the placental burden rather than systemic fetal exposure. Factors such as heterogeneous vascularization, differential transporter expression, and cellular sequestration can complicate interpretation and reduce consistency across samples. Moreover, as the placenta can only be accessed post-delivery, its utility is limited to retrospective assessments. While highly informative for mechanistic and pharmacokinetic studies, the placenta is not commonly used in routine neonatal drug screening, where matrices like meconium and umbilical cord tissue are preferred for their direct relevance and accessibility [37].

Amniotic fluid, in contrast, provides a more direct means of evaluation of fetal drug exposure, particularly during mid-gestation. Early in pregnancy, amniotic fluid primarily consists of maternal plasma ultrafiltrate; however, following the fusion of the amnion and chorion around weeks 15 and 16, fetal renal output becomes the predominant contributor to fluid volume [76,77]. As a result, drugs and metabolites excreted in fetal urine can accumulate, providing insight into fetal drug exposure during critical developmental windows [78,79]. Studies have reported variable correlations between amniotic fluid concentrations and those found in fetal plasma, fetal tissues, and maternal blood, depending on drug characteristics, gestational age, and the extent of fetal and placental metabolism [80,81]. For example, ritodrine and quetiapine levels in amniotic fluid have shown strong concordance with umbilical cord blood, while other compounds such as diclofenac and amikacin display lower concentrations in amniotic fluid relative to fetal tissue [82,83]. Despite its diagnostic potential, the use of amniotic fluid is constrained by the procedural risks associated with amniocentesis, including miscarriage, membrane rupture, fetal injury, and infection. These risks are elevated when the procedure is performed before 15 weeks of gestation [84,85]. Additionally, the majority of clinically indicated amniocenteses occur in pregnancies with suspected fetal anomalies, which may alter drug metabolism and confound interpretation [86]. Sampling opportunities are further limited in early and late pregnancy. Consequently, while amniotic fluid can provide essential data on in utero drug exposure and maternal-fetal pharmacokinetics, its use is largely confined to research settings or specific clinical contexts where amniocentesis is already warranted.

Human breast milk offers a non-invasive and ethically acceptable matrix for assessing short-term maternal drug use during the lactation period and post-natal exposure to a newborn. Drug transfer into breast milk occurs primarily through passive diffusion, influenced by maternal plasma concentration, drug lipophilicity, protein binding, molecular weight, and the milk's lipid content. Due to its dynamic composition and continuous production, breast milk can reflect acute maternal drug exposure over a short timeframe, typically hours after ingestion [44]. Several drugs of abuse, therapeutic agents, and environmental contaminants have been detected in breast milk, highlighting the importance of understanding lactational transfer and infant safety. However, this matrix also presents limitations, including the variability in milk composition over time (foremilk vs hindmilk), diurnal fluctuations, and inter-individual differences which complicate interpretation [79]. Moreover, comprehensive studies on the full range of drugs in breast milk remain limited, and validated methods for many analytes are still under development [80]. While breast milk can be informative for specific clinical or forensic purposes, its utility is currently restricted to certain drug classes and timeframes [2,66].

9. Effects of prenatal drug exposure on child development, neurobehavior, and risk of future substance use

Prenatal drug exposure is associated with adverse effects on child development, particularly in neurobehavioral and cognitive domains, and also increases the risk of substance use disorders later in life. Exposure to substances such as cocaine, alcohol, cannabis, benzodiazepines, and opioids during critical periods of fetal brain development can disrupt neurodevelopmental processes, leading to lasting physical, cognitive, and behavioral deficits. Neuroimaging and developmental studies report altered brain structure and function in exposed children, often presenting as impairments in attention, emotional regulation, and learning [87]. Environmental factors, including parental substance use and socioeconomic disadvantage, further compound these outcomes [88].

Evidence from multiple studies highlights significant associations between prenatal substance exposure and developmental delays [87]. For example, Jarque et al. (2024) found that male infants exposed to cannabis exhibited more pronounced delays in language acquisition and lower cognitive scores than females [89]. Similarly, cocaine exposure in utero has been linked to impaired executive function, particularly in males [90]. Sanlorenzo et al. (2019) reported a 50 % increase in the odds of pharmacologically treated neonatal abstinence syndrome (NAS) in infants exposed to benzodiazepines [89]. At the same time, Benninger et al. (2020) found that infants with opioid withdrawal syndrome displayed worsening cognitive and language outcomes by the second year of life [90]. Prenatal exposure to cocaine and methadone has also been associated with difficulties in cognitive regulation and processing, poorer perceptual reasoning, impaired physical growth, and delayed sensorimotor development [91,92].

Behavioral disturbances have been linked to elevated biomarkers of prenatal alcohol and drug exposure, with studies reporting increased aggression, delinquency, and early initiation of substance use in adolescence [93]. Similarly, Min and co-workers (2021) identified elevated levels of FAEEs as predictors of increased marijuana use and substance-related problems later in life, linking prenatal alcohol exposure to adverse behavioral outcomes [94]. Two years later, another study found that prenatal cocaine exposure was related to greater adolescent substance use, which was associated with substance use

disorders in emerging adulthood [95]. However, Benett and colleagues (2020) presented a contrasting view, indicating that prenatal cocaine exposure did not predict adolescent substance use [96], suggesting variability in outcomes based on individual circumstances or environmental factors.

Physical health consequences are also evident. Studies have reported associations between prenatal drug or tobacco exposure and altered inflammatory markers [97], as well as elevated blood pressure in adolescents [98]. Additionally, in terms of anthropometric outcomes, three studies identified adverse effects on body development associated with prenatal exposure to cannabis and tobacco. Roca et al. (2021) reported marked differences in the exposed neonates' group to cannabis when compared to the non-exposed neonates [99]. Similarly, Jones and coworkers (2022) noted that prenatal marijuana exposure significantly decreased birth weight, length, and head circumference [101]. Additionally, Hamilton and collaborators (2024) reported that children born to mothers maintained on methadone were almost twice as likely to have significant visual abnormalities [102]. These findings collectively emphasize the importance of addressing physical health risks associated with prenatal substance exposure (Table 2).

10. Conclusion and future directions

Meconium represents a retrospective, non-invasive, and highly informative matrix for assessing prenatal exposure. Its capacity to capture cumulative drug use across late pregnancy makes it invaluable for neonatal care, maternal intervention, and long-term child health monitoring. Current evidence supports its value in detecting both licit and illicit drugs and capturing chronic or repeated maternal use that may otherwise go undetected by conventional matrices such as blood or neonatal urine. As interest in early-life toxicological analysis continues to grow, meconium is expected to remain the preferred matrix for identifying prenatal exposures with implications for neonatal care and long-term child health.

Future research should focus on refining analytical methods to improve sensitivity, selectivity, and, particularly for detecting emerging substances such as NPS (e.g., synthetic opioids, synthetic cannabinoid receptor agonists, semi-synthetic cannabinoids). As a complex biological matrix, sample preparation is imperative to isolate the analytes of interest and remove endogenous components. Likewise, effective extraction methods, combined with highly sensitive and selective analytical techniques, can assist in detecting low concentrations of drugs of clinical or forensic interest.

While advanced techniques such as LC-MS/MS and HRMS have enhanced the ability to identify a broad spectrum of compounds, standardized protocols and harmonized cut-off thresholds are needed to improve cross-study comparability and clinical interpretation. Additionally, elucidating the biological mechanisms of drug incorporation into meconium and the influence of factors such as fetal metabolism, placental transfer dynamics, and gestational age will be essential for improving the interpretative accuracy of results. Furthermore, studies aiming to compare meconium to other biological matrices (e.g., umbilical cord, amniotic fluid) are valuable, but their application can be complex, given that paired samples from mother and child might not be readily available for collection.

Integrating untargeted screening strategies with multi-omics

Table 2
Synoptic Summary of Longitudinal Studies Examining Physical, Neurobehavioral and Cognitive Disorder Outcomes in Infants Prenatally Exposed to Toxic Substances.

Substances	Study design	Exposure assessment	Outcome disorder	References
Alcohol and other psychoactive substances	Cohort study (from birth to 12 years old adolescents)	Meconium and mother self-report	Aggressive and delinquent behavior	[92]
Alcohol and other psychoactive substances	Longitudinal study (from birth to adolescence)	Meconium analyses, maternal self-report of substance use and hospital records	Increased likelihood of substance use problems	[94]
Although detecting several psychoactive substances, focus on prenatal cocaine exposure	Prospective study (from birth to 21 years old adults)	Meconium, urine, and mother self-report	Adolescent substance use disorders	[95]
Cannabis, cocaine, ethanol, and other psychoactive substances	A prospective matched case-control study (from birth to 3 years old)	Meconium toxicological analyses and parents' responses to a questionnaire	Severe neurodevelopmental delays	[103]
Cocaine, alcohol, cannabis, and tobacco	Prospective cohort study (from birth to 21 years old)	Urine and meconium analyses and maternal self-report.	Adult perceptual reasoning IQ	[93]
Focus on cocaine, but records the use of other substances	Longitudinal cohort study (from birth and over the years)	Maternal interview and/or newborn meconium sample analysis.	Poor executive functioning	[87]
Focus on prenatal cocaine exposure	Longitudinal study (from birth to almost 18 years old)	Meconium analyses for cocaine and mother's self-report for other drugs	No predictive relationship for substance use	[96]
Cannabis	Retrospective cohort study	Meconium toxicological analyses	Anthropometric deficits	[101]
Methadone	Observational cohort study (birth and 8–10 years old children)	Toxicological analyses (urine and meconium) from mother and child, and maternal self-report/interview	Visual abnormalities	[102]
Methadone	Prospective study (from birth to 10 years old children)	Maternal urine, infant urine and meconium, maternal case-note review, and confidential interview	Cognitive regulation challenges	[91]
Opioid and benzodiazepine (as a medication or misused)	Retrospective cohort study	Meconium and hospital data about the mother	Increased odds of NAS	[89]
Opioids, benzodiazepines, barbiturates, cannabis, or stimulants (amphetamines, methamphetamines, and cocaine)	Retrospective cohort study (from birth to the first years of life)	Toxicology testing of maternal urine, infant urine, meconium, or umbilical cord or by maternal report.	Children presenting worse cognitive and language outcomes during the second year	[90]
Polysubstance use	Cohort study (from birth to 16 years old adolescents)	Meconium, urine, and mother self-report	Elevated blood pressure	[98]
Several substances	Prospective observational study	Meconium, urine, and hair	Anthropometric deficits	[99]
Tobacco	Prospective study (from birth to 24 months old children)	Meconium analysis and maternal interview	Low birth weight	[100]
Tobacco and cannabis	Longitudinal study (from prenatal to 60-month-old children)	Meconium, oral fluid, and mother self-report	Inflammatory pathway alterations	[97]

approaches may lead to identifying novel biomarkers indicative of exposure, susceptibility, and potential adverse outcomes. Longitudinal studies linking toxicological findings with neurodevelopmental, cognitive, and behavioral trajectories are warranted. These investigations will support early risk stratification and the development of targeted intervention strategies that improve child health outcomes. Looking ahead, incorporating meconium testing into broader maternal and child health frameworks, including digital health platforms, predictive analytics, and policy-driven support pathways, will be critical to maximizing its utility. Continued interdisciplinary collaboration among toxicologists, clinicians, analytical chemists, and public health professionals will be essential to address current challenges and drive innovation. Ultimately, advancing the science and application of meconium analysis holds the promise of improving prenatal care, enhancing child wellbeing, and contributing to evidence-based public health and legal frameworks in the context of substance use during pregnancy.

CRediT authorship contribution statement

Dayanne Mozaner Bordin: Writing – review & editing, Writing – original draft, Supervision, Investigation, Conceptualization. **Eduardo Geraldo de Campos:** Writing – review & editing, Writing – original draft, Conceptualization. **Vítor Luiz Caleffo Piva Bigão:** Writing – review & editing, Writing – original draft. **Nayna Cândida Gomes:** Writing – original draft. **Júlia Soares Melo:** Writing – original draft. **R. Deanna Brambila:** Writing – review & editing, Writing – original draft, Visualization. **Monique G. Mello:** Writing – review & editing, Writing – original draft. **Bruno Spinosa De Martinis:** Writing – review & editing.

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.

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Data availability

No data was used for the research described in the article.

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