Biological Matrices for the Evaluation of In Utero Exposure to Drugs of Abuse

Jaime Lozano, MD, Oscar García-Algar, MD, PhD,* Oriol Vall, MD, PhD,* Rafael de la Torre, PhD,† Giulia Scaravelli, MD, PhD,‡ and Simona Pichini, PhD§*

Abstract: In recent years, the evaluation of in utero exposure to drugs of abuse has been achieved by testing biological matrices coming from the fetus or newborn (eg, meconium, fetal hair, cord blood, neonatal urine), the pregnant or nursing mother (eg, hair, blood, oral fluid, sweat, urine, breast milk), or from both the fetus and the mother (placenta, amniotic fluid). Overall, these matrices have the advantage of noninvasive collection (with the exception of amniotic fluid) and early detection of exposure from different gestational periods. Matrices such as amniotic fluid, meconium, fetal hair, and maternal hair provide a long historical record of prenatal exposure to certain drugs and can account for different periods of gestation: amniotic fluid from the early pregnancy, meconium for the second and third trimester of gestation, fetal hair for the third, and finally maternal hair (when long enough) for the whole pregnancy. Placenta may reveal the passage of a substance from the mother to the fetus. Cord blood and neonatal urine are useful for determining acute exposure to drugs of abuse in the period immediately previous to delivery. Drug detection in maternal blood, oral fluid, and sweat accounts only for acute consumption that occurred in the hours previous to collection and gives poor information concerning fetal exposure. Different immunoassays were used as screening methods for drug testing in the above-reported matrices or as unique analytical investigation tools when chromatographic techniques coupled to mass spectrometry were not commonly available. However, in the last decade, both liquid and gas chromatography-mass spectrometric methodologies have been routinely applied after appropriate extraction of drugs and their metabolites from these biological matrices.

Key Words: drugs of abuse, exposure, fetus, biological matrices

(*Ther Drug Monit* 2007;29:711–734)

BACKGROUND

In utero exposure to drugs of abuse may have consequences not only for the development of the fetus but also for the physiology of some biological systems and neurocognitive aspects of children during later stages in life.[1,2] More than 75% of infants exposed to drugs have major medical problems as compared with 27% of unexposed infants.[4] The cost of treating drug-affected infants is twice the cost of nonaffected infants. Also, obstetric complications are higher among drug abusing mothers,[3] so that assessment of in utero drug exposure is relevant for the care of the mother and the offspring.

In this sense, several approaches are possible, such as monitoring maternal drug consumption by urinalysis (approximately 3 times per wk) and weekly sweat analysis through patches or hair testing (approximately once per trimester of pregnancy). Except for hair analysis, the window of detection of urine and sweat is short, reflecting drug use during the previous few days.[3,4] Moreover, the 3 times a week urine testing and weekly sweat testing are both unrealistic and expensive. Alternatively, the assessment of maternal drug consumption may be investigated by the use of questionnaires regarding the use of drugs of abuse. This method, however, has already shown limitations in a clinical series of approximately 3000 newborns, 43% of whom tested positive for drugs of abuse in different biological matrices, whereas only 11% of mothers recognized drug consumption.[32] Another approach is monitoring exposure to drugs of abuse by testing alternative (also defined as nonconventional) biological matrices coming from the fetus or the newborn (eg, meconium, fetal hair, cord blood, neonatal urine), from the pregnant or nursing mother (eg, hair, blood, oral fluid, sweat, urine, breast milk), or from both fetus and mother (placenta, amniotic fluid). Overall, these matrices have the advantage of noninvasive collection (with the exception of amniotic fluid) and early detection of exposure from different gestational periods. The interrelationship between maternal and fetal exposure to drugs is presented schematically in Figure 1. Biological matrices (other than urine) used for monitoring in utero drug exposure are outlined in the figure, and the respective time windows of drug detection as well as their usefulness in retrospective assessment of in utero exposure to illicit drugs are shown in Figure 2.

The present review focuses on the feasibility and clinical significance of the use of alternate biological matrices for the assessment of in utero exposure to drugs of abuse. Each biological matrix and the respective analytical methodologies

Received for publication June 1, 2007; accepted August 20, 2007.

From the *Unitat de Recerca Infància i Entorn (URIE), Paediatric Service, Hospital del Mar, and Departament de Pediatria, Ginecologia i Obstetricia, i Medicina Preventiva, Universitat Autònoma, Barcelona, Spain; †Unitat de Recerca en Farmacologia, Institut Municipal d'Investigació Mèdica-Hospital del Mar and Departament de Ciències Experimentals i de la Salut, Universitat Pompeu Fabra, Barcelona, Spain; ‡National Centre for Epidemiology, Surveillance and Health Promotion, Istituto Superiore di Sanità, Rome, Italy; and §Department of Therapeutic Research and Medicines Evaluation, Istituto Superiore di Sanità, Rome, Italy.

Reprints: Simona Pichini, PhD, Department of Therapeutic Research and Medicines Evaluation, Istituto Superiore di Sanitá, V.le Regina Elena 299, 00161, Rome, Italy (e-mail: simona.pichini@iss.it).

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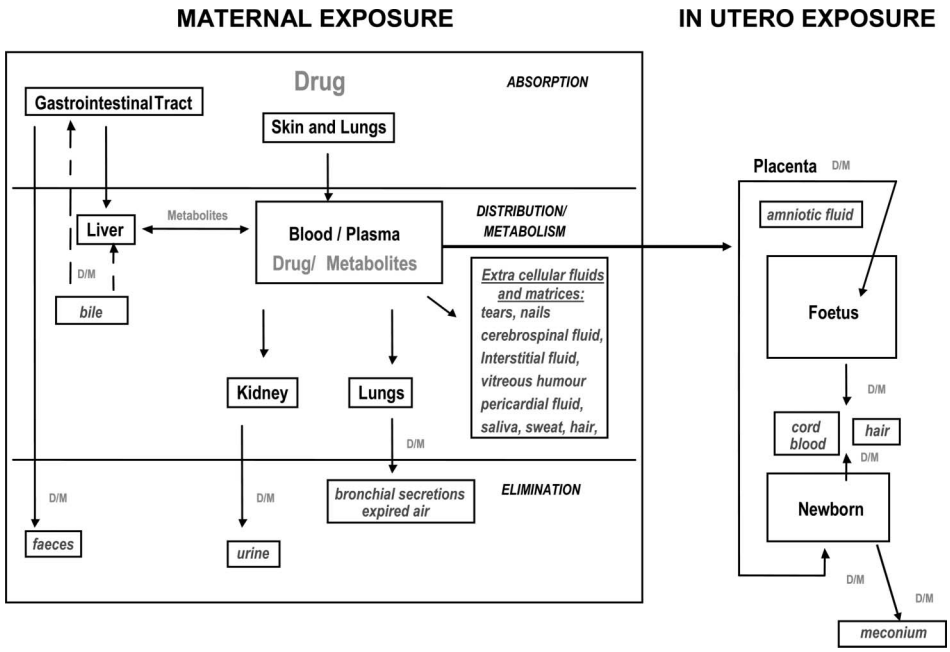

FIGURE 1. Interrelationship between maternal and fetal exposure to drugs.

used for measuring xenobiotics (parent drugs or metabolites) selected as biomarkers of exposure with subsequently related clinical outcomes are briefly described. Matrices are ordered according to first availability in the prenatal and perinatal periods. For each biological fluid and matrix, illicit drugs investigated, parent drugs or metabolites measured, concentrations found, applied methodologies and principal analytical details, and eventual clinical correlates are reported in Table 1.

PLACENTA

The basic structure of human mature placenta formed from trophoblast is complete at 4 weeks of pregnancy. This tissue, a source of fetoplacental circulation, is an interface between maternal and fetal blood, acting as a nutrient and waste exchanger.

It is well known that transplacental passage of drugs and other chemicals is influenced by physicochemical properties of the drug, mechanisms of placental transport, and metabolism of the substances. Fetal blood concentration of drugs given to the mother is dependent on the time elapsed since the maternal drug use, dose, placental blood flow and permeability, plasma protein binding, blood pH, placental biotransformation, and fetal elimination.[5] Moreover, drugs and chemicals can affect the nutrient transport systems in the placenta, modifying placental physiology.[6]

Placental research showed that fatty acid ethyl esters (FAEEs), the biomarkers of ethanol intake during pregnancy originating from alcoholic mothers, are not transferred to the fetus because they are taken up and degraded extensively by the human placenta. Therefore, FAEEs detected in neonatal matrices are likely produced by the fetus from ethanol that has been transferred to and metabolized by the fetus him herself, rendering FAEEs powerful direct biomarkers reflective of true fetal exposure to ethanol in utero.[7]

Placental studies also serve to prove the effect of chronic drug passage through this tissue and the effects on placental morphology as well as the possible subsequent effects on fetal development. Although placenta is not perceived as a metabolizing organ, there have been instances when the enzyme activity in the placenta has been induced, for instance, in the case of maternal smoking.[8] Ostrea et al[9] studied the effect of chronic maternal drug addiction on placental drug metabolism and concluded that, for the most common drugs of abuse, placental metabolism is not induced even in the drug-dependent mother.

Two adverse birth outcomes were observed among six newborns prenatally exposed to arecoline, the principal alkaloid of the sliced nut of the areca palm (*Areca catechu*), the fourth most commonly used drug in the world after tobacco, alcohol, and caffeine.[10,11] Placental concentration of arecoline was measured by using liquid chromatography-mass spectometry (LC-MS), and the analyte was found in five of the six samples analyzed. Focal inflammatory changes in the amniochorial membranes were observed in the placentas in two cases, and, in one case, a decreased median diameter of the vessels in both maternal and fetal surface villi was also observed.

AMNIOTIC FLUID

Up to 20 weeks gestation, maternal secretions are the most important mechanism of amniotic fluid formation, with some additional secretion components from the fetus. After this period, fetal urine and lung fluid secretion become the two primary sources of amniotic fluid, with fetal swallowing and intramembranous absorption as the two primary routes of amniotic water clearance. More than any other compartment, the amniotic fluid accumulates water soluble drugs; it may also contain to some extent parent compounds and their

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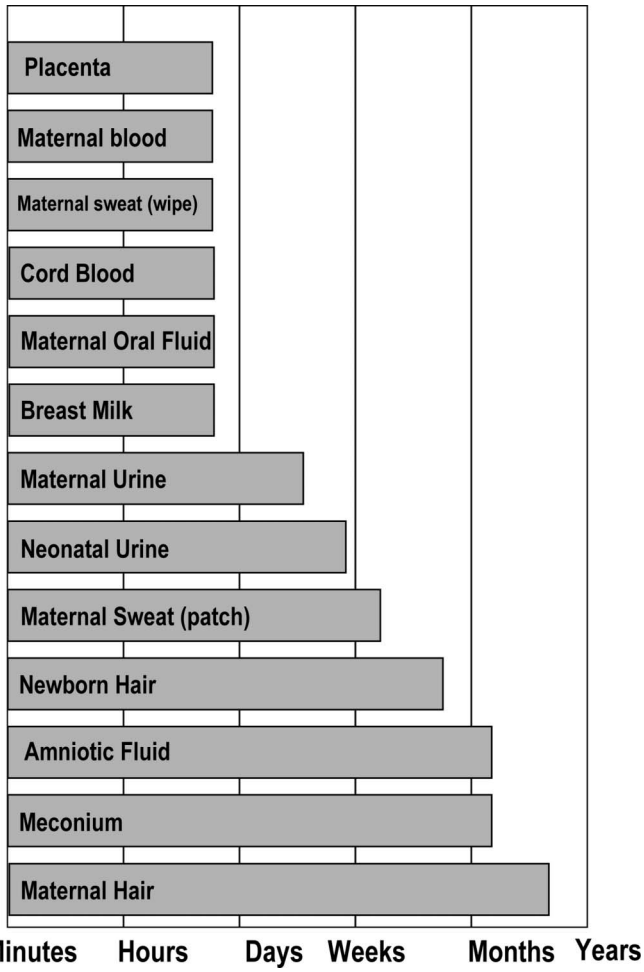

FIGURE 2. Time window of drug detection for different maternal, fetal, and neonatal biological matrices in the assessment of in utero exposure to drugs.

metabolites that are not water soluble. At 10 weeks gestation, the volume of amniotic fluid is 30 mL, which increases up to 800 to 1000 mL at week 37.[12] Because amniotic fluid is already formed in the first weeks of pregnancy, the presence of drugs in this fluid can account for an exposure during the early fetal life.[13–15] Although it is quite dangerous for the fetus, amniotic fluid can be sampled at any time during pregnancy so that the detection of parent drugs and metabolites in the fluid can indicate that the fetus is continuously exposed to the substances detected in the maternal circulation. Studies have been focused on detection of prenatal exposure to cocaine (COC). Both parent drugs and metabolites have been identified with concentrations in the range of nanograms per milliliter of fluid. Although theoretically there are no restrictions in the amount of fluid to be sampled, normally only a few milliliters of fluid collected during amniocentesis are tested.

For the first time in 1992, Ripple et al[16] collected 450 human amniotic fluid samples after 13 to 39 weeks of gestation and screened for the COC metabolite benzoylecgonine (BZE) using fluorescence polarization immunoassay (FPIA). All positive samples, as well as any accompanying maternal serum, were confirmed by gas chromatography (GC)-MS for COC and its metabolites. Five of 450 samples were positive for COC, BZE, and ecgonine methyl ester (EME), whereas one sample showed traces of cocaethylene (CE).

COC and it metabolites were also detected in amniotic fluid of exposed fetuses at extremely high concentrations by Jain et al.[17] With use of a modified solid phase extraction (SPE) procedure, COC and its metabolites were isolated from amniotic fluid to be subsequently analyzed by high-performance liquid chromatography (HPLC). COC or its principal metabolite BZE were detected in 74% of amniotic fluid samples collected from the 23 abusing mothers. Also, 61% and 35% of maternal and neonatal urine samples, respectively, were positive for BZE on routine urine testing. Concentrations in amniotic fluid ranged from 400 to greater than 5000 ng/mL for BZE and from traces to 250 ng/mL for COC.

Recently, Eyler et al[18] found that 56% of a series of 16 amniotic fluid samples were positive for COC and its metabolites. After a SPE procedure, all samples were first screened for the presence of drugs using HPLC, and those that were positive were confirmed with GC-MS using a methodology previously reported by the same authors.[19] Of the 11 mothers who admitted to COC use, 64% had positive specimens, and of the 5 women denying use, 40% were positive. The authors also analyzed cord blood, infant urine, meconium, and maternal hair from the same mother-infant dyads and concluded that none of the different biological specimens had the ability to identify all drug users, but each matrix had different advantages and disadvantages.

Although amniotic fluid analysis has been used to confirm fetal exposure to COC, this biological fluid has not gained popularity as a practical tool for identifying prenatal drug exposure. The most important reason is the difficulty encountered in the collection of this specimen, which requires an invasive procedure that can be harmful to the fetus, unless it is collected at birth.

MECONIUM

Meconium is the first fecal matter passed by a neonate and pertains to a large time window in prenatal metabolism.[20] Its formation starts between the 12th and 16th week of gestation, and it consists of materials ingested during the time the infant spends in the uterus: intestinal epithelial cells, lanugo, mucus, amniotic fluid, bile, and water. It is usually accumulated and confined in the fetal bowel until birth. Meconium can be collected between 1 and 5 days after birth, and its analysis allows the detection of maternal drug use during the last 20 weeks of gestation, approximately, and therefore provides information on fetal chronic drug exposure.[21–23] In this respect, meconium testing provides more complete information on drug exposure during pregnancy than neonatal urine or cord blood analyses. In recent years, drug determination in meconium has been successfully applied to assess intrauterine exposure to drugs and has provided the basis for appropriate treatment and follow-up of newborns who present signs of drug withdrawal and impairment of physical and mental development.[10,24–26]

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TABLE 1. Biological Matrices for the Evaluation of In Utero Exposure to Drugs of Abuse, Illicit Drugs Investigated, Parent Drugs and/our Metabolites Measured, Concentrations Found, Applied Methodologies and Principal Analytical Details, Possible Clinical and Socio-demographic Correlates

Drug	Range of Concentration	Extraction Method	Assay Method	Limit of Detection - LOD, and/or Limit of Quantification - LOQ (Cut off in Case of Immunological Methods)	Correlation With Clinical Outcomes and/or Socio-demographic Status	Reference
Placenta						
Arecoline	Arecoline: 0.009–0.015 μg/g	Liq-Liq	LC-MS	LOQ: 0.004 μg/g	Neonatal withdrawal, low birth weight	10
Amniotic fluid						
Cocaine	BZE: 0–836 ng/mL COC: 0–24 ng/mL EME: 0–34 ng/mL CE: traces	SPE	FPIA/GC-MS	LOD: 5 ng/mL LOQ: 10 ng/mL	NA	16
Cocaine	COC: trace to 250 ng/mL BZE: 400–5000 ng/mL	SPE	HPLC	NA	NA	17
Cocaine	NA	SPE	HPLC/GC-MS	LOD/LOQ (ng/mL) BZE: 5/10 EME: 5/10 EEE: 50/50 NCOC: 5/10 CE: 5/10 m-OH-BZE: 10/25	NA	18, 19
Meconium						
Cocaine	NA	NP	RIA	Cut off: 15 ng/mL†	Marital status Obstetric/perinetal antecedents	34
Opiates	NA	NP	RIA	Cut off: 25 ng/mL†	Marital status Obstetric/perinetal antecedents	34
Cannabis	NA	NP	RIA	Cut off: 50 ng/mL†	Marital status Obstetric/perinetal antecedents	34
Cocaine	NA	Liq-Liq	RIA/EMIT/FPIA	Cut off: 50 ng/mL†	NA	35
Opiates	NA	Liq-Liq	RIA/EMIT/FPIA	Cut off: 60 ng/mL†	NA	35
Cannabis	NA	Liq-Liq	RIA/EMIT/FPIA	Cut off: 50 ng/mL†	NA	35
Cocaine	NA	SPE	HPLC/GC-MS	LOD: 200 ng/mL$	NA	36
Opiates	NA	SPE	HPLC/GC-MS	LOD: 200 ng/mL$	NA	36
Cocaine	NA	NA	RIA/GC-MS	Cut off: 25 ng/mL†	NA	38
Opiates	NA	NA	RIA/GC-MS	Cut off: 25 ng/mL†	NA	38
Cannabis	NA	NA	RIA/GC-MS	Cut off: 15 ng/mL†	NA	38
Cocaine	NA	—	RIA	Cut off: 300 ng/mL§§	Pregnancy complications	39
Opiates	NA	—	RIA	Cut off: 300 ng/mL§§	Pregnancy complications	39
Cannabis	NA	—	RIA	Cut off: 100 ng/mL§§	Pregnancy complications	39
Cocaine	COC: 0.1–0.78 μg/g	SPE	HPLC/GC-MS	NA	Prematurity	40
Cocaine	COC: 0.24–0.78 μg/g NCOC: 0.10–0.56 μg/g CE: 0.12 μg/g	SPE	HPLC/GC-MS	NA	Prematurity Low birth weight	41
Cocaine	BZE: 0.04–1.9 μg/mL*	SPE	FPIA/GC-MS	Cut off: 0.10 μg/mL*	Prenatal care Low birth weight	42

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TABLE 1. (*continued*) Biological Matrices for the Evaluation of In Utero Exposure to Drugs of Abuse, Illicit Drugs Investigated, Parent Drugs and/our Metabolites Measured, Concentrations Found, Applied Methodologies and Principal Analytical Details, Possible Clinical and Socio-demographic Correlates

Drug	Range of Concentration	Extraction Method	Assay Method	Limit of Detection - LOD, and/or Limit of Quantification - LOQ (Cut off in Case of Immunological Methods)	Correlation With Clinical Outcomes and/or Socio-demographic Status	Reference
					Obstetrics antecedents Demographic variables	
Cocaine	NA	Liq-Liq	EMIT/GC-MS/TLC	Cut off: 150 ng/g	Low birth weight Low head circumference Prematurity	44
Cannabis	NA	Liq-Liq	EMIT/GC-MS/TLC	Cut off: 50 ng/g	ND	44
Cocaine	NA	SPE	FPIA/GC-MS	Cut off: 0.06 μg/g	Race, social level Mother antecedents Obstetric antecedents	45
Cocaine	NA	Liq-Liq	FPIA/GC-MS	Cut off: 5 ng/g	NA	45
Opiates	NA	Liq-Liq	FPIA/GC-MS	Cut off: 5 ng/g	NA	46
Amphetamine	NA	Liq-Liq	FPIA/GC-MS	Cut off: 5 ng/g	NA	46
Cannabis	NA	Liq-Liq	FPIA/GC-MS	Cut off: 2 ng/g	NA	46
Benzodiazepines	NA	Liq-Liq	EMIT/GC-MS	Cut off: 200 μg/mL†	NA	47
Cocaine	NA	Liq-Liq	EMIT/GC-MS	Cut off: 300 μg/mL†	NA	47
Opiates	NA	Liq-Liq	EMIT/GC-MS	Cut off: 300 μg/mL†	NA	47
Cannabis	NA	Liq-Liq	EMIT/GC-MS	Cut off: 50 μg/mL†	NA	47
Methadone	MET: 127–10.222 ng/g EDDP: 153–74.336 ng/g	SPE	FPIA/HPLC	LOD (ng/g) MET: 99 EDDP: 113	NA	48
Cocaine	AEME: 12.4–177.9 ng/g EME: 76.5–5672.1 ng/g EEE: 0–36.7 ng/g COC: 44.2–1294.3 ng/g CE: 0–31.4 ng/g BZE: 52.7–6370.0 ng/g NCOC: 0–122.9 ng/g NCE: 0–28.6 ng/g BNE: 0–519.3 ng/g m-HOCOC: 0–167.8 ng/g p-HOCOC: 0–30.7 ng/g m-HOBZE: 47.8–1061.1 ng/g p-HOBZE: 0–709.3 ng/g	SPE	GC-MS	LOD: 7.5 ng/g for all the analytes under investigation	NA	49
Cocaine	NA	SPE	HPLC/GC-MS	NA	NA	18
Cocaine	AECG: 0–12.905 μg/g AEME: 0–0.4194 μg/g ECG: 0–129.580 μg/g EME: 0–6.885 μg/g EEE: 0–0.145 μg/g COC: 0–0.570 μg/g CE: 0–0.582 μg/g BZE: 0–4.091 μg/g BN: 0–59.995 μg/g	SPE	LC-MS	NA	NA	50

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TABLE 1. (*continued*) Biological Matrices for the Evaluation of In Utero Exposure to Drugs of Abuse, Illicit Drugs Investigated, Parent Drugs and/our Metabolites Measured, Concentrations Found, Applied Methodologies and Principal Analytical Details, Possible Clinical and Socio-demographic Correlates

Drug	Range of Concentration	Extraction Method	Assay Method	Limit of Detection - LOD, and/or Limit of Quantification - LOQ (Cut off in Case of Immunological Methods)	Correlation With Clinical Outcomes and/or Socio-demographic Status	Reference
	NCOC: 0–2.145 μg/g NCE: 0–1.516 μg/g m-OHCOC: 0–0.803 μg/g p-OHCOC: 0–0.683 μg/g m-OHBZE: 0–4.337 μg/g p-OHBZE: 0–3.464 μg/g CNO: 0–0.538 μg/g					
Cannabis	THC: 0–7 ng/g THC-OH: 0–929 ng/g diTHC-OH: 0–68.6 ng/g THC-COOH: 0–219 ng/g	Liq-Liq	EMIT/GC-MS	Cut off = 20 ng/g LOD (ng/g) THC: 5 THC-OH: 10 diTHC-OH: 5 THC-COOH: 2	NA	28
Cannabis	THC-OH: 0–48.1 ng/g diTHC-OH: 0–8.9 ng/g THC-COOH: 0–30.2 ng/g	Immuno-Affinity extraction	GC-MS	LOD (ng/g) THC-OH: 1 diTHC-OH: 2.5 THC-COOH: 1	NA	30
Cannabis	NA	SPE	GC-MS	LOD (ng/g) THC-OH: 5 THC-COOH: 5	NA	51
Cocaine	NA	SPE	EMIT/GC-MS	Cut off = 200 ng/g LOD (ng/g) COC and metabolites: 50	Birth weight Maternal medical conditions Pregnancy/labor and delivery characteristics Maternal report of marijuana, alcohol and tobacco	52–54
Opiates	NA	SPE	EMIT/GC-MS	Cut off = 200 ng/g LOD (ng/g) Opiate metabolites: 50	Birth weight Maternal report of marijuana, alcohol and tobacco	52–54
Cannabis	NA	SPE	EMIT/GC-MS	Cut off = 20 ng/g LOD (ng/g) THC-COOH: 5	NA	52
Amphetamines	NA	SPE	EMIT/GC-MS	Cut off = 200 ng/g LOD (ng/g) Amphetamine metabolites: 50	NA	52
Phencyclidine	NA	SPE	EMIT/GC-MS	Cut off = 20 ng/g LOD (ng/g) PCP: 3	NA	52

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TABLE 1. (*continued*) Biological Matrices for the Evaluation of In Utero Exposure to Drugs of Abuse, Illicit Drugs Investigated, Parent Drugs and/our Metabolites Measured, Concentrations Found, Applied Methodologies and Principal Analytical Details, Possible Clinical and Socio-demographic Correlates

Drug	Range of Concentration	Extraction Method	Assay Method	Limit of Detection - LOD, and/or Limit of Quantification - LOQ (Cut off in Case of Immunological Methods)	Correlation With Clinical Outcomes and/or Socio-demographic Status	Reference
Cocaine	COC: 0–0.903 μg/g BZE: 0–0.847 μg/g CE: 0–0.051 μg/g m-OHBZE: 0–0.312 μg/g p-OHBZE: 0–0.319 μg/g	SPE	LC-MS	LOD/LOQ (μg/g) COC: 0.0009/0.003 BZE: 0.0012/0.004 CE: 0.0012/0.004 m-OHBZE: 0.0004/0.0013 p-OHBZE: 0.0015/0.0045	Active tobacco smoking, higher number of smoked cigarettes, cannabis use, lower birth weight for simultaneous COC and opiates exposure	24, 27, 55
Opiates	6-MAM: 0–0.142 μg/g MOR: 0–0.397 μg/g COD: 0–0.048 μg/g M3G: 0–0.120 μg/g M6G: 0–0.091 μg/g	SPE	LC-MS	LOD/LOQ (μg/g) 6-MAM: 0.0003/0.001 MOR: 0.0012/0.004 M3G: 0.0012/0.004 M6G: 0.0003/0.001 COD: 0.0012/0.004	Active tobacco smoking, higher number of smoked cigarettes, cannabis use lower birth weight for simultaneous COC and opiates exposure	24, 55
Methylenedioxy derivates of amphetamine	MDMA: 0.012 ug/g	SPE	LC-MS	LOD/LOQ (μg/g) 0.001/0.004	NA	26
Arecoline	Arecoline: 0.006–0.022 μg/g	Liq-Liq	LC-MS	LOD/LOQ (μg/g) 0.001/0.005	low birth weight, low intrauterine growth, small for gestational age, hyporeflexia, hypotonia, withdrawal syndrome	10, 25
Cannabis	THC: 25.6–81.2 ng/g THC-OH: 20.7–493.3 ng/g THC-COOH: 39.2–182.1 ng/g	Liq-Liq	GC-MS	LOD/LOQ (ng/g) THC:7/20 THC-OH: 7/20 THC-COOH: 7/20	Maternal profession	56, 57
Cocaine	EME: 286–392 ng/g BZE: 241–341 ng/g	Liq-Liq	FPIA-EMIT/GC-MS	Cut off: 300 ng/mL†	NA	58
Opiates	6-MAM: ND MOR: 12–2125 ng/g COD: 15–162 ng/g	Liq-Liq	FPIA-EMIT/GC-MS	Cut off: 300 ng/mL†	Neonatal withdrawal syndrome	58, 59
Cannabis	THC-COOH: 3.5–184ng/g	Liq-Liq	FPIA-EMIT/GC-MS	Cut off: 50 ng/mL†	NA	58
Methadone	MET: 171–1843 ng/g EDDP: 992–9851ng/g		FPIA-EMIT/GC-MS	Cut off: 300 ng/mL†	Neonatal withdrawal syndrome	58
Cocaine	NA	Liq-Liq	ELISA/GC-MS	LOD: 50 ng/g	NA	60
Opiates	NA	Liq-Liq	ELISA/GC-MS	LOD: 50 ng/g	NA	60
Cannabis	NA	Liq-Liq	ELISA/GC-MS	LOD: 50 ng/g	NA	60
Methadone	NA	Liq-Liq	ELISA/GC-MS	LOD: 50 ng/g	NA	60
Benzodiazepines	NA	Liq-Liq	ELISA/GC-MS	LOD: 50 ng/g	NA	60
Barbiturates	NA	Liq-Liq	ELISA/GC-MS	LOD: 50 ng/g	NA	60

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Drug	Range of Concentration	Extraction Method	Assay Method	Limit of Detection - LOD, and/or Limit of Quantification - LOQ (Cut off in Case of Immunological Methods)	Correlation With Clinical Outcomes and/or Socio-demographic Status	Reference
Amphetamines	NA	NA	EMIT/GC-MS	Cut off: 500 ng/g	Birth weight Gestational age Marital status Maternal age Obstetrics antecedents	61
Cocaine	NA	NA	EMIT/GC-MS	Cut off: 75 ng/g	Birth weight Gestational age Marital status Maternal age Obstetrics antecedents	61
Cannabis	NA	NA	EMIT/GC-MS	Cut off: 40 ng/g	Birth weight Gestational age Marital status Maternal age Obstetrics antecedents	61
Alcohol	Laurate:0–0.429 μg/g Myristate: 0–0.794 μg/g Palmitate: 0–1.746 μg/g Stearate: 0–0.934 μg/g Oleate: 0–0.16.58 μg/g Linoleate: 0–5.715 μg/g Arachidonate: 0–1.168 μg/g	SPE	GC-MS	LOD: 0.05–0.2 μg/g	Maternal drinking history	66
Alcohol	Total FAEEs: 1059–62115 ng/g 1139–50143 ng/g	SPE	GC-MS	LOD: 50 ng/g	NA	64
Alcohol	Total FAEEs: 0–362.9 nmol/g	SPE	GC-FID	LOD: 0.16 to 0.22 nmol/g LOQ: 0.32 to 0.44 nmol/g	Maternal drinking history	72
Alcohol	Myristate: 0–21461 ng/g Palmitate: 0–27245 ng/g Palmitoleate: 65–50241 ng/g Oleate: 0–344047 ng/g Linoleate: 0–627705 ng/g Linoleneate: 0–173558 ng/g Arachidonate: 0–23560 ng/g	Liq-Liq/SPE	GC-FID	NA	Maternal drinking history	65
Neonatal hair						
Cocaine	BZE: 0.71–2.47 ng/mg	Liq-Liq	GC-MS	LOD: 0.1 ng/mg	Gestational history of drug abuse	79
Heroin	MOR: 0.61–3.47 ng/mg	Liq-Liq	GC-MS	LOD: 0.1 ng/mg	Gestational history of drug abuse	79
Benzodiazepines	Diazepam: 3.36–17.55 ng/mg Oxazepam: 0.78–31.83 ng/mg	Liq-Liq	GC-MS	LOD: 0.1 ng/mg	Gestational history of drug abuse	79
Amphetamine	AP: 1.21 ng/mg	Liq-Liq	GC-MS	LOD: 0.1 ng/mg	Gestational history of drug abuse	79
Cocaine	BZE: 0.72–5.44 ng/mg	Liq-Liq	RIA	LOD: 32 ng/mL†	Weight Head circunference Apgar scores	80
Cocaine	BZE: 0–5.54 ng/mg	Liq-Liq	RIA/GC-MS	LOD: 5 ng/mL†	Maternal age	81

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Drug	Range of Concentration	Extraction Method	Assay Method	Limit of Detection - LOD, and/or Limit of Quantification - LOQ (Cut off in Case of Immunological Methods)	Correlation With Clinical Outcomes and/or Socio-demographic Status	Reference
					Obstetric antecedents Somatometric values	
Cocaine	COC: 2.5–4 ng/mg	Liq-Liq/SPE	RIA GC-MS	Cut off: 2 ng/mg	Gestational age Birth weight Respiratory distress Malformations	82
Cocaine	BZE: 4.37 + 12.5 ng/mg	Liq-Liq	RIA	LOD: 0.25 ng/mg	Gestational history of drug abuse Maternal medical conditions Newborn somatometry	83
Cocaine	NA	Liq-Liq	RIA	LOD: 0.10 ng/mg	Perinatal complications Maternal smoking Impaired neurobevavioral development	84
Cocaine	NA	SPE	LC-MS	LOQ: COC: 0.05 ng/mg BZE: 0.02 ng/mg	Gestational age Maternal age education level race	85
Cocaine	NA	Liq-Liq	EMIT/GC-MS	LOD: 300 μg/mL	NA	47
Opiates	NA	Liq-Liq	EMIT/GC-MS	LOD: 300 μg/mL	NA	47
Cannabis	NA	Liq-Liq	EMIT/GC-MS	LOD: 50 μg/mL	NA	47
Benzodiazepines	NA	Liq-Liq	EMIT/GC-MS	LOD: 200 μg/mL	NA	47
Cocaine	COC: <2–83.5 ng/mg EME: 2–8 ng/mg	NA	FPIA/EMIT GC-MS	LOD: 1 ng/mg LOQ: 2 ng/mg	NA	56
Opiates	6-MAM: <2–32.6 ng/mg MOR: <2–16.9 ng/mg COD: <2–41 ng/mg	NA	FPIA/EMIT GC-MS	LOD: 1 ng/mg LOQ: 2 ng/mg	Neonatal withdrawal syndrome	56, 57
Cannabis	THC: <0.2–7.18 ng/mg CBN: <0.2–4.01 ng/mg CBD: <0.2–26.22 ng/mg	NA	FPIA/EMIT GC-MS	LOD: 0.1 ng/mg LOQ: 0.2 ng/mg	NA	56
Methadone	MET: <12.5–46.4 ng/mg EDDP: <12.5 ng/mg	NA	FPIA/EMIT GC-MS	LOD: 10 ng/mg LOQ: 12.5 ng/mg	Neonatal withdrawal syndrome	56
Cocaine	NA	NA	ELISA/GC-MS	LOD: 0.2 ng/mg	NA	58
Opiates	NA	NA	ELISA/GC-MS	LOD: 0.2 ng/mg	NA	58
Cannabis	NA	NA	ELISA/GC-MS	LOD: 0.2 ng/g	NA	58
Methadone	NA	NA	ELISA/GC-MS	LOD: 0.2 ng/g	NA	58
Benzodiazepines	NA	NA	ELISA/GC-MS	LOD: 0.2 ng/g	NA	58
Barbiturates	NA	NA	ELISA/GC-MS	LOD: 0.2 ng/g	NA	58
Neonatal urine						
Cocaine	NA	Liq	EMIT/GC-MS/LTC	Cut off: 300 ng/mL	Gestational Age Somatometric values	44

(*continued on next page*)

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TABLE 1. (*continued*) Biological Matrices for the Evaluation of In Utero Exposure to Drugs of Abuse, Illicit Drugs Investigated, Parent Drugs and/our Metabolites Measured, Concentrations Found, Applied Methodologies and Principal Analytical Details, Possible Clinical and Socio-demographic Correlates

Drug	Range of Concentration	Extraction Method	Assay Method	Limit of Detection - LOD, and/or Limit of Quantification - LOQ (Cut off in Case of Immunological Methods)	Correlation With Clinical Outcomes and/or Socio-demographic Status	Reference
Cannabis	NA	Liq	EMIT/GC-MS/LTC	Cut off: 100 ng/mL	Gestational Age Somatometric values	44
Cocaine	NA	Liq-Liq	EMIT/GC-MS	Cut off: 300 μg/mL	NA	47
Opiates	NA	Liq-Liq	EMIT/GC-MS	Cut off: 300 μg/mL	NA	47
Benzodiazepines	NA	Liq-Liq	EMIT/GC-MS	Cut off: 200 μg/mL	NA	47
Cannabis	NA	Liq-Liq	EMIT/GC-MS	Cut off: 50 μg/mL	NA	47
Methadone	MET: 168–3360 ng/mL	NP	RIA	Cut off: 5 ng/mL	Neonatal withdrawal syndrome	91
Cocaine	EME: 545 μg/g creatinine BZE: 13736 μg/g creatinine	Liq-Liq	FPIA-EMIT/GC-MS	Cut off: 300 ng/mL	NA	58
Opiates	6-MAM: ND MOR: ND–4854 μg/g creatinine COD: ND–6416 μg/g creatinine	Liq-Liq	FPIA-EMIT/GC-MS	Cut off: 300 ng/mL	Neonatal withdrawal syndrome	58, 59
Cannabis	THC-COOH: ND–37.5 μg/g creatinine	Liq-Liq	FPIA-EMIT/GC-MS	Cut off: 50 ng/mL	NA	57
Methadone	MET: ND–9042 μg/g creatinine EDDP: :ND–20841 μg/g creatinine		FPIA-EMIT/GC-MS	Cut off: 300 ng/mL	Neonatal withdrawal syndrome	57
Cocaine	NA	SPE	HPLC/GC-MS	NA	NA	18
Arecoline	Arecoline: 0.01 μg/mL	Liq-Liq	LC-MS	LOD/LOQ (μg/mL) 0.0004/0.001	Low birth weight, low intrauterine growth, small for gestational age, hyporeflexia, hypotonia, withdrawal syndrome	25
Cord blood						
Cocaine	COC: ND BZE: traces	SPE	HPLC	NA	Premature delivery	94
Cocaine	BZE: 0–1237 ng/mL EME: 0–52 ng/mL EEE: ND NCOC: 0–172 ng/mL CE: ND m-OH-BZE: 0-trace	SPE	HPLC/GC-MS	LOD/LOQ (ng/mL) BZE: 2.5/2.5 EME: 5/10 EEE: 25/50 NCOC: 10/25 CE: 2.5/5 m-OH-BZE: 5/10	NA	19
Cocaine	COC: 5–88 ng/mL BZE: 74–3880 ng/mL CE: ND	Liq-Liq	GC-MS	LOQ (ng/mL) COC: 5 BZE: 10 CE: 10	Birth weight Head circumference African-American race Prenatal care	96

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TABLE 1. (*continued*) Biological Matrices for the Evaluation of In Utero Exposure to Drugs of Abuse, Illicit Drugs Investigated, Parent Drugs and/our Metabolites Measured, Concentrations Found, Applied Methodologies and Principal Analytical Details, Possible Clinical and Socio-demographic Correlates

Drug	Range of Concentration	Extraction Method	Assay Method	Limit of Detection - LOD, and/or Limit of Quantification - LOQ (Cut off in Case of Immunological Methods)	Correlation With Clinical Outcomes and/or Socio-demographic Status	Reference
Arecoline	Arecoline: 0.005–1.00 μg/mL	Liq-Liq	LC-MS	LOD/LOQ (μg/g) 0.001/0.004	Low birth weight, low intrauterine growth, small for gestational age, hyporeflexia, hypotonia, withdrawal syndrome	25
Maternal hair						
Cocaine	COC: 38.0 + 37.2 ng/mg	Liq-Liq	RIA	NA	NA	98
Cocaine	BZE: 0.8–2.3 ng/mg	Liq-Liq	RIA	NA	Thermal desorption GC-MS	99
Cocaine	BZE: 2.38–23.7 ng/mg	Liq-Liq	RIA/GC-MS	LOD: 5 ng/mL†	Maternal age Obstetric antecedents Somatometric values	81
Maternal urine						
Cocaine	NA	Liq-Liq	EMIT/GC-MS/LTC	Cut off: 300 ng/mL	Gestational age Somatometric values	44
Cannabis	NA	Liq-Liq	EMIT/GC-MS/LTC	Cut off: 100 ng/mL	Gestational age Somatometric values	44
Cocaine	EME: 32.7–48 μg/g creatinine BZE: 312–1965 μg/g creatinine	Liq-Liq	FPIA-EMIT/GC-MS	Cut off: 300 ng/mL	NA	58
Opiates	6-MAM: ND MOR: ND–2909 μg/g creatinine COD: ND–8666 μg/g creatinine	Liq-Liq	FPIA-EMIT/GC-MS	Cut off: 300 ng/mL	Neonatal withdrawal syndrome	58, 59
Cannabis	THC-COOH: 13.5–685 μg/g creatinine	Liq-Liq	FPIA-EMIT/GC-MS	Cut off: 50 ng/mL	NA	58
Methadone	MET: 98–2925 μg/g creatinine EDDP: ND–13575 μg/g creatinine		FPIA-EMIT/GC-MS	Cut off: 300 ng/mL	Neonatal withdrawal syndrome	58
Maternal blood						
Methadone	MET: 183 ± 118 ng/mL	Liq-Liq	GC-MS	NA	Neonatal withdrawal syndrome	100
Cocaine	NA	NA	EMIT	Cut off: 300 ng/mL	Maternal nutritional status	101

(*continued on next page*)

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TABLE 1. (*continued*) Biological Matrices for the Evaluation of In Utero Exposure to Drugs of Abuse, Illicit Drugs Investigated, Parent Drugs and/our Metabolites Measured, Concentrations Found, Applied Methodologies and Principal Analytical Details, Possible Clinical and Socio-demographic Correlates

Drug	Range of Concentration	Extraction Method	Assay Method	Limit of Detection - LOD, and/or Limit of Quantification - LOQ (Cut off in Case of Immunological Methods)	Correlation With Clinical Outcomes and/or Socio-demographic Status	Reference
Cannabinoids	NA	NA	EMIT	Cut off: 100 ng/mL	Maternal nutritional status	101
Phencyclidine	NA	NA	EMIT	Cut off: 25 ng/mL	Maternal nutritional status	101

NA, not available; ND, not detected; NP, not performed.

beta,11-dihydroxy-Δ^9-tetrahydrocannabinol (diTHC-OH), 11-hydroxy-Δ^9-tetrahydrocannabinol (THC-OH), 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH), 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), 6-monoacetylmorphine (6-MAM), amphetamine (AP), anhydroecgonine methyl ester (AEME), anhydroecgonine (AECG), benzoylnorecgonine (BN), benzodiazepines (BZDs), benzoylecgonine (BZE), benzoylnorecgonine (BNE), caffeine (CAF), cannabidiol (CBD), cannabinol (CBN), cocaethylene (CE), cocaine (COC), cocaine-N-oxide (CNO), codeine (COD), cotinine (COT), cotinine-N-oxide (COT-N-OX), ecgonine (ECG) ecgonine ethyl ester (EEE), ecgonine methyl ester (EME), electrospray ionization (ESI), enzyme immunoassay (EIA), enzyme linked immuno-sorbent assay (ELISA), enzyme multiplied immunoassay technique (EMIT), fatty acid ethyl esters (FAEEs), fluorescence polarization immunoassay (FPIA), gas chromatography-flame ionization detection (GC-FID), gas chromatography-mass spectrometry (GC-MS), high performance liquid chromatography mass spectrometry and liquid chromatography mass spectrometry (LC-MS), high-performance liquid chromatography (HPLC), hydrocodone (HYC), hydromorphone (HYM), liquid-liquid (Liq-Liq), electron impact ionization (EI), methadone (MET), methamphetamine (MA), methylendioxymethamphetamine (MDMA), m-hydroxybenzoylecgonine (m-OH-BZE), m-hydroxycocaine (m-OH-COC), morphine (MOR), morphine-3-glucuronide (M3G), morphine-6-glucuronide (M6G), nicotine (NIC), norcocaethylene (NCE), norcocaine (NCOC), p-hydroxybenzoylecgonine (p-OH-BZE), phencyclidine (PCP), p-hydroxycocaine (p-OH-COC), positive chemical ionization (PCI), radioimmunoassay (RIA), selected ion-monitoring (SIM), solid phase extraction (SPE), delta-9-tetrahydrocannabinol (THC), thin layer chromatography (TLC), trans-3-hydroxycotinine (TRANS-3-OH-COT).

†Cut-offs given were those for urine samples and they were not report.

$LOD given in ng/mL and not in grams of meconium.

§§Cut-offs given were those for urine samples and those for grams of meconium were not reported.

*Concentrations in μg/mL were obtained by extraction of 0.5 g meconium in 2.5 mL methanol.

Early studies demonstrated that parent drugs and their principal metabolites are both present in meconium. However, subsequent investigations with more specific and sensitive techniques revealed that particular metabolites can be present only (or mainly) in this biological matrix, such as m-hydroxybenzoylecgonine and p-hydroxybenzoylecgonine in case of COC and 11-hydroxy-Δ^9-tetrahydrocannabinol (THC-OH) in case of cannabis.[27–30] Substances are present in the range of nanograms to micrograms per gram of meconium, and, normally, several grams of matrix can be collected during the first 24 to 48 hours of the baby's life. In some cases, the aliquots collected within the first and the second day are examined separately because in the opinion of some investigators, they represent the nearest and the furthest period of the last two pregnancy trimesters.[31]

Since 1980, when Ostrea et al[32] reported for the first time the tissue distribution of morphine (MOR) at autopsy of six addicted monkey fetuses and two neonates of drug-dependent mothers, a large number of studies have focused on the analysis of this matrix.[33] During the 1990s, several studies, most of them performed by Ostrea et al,[34–38] included meconium testing in the screening for prenatal drug exposure. These authors started with epidemiologic studies in which they screened for opiates, COC, and cannabinoids using immunologic techniques [radioimmunoassay (RIA) and enzyme multiplied immunoassay technique (EMIT)] available at that time as routine tests for this type of large-scale analysis. Only in the most recent studies by this research group were chromatographic techniques (HPLC and GC-MS) introduced to confirm screening tests not only for illicit drugs but also for other xenobiotics, showing a substantial rate of exposure of neonates to different agents such as food additives, over-the-counter medications, as well as drugs of abuse.[36,38]

Similarly, in 1991, Maynard et al[39] used a commercial RIA kit for the analysis of 28 meconium specimens of newborns from mothers suspected of active drug use by history or pregnancy complications associated with drug use. Seventeen (61%) of the 28 samples tested positive: 16 for BZE, 1 for opiates, and 1 for cannabinoids. Compared with the combination of maternal and newborn urine test results, meconium testing showed an 82% positive predictive value (14/17) and a 91% negative predictive value (10/11). On the basis of these results, the authors concluded that the usefulness of meconium testing warrants its incorporation into the routine practice of a toxicologic laboratory.

In 1992, Browne et al[40] successfully used HPLC and GC-MS to detect COC in 34 meconium samples from premature infants of COC-dependent mothers. BZE was the measured biomarker, and EME and ecgonine (ECG) were not present in the samples, suggesting a limited COC metabolism in the premature neonates. However, in 1994, when examining meconium from 106 very low birth weight premature babies, Browne et al[41] found not only 19.8% of samples positive for COC, but 6.6% were also positive for norcocaine and 0.9% also for CE, whereas BZE was not detected in any of the samples.

Although the above-reported studies were based on a limited number of examined samples, in 1993, Rosengren et al[42] evaluated the prevalence of prenatal COC use in 621 racially mixed neonates of urban and suburban mothers from Hartford, CO and correlated its use with maternal demographics and newborn measurements. BZE was screened

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in meconium using FPIA and was confirmed after SPE with GC-MS in selected ion-monitoring (SIM) mode. A 3.4% positivity to BZE was statistically correlated with multiparity, multigravidity, late-onset and evidence-based prenatal care, public assistance, race other than Caucasian, and low academic achievement. COC-exposed infants were significantly smaller, and this correlated best with nonCaucasian ethnicity.

These early studies supporting the use of meconium as a valuable investigation tool for prenatal exposure to drugs were in contrast with the findings reported by Wingert et al[43] in 1994. After examining 345 meconium specimens in a large metropolitan obstetrical population from Bronx, New York City as well as maternal and neonatal urine samples for principal drugs of abuse, they concluded that meconium did not appear to offer any advantage over maternal or neonatal urine testing for the assessment of cannabinoids, codeine (COD), MOR, or methadone (MET) exposure. The positive rate for BZE (12%) was virtually identical for meconium and for maternal as well as neonatal urine.

Likewise, in 1995, Bibb et al[44] screened 386 meconium samples from 580 pairs mother/infants in Louisville, KY by EMIT and confirmed positive samples by GC-MS and thin layer chromatography (TLC). They found that meconium analysis showed equal sensitivity with the interview and maternal urine analysis, whereas with the newborn urine, the correlation was poor. Their percentage of positivity was 3.4% to COC, 1% to delta-9-tetrahydrocannabinol (THC). In contrast with these two studies, Ryan et al[45] demonstrated that meconium testing was more specific and sensitive than urine testing to identify in utero drug exposure. By examining a large cohort of mother-infants pairs (1030 meconium samples) from Rochester, NY, a 5.5% intrauterine exposure to COC was reported by this study; BZE was measured by FPIA with confirmation by GC-MS (SIM mode).

In 1995, another large epidemiologic study performed by Lewis et al[46] using FPIA and confirmation with GC-MS (operated in SIM mode) detected COC, opiates, cannabinoids, and amphetamine (AP) in 1175 meconium samples from Rockford, IL. Confirmation cutoff values were lower than the previous ones: 5 ng/g for COC metabolites, AP, opiates, and phencyclidine (PCP) and 2 ng/g for cannabinoids. Total prevalence of newborns exposed to illicit drugs in this North American midsize Midwestern city was 12.9%. COC-exposed neonates had the highest positive rate (5.4%), followed by cannabis (4.4%), CE (1%), and AP (0.1%). Nine (0.8%) patients had multiple drugs present in the meconium. The interesting point of this study was that the authors included meconium assays from 23 sets of multiple births (21 twins, 2 triplets) as a quality control for the assay. There were 20 sets of multiple births (42 patients) all testing negative. Three sets of twins had concordance in testing positive, with one twin testing positive for COC, whereas the other twin tested positive for COC and marijuana. No absolute discordance of twin assays were noted.

In addition to comparison with neonatal and maternal urine samples, Montgomery et al[33] investigated the rate of agreement of testing for fetal exposure to illicit drugs (APs, opiates, COC, cannabinoids, and PCP) using specimens of meconium versus umbilical cord tissue. Paired samples of meconium and umbilical cord tissue from 118 pregnancies with high suspicion of illicit drug use by the mothers were obtained. The agreement of drug screening between cord and meconium was above 90% for all drugs tested. Umbilical cord tissue performs as well as meconium in assessing fetal drug exposure to AP, opiates, COC, and cannabinoids. With these results, the authors recommended that the analysis of umbilical cord tissue may lead to more rapid test results than meconium analysis because the passage of meconium by the infant may be delayed for several days.

During the 1990s, in addition to the principal drugs of abuse, clinical investigations focused on identifying other specifically abused drugs and all the possible metabolites useful as biomarkers of in utero exposure. Prenatal exposure to benzodiazepines (BZDs) was, for the first time, studied by meconium analysis by Samperiz et al[47] in 1996 in 31 infants whose mothers were confirmed (n = 18) or suspected (n = 13) to be addicted to the drug. In this cohort COC, opiates and cannabis exposure were also investigated. The authors reported 1 positive sample for oxazepam, 1 for BZE, 15 for MOR, and 2 for THC using EMIT as the screening method and confirmation with GC.

In 1997, Stolk et al,[48] using FPIA and HPLC, analyzed 16 meconium samples from neonates of opiate-dependent mothers in treatment with MET. The parent drug and its principal metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine were found in 14 and 15 positive samples, respectively. The amount of 2-ethylidene-1,5-dimethyl-3, 3-diphenylpyrrolidine in meconium was much higher than the amount of MET (ratio: 9.6), showing that this metabolite could be the potential biomarker of fetal exposure to this drug.

All the possible COC metabolites present in meconium of newborns prenatally exposed to the drug were for the first time identified by Oyler et al[49] Six meconium samples of exposed infants were analyzed using GC-MS after SPE. Principal COC metabolites such as BZE and EME were found in high concentrations together with m-hydroxybenzoylecgonine, which was shown to be a specific biomarker of COC exposure in this neonatal matrix.

This interesting study, which achieved the identification of several COC metabolites, was supported by subsequent studies of Eyler et al[18] and Xia et al[50] This group of investigators (Xia et al) developed an accurate LC-MS method that had the ability to analyze COC and multiple metabolites from suspected drug-positive meconium samples using SPE. With respect to the study of Oyler et al, further COC metabolites were identified such as AEGC, ECG, benzoylnorecgonine, and cocaine-N-oxide (CNO). In addition, some variability in metabolite concentration between the two studies can be observed, although the order of magnitude of the amount was similar.[49] Metabolites identified varied qualitatively and quantitatively among the samples. Interestingly, ECG and benzoylnorecgonine detected for the first time were the highest measured. ECG appeared to hold the most promise as a diagnostic marker for neonatal COC exposure because this metabolite was present in 100% of the 21 positive samples tested at a relatively high median concentration. However, a core group of eight metabolites (present in at least 20 of 21 positive samples) was identified that appeared to possess the greatest utility for

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determining COC exposure. Ultimately, pyrolytic products of COC, anhydroecgonine methyl ester, and anhydroecgonine were suggested as biomarkers of crack COC consumption.

As previously mentioned, the identification of all the possible cannabis metabolites present in meconium was successfully carried out by the research group of ElSohly and Feng[28] and Feng et al[30] When examining specimens collected from neonates born to mothers with a history of cannabis use, these authors looked for the presence of not only 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) but also THC and its 8- and 11-hydroxy metabolites.[28] Delta9-THC and its 8-hydroxy metabolites were basically absent in 19 analyzed meconium specimens, whereas 11-OH-delta9-THC and 8 beta 11-dioh-delta9THC contributed significantly to the immunoassay response of meconium extracts. Analysis of meconium specimens that screened positive for cannabinoids, but failed to confirm for THC-COOH, showed significant amounts of 8 beta,11-dihydroxy-Δ^9-tetrahydrocannabinol and THC-OH.[28] This latter metabolite was indeed confirmed as a major meconium metabolite (present in 75% of 24 analyzed meconium samples) in a subsequent study.[30]

In 2005, Coles et al,[51] with the analysis of 246 specimens with positive cannabinoid screen, confirmed the importance of including THC-OH in a meconium cannabis confirmation procedure. Sixteen specimens were confirmed positive for THC-OH only, resulting in a 6.5% increase in the positivity rate compared with THC-COOH alone.

Concerning large epidemiologic studies, in 2002, an important North American study (The Maternal Lifestyle Study) investigated the prevalence of fetal drug exposure in an initial cohort of 11,811 mother-infants dyads from different parts of the country using meconium as the biological matrix of choice to assess maternal self reporting of drug consumption.[52–54] In fact, exposure was defined as the recognition of consumption of COC, opiates, cannabinoids, APs, and PCP by the mother or the presence of drug or metabolites in meconium. The EMIT method was used for initial screening followed by GC-MS as a confirmation assay for positive samples after SPE. With use of the meconium drug screen, of the 8527 analyzed samples, 9.5% were positive for COC or its metabolites, 2.3% for opiates, and 7.2% for cannabinoids. APs and PCP analysis were discontinued during the study because of low prevalence. This observational study confirmed many of the reported adverse social and serious medical perinatal complications of newborns exposed to COC or opiates during pregnancy. COC exposure was associated with significant deviation in all growth measurements, including birth weight, length, and head circumference, whereas opiates had a significant effect on birth weight. Statistically significant differences between COC-opiate exposed and nonexposed (n = 7442) mothers included race (African-American 74.6% and 47.0%, respectively), mean age (29.6 and 26.1 years, respectively), and polydrug use, including any combination of alcohol, tobacco, or marijuana (93% and 42%, respectively). Exposed mothers had a significantly higher risk of medical complications, psychiatric, nervous, and emotional disorders, and placental abruption. In the same period, for the first time in Europe, the Meconium Project sought to estimate the prevalence of drug use by pregnant women and the effects of prenatal exposure on the fetus and infant.[55] A cohort of 1151 mother-infant dyads from the Spanish city of Barcelona were recruited and were examined for drug exposure using a maternal self-reported questionnaire and meconium analysis. Opiates (6-monoacetylmorphine, MOR, morphine-3-glucuronide, morphine-6-glucuronide, COD), COC, and its metabolites (BZE, CE), APs, and methylenedioxy derivatives (3,4-methylendioxymethamphetamine, 3,4-methylendioxyamphetamine), and cannabis metabolites were investigated by LC-MS.[24–27,55–57] Meconium analysis showed that the prevalence for COC, opiates, both drugs, and cannabis were 8.7%, 4.4%, 2.2%, and 5.3%, respectively, and disclosed a single case of 3,4-methylendioxymethamphetamine use. In addition, arecoline, the main areca nut alkaloid, was found in meconium specimens from four Asiatic newborns whose mothers declared beetle nut consumption during pregnancy. In this cohort, parental ethnicity was not associated with drug use nor was the social class, although the professional and partly skilled mothers showed a higher tendency toward COC and opiate consumption than the nonprofessional ones, and a significantly higher percentage of cannabis consumer mothers had a managerial professional job compared with nonconsuming mothers.

Women who used drugs of abuse during pregnancy showed a higher number of previous pregnancies and abortions when compared with nonconsumer mothers (meconium negative test) probably because of a lack of family planning. Consumption of opiates and COC during pregnancy was associated with active tobacco smoking, a higher number of smoked cigarettes, and cannabis use. Exposure status and smoking behavior correlated with significantly lower birth weight in newborns from mothers exposed only to COC and to opiates and COC simultaneously. Of the newborns exposed to arecoline, one showed a low birth weight, low intrauterine growth, hyporeflexia, and hypotonia, and another presented a severe withdrawal syndrome.[10] COC, 6-monoacetylmorphine, and THC-COOH were the substances most frequently found in samples positive for COC, opiates, and cannabis (86.5, 88.5, and 80.8%, respectively). None of the combinations of different metabolites showed a higher percentage of positivity.

Although some authors compared meconium with maternal or neonatal urine analysis for detection of prenatal exposure to illicit drugs, others compared the sensitivity and the predictive power of two biological matrices accounting for chronic exposure: meconium and neonatal hair. Vinner et al[58,59] used both meconium and neonatal hair analysis to establish a drug exposure profile and predict withdrawal syndrome in exposed newborns. Cannabinoids, opiates, COC, and MET were determined by immunologic screening methods and positive results confirmed by GC-MS after extraction of analytes from the respective matrix. Examining 17 mother/neonate pairs with a history of drug abuse, the authors concluded that, in spite of some biases that might hinder an accurate interpretation, neonatal hair analysis made it possible to confirm fetal drug exposure and to reinforce the diagnosis of the withdrawal syndrome, particularly when results obtained in meconium were negative.

Different evidence was obtained by Bar-Oz et al,[60] who compared meconium and neonatal hair as biological markers

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to screen in utero exposure for COC opiates, MET, and cannabinoids in 185 babies with clinical suspicion of maternal drug abuse. On the basis of the results of the study, Bar-Oz et al[60] concluded that meconium is marginally more sensitive than neonatal hair for detection of COC and cannabis. The authors attributed this observation to the fact that meconium detects second trimester exposure, whereas hair grows only during the third trimester of pregnancy. Because a significant correlation was observed between hair and meconium concentrations of COC, cannabis, and opiates, both matrices were considered effective biological markers of in utero illicit drug exposure: meconium was more sensitive, but neonatal hair is available for up to 4 to 5 months after birth. It is important to mention, however, that the use of meconium, being a discarded material, is more acceptable to some parents than hair testing, which entails cutting scalp hair from the newborn.

As illustrated in the above reports, COC, opiates, and cannabinoids were the substances mostly consumed during pregnancy and therefore investigated in meconium. With respect to APs, the widespread abuse of methamphetamine (MA) in the adult female population and consequently during pregnancy was an occurrence prevalently confined to the West, Midwest, and Southwest of the United States. Using meconium testing, Buchi et al[61] estimated in 2003 the prevalence of prenatal exposure to MA and other drugs of abuse among 1519 infants born in Utah and compared the results with those observed in a study performed in 1991 in the same geographic area. Meconium samples were collected and analyzed from 1202 infants from well-baby nurseries and 317 from neonatal intensive care unit infants using EMIT and confirmation by GC-MS. Rates of positivity were 0.6% for MA, 2.9% for cannabis, and 0.3% for COC. There were no significant differences in the rates of positivity for MA or cannabis between the 1991 and 2000/2001 studies. Conversely, COC prevalence declined from 1.1% in 1991 to 0.3% in 2000/2001. The overall prevalence of positivity for any of these three drugs declined over the 10-year period from 4.4% to 2.4%; in comparing the two cohorts, the most recent study showed that the prevalence of positivity for any of these three drugs was higher in the newborns from the neonatal intensive care unit (4.7%) than in the well-baby nurseries.

Whereas in 2001 the prevalence of prenatal exposure to MA was approximately 0.1% in the above-reported population, in the 2006 Infant Development, Environment, and Lifestyle study, Arria et al[62] reported a 5.2% MA exposure in a cohort of 1632 women and their newborns from some specific areas of United States known for a high rate of MA misuse: Los Angeles, CA, De Moines, IA, Tulsa, OK, and Honolulu, HI. A 6.0% exposure to cannabis, 1.3% to barbiturates, and 22.8% self-reported exposure to alcohol was also highlighted.[62] Conversely, less than 1% of this cohort used heroin, BZDs, and hallucinogens.

Apart from the above-reported illicit drugs that have been tested in meconium, investigators have also focused on the assessment of prenatal exposure to the most consumed licit drug of abuse: alcohol. Alcohol is indeed the most popular legal drug of abuse used in our society, and consumption by women at child-bearing age remains an important public health concern. FAEEs are nonoxidative metabolites of alcohol that are formed by the conjugation of ethanol to endogenous fatty acids, and they have been proposed as biological markers of acute and chronic alcohol consumption in adults.[63] Moreover, in recent years, FAEEs in fetal and neonatal biological matrices have been reported to be potential biomarkers to assess the extent of intrauterine exposure to alcohol.[63–66]

Because it was evident from preliminary studies that certain FAEEs could be found in the meconium of neonates without prenatal alcohol exposure, early studies tried to find out which FAEEs could better differentiate between exposed and nonexposed newborns.[67–70] Furthermore, as extensively reviewed by Chan et al,[63] different proposals appeared in the international literature to standardize endogenous FAEEs levels and to validate a positive screening cutoff to confidently establish prenatal alcohol exposure. A positive test was defined as the presence of one particular FAEE, ethyl linoleate (E18:2), above the limit of detection (1 nmol/g) in one study[67] or the detection of ethyl oleate (E18:1) above the cutoff value of 32 ng/g[68] in another study, whereas cumulative presence of six individual FAEEs, palmitate (E16), palmitoleate (E16:0), stearate (E18), oleate (E18:1), linoleate (E18:2), and arachidonate (E20:4), excluding ethyl laurate (E12) and myristate (E14), above 50 ng/g constituted a positive test in another laboratory.[69,70] The first population-based study to determine basal levels of meconium FAEEs was conducted in two distinct populations of nondrinking mothers and their neonates from Toronto (n = 99) and Jerusalem (n = 101).[71] Mothers (n = 17) from both populations who admitted to drinking socially were eliminated from the baseline cohort and analyzed as a separate group. In addition, meconium samples from six infants born to mothers who were confirmed heavy drinkers were also tested. Six individual FAEEs including ethyl laurate (E12), myristate (E14), palmitate (E16), stearate (E18), oleate (E18:1), and linoleate (E18:2) were extracted from meconium samples by SPE and analyzed by GC with flame ionization detection (FID). A positive cutoff of 2 nmol cumulative FAEEs/g meconium, when ethyl laurate and myristate were excluded, was established with 100% sensitivity and 98.4% specificity.

With use of the above-reported cutoffs, epidemiologic studies have been conducted to investigate the prevalence of prenatal exposure to ethanol in different mother-newborn cohorts. Moore et al,[64] in 2003, analyzed the prevalence of six FAEEs, palmitate (E16), palmitoleate (E16:0), stearate (E18), oleate (E18:1), linoleate (E18:2), and arachidonate (E20:4), in the meconium of two separate groups of neonates: 436 babies born in a large, regional perinatal center in Hawaii and 289 infants admitted to six different newborn intensive care units. By the use of SPE, analysis by GC-MS in the chemical ionization mode, and a cutoff of 50 ng/g total FAEEs, the authors found a prevalence of exposure of 16.7% and 12.1% of babies from the two groups. Considering the quantitative results obtained, the authors concluded that in an adequate meconium specimen, a total FAEE concentration greater than 10,000 ng/g may indicate that the newborn has been exposed to significant amounts of alcohol during pregnancy.

In 2004, Chan et al investigated the potential use of meconium FAEE testing in a Canadian high-risk neonatal

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population in the absence of maternal drinking history. One hundred forty-two neonates were analyzed by enzyme-linked immunosorbent assay for illicit drugs and FAEEs (including laurate, myristate, palmitate, stearate, oleate, and linoleate) by GC-FID.[72] A total of 71% of the samples tested positive for at least one illicit drug, with cannabis being the most prevalent (52.3%), and 14% of all samples were positive for prenatal alcohol exposure, as evidenced by cumulative meconium FAEEs of 2 nmol/g. Ethyl oleate, linoleate, palmitate, and arachidonate were detected most often and at the highest levels.

Finally, in 2005, Bearer et al[65] compared concentrations of ethyl myristate, palmitate, palmitoleate, oleate, linoleate, linoleneate (E18:3), and arachidonate in 248 infants from a large urban hospital in Cleveland, OH with varying exposure status and 30 Muslim infants from an urban hospital in Amman, Jordan (abstaining mothers). FAEEs were quantified with GC-FID and compared between abstainers and nonabstainers to identify FAEEs of interest. Six of seven FAEEs were significantly different between the nonabstainers and at least one of two of the abstaining groups. FAEEs best predicted drinks per drinking day, and ethyl linoleate showed the highest sensitivity and specificity for identifying infants not exposed in utero to high levels of alcohol in a high-risk, substance-abusing, clinic-based sample.

As demonstrated by a large number of published studies, meconium has been the fetal matrix most used to assess prenatal exposure to drugs of abuse. The main advantages of meconium analysis are the easy and noninvasive manner of collection, which is generally well accepted by the parents of the newborn, the considerable amount of matrix that can be collected, and finally the fact that although available only for few days after birth, it is the fetal matrix that accounts for the largest exposure period.

NEONATAL HAIR

Neonatal hair is a sensitive biological marker that can define cumulative exposure to drugs during the last trimester of intrauterine life.[13,73] In the fetus, hair starts growing during the last 3 to 4 months of pregnancy and therefore accounts for exposure occurring in the last trimester. Although the detection window is smaller than for meconium, hair has the advantage of being available for as long as 4 to 5 months of postnatal life.[60] However, hair samples that can be obtained in newborns are often sparse, and collection in those particular cases can be considered "almost" invasive. Newborn hair samples are externally contaminated by the amniotic fluid, which not only reaches hair but also the fetus by way of the transdermal route.[74] Nevertheless, this contamination should not be considered as external in the context of the diagnosis of intrauterine drug exposure.

Hair is composed of approximately 65% to 95% protein, 1% to 9% lipid, small quantities of trace elements, polysaccharides, and water.[75] It is well known that drugs, including drugs of abuse, can be incorporated into the hair where they remain indefinitely.[76] Possible pathways for drug incorporation into hair can be summarized as follows: 1) passive diffusion from arterial blood capillaries into the hair follicle; 2) excretion onto the surface of hair from sweat and sebum; and 3) external contamination.

The main analytes generally found in hair are the parent compounds rather than their more polar metabolites, which usually predominate in cord blood, neonatal urine, and in some cases in meconium.[77] Drugs are present in the range of nanograms per milligram of hair, which means a μg/g matrix and therefore theoretically a concentration similar to that found in urine (μg/mL) and one or two orders of magnitude higher than that found in meconium. Although in adults the sample supply appears unlimited, practically, the best extraction and subsequent analysis is achieved when 20 to 50 mg of hair are used. This is not always possible in newborns because the hair supply is limited, and sometimes not more than 5 to 10 mg of hair is available.

Because hair is a solid biological matrix, a chemical or enzymatic digestion is required to extract xenobiotics from the hair shaft. Both alkaline and acid digestions have been performed at temperatures ranging from 37 to 100°C and for times varying from 1 hour to overnight.[76,77]

In case of enzymatic digestions, glucuronidase and arylsulphatase are commonly used enzymes at slightly acidic pH and temperatures at approximately 37°C for 1 to 18 hours.[77] Once the hair is digested, the analytes of interest are extracted either by solvents or SPE, with the exclusion of methanol hair treatment, which includes digestion and extraction as a unique procedure.[58,77]

Since 1989, it was established by the research group of Graham et al[78] that, in studies reporting reproductive risks of COC, hair analysis might identify intrauterine exposure to COC in babies when a maternal drug history was not available or of doubtful truthfulness. COC has been the illicit drug mostly investigated in neonatal hair for confirmation of gestational exposure and subsequent association with birth outcomes. Indeed, in the early studies, hair testing focused more on confirming prenatal exposure in relatively small groups of newborns from suspected consumers rather than on examining the prevalence of the exposure in large mother-infant cohorts.

In 1993, the work team of Kintz and Mangin,[79] one of the pioneers of hair testing, analyzed hair samples collected at the time of delivery from 57 French neonates whose mothers were known users of COC (2 cases) and also of heroin (9 cases), BZDs (11 cases), and AP (1 case). In all cases, the corresponding drug or its metabolite was found in neonatal hair from the infants in a concentration range similar to those found in addicted adults.

During the 1990s, immunologic methods were often applied for hair testing of COC and other illicit drugs. Using RIA, Salle et al,[80] in 1995, investigated the presence of BZE in hydrolyzed hair samples from 34 infants born to mothers with urine positive for COC at delivery compared with 33 infants born to urine-negative mothers from South Carolina at Charleston University Hospital. Twenty-eight neonates' hair tested positive for BZE and differed significantly from the control infants in head circumference and head growth percentiles. A negative correlation approaching significance was found between mean BZE and head circumference in the group of newborns with positive hair for BZE (n = 28). The study

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associated for the first time head growth abnormalities with levels of prenatal COC exposure. Seven years later, the same research group confirmed those results when examining 251 neonatal hair samples from babies born at the same hospital.[81] In this study, the authors not only screened the hair using RIA but also confirmed positive results by GC-MS. After controlling for gestational age, higher BZE levels correlated significantly with smaller head circumference and birth weight.

In agreement with the above-mentioned reports, when examining hair from 123 Italian newborns with malformations, low gestational age, low birth weight, and respiratory distress admitted to an intensive care division, 3 (2.4%) infants were positive for COC compared with none in the control group of 39 healthy newborns.[82] Although the low number of examined cases precluded any general conclusion, this was the first evidence of a correlation between prenatal exposure to COC and adverse outcomes using an objective biomarker.

Ursitti et al,[83] also using RIA, demonstrated that the use of the hair test in cases of clinical suspicion but negative urine test yielded a substantially higher rate of positivity than expected in the general population. In a period of 5 years, 192 neonatal hair samples from a Canadian urban population were analyzed to confirm clinical suspicions of intrauterine exposure to COC. Thirty percent of the samples were positive for BZE, a rate 5.5-fold higher than the 5.5% found previously by the same investigators in a population-based research study in three nurseries in Toronto ($P < 0.001$), thus documenting the efficiency of this test in confirming clinical suspicions of fetal exposure to COC. These results were confirmed by the subsequent study of the same research group: 32% positive hair samples from 392 babies in a 6-year span of clinical use of hair testing for COC.[84]

The fact that hair COC and BZE were the most sensitive biomarkers of COC exposure with respect to maternal self report and urine screen was corroborated by the study of Savitz et al.[85] On the basis of hair analysis, these authors reported 13% to 15% COC exposure (vs. 2% based on self report and 6% based on urine screen) in 604 neonates from prenatal care clinics affiliated with University of North Carolina hospitals. Hair COC and BZE were associated with black ethnicity, lower education, and poverty but not with preterm birth, with the possible exception of higher levels of COC and BZE and birth before 34 weeks of completed gestation.

Neonatal hair analysis was not limited to assess intrauterine exposure to COC. Similar to Kintz and Mangin,[79] other investigators used it to assess intrauterine exposure to other illicit drugs. For example, in 1996, Samperiz et al[47] tested not only meconium, as previously discussed, but also neonatal hair in addition to urine to improve diagnosis of fetal drug exposure. Both alternative neonatal matrices performed better than urine in detecting prenatal exposure. Among 31 French newborns from the Hôpital d'Enfants de la Timone, Marseille, hair analysis detected one positive sample for COC, eight positives for opiates (MOR), one for cannabis (THC), and two for oxazepam.

In addition to the comparison of Samperiz et al of the diagnostic power of neonatal hair versus meconium, Vinner et al[58,59] and Bar-Oz et al,[60] both in 2003, also made the same comparison. Although the latter authors had a confirmation method available with a higher sensitivity for all the analytes in hair compared with the first authors, they obtained a better estimate of prenatal exposure to drug by meconium analysis. Conversely, the results presented by Vinner et al demonstrated that only the neonatal hair concentration of COC (and its metabolites), opiates, and cannabinoids was associated with neonatal withdrawal syndrome.

NEONATAL URINE

Detection of intrauterine drug exposure has traditionally been accomplished through neonatal urine testing. This was especially useful during the last 2 decades when the sampling of neonatal biological matrices other than urine could not be easily accomplished or analytical methodologies to extract substances from these matrices and subsequently measure them with correct sensitivity and specificity were not routinely available.

The major disadvantage of assessing in utero exposure to illicit drugs by neonatal urinalysis is that the time window of detection is short, reflecting drug use only a few days before delivery. In addition, analysis of the neonate urine may produce false-negative results depending not only on the time of the last ingestion of the drug by the mother but the length of time after birth when the specimen was collected.[44] Urine formation starts in the fetus at the 8th week of gestational age, but it is not until the 16th week that urine production is sufficient to account for most of the amniotic fluid.[86] Excretion of drugs of abuse into urine occurs in different parts of the fetal renal nephron. Some drugs are filtered by glomerular filtration and others by transport and tubular secretion or sometimes by a combination of both. Therefore, although the xenobiotics to which the fetus is exposed are excreted through urine, this latter fluid is continuously eliminated into amniotic fluid, which is the ultimate repository of those substances. Thus, the information obtained by examining neonatal urine after delivery is the assessment of the possible acute exposures (which may or may not reflect the chronic exposure) in the days preceding delivery.

Originally, systematic investigations of illicit drug monitoring in neonatal urine were performed using mainly immunologic methods and included rather large mother-infant cohorts. A first study by Halstead et al[88] established that neonatal urine drug screening without strict protocols for specimen collection was of limited usefulness for management of drug abuse in pregnancy and neonatal drug withdrawal. These authors compared the results of maternal and neonatal urine drug analysis for opiates, COC, BZDs, cannabinoids, and PCP with clinical data and history to test the usefulness of peripartum drug screening and to establish guidelines for optimal testing. Urine from 28 mothers and 52 babies from Children and Vancouver Hospital, Vancouver, Canada were analyzed by EMIT, TLC, and GC-MS. Drugs not suspected by history were found in 10 mothers and 6 babies. Results assisted in the management of neonatal withdrawal in three babies. Drugs suspected by history were not found in 23 of 35 babies. Approximately half of these results were associated with delayed urine collection. The authors concluded in favor of a protocol that imposed neonatal urine collection on the first day of life.

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In 1989, Osterloh and Lee[89] carried out general urine drug screening in mothers (n = 601) and their newborns (n = 339) from the Neonatal Nursery and Obstetrics/Gynecology wards at San Francisco General Hospital. Apart from principal drugs of abuse (COC, opiates, APs, BDZs), approximately 80 different drugs and pharmaceuticals were screened for by combined analytical procedures (EMIT, spot test, TLC, GC-FID, GC–nitrogen-phosphorus detection). In newborns, urine drug screens were ordered when there was suspicion or drug effect, drug withdrawal, or history of drug abuse or a positive drug screen in the mother. The results showed COC as the most common detectable drug in the perinatal period. A percentage of 63.1% of urine samples of at-risk newborns were positive for any drug (41.6% were positive for COC and 21.1% for more than 1 drug). In addition, 11 samples of neonatal urine were positive for MOR, 5 for COD, 8 for MET, and 1 for BZDs.

With the use of EMIT as a detection method, Schulman et al[90] screened the urine of newborns from a large public hospital in the Bronx, New York City, where 95% of women were members of minority groups. Of the 1196 live-born infants delivered during a 5-month period, urine toxicology screening was performed in 304 infants with manifestations suggestive of maternal drug use, poor prenatal care, or maternal history of drug abuse. COC, opiates, MET, barbiturates, APs, and BZDs were investigated. An apparent prevalence of COC exposure of 4.9% was found, with only two samples positive for opiates, three for MET, one for barbiturates, and none for AP and BZDs. However, in the opinion of the authors, selective infant testing failed to identify 42.1% of newborns of COC-positive women. For this reason, the authors advocated universal neonatal screening for drugs of abuse for immediate clinical treatment, subsequent follow-up, and possible social service intervention.

As previously mentioned above in the section on meconium, although Wingert et al[43] were in favor of neonatal urinalysis (12% positivity to BZE, 1.4% for opiates, and 0.6% for MET) for assessment of prenatal exposure to drugs compared with meconium (11.9% positivity to BZE, 0.6% for opiates, and 0.6% for MET), Bibb et al[44] strongly supported the use of meconium (4.5% and 1.4% positivity to COC and cannabis) over neonatal urine (2.7% and 0% positivity to COC and cannabis) for neonatal drug screening. In both cases, an immunologic screen with confirmation by GC-MS was used.

In agreement with Bibb et al, in 1996, Samperiz et al[47] demonstrated in a cohort of 31 French infants whose mothers were confirmed or suspected drug users that urinary detection of prenatal drug exposure in neonates could give false-negative results. Meconium and neonatal hair were tested, in addition to urine, to improve diagnosis of fetal drug exposure. Drugs and their metabolites were detected by EMIT, and positive results were confirmed by GC-MS. Ten of 31 infants had a positive urine test (8 opiates, 1 COC, 1 cannabis); 10 of 19 had a positive hair test (8 opiates, 1 COC, 1 cannabis); and 11 had a positive meconium test (9 opiates, 1 COC, 1 cannabis). The authors concluded that urine, meconium, and hair testing versus urine testing alone increased the sensitivity of detection of prenatal drug exposure.

In 1991, Mack et al[91] assessed for the first time MET concentration in neonatal urine and cord blood to determine whether the MET concentration was predictive of the occurrence of neonatal withdrawal syndrome in newborns from addicted mothers in MET maintenance therapy. All of the 10 analyzed urine samples were positive for MET, with concentrations that were 6- to 87-fold higher than in cord blood. However, no relationship was found between maternal dose, MET urine concentration, and occurrence of abstinence syndrome.

In 2003, Vinner et al[58,59] analyzed the feasibility of neonatal urine, among other matrices, for establishing exposure profiles and for predicting withdrawal syndrome in 11 neonates from addicted mothers in MET maintenance. At this time, other neonatal matrices such as meconium or hair were also available for drug testing. Urine analysis had the lowest predictive ability and the highest sampling difficulty.

In recent years, there had been less reliance on neonatal urine analysis alone for the detection of prenatal exposure to drugs of abuse. Urine testing has been conducted in conjunction with other fluids or matrices to determine the ability of the different biological specimens to detect in utero exposure to drugs.

Eyler et al[18] examined neonatal urine together with amniotic fluid, cord blood, meconium, and maternal hair to assess prenatal exposure to COC in 115 mother-infant pairs with a maternal history of probable COC use at the Shands Hospital at University of Florida. Among 51 COC users, urine specimens identified the largest percentage of users: 69% overall and 62% of users who denied COC use. Meconium showed nearly equal sensitivity in detecting exposure. Interestingly, in this population of women who were selected if they admitted to COC use during pregnancy, maternal self report was the single best method to identify COC use during pregnancy. Therefore, the authors of the study supported the use of interviews rather than biologic specimen analysis to identify COC users. Although, traditionally, the maternal interview is not considered very accurate, the cohort that was used in this study was unique in that women with a known history of drug use before pregnancy were studied. It was therefore easy for these mothers to admit to drug use during pregnancy.

Neonatal urinalysis had also been used to assess prenatal exposure to arecoline, the principal alkaloid of the areca nut, the seed of the betel palm (*Areca catechu*). The nut mixed with piper beetle and tobacco are smoked or chewed by many adults and pregnant women in Asia and is the third most consumed drug (after caffeine and nicotine) worldwide.[88,89] Although not an illicit drug, areca nut presents a considerable abuse potential, and toxic effects to the fetus have been demonstrated.[10,11] In 2003, Pichini et al,[25] using the LC-MS method, determined for the first time arecoline in newborn urine, meconium, and cord serum from six infants born to areca nut consuming mothers. Urine from one neonate who suffered from severe withdrawal syndrome tested positive for the drug, indicating that the mother may have used the substance up to a few hours preceding delivery.[10,25]

CORD BLOOD

Drugs administered to pregnant women have the potential to cross the placenta and reach the fetus.[15,16]

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Measuring concentrations in fetal blood or amniotic fluid can be indicative of transplacental passage of drugs and metabolites during pregnancy, and, comparison of the drug concentrations in fetal and maternal blood allows calculation of the extent of such passage and an estimation of the quantity of fetal exposure to drugs.[13] Because both amniotic fluid and fetal blood collection are invasive procedures that can be harmful to the fetus, the development of new techniques for the sampling of blood from the cord vein and artery has permitted safer sampling of cord blood from mid-gestation to term or at delivery.

As with maternal blood, measuring levels of drugs and their metabolites in cord blood accounts only for fetal drug exposure during the previous hours or days before collection and not for chronic exposure during the entire gestation. With respect to illicit drugs, both parent drug and metabolites have been found in cord blood, with concentrations ranging between nanograms per milliliter to μg/mL.

Although several studies have been published in the last 2 decades, mostly with COC, limitations of cord blood as a matrix predictive of prenatal exposure has been recognized. Moore et al[94] analyzed cord blood, amniotic fluid, and neonatal urine for COC and its metabolites by HPLC-ultraviolet light after a premature delivery Because only trace amounts of BZE were found in cord blood compared with neonatal urine and amniotic fluid (0.29 and 1.95 μg/mL in urine and amniotic fluid, respectively) plus COC (0.07 and 0.04 μg/mL in urine and amniotic fluid, respectively), the authors did not recommend cord blood testing. Winecker et al[19] also preferred amniotic fluid compared with cord blood for drug analysis. They analyzed 32 and 70 amniotic fluid and cord tissue specimens, respectively, from 90 women admitted to delivery at Shands Hospital in Gainesville, FL. Half of them admitted COC use during pregnancy. COC metabolites (predominantly BZE) were detected by HPLC/GC-MS in 28.1% and 18.5% of the amniotic fluid and umbilical cord tissue specimens, respectively. BZE was the most common analyte detected, followed by EME. Cord blood showed analyte concentrations two or three orders of magnitude lower than those detected in the amniotic fluid. Later, the authors concluded that infant urine and meconium were more useful and easier to collect matrices compared with cord blood.[18]

Konkol et al[95] reported that compared with cord blood, meconium showed a greater number of COC metabolites and at higher concentration, including the previously undetectable norcocaine and BZE derivatives. BZE was the most common metabolite found in both matrices and was usually lower in concentration in cord blood. This biological matrix (cord blood) suitable for acute fetal exposure gave less information than meconium as used to assess chronic fetal exposure. Nonetheless, Dempsey et al,[96] in 1998, measured cord blood levels of COC in 36 neonates at risk for prenatal COC exposure at San Francisco Hospital, CA to determine clinical effects during the early neonatal period. Cord blood from 18 neonates, analyzed by GC-MS, was positive for BZE, and 50% of it was also positive for COC. CE was not found. The authors considered that the cord blood COC concentrations were consistent with pharmacologic effects and were similar to those from acute and chronic COC intoxications in adults. They concluded that concentrations of COC and BZE in their neonates were high enough to raise the possibility of in utero pharmacologic effects.

Finally, as previously reported, cord blood was one of the neonatal matrices investigated to assess in utero exposure to arecoline in association with possible adverse neonatal outcomes.[24] A recent study by Montgomery et al[33] proposed the use of umbilical cord tissue, instead of blood, as an alternative to meconium drug testing. Paired samples of meconium and umbilical cord tissue were obtained from 118 pregnancies with high suspicion of maternal illicit drug use by McKay Dee Hospital from Ogden, UT. Each specimen was tested for APs, opiates, COC, and cannabinoids using drug–class-specific immunoassays and GC-MS confirmation. The agreement of drug screening results between cord and meconium was above 90% for all drugs tested. The authors concluded that umbilical cord tissue performed as well as meconium in assessing fetal exposure to drugs and may have a faster availability of results because passage of meconium by the infant may take several days, particularly in premature infants or when meconium is passed in utero.

MATERNAL HAIR

Maternal hair testing has been considered the "gold standard" to assess chronic maternal drug use during pregnancy because collection of hair is relatively noninvasive, a large quantity of hair can be collected, and obtaining information on early drug use during pregnancy is possible. On the other hand, as for all the other maternal biological matrices, maternal hair gives a direct estimate of maternal exposure to drugs (active or passive) but only an indirect estimate of those reaching the fetus. Similar to neonatal hair, parent drugs prevail in this matrix and are present in the range of nanograms per milligram of hair.

Maternal hair was first used by the Canadian group of Graham et al[78] to determine gestational COC exposure in seven pregnant women seeking prenatal counseling at the Motherisk Clinic of Toronto, Canada. Subsequently, Callahan et al,[97] in 1992, evaluated the relationship between the presence of COC in 59 paired samples of newborn and maternal hair. The quantity of COC in the newborn infants hair correlated best with the proximal segment of maternal hair ($r = 0.77$, when excluding 9 prematures), representing the last 12 weeks of antepartum hair growth. Approximately half of the variation in the amount of COC in infants' hair was explained by variation in the proximal maternal hair segment. The nearly equal amount of unexplained variability implies the existence of other determinant factors in COC deposition in fetal hair.

One of these factors was suggested by Marques et al,[98] in 1993, when comparing COC in the hair of mother-infant pairs from Landover, MA. A group of mothers who had used crack COC while pregnant was evaluated during the postpartum period. Self-report questionnaires and paired hair samples were acquired from 63 mothers and 63 infants together with maternal urine, which were screened for COC and BZE. The initial correlation between concentrations of COC in mother and infant hair ($r = 0.41$, $P = 0.001$) was strengthened ($r = 0.62$, $P < 0.0005$) by the removal from the dataset of 30 samples of maternal hair independently judged to be

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damaged. Damage to hair was associated with certain types of hair care products. The authors concluded that hair analysis might provide an index of exposure when hair is not damaged and therefore highlighted a the key problem in hair analysis, that of the influence of cosmetic hair treatments on the variability in hair concentration of a consumed drug.

However, a limiting factor to be considered when correlating drug concentrations in infant and maternal hair is the variability in transplacental passage of illicit drugs in general and COC in particular. The variability of fetal drug metabolism and drug deposition in fetal hair should be taken into consideration as well. In support of this statement, Potter et al,[99] in 1994, reported for the first time a case of lack of fetal exposure to COC, after extensive maternal use of COC, both evidenced by hair analysis. BZE was found in the different segments of maternal hair samples corresponding to different pregnancy trimesters, whereas it was not found in baby hair. These data suggest that the mode of maternal use of COC and individual differences in placental handling of the drug may protect some fetuses and highlight the need to address interpatient variability.[98]

In agreement with this, Katikaneni et al[81] reported that 5 of 18 studied newborns had no evidence of hair BZE, although they were prenatally exposed to high concentrations of COC, as indicated by maternal hair BZE ranging from 2.3 to 9.9 ng/mg of hair. Because of this occurrence, no significant linear correlation between maternal and neonatal hair concentration of BZE was found in the 18 COC consumer mother-newborn pairs from the Medical University Hospital in Charleston, SC.

Finally, Eyler et al[18] included measurement of COC in maternal hair in a study concerning the relative ability of biological specimens and interviews to detect prenatal exposure to COC. Of the 115 mother-infant pairs with a probable history of COC use, 43 maternal hair specimens could be obtained and analyzed for COC and metabolites by GC-MS. A 65% positivity was obtained, with 6 positive cases among women who denied drug use (50% of all women in this group) and 22 in the group of mothers who admitted to drug use (71% of mothers in this group).

MATERNAL URINE

As previously stated, maternal urine was used mainly in conjunction with neonatal urine to assess maternal and possibly fetal exposure in the days before delivery. As in cases of neonatal urine, the window of detection is extremely short, although this matrix is easy to collect and always available, with no limitations in the collected volume and with parent drugs and metabolites present in concentrations in the range of nanograms per milliliter to μg/mL.[87] Published studies were performed mostly at the end of the 1980s and in the early 1990s when maternal urinalysis and self report were still the two most commonly used methods for identifying infants prenatally exposed to drugs and when there were still methodologic and technical limitations in the collection and analysis of nonconventional fetal and neonatal matrices. Currently, maternal urinalysis for illicit drugs is used to monitor drug use during pregnancy.[112]

As reported in the section above on neonatal urine, the early study of Halstead et al[88] on maternal and neonatal urine provided evidence that, for good, cost-effective drug screening, maternal urine should be collected at the time of admission for labor. Indeed, in case of delayed collection, drugs suspected by history were not found in 11 of 22 mothers of the study, whereas, in 12 of 28 mothers, drugs administered in the hospital at delivery created a confusion in the interpretation of screen results.

In the study of Osterloh and Lee[89] on general urine drug screening in 601 mothers and 339 newborns, 68.2% of maternal urine had a positive drug screen, and of that, 38.8% was positive to more than one drug. In mother-newborn urine pairs (n = 191), 84% and 67% concordance was shown for COC and MET, respectively, but for the other drugs, concordance was lower than 21%. COC was found to be the most common detectable drug in the perinatal period, with mother and newborn urinalysis recommended to confirm the suspicion of drug effect or withdrawal symptoms in the newborn.

In 1993, Schulman et al[90] investigated the prevalence of drug use in a population of parturient women at a municipal institution in the Bronx and its relationship with infant toxicology. Of the 204 screened women, 9.3% were positive for illicit drugs, 74% were positive for COC, and 21% showed polydrug use. Only 28.6% of COC-positive mothers gave a history of use. Nevertheless, because of the anonymous nature of maternal screening during this study, there were mothers with positive toxicology whose infants did not meet clinical criteria for testing. Indeed, as reported in the Section on Neonatal Urine, infant testing criteria missed 42.1% of infants whose mothers were exposed to drugs at the time of delivery, and, therefore, the authors required universal infant urine screening.

Marques et al[98] compared COC in the hair of mother-infant pairs and maternal urine and found that maternal urine BZE correlated with that in maternal hair ($n = 60$, $r = 0.41$, $P = 0.001$) and with maternal urine COC ($n = 60$, $r = 0.63$, $P < 0.0005$). None of the three self-report measures (use in past 30 days, duration since first use, average regular use) significantly correlated with any of the hair or urine measures. The amount of self-reported drug use could not be corroborated with any of the analytical drug measures in hair or urine. As previously mentioned, Wingert et al[43] in 1994 and Bibb et al[44] in 1995 included maternal urine together with neonatal urine and meconium in their studies on maternal drug use during pregnancy. Because the rate of positivity in maternal urine was similar to that of meconium in the first study (12% for BZE, 6% for THC, 1.3% for opiates, and 1% for MET) and higher than that of meconium in the second study (3.4% for COC and 2.5% for THC), at that time, meconium analysis was not considered more suitable than the other two.

In 2003, Vinner et al[58,59] used maternal urine analysis to establish in utero exposure to drugs and to predict withdrawal syndrome in exposed infants. Maternal urine was collected and analyzed by EMIT and GC-MS together with neonatal urine and hair. From the initial 17 addicted mother/neonate couples, 10 maternal urine samples were available for analysis of COC, opiates, cannabis, and MET. Maternal urine was shown to have less predictive power to detect drug use during pregnancy compared with neonatal hair.

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MATERNAL BLOOD AND ORAL FLUID

Maternal blood was one of the first matrices analyzed to detect drug use during pregnancy and fetal exposure to drugs.[5,6] However, toxicologic findings were of limited interest because drug concentrations in this matrix are related to drug intake occurring a few days or hours before blood sampling, thus disclosing acute consumption, and sample procurement is invasive and not well accepted by subjects. For this reason, the practical applications of using maternal blood for monitoring the presence of drugs of abuse at any time during pregnancy are essentially lacking.[13]

To our knowledge, there are only two studies that determined drugs of abuse using maternal blood. Doberczak et al[100] determined MET concentration in blood of MET-dependent women and their newborns in association with neonatal opiate withdrawal. Twenty-one mothers and their neonates born at the Israel Medical Center in New York City, NY formed the study population. MET was determined by GC-MS after liquid-liquid extraction from serum obtained by blood centrifugation. The maternal MET dose at delivery correlated significantly with the maternal plasma level drawn at 16 hours postpartum ($r = 0.512$, $P < 0.05$), and the maternal plasma MET level in turn correlated significantly with the neonatal plasma MET level at day 1 of life ($r = 0.545$, $P < 0.05$). A positive correlation was found between the severity of central nervous system signs of withdrawal and the rate of decline of the neonatal plasma MET level from day 1 to day 4 of life ($r = 0.550$, $P < 0.05$). From the results obtained, the authors concluded that careful reduction of the maternal MET dose during pregnancy under intensive medical and psychosocial surveillance may benefit the drug-exposed newborn infant. However, after this observation, no further studies supported the authors' suggestion.

In 1994, Knight et al[101] correlated illicit drug concentration in maternal blood during pregnancy and maternal nutritional status in a subset of African-American subjects in Washington, D.C. who participated in a project designed to study nutrition as a factor in the outcome of pregnancy. Fasting blood samples, drawn during each trimester of pregnancy and at delivery, were screened by EMIT for COC, PCP, and cannabinoids. These samples were also analyzed for serum folate, vitamin B12, ferritin, and ascorbic acid. The mothers whose blood levels were above the cutoffs for COC (300 ng/mL), cannabinoids (100 ng/mL), and PCP (25 ng/mL) had concentrations of folate and ferritin significantly less than those of subjects with lower drug levels ($P \leq 0.05$). High maternal blood concentrations of illicit drugs were also associated with a significant increase in leukocyte count ($P < 0.05$). Taken together, these results indicate that addicted mothers present with poor nutritional status during pregnancy, which contributes together with drug use to poor pregnancy outcome.

Oral fluid (saliva) collection is less invasive and more cost effective than blood, and, for this reason, this biological matrix has gained popularity as an alternative matrix. Drugs are incorporated in saliva by passive diffusion produced by gradient concentration. Studies on drugs of abuse in oral fluid[102] have shown that weak bases, such as COC, opiates, BZDs, or nicotine, tend to concentrate in oral fluid because their pH is slightly acidic as compared with plasma.[102–104] Although some metabolites have been detected, the parent drug is usually the main analyte found in oral fluid.[105] Similarly to maternal blood, maternal saliva accounts only for acute consumption that occurred in the hours previous to collection, and repetitive sampling is needed to verify a suspected repetitive maternal abuse of drugs. For this reason, although theoretically interesting, maternal oral fluid testing has not yet been applied extensively to assess maternal abuse of illicit drugs during pregnancy and fetal exposure.

MATERNAL SWEAT

Sweat secretion is an important mechanism for maintaining a constant core body temperature[3] through sympathetic nerve stimulation. Multiple mechanisms have been suggested for the incorporation of drugs into sweat, including passive diffusion and transdermal migration.[13,106] Passive diffusion of drugs from blood to sweat is favored among lipid-soluble compounds. Basic compounds may accumulate in sweat from blood because of the pH differential between the two matrices. Sweat testing is relatively noninvasive, and identification of drugs in sweat may serve as a means of monitoring recent drug use with a window of detection that can be somewhat wider than that provided by urine testing.[106]

Two methodologic approaches are currently applied for drug testing in sweat.[107] The first method aims to demonstrate recent drug use (less than 24 hours) and consists of a punctual sweat collection coupled with an immunochromatographic test, which provides a qualitative result,[108,109] or by a cotton wipe, subsequently extracted for drugs and subjected to drug analysis.[110] The second method is based on patch technology and allows for monitoring of illicit drug use for time windows wider than those provided by urine testing. Because the patches can be worn for up to 1 week, drugs tend to accumulate in the collection device, and no drug degradation appears to occur during this time interval.[111] Both approaches benefit from a low invasiveness and use a matrix that is easier to collect than blood or urine. A potential application of patch technology for assessing prenatal exposure to drugs would be in the weekly monitoring of maternal consumption in cases of proven or suspected addiction. Despite this potential application, so far, maternal sweat has never been used to determine fetal exposure to drugs.

CONCLUSIONS

The fetal, neonatal, and occasionally maternal matrices are, to varying degrees, repositories of drugs to which the fetus is exposed in utero. An accurate assessment of fetal exposure to drugs of abuse through the analysis of these matrices is important in identifying infants at risk and for providing treatment and follow-up. Although currently available analytic methods are specific and sensitive to allow the detection up to minute amounts of parent drugs and their metabolites in these various biological matrices, further studies are needed to translate the information into clinical practice. Finally, the mechanism of fetoplacental passage of drugs, drug metabolism, and the

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potential adverse effects of drugs on developing organisms have to be further ascertained in animal and human studies.

REFERENCES

1. Harvey JA. Cocaine effects on the developing brain: current status. *Neurosci Biobehav Rev.* 2004;27:751–764.
2. Feng Q. Postnatal consequences of prenatal cocaine exposure and myocardial apoptosis: does cocaine in utero imperil the adult heart? *Br J Pharmacol*. 2005;144:887–888.
3. Huestis MA, Cone EJ. Alternative testing matrices. In: S. Karch, ed. *Drug Abuse Handbook*. Boca Raton, FL: CRC Press; 1998:799–857.
4. Huestis MA, Choo RE. Drug abuse's smallest victims: in utero drug exposure. *Forensic Sci Int*. 2002;128:20–30.
5. Olsen GD. Placental permeability for drugs of abuse and their metabolites. *NIDA Res Monogr.* 1995;154:152–162.
6. Sastry BV. Placental toxicology: tobacco smoke, abused drugs, multiple chemical interactions, and placental function. *Reprod Fertil Dev.* 1991;3: 355–372.
7. Chan D, Knie B, Boskovic R, et al. Placental handling of fatty acid ethyl esters: perfusion and subcellular studies. *J Pharmacol Exp Ther.* 2004; 310:75–82.
8. Myllynen P. Human placenta: a human organ for developmental toxicology research and biomonitoring. *Placenta*. 2005;26:361–371.
9. Ostrea E, Porter T, Balun J, et al. Effect of chronic maternal drug addiction on placental drug metabolism. *Dev Pharmacol Ther.* 1989;12:42–48.
10. Garcia-Algar O, Vall O, Alameda F, et al. Prenatal exposure to arecoline (areca nut alkaloid) and birth outcomes. *Arch Dis Child Fetal Neonatal Ed*. 2005;90:F276–F277.
11. Lopez-Vilchez MA, Seidel V, Farre M, et al. Areca-nut abuse and neonatal withdrawal syndrome. *Pediatrics*. 2006;117:129–131.
12. Langman J, Sadler TW. *Langman's Medical Embriology.* Philadelphia: Williams & Wilkins; 1990;7:106–123.
13. Pichini S, Altieri I, Zuccaro P, et al. Drugs monitoring in non-conventional biological fluids and matrices. *Clin Pharmacokinet*. 1996;130:211–228.
14. Szeto HH. Kinetics of drug transfer to the fetus. *Clin Obstet Gynecol*. 1993;36:246–254.
15. Pacifici GM, Nottoli R. Placental transfer of drugs administered to the mother. *Clin Pharmacokinet*. 1995;28:235–269.
16. Ripple GM, Goldberger BA, Caplan YH, et al. Detection of cocaine and its metabolites in human amniotic fluid. *J Anal Toxicol*. 1992;16:328–331.
17. Jain L, Meyer W, Moore C, et al. Detection of fetal cocaine exposure by analysis of amniotic fluid. *Obstet Gynecol*. 1993;81:787–790.
18. Eyler FD, Behnke M, Wobie K, et al. Relative ability of biologic specimens and interviews to detect prenatal cocaine use. *Neurotoxicol Teratol*. 2005;27:677–687.
19. Winecker RE, Goldberger BA, Tebbett I, et al. Detection of cocaine and its metabolites in amniotic fluid and umbilical cord tissue. *J Anal Toxicol.* 1997;21:97–104.
20. Gareri J, Klein J, Koren G. Drugs of abuse testing in meconium. *Clin Chim Acta*. 2006;366:101–111.
21. Pichini S, Altieri I, Zuccaro P, et al. Meconium as a matrix. *Clin Pharmacokinet*. 1996;131:81.
22. Ostrea EM Jr, Parks PM, Brady MJ. Rapid isolation and detection of drugs in meconium of infants of drug-dependent mothers. *Clin Chem*. 1988;34:2372–2373.
23. Koren G, Chan D, Klein J, et al. Estimation of fetal exposure to drugs of abuse, environmental tobacco smoke, and ethanol. *Ther Drug Monit*. 2002;24:23–25.
24. Pichini S, Pacifici R, Pellegrini M, et al. Development and validation of a liquid chromatography-mass spectrometry assay for the determination of opiates and cocaine in meconium. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2003;794:281–292.
25. Pichini S, Pellegrini M, Pacifici R, et al. Quantification of arecoline (areca nut alkaloid) in neonatal biological matrices by high-performance liquid chromatography/electrospray quadrupole mass spectrometry. *Rapid Commun Mass Spectrom*. 2003;17:1958–1964.
26. Pichini S, Pacifici R, Pellegrini M, et al. Development and validation of a high-performance liquid chromatography-mass spectrometry assay for determination of amphetamine, methamphetamine, and methylenedioxy derivatives in meconium. *Anal Chem*. 2004;76:2124–2132.
27. Pichini S, Marchei E, Pacifici R, et al. Application of a validated high-performance liquid chromatography-mass spectrometry assay to the analysis of m- and p-hydroxybenzoylecgonine in meconium. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2005;820:151–156.
28. ElSohly MA, Feng S. delta 9-THC metabolites in meconium: identification of 11-OH-delta 9-THC, 8 beta,11-diOH-delta 9-THC, and 11-nor-delta 9-THC-9-COOH as major metabolites of delta 9-THC. *J Anal Toxicol*. 1998;22:329–335.
29. ElSohly MA, Stanford DF, Murphy TP, et al. Immunoassay and GC-MS procedures for the analysis of drugs of abuse in meconium. *J Anal Toxicol.* 1999;23:436–445.
30. Feng S, ElSohly MA, Salamone S, et al. Simultaneous analysis of delta 9-THC and its major metabolites in urine, plasma, and meconium by GC-MS using an immunoaffinity extraction procedure. *J Anal Toxicol*. 2000;24:395–402.
31. Moore C, Negrusz A, Lewis D. Determination of drugs of abuse in meconium. *J Chromatogr B Biomed Sci Appl*. 1998;713:137–146.
32. Ostrea EM Jr, Lynn SM, Wayne RN, et al. Tissue distribution of morphine in the newborns of addicted monkeys and humans. Clinical implications. *Dev Pharmacol Ther.* 1980;1:163–170.
33. Montgomery D, Plate C, Alder SC, et al. Testing for fetal exposure to illicit drugs using umbilical cord tissue vs meconium. *J Perinatol*. 2006; 26:11–14.
34. Ostrea EM Jr, Brady M, Gause S, et al. Drug screening of newborns by meconium analysis: a large-scale, prospective, epidemiologic study. *Pediatrics*. 1992;89:107–113.
35. Ostrea EM Jr, Romero A, Yee H. Adaptation of the meconium drug test for mass screening. *J Pediatr.* 1993;122:152–154.
36 Ostrea EM Jr, Matias O, Keane C, et al. Spectrum of gestational exposure to illicit drugs and other xenobiotic agents in newborn infants by meconium analysis. *J Pediatr.* 1998;133:513–515.
37. Ostrea EM Jr. Testing for exposure to illicit drugs and other agents in the neonate: a review of laboratory methods and the role of meconium analysis. *Curr Probl Pediatr.* 1999;29:37–56.
38. Ostrea EM Jr, Knapp DK, Tannenbaum L, et al. Estimates of illicit drug use during pregnancy by maternal interview, hair analysis, and meconium analysis. *J Pediatr.* 2001;138:344–348.
39. Maynard EC, Amoruso LP, OH W. Meconium for drug testing. *Am J Dis Child*. 1991;145:650–652.
40. Browne S, Tebbett IR, Moore C, et al. Analysis of meconium for cocaine in neonates. *J Chromatogr.* 1992;575:158–161.
41. Browne S, Moore C, Negrusz A, et al. Detection of cocaine, norcocaine, and cocaethylene in the meconium of premature neonates. *J Forensic Sci*. 1994,39:1515–1519.
42. Rosengren SS, Longobucco DB, Bernstein BA, et al. Meconium testing for cocaine metabolite: prevalence, perceptions, and pitfalls. *Am J Obstet Gynecol*. 1993;168:1449–1456.
43. Wingert WE, Feldman MS, Kim MH, et al. A comparison of meconium, maternal urine and neonatal urine for detection of maternal drug use during pregnancy. *J Forensic Sci*. 1994;39:150–158.
44. Bibb KW, Stewart DL, Walker JR, et al. Drug screening in newborns and mothers using meconium samples, paired urine samples, and interviews. *J Perinatol*. 1995;15:199–202.
45. Ryan RM, Wagner CL, Schultz JM, et al. Meconium analysis for improved identification of infants exposed to cocaine in utero. *Pediatrics*. 1994;125:435–440.
46. Lewis D, Moore C, Leikin JB, et al. Multiple birth concordance of street drug assays of meconium analysis. *Vet Hum Toxicol*. 1995;37: 318–319.
47. Samperiz S, Millet V, Arditti J, et al. Value of toxicological research in newborn infants of addicted mothers by the study of several samples (urine, meconium, hair). *Arch Pediatr.* 1996;3:440–444.
48. Stolk LM, Coenradie SM, Smit BJ, et al. Analysis of methadone and its primary metabolite in meconium. *J Anal Toxicol*. 1997;21:154–159.
49. Oyler J, Darwin WD, Preston KL, et al. Cocaine disposition in meconium from newborns of cocaine-abusing mothers and urine of adult drug users. *J Anal Toxicol*. 1996;20:53–62.
50. Xia Y, Wang P, Bartlett MG, et al. An LC-MS-MS method for the comprehensive analysis of cocaine and cocaine metabolites in meconium. *Anal Chem*. 2000;72:764–771.
51. Coles R, Clements TT, Nelson GJ, et al. Simultaneous analysis of the Delta9-THC metabolites 11-nor-9-carboxy-Delta9-THC and

© 2007 Lippincott Williams & Wilkins

11-hydroxy-Delta9-THC in meconium by GC-MS. *J Anal Toxicol*. 2005; 29:522–527.
52. Lester BM, ElSohly M, Wright LL, et al. The Maternal Lifestyle Study: drug use by meconium toxicology and maternal self-report. *Pediatrics*. 2001;107:309–317.
53. Bada HS, Das A, Bauer CR, et al. Gestational cocaine exposure and intrauterine growth: maternal lifestyle study. *Obstet Gynecol*. 2002;100: 916–924.
54. Bauer CR, Shankaran S, Bada HS, et al. The Maternal Lifestyle Study: drug exposure during pregnancy and short-term maternal outcomes. *Am J Obstet Gynecol*. 2002;186:487–495.
55. Pichini S, Puig C, Zuccaro P, et al. Assessment of exposure to opiates and cocaine during pregnancy in a Mediterranean city: preliminary results of the "Meconium Project." *Forensic Sci Int*. 2005;153:59–65.
56. Marchei E, Pellegrini M, Pacifici R, et al. Quantification of Delta-9-tetrahydrocannabinol and its major metabolites in meconium by gas chromatographic-mass spectrometric assay: assay validation and preliminary results of the "meconium project". *Ther Drug Monit*. 2006;28: 700–706.
57. Lozano J, Garcia-Algar O, Marchei E, et al. Prevalence of gestational exposure to cannabis in a Mediterranean city by meconium analysis. *Acta Paediatr*. 2007; (Epub ahead to print).
58. Vinner E, Vignau J, Thibault D, et al. Neonatal hair analysis contribution to establishing a gestational drug exposure profile and predicting a withdrawal syndrome. *Ther Drug Monit*. 2003;25:421–432.
59. Vinner E, Vignau J, Thibault D, et al. Hair analysis of opiates in mothers and newborns for evaluating opiate exposure during pregnancy. *Forensic Sci Int*. 2003;133:57–62.
60. Bar-Oz B, Klein J, Karaskov T, et al. Comparison of meconium and neonatal hair analysis for detection of gestational exposure to drugs of abuse. *Arch Dis Child Fetal Neonatal Ed*. 2003;88:F98–F100.
61. Buchi KF, Zone S, Langheinrich K, et al. Changing prevalence of prenatal substance abuse in Utah. *Obstet Gynecol*. 2003;102:27–30.
62. Arria AM, Derauf C, Lagasse LL, et al. Methamphetamine and other substance use during pregnancy: preliminary estimates from the Infant Development, Environment, and Lifestyle (IDEAL) study. *Matern Child Health J*. 2006;10:293–302.
63. Chan D, Caprara D, Blanchette P, et al. Recent developments in meconium and hair testing methods for the confirmation of gestational exposures to alcohol and tobacco smoke. *Clin Biochem*. 2004;37: 429–438.
64. Moore C, Jones J, Lewis D, et al. Prevalence of fatty acid ethyl esters in meconium specimens. *Clin Chem*. 2003;49:133–136.
65. Bearer CF, Santiago LM, O'Riordan MA, et al. Fatty Acid ethyl esters: quantitative biomarkers for maternal alcohol consumption. *J Pediatr*. 2005;146:824–830.
66. Ostrea E. Hernandez J, Bielawski D, et al. Fatty acid ethyl esters in meconium: are they biomarkers of fetal alcohol exposure and effect? *Alcohol Clin Exp Res*. 2006;30:1152–1159.
67. Bearer CF, Lee S, Salvator AE, et al. Ethyl linoleate in meconium: a biomarker for prenatal ethanol exposure. *Alcohol Clin Exp Res*. 1999; 23:487–493.
68. Bearer CF, Jacobson JL, Jacobson SW, et al. Validation of a new biomarker of fetal exposure to alcohol. *J Pediatr*. 2003;143:463–469.
69. Moore C, Jones J, Lewis D, et al. Prevalence of fatty acid ethyl esters in meconium specimens. *Clin Chem*. 2003;49:133–136.
70. Moore CM, Lewis D. Fatty acid ethyl esters in meconium: biomarkers for the detection of alcohol exposure in neonates. *Clin Chim Acta*. 2001;312: 235–237.
.71. Chan D, Bar-Oz B, Pellerin B, et al. Population baseline of meconium fatty acid ethyl esters among infants of nondrinking women in Jerusalem and Toronto. *Ther Drug Monit*. 2003;25:271–278.
72. Chan D, Klein J, Karaskov T, et al. Fetal exposure to alcohol as evidenced by fatty acid ethyl esters in meconium in the absence of maternal drinking history in pregnancy. *Ther Drug Monit*. 2004;26: 474–481.
73. Klein J, Karaskov T, Koren G. Clinical applications of hair testing for drugs of abuse: the Canadian experience. *Forensic Sci Int.* 2003;10:281–288.
74. Bailey B, Klein J, Koren G. Noninvasive methods for drug measurement in pediatrics. *Pediatr Clin North Am*. 1997;44:15–26.
75. Harkey MR. Anatomy and physiology of hair. *Forensic Sci Int*. 1993;63: 9–18.
76. Kintz P, Mangin P. Evidence of gestational heroin or nicotine exposure by analysis of fetal hair. *Forensic Sci Int*. 1993;63:99–104.
77. Kintz P, Villain M, Cirimele V. Hair analysis for drug detection. *Ther Drug Monit*. 2006;28:442–446.
78. Graham K, Koren G, Klein J, et al. Determination of gestational cocaine exposure by hair analysis. *JAMA*. 1989;262:3328–3330.
79. Kintz P, Mangin P. Determination of gestational opiate, nicotine, benzodiazepine, cocaine and amphetamine exposure by hair analysis. *J Forensic Sci Soc*. 1993;33:139–142.
80. Sallee FR, Katikaneni LP, McArthur PD, et al. Head growth in cocaine-exposed infants: relationship to neonate hair level. *J Dev Behav Pediatr*. 1995;16:77–81.
81. Katikaneni LD, Salle FR, Hulsey TC. Neonatal hair analysis for BZE: a sensitive and semiquantitative biological marker for chronic gestational cocaine exposure. *Biol Neonate*. 2002;81:29–37.
82. Strano-Rossi S, Chiarotti M, Fiori A, et al. Cocaine abuse in pregnancy: its evaluation through hair analysis of pathological newborns. *Life Sci*. 1996;59:1909–1915.
83. Ursitti F, Klein J, Koren G. Clinical utilization of the neonatal hair test for cocaine: a four-year experience in Toronto. *Biol Neonate*. 1997;72: 345–351
84. Koren G, Klein J, McMartin K. Diagnosing intrauterine exposure to cocaine by hair testing: six years of clinical use. *Ther Drug Monit*. 1998; 20:478–480.
85. Savitz DA, Henderson L, Dole N, et al. Indicators of cocaine exposure and preterm birth. *Obstet Gynecol*. 2002;99:458–465.
86. Areses R, Arruabarrena D, Alzueta MT, et al. *Protocolos de Nefrología*. 2002. Available at: http://www.aeped.es/protocolos/nefro/index.htm. Accessed October 2007.
87. Sanaullah F, Gillian M, Lavin T. Screening of substance misuse during early pregnancy in Blyth: an anonymous unlinked study *J Obstet Gynaecol*. 2006;26:187–190.
88. Halstead AC, Godolphin W, Lockitch G, et al. Timing of specimen collection is crucial in urine screening of drug dependent mothers and newborns. *Clin Biochem*. 1988;21:59–61.
89. Osterloh JD, Lee BL. Urine drug screening in mothers and newborns. *Am J Dis Child*. 1989;143:791–793.
90. Schulman M, Morel M, Karmen A, et al. Perinatal screening for drugs of abuse: reassessment of current practice in a high-risk area. *Am J Perinatol*. 1993;10:374–377.
91. Mack G, Thomas D, Giles W, et al. Methadone levels and neonatal withdrawal. *Paediatr Child Health*. 1991;27:96–100.
92. Trivedy CR, Craig G, Warnakulasuriya S. The oral health consequences of chewing areca nut. *Addict Biol*. 2002;7:115–125.
93. Winstock A. Areca nut-abuse liability, dependence and public health. *Addict Biol*. 2002;7:133–138.
94. Moore CM, Brown S, Negrusz A, et al. Determination of cocaine and its major metabolite, benzoylecgonine, in amniotic fluid, umbilical cord blood, umbilical cord tissue, and neonatal urine: a case study. *J Anal Toxicol*. 1993;17:62.
95. Konkol RJ, Murphey LJ, Ferriero DM, et al. Cocaine metabolites in the neonate: potential for toxicity. *J Child Neurol*. 1994;9: 242–248.
96. Dempsey DA, Partridge JC, Jones RT, et al. Cocaine, nicotine, caffeine, and metabolite plasma concentrations in neonates. *J Anal Toxicol*. 1998; 22:220–224.
97. Callahan CM, Grant TM, Phipps P, et al, Measurement of gestational cocaine exposure. Sensitivity of infant's hair, meconium, and urine. *J Pediatr*. 1992;120:763–768.
98. Marques PR, Tippetts AS, Branch DG. Cocaine in the hair of mother-infant pairs: quantitative analysis and correlations with urine measures and self-report. *Am J Drug Alcohol Abuse*. 1993;19:159–175.
99. Potter S, Klein J, Valiante G, et al. Maternal cocaine use without evidence of fetal exposure. *J Pediatr*. 1994;125:652–654.
100. Doberczak TM, Kandall SR, Friedmann P. Relationship between maternal methadone dosage, maternal-neonatal methadone levels, and neonatal withdrawal. *Obstet Gynecol*. 1993;81:936–940.
101. Knight EM, James H, Edwards CH, et al. Relationships of serum illicit drug concentrations during pregnancy to maternal nutritional status. *J Nutr*. 1994;124:973S–980S.
102. Cone EJ. Saliva testing for drugs of abuse. *Ann N Y Acad Sci*. 1993;694: 91–127.

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103. Kidwell DA, Holland JC, Athanaselis S. Testing for drugs of abuse in saliva and sweat. *J Chromatogr B Biomed Sci Appl*. 1998;713:111–135.
104. Jenkins AJ, Oyler JM, Cone EJ. Comparison of heroin and cocaine concentrations in saliva with concentrations in blood and plasma. *J Anal Toxicol*. 1995;19:359–374.
105. Moolchan ET, Cone EJ, Wstadik A, et al. Cocaine and metabolite elimination patterns in chronic cocaine users during cessation: plasma and saliva analysis. *J Anal Toxicol*. 2000;24:458–466.
106. Wolff K, Farrell M, Marsden J, et al. A review of biological indicators of illicit drug use, practical considerations and clinical usefulness. *Addiction*. 1999;94:1279–1298.
107. de la Torre R, Pichini S. Usefulness of sweat testing for the detection of cannabis smoke. *Clin Chem*. 2004;50:1961–1962.
108. Kintz P, Cirimele V, Ludes B. Codeine testing in sweat and saliva with the Drugwipe. *Int J Legal Med*. 1998;111:82–84.
109. Samyn N, van Haeren C. On-site testing of saliva and sweat with Drugwipe and determination of concentrations of drugs of abuse in saliva, plasma and urine of suspected users. *Int J Legal Med*. 2000;113: 150–154.
110. Samyn N, de Boeck G, Verstraete AG. The use of oral fluid and sweat wipes for the detection of drugs of abuse in drivers. *J Forensic Sci*. 2002; 47:1380–1387.
111. Caplan YH, Goldberger BA. Alternative specimens for workplace drug testing. *J Anal Toxicol*. 2001;25:396–399.
112. Land DB, Kushner R. Drug abuse during pregnancy in an inner-city hospital: prevalence and patterns. *J Am Osteopath Assoc*. 1990;90:421–426.

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