

Effects of Addictive Substances During Pregnancy and Infancy and Their Analysis in Biological Materials

Justyna Płotka, Sylwia Narkowicz, Żaneta Polkowska,
Marek Biziuk, and Jacek Namieśnik

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1 Introduction

Addictive substance use is most prevalent in people who are of reproductive-age. In a national prevalence survey performed among pregnant women aged 15–44 years, 10.8% reported using alcohol, 17% reported smoking during pregnancy, and 4.4% reported abusing one or more illicit substances (Fig. 1) (SAMHSA 2011). Substance abuse for a pregnant woman is twice as dangerous as for others, because:

- She may harm her own health and impair her ability to support a successful pregnancy
- In utero exposure to substances of abuse either may affect fetal development or may induce physiological changes (e.g., organic and/or neurocognitive) to the child later in life (Narkowicz et al. 2012)

More than 75% of infants exposed to drugs later suffer from major medical problems. Similar problems result from excessive use of tobacco (cigarettes) during

J. Płotka (✉) • S. Narkowicz • Ż. Polkowska • M. Biziuk • J. Namieśnik
Department of Analytical Chemistry, Chemical Faculty, Gdansk University of Technology
(GUT), 11/12 Narutowicza Street, 80-233 Gdańsk, Poland
e-mail: plotkajustyna@gmail.com; chemanal@pg.gda.pl

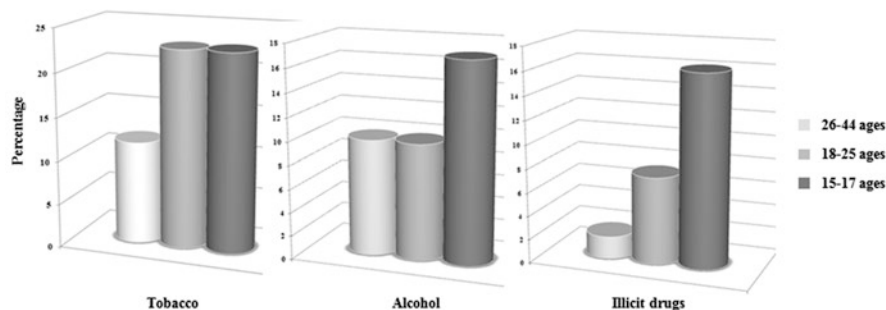


Fig. 1 Addictive substance abuse among pregnant women aged 15–44, by age, 2009–2010 combined (SAMHSA 2011)

pregnancy. The cost of treating drug-affected infants is twice the cost of medical care for non-affected infants (Huestis and Choo 2002). The incidence of obstetric complications are also higher among drug abusing mothers; therefore, assessing in utero drug exposure is quite relevant and important in providing for adequate care of the mother and the offspring of a fair segment of the population (Huestis and Cone 1998). The problem is quite serious, since 3% of women use illicit substances during pregnancy and about 54% of women use legal substances, including alcohol and tobacco, which could be harmful to a fetus (Scharnberg 2003).

When one wishes to assess the incidence of addition, several approaches are possible. For example, one can monitor maternal drug or cigarette consumption by performing periodic urinalysis, weekly sweat analysis or by analyzing patches or hair samples (Huestis and Cone 1998; Huestis and Choo 2002). Another approach is to monitor addictive substance exposure, or exposure to tobacco smoke by testing alternative (also defined as nonconventional) biological specimens from the fetus or the newborn, from the pregnant or nursing mother, or from both fetus and mother. The advantages of such specimen types are that they can be collected in a noninvasive way (except for amniotic fluid), and offer early exposure detection at different gestational periods. Obviously, several factors that concern both specimen and analyte need to be taken into account when selecting biological material for determination of addictive substances by a chosen analytical technique. Toxic substances that are absorbed circulate within the physiological fluids of the body, accumulate in tissues, or are excreted unchanged or as polar metabolites. Biological fluids are typically complex matrices, and require special procedures for sample preparation (Polkowska et al. 2004).

In this article, we present information on the effects of prenatal exposure to addictive substances, and on the prospects and difficulty of using different biological specimens for monitoring and assessing in utero exposure to illegal drugs, tobacco, and alcohol.

In Table 1 we describe the abbreviations used in this paper.

Table 1 Description of abbreviations used in this paper

Abbreviation	Description
6-AM	6-Acetylmorphine
AIDS	Acquired immunodeficiency syndrome
AMP	Amphetamine
BAR	Barbiturates
BE	Benzoyllecgonine
BENZ	Benzodiazepines
BNE	Benzoylnorecgonine
C6G	Codeine-6-glucuronide
CNS	Central nervous system
COC	Cocaine
COCE	Cocaethylene
COMT	Catechol-O-methyltransferase
DI	Direct immersion
EC	Electrophoresis
ECG	Ecgonine
EDDP	2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine
EEE	Ecgonine ethyl ester
EMDP	2-Ethyl-5-methyl-3,3-diphenylpyrrolidine
EME	Ecgonine methyl ester
EMIT	Enzyme multiplied immunoassay test
ETARA	Ethyl arachidonate
ETLAU	Ethyl laurate
ETLIN	Ethyl linoleate
ETMIR	Ethyl myristate
ETOLE	Ethyl oleate
ETPAL	Ethyl palmitate
ETSTE	Ethyl stearate
FAEE	Fatty acid ethyl esters
FAS	Fetal alcohol syndrome
FASD	Fetal alcohol spectrum disorders
FPI	Fluorescence polarization immunoassay
GC	Gas chromatography
HER	Heroin
HIV	Human immunodeficiency virus
HPLC	High performance liquid chromatography
HS	Headspace
IC	Ion chromatography
LC	Liquid chromatography
LLE	Liquid-liquid extraction
LOD	Limit of detection
LOQ	Limit of quantification
M3G	Morphine-3-glucuronide
M6G	Morphine-6-glucuronide
MAMP	Methamphetamine
MDA	3,4-Methylenedioxyamphetamine
MDEA	3,4-Methylenedioxy-N-ethylamphetamine
MDMA	3,4-Methylenedioxymethamphetamine

(continued)

Table 1 (continued)

Abbreviation	Description
METH	Methadone
MOR	Morphine
MS	Mass spectrometry
NAS	Neonatal abstinence syndrome
NCOC	Norcocaine
NMOR	Normorphine
RIA	Radio immunoassay
SIDS	Sudden infant death syndrome
SPE	Solid-phase extraction
SPME	Solid-phase microextraction
THC	Δ -9-Tetrahydrocannabinol
THC-COOH	11-Nor- Δ -9-tetrahydrocannabinol-9-carboxylic acid

2 Addictive Substance Use by Pregnant Women: A Social Problem

The term “addictive substances” normally refers to compounds that are illicit drugs, nicotine or alcohol. Addiction to these substances produces physical and psychological dependence in ways that cause health deterioration of the addict. In short, the drug user has a compulsive need to use the controlled substances for the purpose of functioning normally (SAMHSA 2011).

The percentage of women who use addictive substances is constantly growing. It has been shown that women aged 15–44 years (women of childbearing age) are the group that is the most frequent abusers of addictive substances. Such abuse is a major problem, not only because of the health impact to the pregnant woman and her offspring, but also because of the social costs it breeds, e.g., childcare neglect when the mother is dependent on addictive drugs (SAMHSA 2011).

As a result of drinking excessive alcohol or abusing illicit drugs, mothers are often deprived of their children, and consequentially the entire family suffers from the effects of the mother’s addiction (Kissin et al. 2001). Infants born to mothers dependent on addictive substances often end up with Foster Families. Children of alcohol- or illicit drug-addicted women often do not receive adequate care during their first months of life, and are exposed to maltreatment. In addition, the costs of medical care for children born to addictive drug-abusing mothers, who used drugs during pregnancy, are higher than for children born to women not using drugs. Surveys conducted among the general public, show that up to 92% of respondents said that the mother has an ethical obligation during pregnancy to behave in a way that is not detrimental to the health and life of the fetus (Anderson et al. 1997).

A pregnant woman is a special organ of society, and therefore requires special care during pregnancy and during the course of her treatment for addiction. Indeed, specialized programs have been created to help pregnant women in their fight against addiction (Narkowicz et al. 2012). Such programs include the one being used at the Johns Hopkins Bayview Medical Center in Baltimore; this program

provides psychiatric and medical care to addicted pregnant women and these patients are under continuous ambulatory care.

Kissin et al. (2001) conceived and provided an index called the Addiction Severity Index, which characterizes seven basic factors that are involved with the life and social functioning of addicted individuals. These areas are (Kissin et al. 2001; SAMHSA 2011):

- Medical
- Employment/Support
- Drug
- Alcohol
- Legal
- Family/Social, and
- Psychiatric.

These factors often are extended to include child care, assistance and help with the *upbringing of children*, vocational training, education and counseling (Daley et al. 1998). Pregnancy can be a good moment in a woman's life, in which care for the offspring acts as a stimulus to facilitate the cessation of addiction (Huestis and Choo 2002).

3 The Effects of Prenatal Exposure to Addictive Substances

The effects of addictive substances on pregnant women may be classified into a chronology of categories: maternal effects; effects on the course of pregnancy and delivery, and effects on the fetus, newborn, and developing child (Marx et al. 2002). Substance abuse by pregnant women is one of the major problems of modern civilization and users of these substances can also be categorized according to what they abuse. These are women who:

- Use illicit drugs
- Smoke tobacco or utilize the so-called nicotine replacement therapy, and/or
- Drink alcoholic beverages

Each of these substance categories may adversely affect both the woman and her offspring. Illicit drugs are usually potent central nervous system stimulants, and their long-term consumption may destroy the whole organism. Excessive or chronic intake of illicit drugs and other addictive substances damage cells of the central nervous system. Neurons, in contrast to the other cells of the human body, do not easily regenerate, and therefore, one-time illicit drug consumption may produce lasting toxic damage to the body.

Tobacco smoking and its effects on mother and developing fetus is a common problem among pregnant women. A pregnant woman can either be exposed to tobacco smoke components by actively smoking or by being a passive smoker. An actively smoking woman is mainly exposed to mainstream smoke, which is absorbed via inhalation by mouth. In contrast, a passive smoker is exposed to the components of environmental tobacco smoke (ETS), which is a mixture of side stream and

exhaled mainstream smoke. ETS diffuses into the atmosphere and is diluted in ambient air, and undergoes various physical and chemical transformations that include reactions with the mouth during smoke inhalation. Another exposure source is side stream smoke that enters the environment from chemical substances not generated from burning tobacco (from the lit end of the cigarette between puffs) (Borgerdinga and Klusb 2005).

Excessive consumption of alcoholic beverages by pregnant women is another common societal problem. Ethyl alcohol acts primarily on the central nervous system, and at sufficient intake levels is poisonous. Alcohol poisoning is a life-threatening consequence if large amounts are consumed in a short period of time. This is because alcohol quickly moves from the bloodstream into every part of the body that contains water, including major organs like the brain, lungs, kidneys, and heart, and distributes itself equally both inside and outside of cells. Ethyl alcohol is rapidly metabolized to acetaldehyde, which is the most toxic compound arising from the decomposition of alcohol. Acetaldehyde, at sufficient levels in humans, may cause nausea, vomiting, and headache (Quertemont and Didone 2006). In Table 2, we summarize the health effects that may result from prenatal exposure to several addictive substances.

Table 2 Effects on pregnant mothers, fetuses, and newborns that result from in utero exposure to addictive substances

	Maternal effects	Effects during the course of pregnancy and delivery	Effects on the fetus the newborn and the developing child
Illicit drugs	Anemia-results from iron and folic acid deficiency	Obstetric complications	Over 75% of infants exposed to drugs have major medical problems versus only 27% of unexposed infants
	Central mechanism that controls appetite and hunger is inhibited	Increased morbidity and mortality	Almost 20% of drug-exposed babies are delivered prematurely
	Narcotics affect the absorption or utilization of ingested nutrients	Abortion and spontaneous abortion	Sudden infant death syndrome (SIDS)
	Illegal drug abuse during pregnancy increases a mother's risk of blood, heart, and skin infections, and other infectious diseases such as sexually transmitted diseases and human immunodeficiency virus (HIV)	Intrauterine death, placental insufficiency, placenta previa and abruptio placenta	Neonatal abstinence syndrome (NAS)
	Increased incidence of psychiatric disorders, for example chronic anxiety and depression, psychosis, personality changes, and delusions of paranoia	Premature rupture of membranes and premature delivery	Respiratory distress syndrome, congenital anomalies, and neurobehavioral changes

(continued)

Table 2 (continued)

Maternal effects		Effects during the course of pregnancy and delivery	Effects on the fetus the newborn and the developing child
Environmental tobacco smoke	47–72% of women from various age groups suffer from dysmenorrhea Both active and passive smoking adversely affects fertility	Eclampsia	Fetal death
		Gestational diabetes	Premature birth
		Post partum hemorrhage and septic thrombophlebitis and intrauterine growth retardation	Birth defects
			Low birth weight, growth retardation
			Development disorders
Environmental tobacco smoke	47–72% of women from various age groups suffer from dysmenorrhea Both active and passive smoking adversely affects fertility	Smoking aggravates the symptoms of pregnancy	Long-term effects of illicit drugs abused during pregnancy, which are seen in older children, include: poor social adjustment, exhibit cognitive deficits, and learning disabilities
		Smoking increases the risk of the child being lost	Children and teenagers who were exposed to illicit drugs prenatally can be more irritable, have difficulty focusing attention, and have more behavioral problems
		Extrauterine pregnancies are more frequent	Neurodevelopmental and behavioral disturbances (from changes in the child’s brain following fetal hypoxia)
		Morphological damage to the placenta may become apparent as early as the first trimester of pregnancy, and irreversible changes, such as necrosis, are recognizable after the 9th week	Low birth weight (the link between maternal smoking and birth weight is weaker during the early stages of pregnancy, becomes stronger as the pregnancy advances, and is strongest in the third trimester)
			The thiocyanate ion, a metabolite of cyanide ions, inhibits iodine capture, which may inhibit the proper development of the brain and nervous system in infants
			Hyperactivity, reduced concentration

(continued)

Table 2 (continued)

Maternal effects		Effects during the course of pregnancy and delivery	Effects on the fetus the newborn and the developing child
Alcohol		Nicotine binds to acetylcholine, which controls the absorption of nutrients, volume of fluid, blood flow, and the vascularization of the placenta	Weak reaction to auditory stimuli in infants in the first week of life
		Chronic exposure to nicotine may cause the various known effects of tobacco smoking to manifest themselves in the fetus	Lower intelligence at preschool age
			Fetal exposure to nicotine can lead to addictive behaviors, and thus to smoking in adult life
			The action of irritants present in ETS may lead to chronic inflammation of the child's respiratory tract, which in turn may cause asthma
Alcohol	When women consume alcohol during pregnancy, the blood-alcohol content in the fetus reaches the same level as it does in the mother	Fetus is exposed not only to the teratogenic effects of alcohol, but also to the negative effects of the other factors that coexist in its mother's life	Urinary tract disorders Sudden infant death syndrome (SIDS) Sudden infant death syndrome
	A pregnant woman who consumes alcohol is also likely to follow a poor diet and exercise plan	Pregnant alcohol-exposed women are more likely to experience obstetric complications, and increased morbidity and mortality	FASDs (Fetal alcohol spectrum disorders) include physical, mental, behavioral, and/or learning disabilities with possible lifelong implications

(continued)

Table 2 (continued)

Maternal effects	Effects during the course of pregnancy and delivery	Effects on the fetus the newborn and the developing child
She may also have several other problems, including comorbid medical or psychiatric disorders such as depression, and social problems		Fetal alcohol syndrome (FAS) is the most clinically recognizable form of FASD characterized by: <ul style="list-style-type: none">• Prenatal and postnatal growth retardation• Functional or structural central nervous system (CNS) abnormalities such as mental retardation and behavioral problems• A pattern of minor facial and skull anomalies including small eye openings, altered nose and forehead structure, an absent or elongated groove between the upper lip and nose, a thin upper lip, a flattened mid face, and under development of the upper or lower jaw FAS consequences are lifelong, and behavioral and learning difficulties are often greater than the degree of neurocognitive impairment

Sources: Chen et al. 2000; Dejmek et al. 2002; Eskenazi and Castorina 1999; Finnegan 1994; Gilmour et al. 2006; Huestis and Choo 2002; Jauniaux and Burton 2007; Jones 1974; Larkby and Day 1997; Marx et al. 2002; Miller and Hyatt 1992; Niemann and Anderson 2008; Otero et al. 2004; Phibbs et al. 1991; Rogers 2009; Vogel 1997; Zuckerman et al. 1989

4 Biomonitoring to Assess In Utero Exposures to Addictive Substances

Accurate identification of in utero exposure to addictive substances has important implications for the care of mothers and children. Maternal illicit drug use during pregnancy can be monitored by performing analyses on several key media; such media includes urine, sweat, oral fluid, and/or hair. The rate of drug absorption and disposition of addictive substances and metabolites into different matrices is dependent on the route of drug administration and on the physiochemical characteristics of the drug (Huestis and Choo 2002).

Maternal blood was one of the first types of biological material that was analyzed to detect drugs. The analytical targets were either for illicit drug use during pregnancy, or for fetal exposure to drugs. However, the value of testing blood for drugs of abuse is limited because the window of detection is short and the fact that obtaining the sample is invasive (Lozano et al. 2007).

Other potential specimen types that can be monitored to evaluate the degree and type of drug exposure include the following: neonate cord blood, placenta, vernix, amniotic fluid, neonatal hair and urine, and meconium. As with maternal blood, measuring levels of drugs and their metabolites in cord blood reflects only fetal drug exposure during the previous hours or days before collection, and does not reflect chronic exposure during the entire gestation period. However, collecting other specimen types is preferred because obtaining neonatal plasma is invasive and difficult. The placenta and vernix have been rarely sampled for testing, but are currently under evaluation to determine their usefulness (i.e., noninvasiveness of collection and ready availability at delivery; Esteban and Castaño 2009; Lozano et al. 2007).

Because amniotic fluid is already formed in the first weeks of pregnancy, the presence of drugs in this fluid can reflect exposure during the early fetal life. Although it is dangerous for the fetus, amniotic fluid can be sampled at any time during pregnancy, if detecting either parent drugs or their metabolites are essential for protecting the fetus. Meconium testing has also been shown to be an effective and practical means of detecting in utero drug exposure. Analysis of meconium provides more complete information on drug exposure during pregnancy than does analysis of neonatal urine or cord blood. Recently, drug determination in meconium has been successfully applied to assess intrauterine exposure to addictive substances (Lozano et al. 2007).

5 Role of the Placenta in Biomonitoring of Addictive Substances

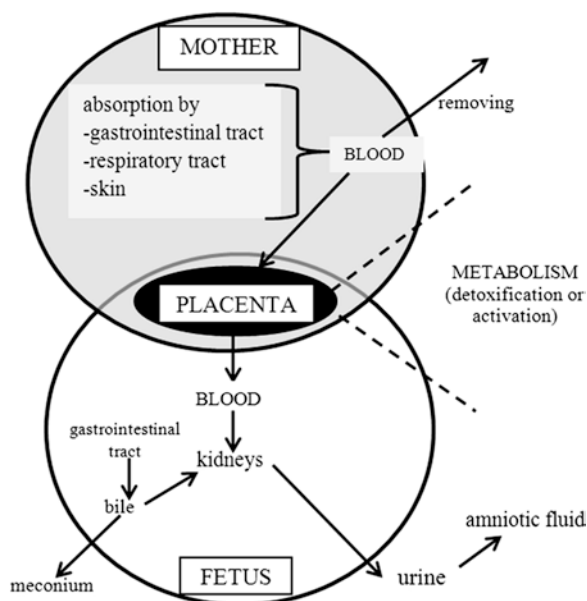
When performing biomonitoring studies on toxic substances, several liquid and solid tissue types are suitable for sampling. The particular tissues or fluids selected will depend on the goals of the experiment and what is available. Theoretically, the biological material that is selected for further research should fulfill the following criteria:

- Sample collection will not pose a risk to the health or life of the donor
- The amount of analyte to be sampled will be determined by currently available techniques
- The sample size is sufficient for analysis
- Sample collection is convenient
- Samples can be easily stored until analysis

Placental tissue has a key role, when used for biomonitoring and assessing the degree of in utero exposure to addictive substances. The placenta is an organ that connects the developing fetus to the mother, and provides the following main functions (Van der Aa et al. 1998):

- Nutrition
- Excretion
- Immunity
- Endocrine function, and
- Cloaking of the fetal immune system from that of the mother

Fig. 2 Schematic of how xenobiotics are transferred from mother to child through the placenta



Unfortunately, the women who consume toxic illicit drugs, alcohol and their metabolites will transport these substances across their placentas, which can cause serious harm to the fetus (Leino et al. 2011). Figure 2 schematically depicts the manner in which addictive substances can be transferred from mother to child through the placenta.

Tobacco smoke contains toxic compounds that are readily soluble in water and may easily transgress the placental barrier. Although most of these substances can be removed by xenobiotic detoxification enzymes, the smoke components in tobacco may directly affect the villous cytotrophoblast.

The placenta, like the liver, may play an important role in metabolizing toxic substances. The cytochrome P450 system (CYP) is a family of enzymes that control the concentrations of many endogenous and exogenous substrates. CYP fulfill their role by actively metabolizing a wide variety of xenobiotics (e.g., drugs and other toxic chemicals). CYP also metabolize endogenous compounds, such as steroid hormones and arachidonic acid. This family of enzymes is composed by multiple subunits that differ in their amino acid sequences. In the human body, 19 enzymes from subfamily of CYP P450s have been discovered, most of which are located in the liver. However, several enzymes such as CYP1A1, CYP2F1, and CYP4B1 are associated largely with extrahepatic organs. The activity of CYP enzymes may lead to the formation of reactive metabolites with toxic consequences (sometimes carcinogenic). To date, the mechanism and function of particular forms of CYP enzymes in human placental tissue are not well known. However, the appearance of the CYP1A1 enzyme has been observed in placental samples from women who smoked during pregnancy. In addition, the mRNA and protein of CYP3A7, the prominent

form in fetal liver, have been observed to also exist in the early-term placenta (Hakkola et al. 1996).

The use of addictive substances by woman during pregnancy results in changes to the placenta. Due to prenatal exposure to addictive substances, morphological damage of the placenta may be observed in the first trimester of pregnancy, and irreversible changes (e.g., necrosis) may be seen after the first 9 weeks of pregnancy (Jauniaux and Burton 2007). Hakkola et al. (1996) studied the expression of CYP P450 forms and they described the external appearance of the placenta. It has been proved that the placenta from women who smoked during pregnancy were calcified or thick. Similarly, the placentas from women who abused illicit drugs during pregnancy have been reported to be calcified.

The placenta is a good biological entity to use for biomonitoring and assessing the effects of prenatal exposure to toxic and addictive substances (Al-Saleh et al. 2011); the biomonitoring value of the placenta over blood or urine is that it can be used to assess long-term exposures (Myllynen et al. 2005). The placenta has other advantages for biomonitoring of toxic substances as well (Esteban and Castaño 2009), viz., samples can be taken noninvasively and it is a matrix that reflects the character of constant contact with both the mother and the fetus.

Protecting the fetus and serving as a barrier to entry of xenobiotics are not the only placental functions. Another function of the placenta is to transfer nutrients and oxygen from the mother to the fetus. The placenta also metabolizes chemical compounds, and thereafter assists in removing metabolites and waste products from the fetus.

There are several mechanisms responsible for the transport of addictive substances that are taken in by the mother. Among these mechanisms are passive diffusion, facilitated transport, active transport, pinocytosis, and phagocytosis. Most compounds enter the placenta by passive diffusion, which process is described by Fick's law (Myren et al. 2007). Myren et al. (2007) and Van der Aa et al. (1998) defined the factors that affect the rate of transport as including the:

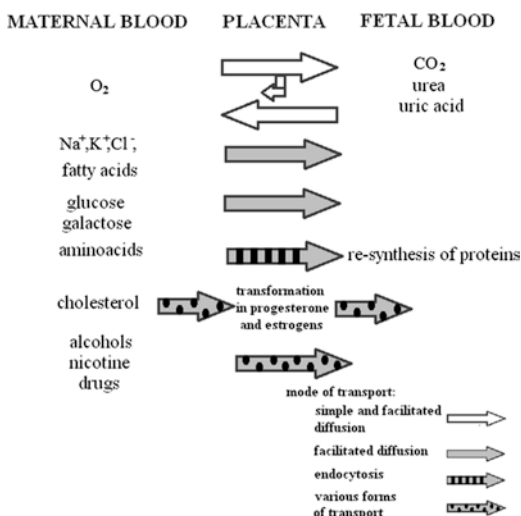
- Xenobiotic concentration gradient between the circulatory system of the mother and that of the child
- Surface area of the exchange membrane
- Thickness of the endothelio-syncytial membrane
- Blood flow rate through the placenta
- pH of both the maternal and fetal blood
- Physicochemical properties of the individual chemical compounds
- Status of maternal and child health, and
- Rate of the metabolism of the xenobiotics present

Xenobiotics can also enter and pass through the placenta via facilitated transport. The diffusion processes is facilitated by carrier-mediated mechanisms that operate along a concentration gradient, without making use of an outside energy source. Only a few drugs are known to be transported by this mechanism (Myren et al. 2007).

Substances may cross the placenta by active transport as well. Active transport takes place against an electrochemical or concentration gradient, but extracts an energy cost during the process. Active transport is also carrier-mediated. A total of 20 different transport proteins have been detected in the human placenta (Myren et al. 2007).

Finally, transport may occur by pinocytosis or phagocytosis, in which the substance is invaginated into a cell membrane and is transferred to the other side of a

Fig. 3 Schematic of how various substances are transported to and through the placenta



membrane as an enclosed vesicle. This route of placental transfer of xenobiotics is the least important, mainly because this process is very slow (Myren et al. 2007).

In Fig. 3 we diagram the various mechanisms by which xenobiotics are transported between the circulatory systems of the mother and child.

6 Analysis of Addictive Substances in Biological Media

Above, we have described the important uses to which analytical drug residue data on pregnant mothers, their fetuses or the newborn can be put (Fig. 4). The methods used to perform these analyses (e.g., GC-MS and LC-MS) are quite sensitive and can be used to accurately measure addictive substances and their metabolites that are biomarkers of in utero drug exposure. Those abused substances and their metabolites that are most commonly used as biomarkers of in utero drug exposure are summarized in Table 3.

In Fig. 5, we show the stages that are involved when analyzing for xenobiotic residues of interest in biological media. Sample preparation is critical because the addictive substance analytes must be isolated from complex biological matrices such as tissues, or bodily fluids. The direction taken in sample preparation is determined by the physicochemical properties of the analytes and the complexity of the sample tissue or fluid from which they are to be extracted. The methods used to separate and purify the analytes in these specimens commonly utilize methods such as LLE, SPE, and SPME. If the parent compound or metabolites are polar, and must be detected by gas chromatography, appropriate derivatization to form volatile analytes must be added to the sample preparation steps.

Before identifying and quantifying analytes of interest, immunoassay methods may also be used as screening tests. The most popular immunoassay tests are: EMIT, RIA, and FPI. After a rapid screening is completed, specific and sensitive chromatographic methods are used to obtain more detailed information (Gray and

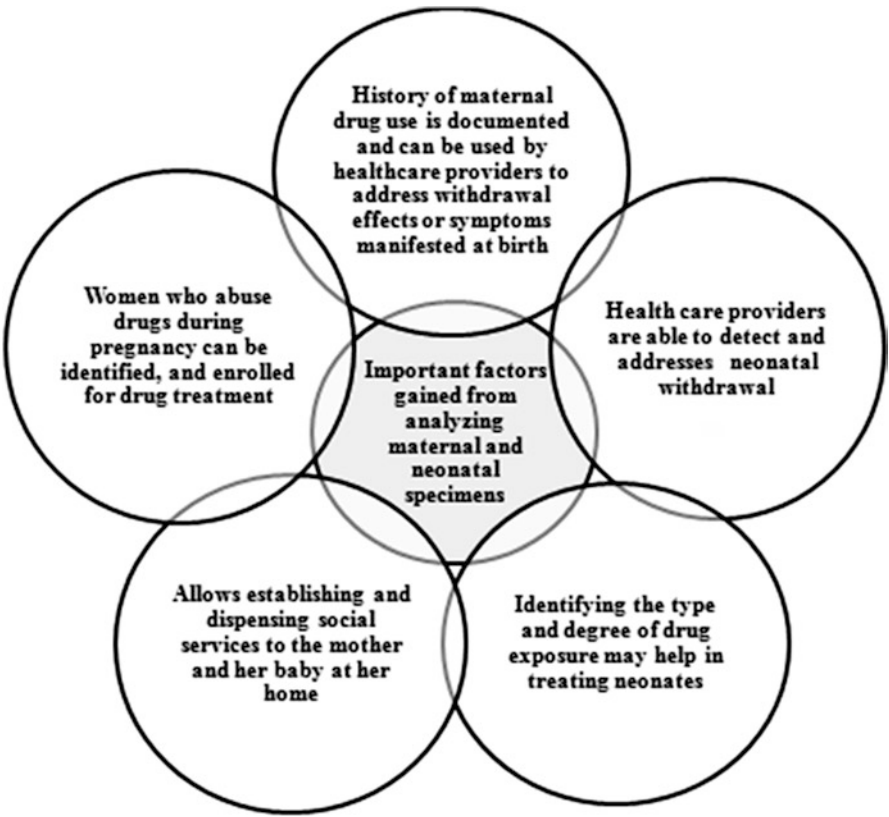


Fig. 4 Important factors gained from analyzing biological specimens from fetuses, newborns, and pregnant women (when these women have abused additive substances)

Table 3 Abused substances and their metabolites as biomarkers for biomonitoring of these substances

Abused substance	Toxic substance	Excretion substances—biomarkers
Tobacco smoke	Nicotine, hydrogen cyanide, formaldehyde, kadm, PHA, benzene	Nicotine, kotonina, <i>trans</i> -3'-hydroksykotynina, thiocyanate ion, formaldehyde, kadm, 1-hydroksy-benzo(a)piren, benzene, muconic acid, and S-phenyl mercapturic acid
Amphetamine group substances	Methamphetamine/amphetamine	Methamphetamine, p-hydroxymethamphetamine, amphetamine, p-hydroxyamphetamine, glucuronide or glycine (hippuric acid) conjugate, benzoic acid, acid-labile precursor of benzyl methyl ketone, norephedrine, p-hydrohynorephedrine

(continued)

Table 3 (continued)

Abused substance	Toxic substance	Excretion substances—biomarkers
Opioids	MDMA/MDA/MDE	MDMA, MDA, MDE, 3,4-dihydroxymethamphetamine, 3,4-dihydroxyamphetamine, 4-hydroxy-3-methoxyamphetamine, 4-hydroxy-3-methoxymethamphetamine, 3,4-dihydroxyethylamphetamine
	Heroin	Heroin, morphine, 6-acetylmorphine
	Morphine	Morphine, morphine-3-glucuronide, morphine-6-glucuronide, morphine-3-sulfate, normorphine
	Methadone	Methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine, 2-ethyl-5-methyl-3,3-diphenylpyrrolidine
Cocaine	Naloxone	Naloxone, naloxone-3-glucuronide
	Cocaine	Cocaine, benzoylecgonine, ecgonine methyl ester, anhydroecgonine methyl ester, norcocaine
Cannabis	Lidocaine	Monoethylglycinexylidide, glycine, xylidide
	Benzocaine	Benzocaine, acetylbenzocaine
	Marijuana	THC, 11-OH-THC, 8- β -hydroxy THC, THC-COOH
	Cannabidiol	Cannabidiol, THC, 11-OH-THC
	Cannabinol	Cannabinol, THC, 11-OH-THC
Alcohol	THCV	THC, THCV
	Ethanol	Acetaldehyde, ethyl glucuronide, ethyl sulfate, fatty acid ethyl esters

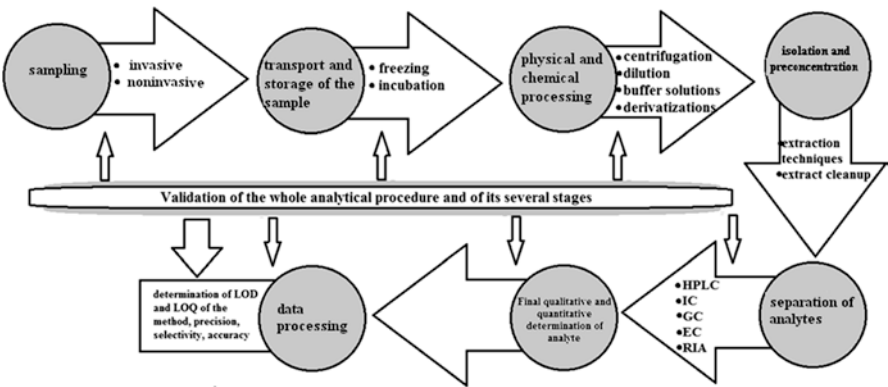


Fig. 5 General scheme for preparing and analyzing biological samples of complex matrices

Huestis 2007; Schütz et al. 2006). Although several chromatographic techniques are used to analyze for addictive compounds in biological materials, the majority are based on GC-MS, LC-MS, or LC-MS-MS. In Table 4, we describe the characteristics of biological media commonly sampled from mothers and fetuses.

Table 4 Characteristics of biological materials normally selected for biomonitoring of addictive substances, and techniques used for their analysis

Biological materials	Characteristic	Advantages	Reference	Parent substances	Analytes	Detection levels	Concentration range	Sample preparation method(s)	Analysis method(s)	Reference
Mother's hair	Abused substance accumulates in hair during hair growth, and thereby is a unique measure of long-term, cumulative exposure to abused substances	Matrix is stable	Esteban and Castaño 2009	AMP, MAMP, BAR, BENZ, Cannabinoids, COC, Opiates, METH	Fatty acid ethyl esters (FAEE)	ng/mg	0.2–1.0	LLE	ELISA	Kulaga et al. 2010
	Possible pathways for drug incorporation into hair include: diffusion from capillaries to hair follicles; excretion onto the hair surface from sweat and sebum; and external contamination	Low cost		Nicotine	Nicotine, cotinine		Nicotine: <LOD-24.9 Cotinine: <LOD-3.3	HS-SPME SPE	GC-MS HPLC	Pichini et al. 1997
		Long prenatal exposure to certain drugs of abuse								
		Can reflect exposures for the entire pregnancy		COC, BE	COC and metabolites, BE		40–2,000	SPE	LC-MS	López et al. 2007
Oral fluid		Large sample size								
		More sensitive and stable than other specimen types								
	Is a composite tissue consisting primarily of saliva, mixed with gingival fluid, buccal and mucosal transudates, cellular debris, bacteria, and residues of ingested products	Easy and noninvasive collection	López et al. 2007	Cannabis	THC	µg/L cut off	4.0	SPE	GC-MS ELISA	Gray et al. 2010
	Most prevalent substance form detected is parent compound, rather than its metabolites	Low cost								
	Several factors affect the abused-substance concentration in oral fluid			Nicotine	Cotinine		10.0		ELISA LC-MS	

Mother's blood	<p>Bodily fluid delivers necessary substances such as nutrients and oxygen to cells and transports metabolic waste products away from those same cells</p> <p>Blood concentrations may closely reflect the amount of abused drug exposure to the fetus</p> <p>Excessive blood collection may produce anemia</p>	<p>The ideal matrix for most chemicals from the constant contact with the entire organism</p> <p>Restricted use in infants and children</p>	<p>López et al. 2007</p>	<p>COC</p> <p>Opiates</p> <p>METH</p> <p>Camabinoids</p>	<p>6-AM, MOR, COD, COC, BE, COCE, AMP, MAMP, MDMA, MDA, THC, THC-COOH</p>	<p>ng/ml</p>	<p>MOR: 0.6–1.0</p> <p>COC and metabolites: 9.2–23.3</p> <p>BE: 3.0–15.5</p> <p>COCE: 0.5–0.8</p> <p>THC: 0.2–2.7</p> <p>THC-COOH: 0.1–0.8</p>	<p>NA</p>	<p>GC-MS</p>	<p>Falcon et al. 2010</p>
Breast milk	<p>A complex physiological liquid that simultaneously provides nutrients and bioactive components</p> <p>Content: certain vitamins proteins, bioactive peptides, oligosaccharides, and organic (including fatty) acids</p> <p>Extracting abused substance from breast milk is analytically challenging, because of its high protein and fat content, and its variable composition during the postpartum period</p>	<p>Noninvasive collection</p> <p>Used for monitoring because it provides information on exposure to both mother and fetus</p>	<p>López et al. 2007</p>	<p>METH</p> <p>METH</p> <p>EDDP</p> <p>EMDP</p> <p>NA</p> <p>BE</p> <p>COC</p> <p>MOR, 6-AM, COD, EDDP</p> <p>MAMP, AMP, MDMA, MDA</p> <p>Nicotine</p>	<p>METH</p> <p>EDDP</p> <p>EMDP</p> <p>NA</p> <p>BE</p> <p>COC</p> <p>MOR, 6-AM, COD, EDDP</p> <p>MAMP, AMP, MDMA, MDA</p> <p>Nicotine</p>	<p>µg/L</p>	<p>SPE</p> <p>EDDP: 5.5–196.0</p> <p>EMDP: <LOD-3.17</p> <p>THC: 86 ng/mL</p> <p>THCOH: 5 ng/mL, METH: 97 ng/mL, EDDP: 8 ng/mL, MOR: 7 ng/mL</p>	<p>SPE</p>	<p>EL-GS-MS</p> <p>LC-MS-MS</p>	<p>Nikolaou et al. 2008</p> <p>Marchei et al. 2011</p>
					<p>Nicotine</p> <p>Cotinine</p> <p><i>trans</i>-3-hydroxycotinine, <LOD-17.3</p> <p>Cotinine-N-oxide <LOD-18.4</p>	<p>µg/L</p>	<p>LLE</p> <p>Nicotine, <LOD-513.5</p> <p>Cotinine, <LOD-344.8</p> <p><i>trans</i>-3-hydroxycotinine: <LOD-17.3</p> <p>Cotinine-N-oxide: <LOD-18.4</p>	<p>LC-MS-MS</p>	<p>Pellegrini et al. 2007</p>	

(continued)

Table 4 (continued)

Biological materials	Characteristic	Advantages	Reference	Parent substances	Analytes	Detection levels	Concentration range	Sample preparation method(s)	Analysis method(s)	Reference
Mother's urine	Sterile liquid by-product of the body secreted	The parent substance of abuse and its metabolites are present in urine	López et al. 2007	Opiates, COC	EME, BE, COCE, COC, mOHBE, pOHBE, NCOC, EEE	µg/L	COD: 25–136	NA	LC-MS	Shakleya et al. 2010
	Cellular metabolism generates numerous by-products, many rich in nitrogen, that require elimination from the bloodstream	Large sample size			MOR, M3G, M6G, 6-AM, NMOR, C6G, HER, COD		Free MOR: 53–3, 359, NMOR: 50–181, NCOD: 31–63			
		Easy and noninvasive collection		COC Opiates METH Cannabinoids	EME, BE 6-AM, MOR, COD METH, EDDP THC-COOH	mg/g creatinine	EME: 32.7–48; BE: 312–1,965; MOR: ND–2,909; COD: ND–8,666; METH: 98–2,925; EDDP: ND–1,275	LLE	FPIA EMIT GC-MS	Vinner et al. 2003
Meconium	The first fecal matter passed by the neonate	Large sample size	López et al. 2007	Alcohol	ETMIR, ETPAL, ETOLE, ETSTE, ETLAU, ETLIN, ETARA	mg/g	ETMIR: 0–0.794; ETPAL: 0–1.746; ETOLE: 0–0.1658; ETSTE: 0–0.934; ETLAU: 0–0.429; ETLIN: 0–5.715; ETARA: 0–1.168	SPE	GC-MS	Ostrea et al. 2006
		Easy and noninvasive collection		COC, heroin	COC, BE	ng/g	40–2,000	SPE	RIA	López et al. 2007
	Composition: bile salt and acids, epithelial cells, lipids, mucopolysaccharides, and water	Reflects exposures from the second and third trimesters of gestation		Nicotine	COD, MOR, 6-AM Nicotine, cotinine, caffeine	ng/g	Cotinine: 20–86 Caffeine: 10–45	LLE, SPE	GC-MS HPLC	Baranowski et al. 1998
				Nicotine	NIC, COT, <i>trans</i> -3-hydroxycotinine, NNIC, NCOT	ng/g	NIC: 101.4; COT: 94.7; <i>trans</i> -3-hydroxycotinine: 196.8; NNIC: 10.2; NCOT: 4.4	SPE	LC-MS-MS	Gray et al. 2008

Fetal hair	Hair residues could come from blood or from amniotic fluid Hair starts growing at ~6 months of gestation and reaches the scalp surface approx. 3 weeks later	Samples can be stored at room temperature Reflects the third trimester of gestational exposure Record of prenatal exposure available for as long as 4–5 months of postnatal life	López et al. 2007	Nicotine Cotinine COC Opiates Cannabinoids METH BENY BAR	ng/mg mg/g NA NA	SPE LLA	HPLC ELISA GC-MS	Pichini et al. 1997 Vinner et al. 2003
Placenta	Source of fetoplacental circulation, between maternal and fetal blood, acts as a nutrient and waste exchanger	Large sample size Easy and noninvasive collection	López et al. 2007	Nicotine Amphetamines, ecstasy COC Cannabinoids Opiates METH, EDDP, MOR, COD, 6-AM, COC, BE COC	NA NA NA COC, BE, COCE THC-COOH METH, MOR	SPE SPE	GC-EI-MS GC-EI-MS	Joya et al. 2010
Amniotic fluid	Reflects a 20-week gestational content of maternal secretions, plus fetal secretions Later, fetal urine and lung secretions are added to the amniotic fluid The amniotic fluid accumulates mainly water-soluble substances Traces of apolar parent compounds and their metabolites may also be present	Reflects long prenatal exposure to certain drugs of abuse Can be sampled at any time during pregnancy The presence of drugs of abuse in this fluid may reflect exposure during the early fetal life	Lozano et al. 2007	BE, EME, EEE, NCOC, COCE, mOHBE	ng/g μg/L NA	NA NA SPE	LC-MS IC-MS/MS HPLC-MS GS-MS	De Castro et al. 2009 Eylar et al. 2005 Loughhead et al. 2006

This description includes advantages and drawbacks, and provides literature references that address analytical procedures for determining markers of in utero drugs exposure in these specimens.

New techniques for analysis of addictive substances and their metabolites are routinely being developed; therefore, extraction efficiency, detection limits for addictive substances or their metabolites are improving, as are methods to analyze for substances in alternative matrices.

Although the wide spectrum of analytical methods available allows collecting important information on in utero exposure of addictive substances, there is still a dearth of knowledge about how addictive compounds are distributed in some human materials. Therefore, we propose that future research be performed to gather more robust data on addictive substances (tobacco smoke, illicit drugs and alcohol) in regard to their pharmacokinetics in humans, and how abused substances are mechanistically diffused among human tissues.

7 Summary

The use of addictive substances during pregnancy is a serious social problem, not only because of effects on the health of the woman and child, but also because drug or alcohol dependency detracts from childcare and enhances the prospect of child neglect and family breakdown. Developing addictive substance abuse treatment programs for pregnant women is socially important and can help ensure the health of babies, prevent subsequent developmental and behavioral problems (i.e., from intake of alcohol or other addictive substances such as methamphetamine, cocaine, or heroine) and can reduce addiction costs to society.

Because women of childbearing age often abuse controlled substances during their pregnancy, it is important to undertake biomonitoring of these substances in biological samples taken from the pregnant or nursing mother (e.g., blood, urine, hair, breast milk, sweat, oral fluids, etc.), from the fetus and newborn (e.g., meconium, cord blood, neonatal hair and urine) and from both the mother and fetus (i.e., amniotic fluids and placenta). The choice of specimens to be analyzed is determined by many factors; however, the most important is knowledge of the chemical and physical characteristics of a substance and the route of its administration. Maternal and neonatal biological materials reflect exposures that occur over a specific time period, and each of these biological specimens has different advantages and disadvantages, in terms of accuracy, time window of exposure and cost/benefit ratio.

Sampling the placenta may be the most important biomonitoring choice for assessing in utero exposure to addictive substances. The use of the placenta in scientific research causes a minimum of ethical problems, partly because its sampling is noninvasive, causes no harm to mother or child, and partly because, in any case, placentas are discarded and incinerated after birth. Such samples, when properly analyzed, may provide key essential information about fetal exposure to toxic

substances, and may provide the groundwork for protecting the fetus or newborn and the mother from further damage.

Several sensitive and specific bioanalytical methods are commonly utilized to accurately measure for drug biomarkers of in utero drug exposure. Moreover, several immunoassay methods are used to rapidly screen for drugs in many biological specimen types. However, results from immunoassays should be carefully interpreted, and should be confirmed by more specific and sensitive chromatographic methods, such as GC-MS or LC-MS. Although techniques for analysis of addictive substances are still being developed or are being refined, current methods are efficient and sensitive and provide valuable information on human exposures to addictive substances and their metabolites.

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