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EXAMINING THE INTERRELATIONSHIP BETWEEN THE OPIOID EPIDEMIC, PUBLIC HEALTH, AND FORENSIC SCIENCE

by

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ADRIANNA U. K. DUROCHER

ABSTRACT

The United States (U.S.) government has been attempting to combat the growing opioid epidemic ravaging the nation. The opioid epidemic has had a significant impact on public health and forensic science laboratories. Moreover, this epidemic has moderate to fatal health consequences for expectant mothers with substance use disorder and their child who may develop Neonatal Abstinence Syndrome (NAS), otherwise known as Neonatal Opioid Withdrawal Syndrome (NOWS). The objective of this thesis is to emphasize that further research is needed for the identification and quantification of opioids in human breastmilk. This topic has public health implications such as discussing the information gaps as it relates to a highly vulnerable group, women and infants, affected by the opioid epidemic. Furthermore, there are implications in forensic science connected to postmortem toxicology and pathology when determining the cause of death and contributing factors in pediatric cases. This emphasis on the need for greater research will be accomplished by highlighting the opioid epidemic, its impact and further understanding of the addictive drug class known as opioids. The history of the crisis, effects on society as well as pharmaceutical knowledge of opioids will assist in development of plans to suppress growth and provide care for the afflicted. Furthermore, this thesis will attempt to demonstrate the need for further research involving opioids will be of significant value for

public health and forensic science. As the forensic laboratories and various medical facilities are at the forefront of the opioid epidemic, there is a need for more robust, validated, inexpensive, and fast drug detection methodologies. Increasing rates of new designer drugs, addiction, and opioid-related deaths has caused a backlog in the forensic laboratories due to the great number of cases. While, the higher instances of maternal substance use disorder (SUD)/ opioid use disorder (OUD) with parallel increases in cases of NAS incidences are a few of the issues that need to be managed by public health leaders. Additionally, this thesis will examine current methodologies for drug quantification of opioids in human breast milk. The valid methodologies developed as well as the findings by the few available studies allowed for the current recommendations related to the acceptability of mothers in MAT programs, using methadone and buprenorphine during pregnancy and postpartum, being able to breastfeed their infant. By examining these studies and the findings, standardization criteria for the development of study designs for new methodologies relating to drug determination in human breastmilk could be developed. The establishment of standardization criteria and acknowledging information gaps in current knowledge will be significant as these findings could influence policies, guidelines and procedures relating to maternal SUD/OUD, NAS/NOWS, and pediatric death determination as well as postmortem toxicology.

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LIST OF ABBREVIATIONS

AAP	American Academy of Pediatrics
ACOG	American College of Obstetricians and Gynecologists
BFHI	Baby-Friendly Hospital Initiative
CDC	Centers for Disease Control and Prevention
CEA	Council of Economic Advisors
DEA	Drug Enforcement Administration
GC-MS	Gas Chromatography – Mass Spectrometry
GC-MS-MS	Gas Chromatography – Mass Spectrometry – Mass Spectrometry
HHS	United States Department of Health and Human Services
HIV	Human Immunodeficiency Virus
LactMed	Drugs and Lactation Database
LC-MS	Liquid Chromatography – Mass Spectrometry
LC-MS-MS	Liquid Chromatography - Mass Spectrometry – Mass Spectrometry
MAT	Medication-assisted Treatment
NAS	Neonatal Abstinence Syndrome
NIH	National Institutes of Health
NLM	National Library of Medicine
NOWS	Neonatal Opioid Withdrawal Syndrome
NPS	Novel Psychoactive Substances
OUD	Opioid Use Disorder
PRGLAC	Task Force on Research Specific to Pregnant Women and Lactating Women

SAMHSA	Substance Abuse and Mental Health Services Administration
SUD	Substance Use Disorder
UDP	Uridine diphosphate
UGT	UDP-glucuronosyltransferase
UNICEF	United Nations International Children's Emergency Fund
U.S.	United States
USFDA	United States Federal Drug Administration
WHO	World Health Organization

1. INTRODUCTION

1.1 The Opioid Epidemic: A Growing Crisis

1.1.1 History

The United States (U.S.) government has been attempting to combat the growing opioid epidemic ravaging the nation. The U.S. opioid epidemic was the product of a distinct set of historical circumstances that escalated into this national public health crisis. Papaver Somniferum (Opium Poppy or Breadseed Poppy) is known for its medicinal use as a pain reliever and recreational use due to its euphoric effects for millennia. The opium poppy has roots as far back as the 3400 B.C. Sumerian, ancient inhabitants of Southern Mesopotamia, culture. It is in the 1700s that opium and opium-smoking made its way to China, which had a devastating impact on the Chinese nation due to its addictive qualities and availability on the drug market [1]. By the 1800s, opium had spread across the globe due to international trading, which led to the discovery of Principium Somniferum (Morphine), the active ingredient in opium, by Friedrich Sertürner of Germany. Morphine became prominently used in the medical field due to its reliability and long-lasting effects. However, morphine was also highly addictive, leading to a significant rise in morphine addiction and abuse during the period of the Civil War with American soldiers. The U.S. Congress attempted to curb the increase in addiction by imposing a tax on opium and morphine, which, later, escalated to banning opium in 1909 [2, 3]. In the late 1800s, English researcher C.R. Wright synthesized diacetylmorphine, which is later coined “heroin” by the Bayer Company of Germany. The Bayer Company, eventually, marketed heroin as an over-the-counter pain-reliever without the common side effects of morphine. However,

heroin was also addictive and more commercially available than morphine and opium, resulting in an alarming rise in heroin addiction. The U.S. Congress passed the Pure Food and Drug Act of 1906, which required addictive and dangerous contents be placed on the label of all medications patented by pharmaceutical companies. The Pure Food and Drug Act resulted in the decreased availability of opioid/opiates, and opioid/opiate consumers significantly declined [2-4].

In 1914, the Harrison Narcotics Act was passed thus requiring prescribers of narcotics to register and pay a tax for each prescription issued. This act aimed to suppress drug addiction and abuse, but the impact was not significant. Thus, the U.S. Treasury Department's Narcotics Division was created as the first federal drug agency and a ban on the sale of all narcotics was one of its first acts in 1923. Black market and illegal street sales drew many opioid-dependent individuals as the ban prohibited licensed sites from stocking heroin [4, 5]. As a result, the number of individuals with heroin addiction in the U.S. grew exponentially due to the U.S. attempts to stop the spread of communism in Asia as well as eradicate opium sources leading to U.S. involvement in the Vietnam War. U.S. involvement in the Vietnam War led to many soldiers using various drugs, including opium due to the popularity of the illegal substance in Vietnam, for a variety of reasons but mainly performance enhancement. The addictive nature of the various drugs resulted in many soldiers returning to the U.S. with addiction.

The interference of the U.S. in Asia resulted in increased smuggling of illegal heroin into the United States by various international parties. In 1973, the Drug Enforcement Administration (DEA) under the Justice Department, was created to

consolidate federal powers of drug enforcement into a single agency. The U.S. managed to suppress the Chinese and Mexican heroin sources causing the drug market to decline. However, a new source of heroin appeared in Iran, Afghanistan, and Pakistan which resulted in a resurgence of production and trade of illegal heroin. During the 1990s, the opioid epidemic experienced a considerable upsurge in the U.S. due to medical misconceptions, poor research, and the American Pain Society "pain as the fifth vital sign" campaign [2-5]. Pharmaceutical companies and other medical societies claimed limited risk of addiction to prescribed opioids thus reassuring prescribers causing a massive increase in opioid prescriptions. The use of opioids for chronic pain and other non-cancer related pain was heavily promoted by pharmaceutical companies despite the lack of appropriate data for a risk-benefits analysis. Purdue Pharmaceuticals is well-known for having aggressively and misleadingly marketed OxyContin as safer and less addictive than other opioids resulting in severe fines and an abuse-deterrent formulation of OxyContin. By the 2000s, there was a rapid increase in deaths from heroin misuse and abuse. Efforts in decreasing opioid prescribing began to show results due to limited availability which caused increased use of heroin as a result of its low cost, availability, and potency [2-5].

According to Rudd RA, "Deaths due to heroin-related overdose increased by 286% from 2002 to 2013, and approximately 80% of heroin users admitted to misusing prescription opioids before turning to heroin" [6]. In 2013, there was an increase in synthetic opioid-related deaths such as fentanyl and fentanyl-related analogs. However, in 2016, the most significant increase of opioid-related deaths occurred with over 20,000 deaths from fentanyl and related drugs [6, 7]. The high death toll continued to increase as

more fatal incidences of opioid use overdoses occurred as a result of the illicit production of fentanyl as an adulterant or substitute. The use of fentanyl and other synthetic opioids as a substitute or adulterants caused opioid use overdoses to continue increasing, and by 2017, the U.S. government declared the opioid epidemic a national public health emergency. Various initiatives, policies, and programs are being developed and implemented to this day to combat the deadly opioid epidemic.

1.1.2 The National Impact of the Opioid Epidemic

The national impact of the opioid epidemic in the U.S. can be observed through the analysis reports published by the National Forensic Laboratory Information System (NFLIS). In 1997, the Drug Enforcement Administration (DEA), Diversion Control Division, established the NFLIS program for the purpose of data collection. NFLIS, or NFLIS-DRUG, involves the systematic collection of drug identification reports, which include controlled substances, non-controlled substances, and associated information from drug cases. NFLIS collects data about drug identifications and analysis reports from participating federal, state, and local forensic laboratories across the United States [8].

By analyzing annual NFLIS reports from 2000 to 2018, an observable trend emerges from the data that shows the expanding damage of the opioid epidemic when comparing the total number of cases related to opioids (Figure 1). The number of cases involving opioids have been increasing since 2000 with minor declines between 2010 to 2014 due to efforts to eliminate illegal sources of opium. Opioids were involved in 28,647 deaths, or 61% of all drug overdose deaths in 2014 which continued to rise in the following years [6]. According to Calcaterra et al., from the article *National Trends in*

Pharmaceutical Opioid Related Overdose Death Compared to Other Substances Related Overdose Deaths: 1999-2009, "from 1999 to 2007, substance abuse treatment admissions for pharmaceutical opioid abuse increased nearly 4-fold. Additionally, emergency department visit rates related to pharmaceutical opioids increased 111% from 2004 to 2008; visit rates were highest for oxycodone, hydrocodone, and methadone" [8]. Drug overdoses reached a new high in 2017, with 47,600 deaths caused by drugs such as fentanyl and heroin as well as prescription drugs, according to the Centers for Disease Control and Prevention (CDC) [9, 10].

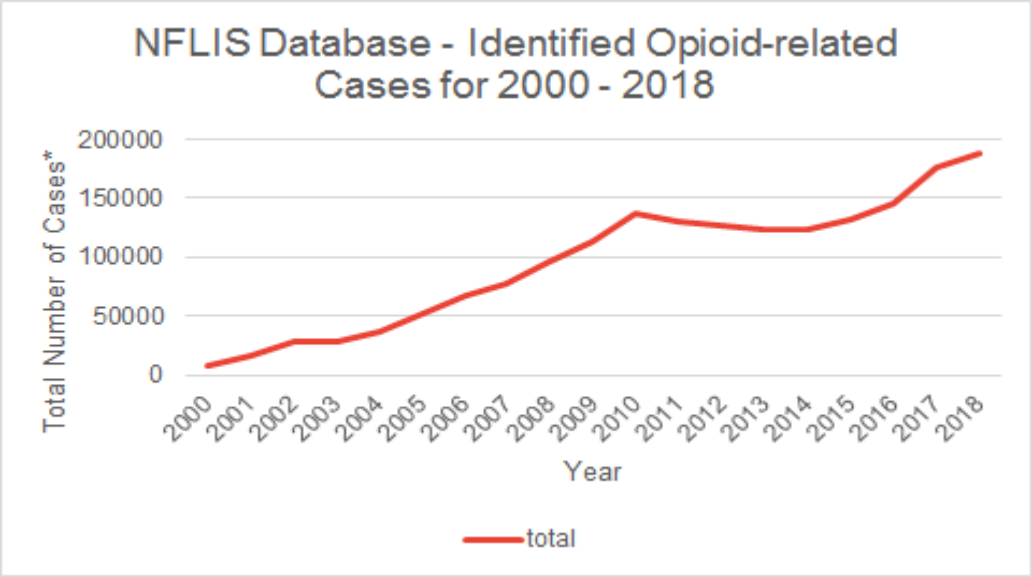

Figure 1. NFLIS Database - Identified Opioid-related Cases for 2000 -2018. The figure above is a line graph showing the total number of identified opioid-related cases throughout the U.S. from 2000 to 2018.

Deaths attributed to opioids in the United States were nearly six times greater in 2017 than they were in 1999. Opioids were involved in more than two-thirds of overdose deaths in 2017, and U.S. overdose death rates linked to synthetic opioids increased more than 45 percent from 2016 to 2017 [9 and 10]. The opioid epidemic puts a significant burden on

the U.S. economy, healthcare system and legal system. According to the U.S. Council of Economic Advisors (CEA) in the published 2017 report *The Underestimated Cost of the Opioid Crisis*, "the measured full cost of the opioid crisis by considering the value of lost lives, as well as increases in healthcare and substance abuse treatment costs, increases in criminal justice costs, and reductions in productivity" [11]. Furthermore, the CEA asserts in that report that the estimated cost of the opioid crisis was more than $2.5 trillion for the four-year period from 2015 to 2018 but may be three times greater in value [11].

The healthcare system faces great challenges in providing treatment and life-saving efforts to individuals with opioid use disorders (OUDs), which causes excess medical and prescription costs on an already strained system. Furthermore, it places medical workers at risk since opioids can be unintentionally administered through numerous routes and could potentially lead to overdose depending on the opioid. The Criminal Justice System also feels the burden of the opioid epidemic due to the increasing number of people facing litigation for illicit dealing of opioids, increasing police to combat drug dealers, and the cost to hold these dealers or abusers until their day in court. Forensic laboratories are feeling the weight as well with the continuous production of new synthetic opioids and the difficulty of distinguishing between prescription and illicit opioids which plays a big role in sentencing.

1.1.3 The Public Health and Forensic Science Implications

The opioid epidemic presents unique, multifaceted challenges for law enforcement, first responders, and medical personnel. Law enforcement such as the forensic science laboratories is struggling with the increasing number of opioid-related cases. The

challenges in the detection, identification, and screening of synthetic opioids mandate the use of multiple analytical techniques and instrumentation, both field-deployable and laboratory-based. The chemical make-up of new synthetic opioids is continually evolving, and forensic laboratories are charged with quickly identifying new drugs to help surveillance, enforcement and to alert officials to new trends in opioid-linked deaths. Reference materials are critical components to analytical method validation and quality control and assurance; they are required to confirm the identity of a compound present in a sample.

Per Morrow et al., from the article *The Opioid Epidemic: Moving Toward an Integrated, Holistic Analytical Response*, "Reference materials are used to enhance the timeliness and accuracy of compound identification; however, access to these reference materials is challenging when emerging illicit fentanyl analogs first entered the drug market. Analytical reference standards for novel compounds that require custom synthesis can take 3 to 6 months from identification to commercial availability." Morrow et al. further asserts that the companies producing reference materials for use in forensic laboratories faces considerable obstacles. Some of these obstacles include supply and demand, the production of newer substances, and policies involving production of inexpensive material as well as distribution. These issues cause significant problems for forensic laboratories as the reference materials are used to validate analytical methods, for quality control and quality assurance, and for chemical comparison to collected evidence. As the epidemic continues with the production of new opioids, the forensic laboratories must handle an increasing backlog of case which poses a serious public health concern.

The public health concern comes from the inability to report out about the increasing number of cases involving a new opioid or other substances due to slow turnaround time of chemical analysis [12].

Public health is also facing devastating consequences as the opioid epidemic is causing high rates of hepatitis C, human immunodeficiency virus (HIV), and other diseases, mainly due to shared syringes. Per the CDC, an estimated one in 23 women and one in 36 men using drugs via injections would have a HIV diagnosis in their lifetime, and opioid use is thought to have contributed to hepatitis C infection transmission, which is estimated to have tripled between 2000 and 2015 [13]. Additionally, there has been a significant impact on the safety of first responders and medical personnel when they encounter highly potent and fast-acting fentanyl and fentanyl analogs during their routine emergency responses. According to Sisco et al., "The DEA issued a warning to law enforcement in June of 2016 to exercise extreme caution when handling possible fentanyl-containing materials. Lethal dosing of fentanyl can be as low as 2 mg, with ingestion, inhalation, and absorption through the skin as possible exposure routes, which puts medical and law enforcement personnel in high levels of danger" [14].

The opioid epidemic presents detrimental and long-lasting effects on the social and economic portions of life. The social and economic impact is derived from the pharmacological effects of opioid intoxication which may produce multiple symptoms that vary in severity such as excessive drowsiness, altered mental status, mood swings, and respiratory problems. These symptoms will influence the individual's responsibilities (i.e., finances, education, and profession), social interactions (i.e., family, friends, and partners),

and health status (i.e., mental, emotional and physical). Infants exposed to opioids in utero are at risk of Neonatal Abstinence Syndrome (NAS), otherwise known as Neonatal Opioid Withdrawal Syndrome (NOWS) and prenatal exposure to opioids has been associated with the risk of short-term to long-term health complications that ranges in severity depending on various factors (i.e., prematurity, comorbidity, etc.), but most often proper management prevents such occurrences. For example, infants diagnosed with NAS may face potential long-term neurodevelopmental consequences. However, these potential health complications may be mitigated with early intervention, proper treatment and post-hospitalization monitoring to improve maternal and neonatal outcome. It is of critical importance that more research is conducted regarding opioids to increase safety measures, reverse the adverse effects, and develop new policies [15].

1.2 Opioids

1.2.1 Background

Opioid exposure can have minor to fatal effects for any individual; however, these effects are variable depending on the individual and opioid administered. Case-by-case observance of impact is necessary as well as comprehensive knowledge of opioids to understand the occurrence of variances. Opioids are a class of drugs that may be a schedule I to V per the DEA based on acceptable capability for medical usage and prospective dependency or addiction. Narcotic tranquilizers, or opioids, act as a depressant on the central nervous system, thus causing brain activity to reduce, which allows for relaxation and sleep. Furthermore, opioids act as an analgesic, or a pain reliever, for moderate to severe pain by affecting the pain signals going through the central nervous system.

Although opioids are mainly prescribed as an analgesic to relieve pain symptoms, these drugs can present adverse effects such as respiratory depression and addiction. However, not all opioids are prescribed thus are available for illegal purchase through illicit production or diversion of pharmaceutical products to non-medical outlets (i.e., street dealers or the black market) [16, 17].

There are a multitude of opioids with varying effects that can be classified by the method of production and pharmacological activity.

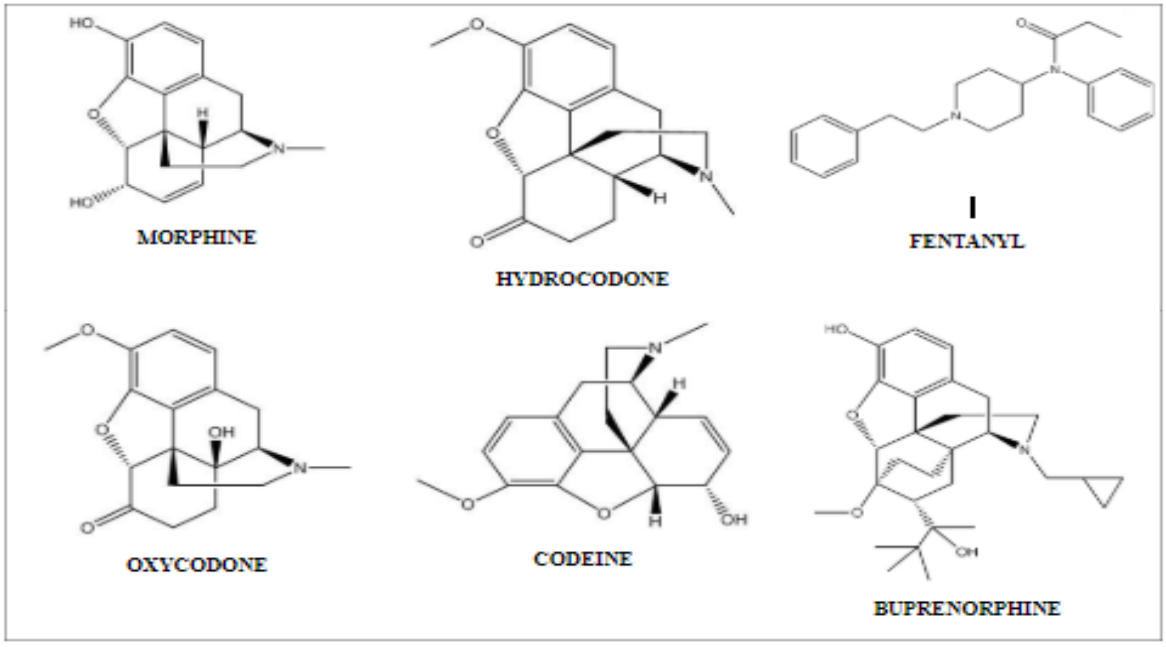

Figure 2. Six Common Prescription Opioids. The figure above shows the molecular structure of morphine, hydrocodone, fentanyl, oxycodone, codeine, and buprenorphine.

There are three classes of production for opioids, which are divided into natural opiates, semi-synthetic analogs, and synthetic analogs. Natural opiates are naturally occurring organic compounds that are derived from the opium poppy plant. Morphine and

codeine are two examples of natural opiates as they are produced from opium poppy extracts. Opiates may also include semi-synthetic analogs such as heroin, a well-known, illicit, and addictive street drug. Semi-synthetic analog production involves making modifications to natural opiates by means of chemical experimentation. For instance, morphine can be converted to heroin through various chemicals, heating, and purification steps. Synthetic analogs, such as fentanyl, are quite different as these substances are completely man-made, but have similar chemical compositions and pharmacological activities of natural opiates and semi-synthetic analogs.

Opioids can be further divided into categories based on the pharmacological activity of the substance. Opioids and opioid-related substances may be classified as a full or partial agonist, and a full or partial antagonist, which is influenced in large part by the receptor binding and affinity of the substance. Full agonist substances can produce the maximal effect within the body by binding to and fully activating available opioid receptors throughout the body. Partial agonists are less effective than full agonists, thus produce partial activity when bound to and activating an opioid receptor. Antagonist substances block full agonists and partial agonists from producing their effects by binding to the opioid receptors thus preventing the agonists from activating the receptor [18-20].

These classifications may provide inferences on the effects of certain opioids, but it is the pharmacology of opioids that will inform on the pharmacokinetics and pharmacodynamics of the substance. Pharmacological understanding has proven vital in determining the potential interactions caused by individuals taking multiple prescribed, over-the-counter, and even recreational medications. These potential interactions are of

great importance in the field of medicine and forensics as it provides information on the dose-response relationship. The dose-response relationship guides the level of therapeutic, toxic, and fatal doses by observing the response of the body to the level of a dose. Determination of the level of a dose and how the body should respond would assist in life-saving efforts but also determining the cause of death.

1.2.2 Pharmacology

Pharmacology involves two branches, pharmacokinetics, and pharmacodynamics, to conceptualize drug action. The two branches are dependent on each other, so any alteration to one branch affects the other. Pharmacokinetics is the study of the movement of a drug into, through, and out of the body. This branch focuses on the absorption, distribution, metabolism, and excretion of a substance which can be variable depending on the opioid. Pharmacokinetics will influence onset, intensity, and duration, although other factors such as inter- and intra-individual variances will play a part as well. Absorption will be determined by a drug's physical properties (i.e., solubility) and chemical properties (i.e., toxicity). Furthermore, the route of administration plays a significant role as well as the form in which the drug is administered, which is dependent on the individual. Opioids can be administered using enteral and parenteral routes due to the various available forms an opioid may be administered. The combination of these factors determines drug absorption as a drug must be absorbed to be transported to the site of action to produce their pharmacological activities [18-22].

Absorption focuses on the movement of a drug from its administration into the body to its entrance into the systemic circulation. The properties of the drug, as well as the route

of administration, must be considered to ensure the most efficient adsorption of the substance. Opioids are characterized by their aromatic core and weak basic nature (pKa 6.5-8.7). Typically, opioids are lipophilic; higher lipophilicity means the increased capability to diffuse through biological barriers, such as the blood-brain barrier or the lining of organs, to enter the bloodstream (Figure 2). Lipophilicity is affected by ionization as ionized compounds will be less capable of diffusion through biological barriers. Due to the weak basic nature of opioids, an alkaline environment such as the small intestine would be better for absorption versus a more acidic environment such as the stomach. In an alkaline environment, opioids would be mostly unionized, thus more lipophilic, which means more readily absorbed. Conversely, opioids in an acidic environment would be protonated, thus less lipophilic, which means poor absorption. After the administered substance, has undergone absorption, the drug is distributed throughout the body through the bloodstream. Lipophilicity, protein binding, and tissue binding are essential factors that indicate the extent to which a drug distributes in the body. Once a drug has entered into the systemic circulation, a drug will be transported unbound or bound to blood components (i.e., plasma proteins) throughout the body. However, only unbound drugs can passively diffuse to the tissue sites, so the pharmacologic activities of the drug can have their occurrence. The degree of binding between the drug and proteins or other components in the body will affect the concentration of unbound drug, which will determine drug concentration at the active site which will indicate level of efficacy [20-26].

As opioids are typically lipophilic, opioids will have a higher propensity to leave the aqueous environment of the blood to more hydrophobic environments such as

cerebrospinal fluid, tissues and adipose. Because of the higher propensity to associate with more hydrophobic environments, there will be minimal drug remaining in systemic circulation. There is a high number of opioid receptors distributed through the brain and CNS, which means that opioids must cross the blood-brain barrier to access, bind to and activate opioid receptors. Opioids will distribute quickly to get to a more lipophilic environment resulting in quicker onset. However, the duration will be determined by the half-life of the specific opioid taken. The other branch of pharmacology, pharmacodynamics, plays a significant role due to the focus on the effect of drugs on the body, such as onset and duration [18-24].

Pharmacodynamics examines the molecular, biochemical, and physiological effects of a drug as well as the mechanism of action. Pharmacodynamics influence opioid effects in the body as there are inter- and intra-individual variances that affect opioid binding as well as the selected opioid may bind differently compared to other opioids. There are three subtypes of opioid receptors to which opioids will bind to enact their pharmacological activities: μ [mu], κ [kappa], and δ [delta]. These opioid receptors are distributed throughout the body but are prominently found in the brain, CNS, and spinal column. The binding of opioids to those receptors in sufficient numbers will generate a collective effect and that receptor binding- effect generation can be relative to the dose-response relationship. The dose-response relationship is constructed from the pharmacology of a substance. This relationship determines the efficacy and safety of a drug by examining the amount and frequency of dosing—furthermore, the therapeutic, toxic, and fatal ranges of a substance. However, inter-, and intra-individual differences (i.e., age,

gender, health) will affect these factors. Factors that influence binding potential and affinity to receptors will also cause alterations to the therapeutic, toxic, and fatal ranges, as well as their effects [22-26].

Opioids produce their effects by interacting with various opioid receptors, but the duration of the pharmacological activity is greatly affected by the mechanism of action. The pharmacological activities of opioids are due to the receptor to which an opioid binds. For instance, agonist opioids will typically bind with the mu, and potentially the kappa receptor, to exert their effect. Interaction with specific receptors will produce certain effects, such as binding with the mu receptor presents potential sedation, euphoria, and physical dependency. Tolerance and physical dependence can occur due to changes in these opioid receptors, thus requiring an increased dosage to achieve the analgesic and euphoric effects of opioids [18-23].

Per Mallappallil et al., from the article *What Do We Know about Opioids and the Kidney,* "Chronic use of opioids results in a higher incidence of toxicity due to the accumulation of metabolites, which could cause unwanted side effects. One reason is that with chronic use, a steady-state of the drug is reached with distribution and accumulation in the various body tissues." Accumulation can extend biological action due to the constant release of the stored drug back into the bloodstream when concentrations decrease. Opioids stored within tissues, and while in equilibrium with blood concentration, will eventually move into the bloodstream as they are eliminated from the body. Elimination of drugs from the body involves metabolism and excretion. Opioids differ with respect to how each one is metabolized, and patients differ in their ability to metabolize varying opioids. Drug

metabolism rates are unique to each individual and will be influenced by various factors such as age, gender, genetics, comorbidity, and co-administration. Drug metabolism focuses on changing the drug to a more suitable form for excretion in bodily waste such as urine or feces. Opioids are metabolized in the liver, which contains a high number of enzymes to break down the opioid for elimination. Metabolism via the liver for opioids provides two pathways for metabolism which are phase 1 involving modification and phase 2 involving conjugation [22-29].

According to Smith, from the article *Opioid Metabolism",* "Phase 1 metabolism of opioids mainly involves the CYP3A4 and CYP2D6 enzymes. Furthermore, the CYP3A4 enzyme metabolizes more than 50% of all drugs; consequently, opioids metabolized by this enzyme have a high risk of drug-drug interactions" [28]. Drug-drug interactions would pose a serious risk for individuals whom misuse, abuse or are addicted to opioids as it could delay, decrease, or enhance absorption of the co-administered drugs. It could interfere with treatment plans for pre-existing physical, mental, or emotional issues that are being managed by various medications, prescription and non-prescription. Furthermore, Smith states that the "[administration] of CYP3A4 substrates or inhibitors can increase opioid concentrations, thereby prolonging and intensifying analgesic effects and adverse opioid effects, such as respiratory depression" [28]. CYP3A4 inhibitors such as antiretroviral, antibiotics, antidepressants and other medications needed for the maintained well-being of an individual with opioid addiction could cause detrimental health effects that range in severity.

As stated by Smith, “Phase 2 metabolism of opioids involves the enzyme uridine diphosphate glucuronosyltransferase (UGT), which acts as a catalyst for glucuronidation. The enzymes responsible for glucuronidation reactions may also be subject to a variety of factors, such as genetic variants that may alter opioid metabolism” [28]. Generally, most opioids would undergo phase 1 metabolism pathway, however inter- and intra-individual differences in the response to varying opioids may influence metabolism. The notation of these inter- and intra-individual variances assist clinicians in their treatment and medicating of the patient. Clinicians should utilize these variances to maximize their treatment plan to provide optimal care, which requires case-by-case examinations to guide their medication therapy plans [28].

Generally, the metabolism of parent drugs forms polar metabolites which will be eliminated via enterohepatic or renal recirculation. Most drug metabolites are polar compounds that are not capable of entering circulation; thus, are excreted via bodily waste. The main organs for excreting metabolized substances such as metabolites or parent drugs that have been processed in the body are the kidneys. Glucuronidated substances can be excreted via feces by way of the biliary system. There are other pathways for excretion such as saliva, sweat, tears, and breastmilk, however excretion by these pathways are generally small. It has proven crucial to understand the pharmacology of a drug as alteration may affect various areas of pharmacokinetics and pharmacodynamics, especially tolerance. Tolerance can be dangerous as abusers of illicit and prescription opioids will develop tolerance quicker, which may cause an increase in their intake, resulting in severe to fatal consequences [25-30].

1.2.3 Opioids, Women, and Infants

The opioid epidemic impacts men and women of the U.S., but the impact is different for each group due to gender-related biology. Between 1990 and 2010, overdose deaths from prescription painkillers increased among men by 265 percent, while the number grew by 400 percent among women, according to the Centers for Disease Control and Prevention (CDC) [31-33]. Women are more likely to suffer chronic pain thus more likely to be prescribed opioid analgesics, which means a greater tendency to develop dependence due to use over a longer time frame. Thus, incidences of opioid use disorder amongst women were greater than men, which lead to greater incidences of women with OUD at labor and delivery. Between 1999-2014, the number of cases of maternal opioid use disorder at delivery hospitalization increased from 1.5 per 1000 delivery hospitalization to 6.5 per 1000 [34, 35].

Crowley et al., in the article *Health and Public Policy to Facilitate Effective Prevention and Treatment of Substance Use Disorders Involving Illicit and Prescription Drugs: An American College of Physicians Position Paper*, asserts that there is an increasing trend of SUD that presents a serious public health issue that has ignited policymakers to put more efforts into expanding accessible and affordable substance use disorder programs. However, it has also ignited efforts to criminalize the use of narcotics while pregnant as chargeable child abuse to act as a deterrent. Those actions have increased stigma which has led to more devastating consequences as expectant women will not seek the care they need in fear of punishment. Maternal OUD is a significant public health concern as maternal opioid exposure has an inverse relationship with maternal and neonatal

well-being. Opioid exposure during pregnancy may be the result of clinically-approved use of prescription opioids, misuse or abuse of prescription opioids, illicit use (i.e., illegally manufactured fentanyl) or medication-assisted treatment (MAT) of OUD. Maternal SUD allows the drug to enter systemic circulation which will also expose the fetus to the drug via the placenta. The exposure may lead to the infant developing NAS, otherwise known as NOWS, while still in utero. Various transient clinical signs associated with NAS, such as a distinct high-pitched cry, high muscle tone and tremors, usually will appear within 48–72 hours. and Feeding difficulties are more prevalent health complications that may present with infants diagnosed with NAS in the immediate postpartum period [30-35].

Between 2009 -2012, the number of incidences of NAS per 1000 hospital births shows an increasing trend from 3.5 per 1000 in 2009 to almost 6 per 1000 in 2012 [36]. Infants with NAS will experience additional health complications ranging from short-term feeding difficulties to long-term neurodevelopmental complications. The incidence of NAS is variable as is its level of severity and it is not quite understood as to the cause of variability in NAS expression. Paucity in research and literature regarding NAS, causation of variability, and accurate reporting of maternal substance exposure has contributed to gaps in the conceptual knowledge regarding maternal substance-use and neonates. The actual figures of occurrence for substance use during pregnancy is hard to verify due to underreporting, which is likely caused by stigmatization, fear of legal repercussion and inaccessible as well as unaffordable health services. Underreporting can place prominent obstacles and other difficulties in the way of women who need care due

to substance use during pregnancy as well as raising awareness about the need for more research geared toward drug-use effects on infants [35-37].

According to McQueen et al., from the article *Maternal Substance Use and Neonatal Abstinence Syndrome: A Descriptive Study*, "current evidence regarding how the onset, duration, and severity of NAS may be impacted by drug type is needed." McQueen et al. further elaborates that "much of the literature regarding opioids and NAS is from studies evaluating methadone or heroin and the effects of opioids such as oxycodone and symptoms of NAS are lacking." Clinically, variations in treatment plans and physical assessments will be needed to manage the presentation of withdrawal symptoms as well as the effects of a substance. The standard of care for OUD during pregnancy in the U.S. would be the application of MAT using buprenorphine or methadone in combination with therapy, counseling and monitoring. Opioid detoxification or withdrawal in pregnancy presents high levels of risk for the mother and child such as decreased birth weight for neonates and high-risk maternal behavior. MAT provides stabilization during pregnancy by minimizing opioid withdrawal, reducing pregnancy-related complications, and decreases risk of relapse. Methadone is a full opioid agonist thus will bind with opioid receptors strongly to produce maximal effect. Buprenorphine, on the other hand, is a partial opioid agonist thus will bind with the opioid receptors but presents a lesser effect than a full opioid agonist such as methadone. Thus, buprenorphine has become more common in use alongside methadone for treating opioid addiction. Methadone and buprenorphine are currently approved for use during pregnancy through medication-assisted treatment (MAT) programs. However, buprenorphine and methadone are classified as category C drugs by

the U.S. Food and Drug Administration (USFDA). Animal reproduction studies involving drugs in category C have shown risk to the fetus. Drugs in category C have insufficient and inadequate studies on use during pregnancy for full risk-benefit assessment in terms of safety. However, the potential benefits may warrant use of the drug in pregnant women despite potential risks such as MAT or comorbidity.

1.3 Human Breast Milk: A Complex Matrix and an Important Source of Nutrients

1.3.1 The Global Focus on Promoting Breastfeeding

The issue of maternal opioid use disorder (OUD)/substance use disorder (SUD) and NAS/neonatal opioid withdrawal syndrome (NOWS) is significant due to its impact on the welfare of the mother and child. Maternal opioid use disorder directly impacts the mortality and development of the child as the neonate needs the nutrients provided by the mother through breast milk. Breast milk is essential for the infant to receive the immunological, developmental, and nutritional needs for better chances of survival. Since the late 1980s, there has been a global effort to protect, promote, and support breastfeeding for the sake of child survival, nutrition as well as development and maternal health. In 1989, the United Nations International Children's Emergency Fund (UNICEF) and World Health Organization (WHO) presented a Joint Statement on the Protection, Promotion, and Support of Breastfeeding: The indispensable role of the Maternity Services. The UNICEF/WHO Joint Statement, based on the Ten Steps to Successful Breastfeeding, opened a pathway that supports and enables women to breastfeed with trained healthcare professional for support [38-40].

These Ten Steps were re-emphasized in 1990 when the policymakers of at least 30 different countries, WHO, UNICEF, and other healthcare organizations joined each other to sign the Innocenti Declaration, which aimed to protect, promote, and support breastfeeding. WHO and UNICEF launched a global strategy named the Baby-Friendly Hospital Initiative (BFHI) in 1991, which aimed to increase the number of exclusively breastfed babies worldwide. The purpose of the Baby-Friendly Hospital Initiative was a global program to guide healthcare workers in the implementation, assessment, and training of the Ten Steps as well as the International Code of Marketing of breast-milk Substitutes for the sake of promoting, protecting and support breastfeeding. The Innocenti Declaration would become integrated with BFHI in hopes that all maternity wards, whether free-standing or in a hospital, become centers of breastfeeding support.

The benefits of breastfeeding for children and mothers have been well documented for many years. These benefits are quite apparent during infancy as the effects promote optimal health throughout the life of the breastfed baby and their mother [38-40]. Many medical organizations, including the American Academy of Pediatrics (AAP), WHO, CDC, and the American College of Obstetricians and Gynecologists (ACOG), strongly recommend exclusive breastfeeding for six months and continued breastfeeding with complementary foods until the child is at least one year. According to WHO on Breastfeeding, “breastfeeding contributes to the health and well-being of mothers; it helps to space children, reduces the risk of ovarian cancer and breast cancer, increases family and national resources, is a secure way of feeding, and is safe for the environment” [39-42].

However, sometimes drugs are essential for the health of the woman and child such as in the case of antibiotics, antiretroviral and MAT. Research shows that illicit substance use or misuse of prescriptions by pregnant and breastfeeding women with OUD, whom are not receiving treatment and counseling, can have severe health consequences for infants. Any substances consumed by the mother will eventually pass to the child, either through the placenta or breast milk. Drug use should be carefully monitored by medical professionals as some substance use may present a great risk to infant health. Infants will face difficulties with drug elimination due to the long half-life of metabolites that could re-enter systemic circulation or may remain bound to various tissues as well as adipose. Research on the risk of harmful effects on infants from drugs in breast milk is sparse. The U.S. National Library of Medicine (NLM) at the National Institutes of Health (NIH) maintains a peer-reviewed Drugs and Lactation Database (LactMed) on the most current information on adverse effects of any substances in breast milk. The LactMed database is critical as it provides some inferences on the effect of substances in breastmilk which provides further clarity on the controversy regarding the occurrence of substance toxicity in breastfed infants. The AAP, USFDA and ACOG recommend against the use of codeine and tramadol in women who are breastfeeding as neonates may present with adverse symptoms such as moderate to severe respiratory issues [40-42].

Most research in NAS has focused on the effects of opioids in terms of outcome. According to the CDC, between 1999 and 2013, the overall NAS incidence in the U.S. increased by 383%, from 1.5 per 1,000 hospital births in 1999 to 6.0 per 1,000 hospital births in 2013. The increased incidences in NAS have become a prime motivation of the

perpetuation of stigma against pregnant women who use opioids and policymaker's efforts to criminalize maternal substance use. The increased rates of opioid use amongst expectant mothers encourages further stigmatization of maternal substance-use disorder and policymaker's efforts to create deterrents as well as assistance for these pregnant women. Some states, particularly those most affected by the epidemic such as Kentucky, have proposed potential laws that could make penalties, criminal and civil, for maternal exposure of drugs to fetuses and breast-fed infants [36-40]. Additionally, it could lead to further examination of policies that focus on assessing the dependability of the mother to provide adequate care for their child when dealing with substance-use disorders. Risk-assessments and case-by-case monitoring could be developed and implemented, but greater understanding is needed as well as research in pregnancy, post-pregnancy, and substance-use disorders. For example, knowledge of the process of lactation and the changing composition of breast milk would be beneficial for determining the extent of adverse results of opioid abuse and addiction by expectant mothers. Conceptualizing the risk of opioids on maternal and neonatal well-being will provide better patient care management, policy revisions, and exploration of novel methodologies for drug quantification with a sparsely studied matrix, human breast milk.

1.3.2 Production and Composition

The process of lactation involves the production of milk that is discharged from the postpartum breast when an infant suckles on the nipple. The mammary gland undergoes stages of development during and post pregnancy in response to various hormones (i.e., estrogen) and proteins in the body. There are five stages to the process of lactation:

Mammogenesis, Lactogenesis, Galactokinesis, Galactopoiesis, and Involution. The first four stages are essential as their occurrence, hormonal properties and functions are the most affected by substance use [43-45]. The first stage, mammogenesis, involves the development of the mammary gland in preparation for the secretion of breast milk, which occurs during the 1st & 2nd trimester. The main hormones involved with this stage are prolactin, estrogen, and progesterone, which promotes development for secretion and suppression of milk. Lactogenesis, the second stage, occurs in two stages, with the initial stage occurring 15 to 20 weeks into gestation and the final stage between late pregnancy to 8 days after birth.

The final stage of lactogenesis is dependent on the transformation of endocrine to autocrine function for the transition from lactogenesis to the third and fourth stage, galactokinesis and galactopoiesis. There is a decrease in progesterone and estrogen levels, thus the onset of abundant milk secretion due to elevated levels of prolactin and other hormones to mobilize nutrients and minerals of breast milk. Prominent hormones during this stage are prolactin, insulin, cortisol, thyroxine, and oxytocin to assist with milk production and ejection. Galactokinesis involves the milk ejection reflex, which takes 30 - 60 seconds for occurrence. This stage is dependent on nipple stimulation that sends signals to the pituitary gland releasing oxytocin, which causes the contraction of muscles surrounding the alveoli to eject milk from the duct system. Galactopoiesis focuses on the maintenance of lactation by milk extraction using various hormones and growth factors to assist the normal physiological maintenance of lactation. Galactokinesis and galactopoiesis are affected by environmental, physical, emotional, and psychological factors. Involution

is the end of breastmilk production and the return of the lactation system into dormancy [43-45]. Human breast milk contains various components that support the developmental, immunological, and nutritional needs of a newborn child. The composition of breast milk changes due to inter- and intra-maternal variances, the preterm/term of the child, and the course of lactation. There are three phases of breast milk change in volume, appearance, and composition. During the initial stage of breastfeeding, large molecules can enter breast milk by utilizing the wide gaps between lactocytes in the breast. Lactocytes are responsible for the synthesis and secretion of milk, which fills alveoli sacs and is squeezed into the ducts that provide a pathway for milk discharge through the nipple. These gaps allow higher-molecular-weight drugs (i.e., heroin) and immunological components to enter breast milk [43-46].

Colostrum is the first fluid produced by postpartum mothers and is, usually, produced from late pregnancy to a few days after delivery in low quantities with a yellowish or creamy color and a very thick consistency. Colostrum is rich in immunologic components and developmental factors. According to Ballard et al., from *Human Milk Composition: Nutrients and Bioactive Factors*, "Levels of sodium, chloride, and magnesium are higher, and levels of potassium and calcium are lower in colostrum than later milk. As tight junction closure occurs in the mammary tissues, the sodium to potassium ratio declines, and lactose concentration increases, indicating secretory activation and the production of transitional milk." Transitional milk shows the highest variability among mothers and has little to no research on drug passage. Transitional milk is so variable due to individual differences of need between infants, which will influence

the concentration of drugs in breast milk. As stated by Ballard et al., “Transitional milk shares some of the characteristics of colostrum but represents a period of “ramped up” milk production to support the nutritional and developmental needs of the rapidly growing infant.” Transitional milk occurs from 5 days to two weeks postpartum, after which milk is considered mostly mature milk which is typical for most individuals [43-46]. By the fourth to sixth week postpartum, human milk would be considered fully mature as appearance and composition have changed. Mature milk consists of fore-milk and hind-milk, which are both necessary for the developmental and nutritional needs of the infant. The initial secretion of mature breast milk releases milk that contains high-levels of water, vitamins, and proteins and this is called fore-milk. The secretion of mature breast milk that follows shows greater levels of components needed for physical development such as fat and is called hind-milk [43-46]. Drug passage into mature milk depends on a variety of factors related to the pharmacology of the opioid administered. Different stages of breastfeeding can affect amount of drug transferred to breast milk as the levels of lipids, fats and carbohydrates will vary from stage to stage. A great number of lipids, fats and carbohydrates means a great potential for lipid-soluble drugs to enter the breast milk thus a greater amount of drug. Maternal exposure leads to infant exposure either in-utero or by way of breastfeeding. Infant-related factors assist in determining drug safety as a dosage produces different responses person to person. Infants may potentially test positive for an extended time (i.e., weeks to months) after maternal exposure because of lesser elimination and metabolism capabilities than an adult [47-49].

1.3.3 Research Relating to Pregnant and Lactating Women

Drug use during pregnancy and lactation should be limited but may be compulsory such as the case with nonpregnancy-related conditions (i.e., HIV), or pregnancy-related conditions (i.e., gestational diabetes). However, insufficient and inadequate data to inform on safety, efficacy and dosing information remains an important issue for forensic science and public health. Currently, there is not enough research regarding drug safety during pregnancy, post-pregnancy and while breastfeeding. Frequently, scientific studies exclude pregnant and lactating women for ethical reasons, research guidelines, and health concerns relating to maternal, fetal and neonatal well-being. According to the NIH, the 21st Century Cures Act (Cures Act) established the Task Force on Research Specific to Pregnant Women and Lactating Women (PRGLAC) which is led by the NIH. NIH asserts that the purpose of PRGLAC was to provide the Secretary of the U.S. Department of Health and Human Services (HHS) advisement on the information gaps relating to the safety and efficacy of drug therapies for pregnant and lactating women. The central theme of the recommendations made by PRGLAC to HHS was the need to expand scientific knowledge of the safety, efficacy, and dosing of drug therapies for pregnant and lactating women.

There are several reasons that could explain the limited interest in studies related to pregnant and lactating women. Some of the main reasons include ethical concerns, litigation, study design (i.e., operational and recruitment) and possible harm to maternal, fetal and neonatal well-being. Furthermore, the investment venture into this area of research would need to be significant, but the revenue generated in relation would be too limited. Studies would have to be designed to examine the efficacy and safety of drugs in

pre-pregnancy, each trimester, and postpartum. For studies involving lactation, the study design would have to examine the different phases of breastmilk, maternal conditions and extensive, long-term monitoring of mother and child. In this manner, interindividual variability would be accounted for but this may make the study design too complex which may impose restrictions on the recruitment or operational components.

Wang et al, in the article *Evaluation of the Safety of Drugs and Biological Products Used during Lactation: Workshop Summary*, outlines the current approaches to data collection on medications used during lactation. Furthermore, the article focuses on various considerations for the design and standardization of clinical lactation studies. According to Wang et al., there have been three main methods for data acquisition regarding the level of drug exposure to neonates and children via breastfeeding; predicative studies, animal models and clinical studies. Predictive studies focus on the physicochemical properties of a drug to provide an explanation of drug transfer into human breast milk. An example of a predictive study would be the maternal milk-to-plasma ratio model. According to Larsen et al., in the article *Prediction of Milk/Plasma Concentration Ratio of Drugs*, the milk-to-plasma ratio is a mathematically calculated estimation of the drug transfer into human breast milk. The initial milk-to-plasma ratio calculation model is focused on static measurements of drug concentration in maternal milk and plasma, while newer models are graphing these concentrations over a period of time. The area under the curve is more representative of the drug concentration in milk as the concentration varies over time and does not remain at a constant concentration. With further studies on drug transfer into breastmilk, the milk/plasma ratio calculation could be improved and use more considerable

variables (i.e., prematurity, prior medical history of the mother, etc.). Thus, a new mathematical model could be developed that allows for a more accurate estimation of drug concentration in breastmilk.

Animal models involve using animals as a replacement for human subjects as they mimic potential human biological and pharmacological responses to substances. Wang et al. asserts that the major issue involving animal studies relates to the lack of data characterizing species differences. As a result, animal studies are mainly used to represent the potential presence or absence of a drug in human breast milk but are insufficient as predictive model for the drug concentration in human milk. However, the differences between species' lactation process presents a challenge as there is a lack of clarity regarding the significant differences. Animals and humans grow at different rates, have different physiologies and different biological processes in drug transfer to breastmilk which must be considered. Furthermore, important information relating to lactation is not recorded such as the amount of drug transferred from mom to milk, amount ingested by the juvenile animals, and how much of the drug concentration is related to prenatal exposure. Information relating to these studies focus more on effects of the exposure rather than the level of exposure leading to those effects on development. Clarification of the difference between the selected animal model and humans is needed to understand, improve, and re-assess the level of substance exposure in breastmilk as well as safety of substance use for lactating women.

Clinical studies focusing on drugs in human breastmilk are derived from the examination of case studies, case series and cohort studies. These studies are informative and useful but are not sufficient in establishing the safety of drugs. Thus, this inadequate and insufficient information prevents firm risk-benefits assessment or confirmation of safety for use in pregnant or lactating women. Wang et al. emphasizes that these clinical studies are not sufficient for interpretation as there is a lack of standardization for clinical lactation studies. Furthermore, with sufficient information relating pharmacology of drugs in a clinical setting including concentrations in human breastmilk to better assess the clinical effect on breastfed-infants. Additionally, Wang et al. explains that most lactation studies are generally post-marketing studies focused on drugs with potential to be in human breastmilk, a safety risk, and used by women of reproductive age.

The current avenues available for studying the level of exposure to infants through maternal milk needs to be improved and expanded. There is a clear significant need for research, especially in the quantification of prescription and illegally manufactured opioids, to create better guidelines and policies on the clinical and toxicological significance of opioid concentrations in human breastmilk. These guidelines will be of importance for public health relating to maternal OUD, NAS/NOWS and clinical treatment planning. Furthermore, these guidelines would assist in pediatric determination of death as they will inform on the clinical and toxicological significance of opioid concentrations in postmortem toxicology. In order to create these guidelines and close the gaps of information in drug safety for pregnant and lactating women, increased knowledge and experience relating to lactation, compositional changes of breastmilk and physiological

changes must occur in the research world. Moreover, we need to examine, develop, and validate methods for the determination of opioid concentrations in human breastmilk for clinical and forensic utilization. These are the areas where information is insufficient and inadequate thus needs to be rectified to develop firm risk-benefit assessments relating to clinical decisions of opioid-related treatments of pregnant and lactating women. Furthermore, the clarification of the full extent of any adverse health complications for breastfed-infants relating to opioid concentrations from prenatal and breastfeeding exposure which is clinically and forensically significant.

2. EXPLORATION OF DRUG ANALYSIS METHODOLOGIES

2.1 Current Methods for Drug Analysis

Laboratory analysis plays a momentous role in the clinical and forensic setting by providing information about substances in an individual's system. Medical personnel utilize the results to better plan for patient care, provide more suitable drug therapies, and allow for risk assessment. Forensic laboratories focus on determining if the substances found in the body may have contributed to impairment or death. Testing methods have been evolving with the increasing trend of opioid misuse and addiction in order to combat its effects on society. A great level of understanding is needed regarding opioid pharmacology and available analytical methods toward different opioids is critical for accurate reporting as well as monitoring. Analysis can be qualitative, quantitative, or both depending on the purpose of the test. There are two main classifications of tests: presumptive tests and confirmatory (definitive) tests. Presumptive tests involve qualitative techniques to identify that a class of drugs may be present in the sample. Presumptive tests are rapid, inexpensive, sensitive, and non-specific. Furthermore, presumptive tests are simplistic, need minimal training to use and can be performed with limited available samples.

Presumptive tests tend to guide further analysis in forensics or medical treatment plans as they are not definitive due to the subjective nature of interpretation of the results. Presumptive tests that are observed as positive should always be followed with confirmatory tests. Sometimes false-positives could occur which could be misleading if observed. Samples where a negative presumptive result is observed is not tested further.

However, false-negatives rarely occur unless the amount of substance is too minuscule to be detected. According to Choodum and Daeid, “the United Nations International Drug Control Programme has recommended four rapid testing methods for opiates, which are the Marquis, Mecke, Nitric acid, and Ferric sulfate tests. These four tests have been widely used as presumptive tests in various forensic science laboratories, however the Marquis and Nitric acid tests are the most reported” [50]. Presumptive tests are, most commonly, color tests in which the combination of the substance and a reagent causes a chemical reaction that produces a color change such as the Marquis and Nitric acid tests. The Marquis test will be varying shades of purple, depending on the opioid (i.e., morphine and heroin will be deep purplish red, while codeine is very dark purple), as a result of the formaldehyde in the reagent attacking the substituted aromatic ring thus forming a carbocation. Further reaction between the two compounds results in a colored dimer product. The nitric acid test can differentiate between morphine (i.e., orange red) and heroin (i.e., yellow) as well as codeine (i.e, orange). This is the result of the opioid compound undergoing nitration at C-2, but only morphine will form a hydrogen bond between the nitro group and a hydroxyl group.

Notwithstanding the positive contributions of presumptive tests in the medical and forensic field, but presumptive tests have some major drawbacks such as cross-reactivity. Cross-reactivity presents issues for the forensic laboratories and the medical field due to potential false-positives. For forensic science, it may mislead analysts who base their next steps of analysis on presumptive testing. In terms of the medical field, a false-positive can have serious consequences from incorrect medical treatment to a subconscious change in

treatment toward a patient. Urine is the most frequently tested sample for opioids. However, urine drug testing containing multiple drugs may escape detection by opiate immunoassays due to limited cross-reactivity with the diversity of opioid drugs [50-52].

Sample collection can be witnessed or escorted (unwitnessed), both face the potential of adulteration or alteration of the sample. There are methods to detect tampering such as temperature, specific gravity, or creatinine concentrations, however these detection methods can still be circumvented. Analysis of opioids has other available methods for analyzing alternate samples such as saliva, sweat, and hair. However, considerable effort has been spent on developing testing methods for alternate specimen types that allow for simple, observed collection. Saliva has shown the most promise to meet these requirements and oftentimes, the concentration of drugs in saliva is equivalent to unbound drug concentration in serum. However, saliva specimens face challenges due to small sampling thus limiting use in multi-drug detection and consistency in collection as dry mouth accompanies opioid usage as well as other drugs. Sweat and hair have been given consideration due their non-invasive collection and capability for drug detection. However, sweat is not generally used for drug detection due to diversity in excretion of different opioids. Hair has a great potential for contamination, intensive labor and time for analysis, and lack of correlation to administered dose [53-56].

Presumptive tests do not detect opioid drugs equally thus separate assays are required for specific opioid compounds or more sensitive and specific instrumental methods. The other category of testing is definitive, or confirmatory, testing which is usually more quantitative or a mix of both qualitative and quantitative techniques.

Confirmatory testing is commonly performed to "confirm," presumptive test results or for substances in which no presumptive tests are available. Highly specific, expensive, and sensitive analytical methods are utilized such as liquid chromatography-mass spectrometry (LC-MS), gas chromatography-mass spectrometry (GC-MS) and other instrumentation such as High-Performance Liquid Chromatography-Ultraviolet [57,58] and Radioimmunoassay [59]. These instruments are capable of providing the chemical composition and concentration of a drug within a matrix, which may be determined by the limit of detection [50-52].

Additionally, confirmatory testing has multi-drug detection capabilities, unlike presumptive tests, allowing detection of various opioids. LC-MS or GC-MS testing is far more sensitive and specific than presumptive testing thus the production of false-negatives or false-positives is uncommon. There are some drawbacks as confirmatory testing requires more time and labor. Typically, multi-step processing for qualitative analysis is required to confirm a positive identification. Furthermore, this process may include quantitative analysis depending on the laboratory requirements. Quantitative analysis is useful for forensics as it determines the concentration and purity of substances which influences sentencing at the local, state, and federal levels. Confirmatory results are not usually available the same day which can contribute to a backlog for forensics as results must be reviewed, interpreted, and placed in an official report. Hospital laboratories usually perform presumptive testing for clinical purposes as time is precious for the sake of patient treatment. Furthermore, confirmatory testing is not routinely performed for clinical purposes and exerts minimal influence on clinical decisions. There are multiple

methodologies available for opioid detection, however the choice is affected by laboratory requirements, collected sample, sample preparation and instrumentation.

2.2 Sample Preparation with Complex Matrices

In most chemical analyses, the collected sample is not suitable for direct analysis thus the sample requires conversion to a more appropriate form through a process known as sample preparation, or extraction. Sample preparation is utilized to remove or mask compounds that could interfere with the analysis. The method of preparation may vary in terms of selectivity, specificity, speed, and simplicity but the instrumental approach will be a significant influence on the results and limitations of the analysis. Sample preparation is used mainly to accomplish one goal which is the separation and detection of target analytes from the substrate or matrices. Biological matrices present a complicated issue for quantitative analysis due to the substantial concentration of diverse endogenous compounds (i.e. proteins, hormones, and lipids) within these complex matrices. Sample preparation is essential for biological matrices because a direct injection of the matrix onto instrumentation would result in contamination and potentially damage occurring to different components of the selected instrument system. Sample preparation can assist in improving performance, decreasing interferences, and enhancing detection capabilities of the instrumental approach. Liquid-Liquid Extraction (LLE) and Solid-Phase Extraction (SPE) are some of the most commonly utilized sample preparation methods.

LLE separates an analyte through partitioning between two immiscible solvents. This method is utilized with biological matrices, but the high solvent consumption, formation of emulsions, labor, and lack of capability to be automated shifted focus to other

methods. SPE involves compounds in a liquid mixture being separated, based on physicochemical properties, using a solid stationary phase. McCarthy et al. [60], Choo et al. [61], and Nikolaou et al. [62] utilized SPE for sample extraction from human breastmilk samples exposed to methadone from mothers in MAT. SPE can bind hydrophobic drugs as well as other hydrophobic materials (i.e., proteins and lipids) thus a multistep extraction will be needed. In the aforementioned studies, centrifugation or protein precipitation was utilized to remove the larger biological hydrophobic materials prior to SPE, but some loss of drug analyte was experienced due to the additional sample extraction. Protein precipitation followed by centrifugation and SPE would be a good method of sample preparation. The protein precipitation would 'crash' the larger biological materials out of solution, and subsequent centrifugation for a limited time would speed the process. Furthermore, centrifugation would assist with the separation of potential interferences (i.e. biological materials) in the solution (i.e., human breastmilk) causing the formation of a pellet at the bottom of the container. SPE would be better able to isolate the hydrophobic target analyte (i.e., opioid such as fentanyl) with the removal of the larger biological materials (i.e., lipids and proteins). Based on these studies, the combination of protein precipitation with either cold methanol or acetonitrile followed by SPE is effective, minimizes matrix effects and viable for preparing a human breastmilk sample for instrumental analysis. SPE extraction technique became a popular and effective method due to its many advantages over LLE such as high extraction efficiency, reduced solvent consumption, and automation capabilities. In cases involving samples with protein rich matrices such as whole blood or human breast milk, sample preparation is a crucial step in

confirmatory drug testing to minimize matrix effects and extend instrument life. The optimization of the extraction process strengthens the analysis by increasing recovery, accuracy, and precision. Sample preparation methods should be simple, fast, robust, inexpensive, and have potential to be automated. Furthermore, the methods should also provide internationally acceptable limits for recovery, precision, and accuracy.

However, these goals require sample preparation techniques to be further developed so single tube extractions could be performed with minimal target analyte loss. Sample preparation research has been gaining increased interest which was produced by the development and introduction of nontraditional extraction techniques such as saponification. These nontraditional techniques focus on reducing solvent usage, automation, and miniaturization in hopes of on-site implementation. Additionally, there has been increasing interest in decreasing cost, lessening waste and being more environmentally-conscious which may be accomplished through nontraditional techniques. Sample preparation is just one step in the overall method; an effective instrument that can analyze the extracted sample with speed, sensitivity and specificity is quite important for qualitative and quantitative analysis.

2.3 Instrumental Analysis with Complex Matrices

There are various instruments with different degrees of capabilities that are able to separate, detect, and quantify a target analyte present in biological matrices. Clinically, the ability to identify and quantitate drugs in body fluids is necessary for the development of drugs, effective therapies and improved assessments. In terms of forensic science, this knowledge is crucial as the data provided assists in determining toxicological significance

and the role of a substance in death or impairment. Current methods for qualitative and quantitative analysis, in general, include GC-MS and Liquid Chromatography coupled to Tandem Mass Spectrometry (LC-MS-MS). GC and LC separates components of a mixture based on their affinity to a stationary or mobile phase and subsequently detects these components using ultraviolet light, fluorescence, or electrical conductivity, which can all affect the analyzed substances. MS and MS-MS involves generating charged ions which can be separated, identified, and quantified due to specific mass-to-charge ratio of the ions. GC-MS and LC-MS-MS are highly utilized for drug detection in matrices collected from an individual, living or deceased. Furthermore, these instruments have high capabilities which allow for a greater level of sensitivity, specificity, and molecular structural information.

GC-MS and LC-MS-MS have advantages and disadvantages, but the capabilities of both instruments will allow for a robust, sensitive, and specific testing of analytes in complex matrices such as whole blood or human breast milk. GC-MS has many advantages which makes this chromatographic-spectrometric technique more utilized in the qualitative and quantitative analysis of drugs in complex matrices such as blood and urine. GC-MS is a simple, rapid, and reproducible technique with, generally, acceptable standards of accuracy and precision as well as valuable data on the molecular structure of the analyte. However, there are some disadvantages to GC-MS such as being of limited capability for directly analyzing drugs that are nonvolatile, polar, or thermally labile. Furthermore, complex sample preparation in the form of derivatization is required to increase the volatility and thermal stability of target compounds thus increasing time and labor related

to the analysis. Blinick et al. [66], Geraghty et al. [67], and McCarthy et al. [60] are some studies that have utilized GC-MS to determine methadone and its metabolites in human breastmilk samples, but were not validated. A validated and highly sensitive methodology with acceptable accuracy and precision for the determination of methadone and its metabolites using GC-MS was established by Nikolaou et al. [62]. These studies findings were similar in that concentration of methadone and its metabolites were of low concentration in breastmilk thus present no risk to the infant and the mother should be allowed to breastfeed. However, some of these studies used spike breastmilk while others used significantly small sample size (i.e. < 10 individuals) of breastfeeding women in methadone-maintenance programs. Furthermore, some of the studies do not fully detail the method, instrumentation parameters, phase of breastmilk, and other important variables such as comorbidity or polysubstance-use. Moreover, these studies were only examining a single opioid, methadone, thus gives no indication about the utilization of GC-MS for the detection of multiple analytes in human breastmilk.

Similar to GC-MS, LC-MS-MS is also good for qualitative and quantitative determination of substances in complex mixtures and matrices. LC-MS-MS has greater selectivity and specificity due to its broader mass range as well as the use of multiple physical properties related to the analyte of interest compared to GC-MS. Moreover, LC-MS-MS allows for a lower limit of detection and limit of quantitation as well as the identification and quantification of multiple analytes simultaneously in a single run. While LC-MS-MS has many advantages, this technique does have some disadvantages. For example, this instrumentation is a highly complex system that requires technical expertise

or training. Additionally, LC-MS-MS has higher operational cost as well as limited sample throughput. Choo et al. [61] established a validated method for the determination of methadone and its metabolite in human breastmilk using LC-MS-MS, which stated similar findings to Nikolaou et al. [62] regarding low concentrations. Choo et al. utilized the breastmilk methadone-maintained breastfeeding mothers and was not as sensitive as the method of Nikolaou et al. [62], but provided acceptable sensitivity, accuracy and precision. Marchei et al. [68] also established a validated for the simultaneous analysis of multiple drugs, prescription and illicit, in breast milk using LC-MS-MS with adequate sensitivity, accuracy and precision. Morphine, codeine and methadone as well as the metabolites 2-ethylene-1,5-dimethyl-3,3-diphenylpyrrolidine and 6-acetylmorphine were the only components related to opioids out of 22 drugs analyzed in the study. Marchei et al. [68] sample size (i.e., n=400) was significant greater compared to the other aforementioned studies in this paper as well as discusses the potential for implementation as a rapid screening for milk samples at a milk bank. Both studies do not detail the phase of collected breastmilk, but are more detailed in comparison to aforementioned GC-MS studies. Choo et al. and Marhei et al. show that LC-MS-MS has flexibility and versatility such that it can be applied in a clinical setting, but may be possible in a forensic setting as well.

These analytical techniques, LC-MS-MS and GC-MS, are important due to their ability to separate, identify, and quantify compounds in complex matrices such as human breast milk. However, LC-MS-MS would be a better choice compared to GC-MS when analyzing human breastmilk for the determination of opioid concentrations. Compared to GC-MS, LC-MS-MS is amenable to most drug analytes thus may not require extensive sample

preparation depending on the sample matrix. Furthermore, the capability of simultaneous drug analysis in human breastmilk would be important in cases of polysubstance use. Moreover, LC-MS-MS has greater analytical specificity, due to the tandem mass spectrometer, in comparison to GC-MS and will not require derivatization. By using these studies, it is possible to establish current limitations, create standardization parameters regarding sample collection, and develop improved methodologies for opioid determination as well as other substances in human breastmilk that could be applied in forensic and clinical laboratories. However, we must examine the appropriate methodological considerations when creating the study design for drug determination in human breastmilk and these current studies will influence that design.

3. DISCUSSION

3.1 Method Considerations for the Analysis of Human Breast Milk

There are some works available that focus on reporting quantitation of drugs, illicit and prescription, in human breast milk. In order to design additional analytical methods for the drug determination in human breast milk with potential for application in a forensic and clinical laboratory, each step of the analytical process must be given significant consideration. Firstly, we must take note of how human milk samples were collected in the few studies available to create a better method for sample collection. Many of the previously discussed studies collected at one stage of lactation, different stages of lactation and pooled their samples or failed to mention when the samples were collected. Comparison between available studies and understanding the effect of the composition would be difficult given variations in sample collection and sampling criteria.

As previously discussed, three phases of breast milk production are colostrum, transitional milk, and mature milk. It would be beneficial to pool samples at the same stage of lactation and phase of breastmilk to account for variances and allow for comparison between different studies. Furthermore, the pooled samples would be representative of the effects of the breast milk composition on the analysis such as matrix effects, co-eluting substances, and detection as well as quantitative limitations. Sample collection methods should also be considered while looking at the sampling as the collection method should be simple, fast, and non-invasive. Breast milk can be collected via a breast pump and can be observed using nurses who usually provide instruction on pumping for new mothers from delivery to discharge of the child. Storage conditions best suited for breast milk are

to store the milk at -20 degrees Celsius for long term storage and a low cooling temperature (0-4 degrees Celsius) for short term. Public Health and Healthcare organizations such as the CDC and AAP provide instruction on proper storage parameters which can be easily implemented in other settings. An extracted sample is a critical component for quantitative analysis as issues will present due to the significant effect of interferences and co-eluting compounds in an un-extracted sample resulting in a poor analysis.

Next, we should consider the best approach to removing matrix interferences with minimal sample dilution. Due to the varying levels and diverse number of endogenous compounds in human milk, it would be best to look at sample preparation methods that focus on removing interferences in the matrix and concentrate the sample. A multi-step sample preparation protocol would be beneficial as a single method would not be able to sufficiently isolate the target analyte. Furthermore, methods that are more simplistic may be easier to automate, improve accuracy, precision and lower detrimental effects on analysts and environment. Traditional and nontraditional methods should be reviewed as each method has its advantages and disadvantages depending on the instrumentation, sample matrix and target compound. For example, a good traditional method would be SPE which has multiple advantages with complex matrices such as isolation, purification, and fraction collection of different target compounds. Furthermore, this method provides consistent retention, great recovery, selectivity, and specificity. A nontraditional method would be saponification which is traditionally used in soap-making but can be used as a novel, rapid and easy sample preparation. Wei et al., in the article *Sensitive Quantification of Cannabinoids in Milk by Alkaline Saponification–Solid Phase Extraction Combined*

with Isotope Dilution UPLC–MS/MS, utilized saponification-solid phase extraction as a sample preparation method for human breastmilk which Wei et al. consider a contributing factor in improving the selectivity and sensitivity of their method. Saponification would involve the conversion of glycerides, which makes up a good portion of a lipid, to water-soluble substances. The target compounds would remain in the organic solvent layer and interfering substances would move into the aqueous layer for removal. Saponification, SPE and combined could be useful in constructing a standard sample preparation protocol for human breast milk due to its various interferences. Both techniques have the capabilities to remove large particulates, proteins, lipids, and other matrix components that would suppress detection of analyte. Optimization of the sample preparation offers better results for the instrumental analysis of a sample such as improved efficiency and performance. Optimization can be performed in multiple areas of the protocol with the purpose of enhancing the capabilities of the analysis.

Instrumentation optimization can occur for different components such as optimizing the instrument's detection capabilities by setting parameters specific to the target analyte. GC-MS and LC-MS-MS are the most commonly used instrumental methods for drug analysis. LC-MS-MS is quite useful for analyzing the chemical composition of a multi-compound sample. LC-MS-MS can be employed with a wide variety of compounds such as non-volatile and thermally-fragile compounds. However, GC-MS has become a preference due to its more targeted detection capabilities, simplistic operation, low-cost and limited upkeep compared to LC-MS-MS. The main advantage of LC-MS-MS is the lack of need for derivatization that must be done for GC-MS in order for some samples to

be suitable for analysis. LC-MS-MS offers greater selectivity, specificity, accuracy, precision and structural information than LC-MS. This enhancement is due to the tandem MS which combines two mass analyzers into a single instrument thus allowing for superior performance. This performance relates to the greater sensitivity and specificity of LC-MS-MS due to its fragmentation of molecules and the tandem mass spectrometer. The greater amounts of structural information than LC-MS that can confirm the structure of the drug analyte. The parameters utilized with the selected instrument will play a significant role in the level of performance as the instrument must be adjusted based on the compounds of interest. Optimization will be vital as the MS must be able to selectively detect the compound of interest using its heightened detection capabilities. Other factors to consider would be the column, solvents, time of run, type of elution (gradient or isocratic) and sample. Examination of GC-MS and LC-MS-MS on drug quantitation in human breast milk would be a great comparison study of the capabilities of each instrument with an uncommon matrix. The choice in instrumentation will impact all other areas of the analysis protocol, but also has a role in validation and potential implementation. Validation studies would need to be performed for possible implementation in a laboratory be it clinical or forensic.

Current methods regarding opioids in human breastmilk are mainly focused on methadone and buprenorphine. Methadone and buprenorphine are the focus due to their utilization in MAT for maternal OUD and relation to Neonatal Opioid Withdrawal Syndrome (NOWS). There are s studies available on the determination of an opioid in human breastmilk and less that involve the development of validated methods versus

literature reviews or clinical studies. Choo et al. [61] and Marchei et al. developed valid methods for the detection of methadone and its major metabolites in human breastmilk with acceptable levels for sensitivity, accuracy and precision using LC-MS-MS. Jansson et al. [63,64], utilizing the valid method of Choo et al., in two studies determined that concentrations of methadone in human breastmilk are low as well as unrelated to maternal dose. Furthermore, Jansson et al. found that breastfed-infants ingest negligible amounts less than 0.2 mg per day with no adverse effects on breastfed-infants. Lindemalm et al. [65] developed a method that could be used for therapeutic drug monitoring due to its sensitivity and precision. Sampling of human breastmilk involved seven women with OUD who breastfeed their infants. Lindemalm et al. found the transfer of buprenorphine and norbuprenorphine (main active metabolite of buprenorphine) into human breastmilk using High-Performance Liquid Chromatography-Atmospheric Pressure Ionization-Mass Spectrometry. Moreover, Lindemalm et al. determined that breastfed-infant exposure to maternal dose was less than 1%. Nikolaou et al. [62] developed a valid method for the quantification of methadone and its two major metabolites in human breast milk using GC-MS. The aforementioned articles contributed to the current recommendation that mothers in MAT programs are approved to breastfeed their child.

These methods are validated meaning they could be potentially utilized in a clinical and forensic setting, but there remain areas in available studies that could be improved. For example, the sample collection of human breastmilk should be at Lactogenesis II stage as that is when lactation occurs. Furthermore, colostrum would not be advisable given it will be of small volume (i.e., < 60 mL) and is a significant source of nutrition and immunology

for neonates. Transitional milk is highly variable but would be a good phase for testing. Additionally, mature milk would also be good as fore-milk, hind-milk or a pooled sample of both could be examined. It is by looking at the standards set by these studies that improvements can be made such as shifting methods to more green chemistry and waste reduction efforts. Furthermore, the studies will assist in guiding standardization and development of future methods. While clinical studies and forensic studies focus on different information, the general findings when combined allow for the creation of new policies and guidelines in public health and forensic laboratories. Clinical studies focus on information that affects maternal, fetal, and neonatal well-being. Such studies would include the bioactive components in breast milk, various contaminants that may be found in breast milk and effects on infants when consumed. Forensic toxicology studies look to create new methods of qualitative and quantitative analysis involving breast milk and other matrices that are not typically analyzed. The information produced from these studies could be beneficial to many areas of society and lead to new advancements in postmortem toxicological analysis as well as the clinical assessments influencing treatment care plans.

3.2 Forensic Science and Neonatal Death

Opioid exposure to children can occur in three general ways: purposeful administration by another party, unintentional self-administration, and intentional self-administration. Forensic laboratories, especially in terms of forensic toxicology and forensic pathology, have been tasked with determining how the exposure occurred and how much that exposure influenced impairment or death. Postmortem toxicology has a significant role as provides information on potential cause of death regarding prescription,

illicit or poisonous substance use. Additionally, it provides a way to determine illicit drugs being used in specific areas or communities. This information would be significant to law enforcement during an investigation as well as public health officials in the development of policies and allocution of funds. Postmortem drug levels are not static as a decedent's body is not a static environment. Postmortem redistribution, life-saving efforts and postmortem biological changes will influence drug levels. Moreover, variations in postmortem blood sampling may affect drug level measurements in significant manner. Postmortem redistribution involves the drug levels equilibrating between adjacent tissues and blood. Pediatric postmortem blood samples are affected by redistribution as blood is usually taken from the hearts in infants for postmortem toxicological analysis as there is so little peripheral blood available for a suitable sample. The validity of these measurements is potentially questionable due to the non-static environment of the decedent's body.

Furthermore, postmortem toxicology analysis does not include all substances and negative results does not necessarily mean that substances can be excluded. There is a significant concern with the targeted screenings that could fail to detect unknown compounds (i.e., new designer drugs, new derivatives). There could be no validated method at the time to detect presence of these new substances, which makes quantification and identification difficult and, on certain occasions, unlikely. Generally, therapeutic ranges for prescription drugs are based on adult studies. The application of those adult standard therapeutic ranges to infancy remains unclear on viability thus presenting a difficulty in determining the significance of drug levels for pediatric postmortem toxicology findings. According to Mistry et al., from the article *Methadone Toxicity in Infants: A Report of Two*

Fatalities, “the interpretation of pediatric post-mortem toxicology relies on the literature published on adult subjects. However, infants and children have important physiological differences compared to adults including slower gastrointestinal absorption, immature renal function and liver enzyme systems leading to prolonged half-life of substances and slower elimination.”

According to Madadi et al., from the article *Forensic Investigation of Methadone Concentrations in Deceased Breastfed Infants*, “There is little known about the inherent ability of a neonate to metabolize and eliminate methadone, and the relationship between blood concentration and toxicity has not been established.” Madadi et al. explains that many drugs do not have an established dose-toxicity relationship in neonates and infants and literary evidence of this relationship is too sparse for adequate assertion. Moreover, the paucity of data to assist in the assessment of clinically and toxicologically significant values for postmortem findings in breastfed infants also calls for more research. Madadi et al. examines two cases of deceased infants exposed to methadone through breastfeeding in which the cause of death was determined to be unascertained. However, interindividual variability related to age, drug interactions and genetic polymorphisms may have contributed to the levels of methadone in the system of the two infants. However, the lack of suitable data to establish the level of significance of those drug levels remains undetermined. Additionally, the maternal pharmacogenetics and drug pharmacological properties may play a role as such in the second case examined in the article. Based on the findings of the postmortem toxicology findings, both R-methadone and S-methadone enantiomers were found in breastmilk, but R-methadone was found in higher

concentrations [69,70]. R-methadone has been associated with the disruption of the heartbeat rhythm as well as increased risk of cardiac arrhythmia and sudden death. Yet, our current level of limited information acts as a barrier to explain how these findings may have been or not been a contribution to cause of death. Madadi et al. asserts that "to aid in the interpretation of elevated postmortem methadone concentrations in infants, one needs to consider exposure to methadone in utero, pharmacological treatment of neonatal withdrawal symptoms in the postpartum period, breastfeeding, neonate sleeping and care conditions, and the capacity of the neonate to metabolize and clear methadone from his or her system as part of the death investigation." There remains a paucity of available literature about the clinical and toxicological significance of substance (i.e., opioids) levels in neonates and infants from maternal breastfeeding.

Additionally, maternal as well as pediatric, including neonatal and infancy, pharmacogenetics should be researched more thoroughly as genetic information can influence the pharmacological actions of a substance. For example, there have been greater incidences of Neonatal Abstinence Syndrome, extreme sleepiness and serious breathing problems in infants of breastfeeding mothers who were taking codeine. There has been one prominent case in which the mother was an ultra-rapid metabolizer of codeine which produces large amounts of morphine from the breakdown of codeine. As a result, toxic accumulation of morphine occurred in her breastfed infant causing respiratory depression and death. This led to a wariness regarding such opioids as codeine and tramadol as well as warning against using the two opioids during pregnancy and children under 12. While opioids and other drugs enter the breast milk at lower concentration than the concentration

in maternal blood, there is not enough research to support any conclusion on the significance of those concentrations.

Furthermore, these issues indicate a need to assess current policies regarding neonates and infants in order to produce more accurate findings. The autopsy performed would have to be re-assessed. For instance, the method of post-mortem blood collection would be different given the vast difference between an adult and an infant in multiple ways. Newer policies would be more beneficial for the sake of cause of death determination and toxicology analysis. Further research and understanding regarding opioids and children are necessary for improving forensic analysis for pediatric and neonatal cases. Even more so, the task of addressing exposure administration is quite important as it will play an important role in cases where child protective services may need to be called. Public health also has a great need for more of these studies to be performed regarding opioids, breast milk and the effect on maternal and neonatal well-being.

3.3 Public Health and Neonatal Drug Exposure

According to Lopes et al., from the article *Quantification of Carbamazepine and its Active Metabolites by Direct Injection of Human Milk Serum using Liquid Chromatography Tandem Ion Trap Mass Spectrometry*, "Human milk has been used to assess neonatal exposure to drugs, with the advantage that it is collected easily and non-invasively. However, the extraction of drugs from breast milk is a great analytical challenge given that the transfer of drugs from plasma into breast milk is related to factors such as plasma protein binding, lipophilicity, ionization, molecular weight, pharmacokinetics, and plasma concentration to name a few." Clinicians must be considerate of various pressures,

such as maternal well-being, infant variances, and drug pharmacology on maternal and neonate well-being in order to allow for breastfeeding and decreased risk toward infant health.

Unfortunately, current clinical risk assessment is heavily affected by a lack of data relating to neonatology and drug toxicity due ethical restrictions. However, there are other methods available that look to quantify the amount of drug excreted in breastmilk by using mathematical equations and pharmacology to relate maternal plasma concentration to breast milk concentration such as the milk-to-plasma ratio. According to Calcaterra, "The milk-to-plasma concentration ratio is the most commonly quoted index of drug distribution into human milk. However, calculation of the daily infant dose of drug ingested in milk is a more relevant indicator of infant exposure to a drug" [9]. Furthermore, Calcaterra asserts that "A better indication of infant exposure to a drug is the steady-state plasma drug concentration in a breast-feeding infant, the major determinants of which are the dose rate (via milk) and the oral availability and clearance in the infant. Clearance, however, is impaired in very young infants, particularly if premature" [9]. An infant's gastrointestinal system can alter drug pharmacokinetics as a result of its lesser development. Furthermore, a variety of factors related to the infant will play a role in the metabolism of drugs. Some of these factors can be connected to genetics, co-administration, comorbidity, and reduced development [60-62]. Numerous factors, such as infant clearance, plasma-to-milk concentrations, and drug toxicity, provide significant information on medication therapies and treatment plans that are safe while breastfeeding. As medicine advances, so should

research to provide the most effective and safe patient care in terms of treatment plans and risk assessments.

4. CONCLUSION

4.1 The Need for Intervention and Research

Development of new methodologies for drug quantification is quite relevant for today's society, especially in terms of the forensic science community and public health concerns. Public health faces unique challenges due to rising rates of infectious diseases, potentially fatal dangers for first responders and the medical field as well as adverse outcomes for different special groups. Two specific groups face greater difficulties with the opioid crisis than most people within the afflicted population: women and infants. There are not enough studies done with the introduction of new drugs, prescription and illicit, or further examination of old drugs, prescription and illicit, that focus on the impact on maternal, fetal, or neonatal health as well as breast milk.

As a result, the consequences of opioid abuse and addiction on women and infants cannot be fully conceptualized and properly managed. In addition to these issues, forensic science faces a serious backlog due to the increasing number of opioid-related cases occurring throughout the United States. There are various causes for this difficulty such as a lack of available reference materials due to increasing diversification of illegally manufactured opioids. Additionally, neonatal death determination requires more research as current standards are based on adult physiology which limits understanding about the effects of drugs on neonatal or pediatric physiology, determining substance exposure and its role in the impairment or death of the child.

Further experimental research is needed to develop new or optimized methods of drug quantification which could be applicable in a clinical and forensic setting. Moreover,

the benefits of the research could present improved clinical treatment and forensic investigation of children exposed to drugs, minor to severe. These findings could push forth new policies and programs geared to maternal substance use disorder, neonatal abstinence syndrome, and fetal drug exposure. Future consideration should be given to the expansion of testing in clinical setting as individualized patient care plans would be better. Furthermore, the development of more tools for drug testing in forensics that could foster more research in pediatric death and toxicology testing. Lastly, greater understanding of the opioid epidemic and how to provide treatment for different groups, such as mothers and infants, will support improved health services, affordable and accessible drug addiction programs, and greater research opportunities.

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