Forensic Technology
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A program of the National Institute of Justice

IN-BRIEF SERIES (Part 3 of 3)

Beyond DNA: The Impact of Toxicological Evidence in Sexual Assault Investigations

Because of the effects that CNS depressants can have on memory and the subsequent reporting of suspected DFSA by potential victims, the true prevalence of this crime may never be fully realized.

- Marc LeBeau, FBI Laboratory

Preface

Sexual assault remains prevalent in the United States, with an average of 300,000 cases reported to law enforcement each year [1]. However, another 600,000 go unreported [2]. The circumstances of and trauma resulting from a sexual assault can pose a challenge to investigators. For example, witnesses are not always present; the impact of trauma or incapacitating substances, such as alcohol, may affect the victim's ability to recount details of the incident; and frequently, corroborating evidence is limited.

DNA evidence, while valuable, is not always probative or present in every case: many DNA samples do not meet the quality standards required to be uploaded into CODIS (38% of profiles were found to be ineligible as noted from recent NIJ-supported research [3]). Even in cases where a DNA profile is present and is CODIS-eligible, a CODIS hit occurs only about half of the time [3]. Additionally, a DNA profile may provide limited probative value in situations where sexual contact is not disputed. Thus, many types of additional physical evidence play a critical role in the investigation and prosecution of sexual assault cases.

Physical evidence collection, submission, and analysis can be an effective and necessary means of reconstructing at least some of the events that occurred during a sexual assault. Physical evidence provides value to investigations even if a DNA profile is developed and probative, as it can be used to corroborate and supplement a greater understanding of the circumstance and make a stronger case. This three-part Beyond DNA In-Brief series highlights types of physical evidence that can provide crucial information about a sexual assault, so that key stakeholders in the criminal justice community ultimately obtain just resolutions for these crimes.

Disclaimer: *This project was supported by Award No. 2016-MU-BX-K110, awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication/program/exhibition are those of the author(s) and do not necessarily reflect those of the Department of Justice.*

Objectives

- To illustrate the impact of toxicological evidence in sexual assault investigations.
- To provide an overview of analytical toxicology analysis techniques used to identify drugs and poisons in blood, urine, and hair samples.
- To identify limitations of and technological advancements in toxicology testing that current research efforts address.

These reports are designed to provide **law enforcement, policymakers, legal professionals, and the public** with an introduction to various types of physical evidence and the roles they may play in sexual assault investigations.

This is the third installment of a three-part series, which also includes (1) *Beyond DNA: The Role of Physical Evidence in Sexual Assault Investigations* and (2) *Beyond DNA: The Role of Biological Evidence in Sexual Assault Investigations*.

Introduction

Toxicology testing is used to detect the presence of drugs and toxins in an individual's body through biological matrices such as blood, urine, and hair. In addition, forensic toxicologists can provide interpretations of the concentration of drugs and toxins in a person's system. Toxicological evidence offers valuable information that can help establish whether the victim of a sexual assault was incapacitated or significantly impaired by a drug or toxin, in what is known as alcohol and drug-facilitated sexual assault (DFSA).

In cases of DFSA, drugs (over-the-counter, prescription, or illegal) and/or other toxicants (e.g., solvents such as chloroform) are used to physically and mentally incapacitate an individual, impairing the decision to consent to sexual activity. This drug-altered decision-making ability is known as 'diminished capacity' in sexual assault cases. Alcohol is the most prevalent substance reported in DFSA cases [4].

The Bureau of Justice Statistics (BJS) estimates that 39% of sexual assaults in the United States from 2005-2010 were linked to a victim being under the influence of alcohol or drugs at the time of the incident [5]. DFSA has been described as occurring in one of two primary categories, "opportunistic" or "proactive"[6].

- **Opportunistic DFSA** occurs when the assailant sexually assaults a victim that is extremely incapacitated due to his or her own voluntary drug or alcohol ingestion "to the point of near or actual unconsciousness."
- **Proactive DFSA** (also referred to as Predatory DFSA) occurs when the victim is intentionally, whether forced or covertly, given a substance with the intent to incapacitate for the purpose of sexual assault. Some of the drugs used in proactive DFSA are colorless, odorless, and tasteless when placed in food or drink.

Historical Use of Drugs in Crime: Mickey Finn
One of the most famous accounts of an assailant drugging an individual with the intent to commit a crime dates to 19th century Chicago. Mickey Finn, the owner of Chicago's Lone Star Saloon, was accused of using chloral hydrate, known as "knock-out drops," to drug and incapacitate his customers to rob them. Throughout history, the term "Mickey" and later "Roofie", the nickname for Rohypnol, have been used to describe any substance used to render a person helpless for the intent of committing a crime (e.g., robbery, sexual assault) [7].

Representative Drug Classes on the SOFT Drug Facilitated Crime (DFC) List with Exemplary Drugs for Each Category

- **Antidepressants**-amitriptyline, citalopram
- **Barbiturates**-amobarbital, butalbital
- **Benzodiazepines**-alprazolam, clonazepam
- **Over-the-counter Medication**-brompheniramine, dextromethorphan
- **Ethanol**
- **GHB and analogs**-gamma-hydroxybutyrate, 1,4-butanediol
- **Narcotic and non-narcotic analgesics**-codeine, oxycodone, propoxyphene
- **Marijuana**-11-carboxy-tetrahydrocannabinol (THC)
- **Miscellaneous**-ketamine, zolpidem, methylenedioxmethamphetamine (MDMA, ecstasy)

Sexual assault victims impaired by various drugs and toxicants, whether voluntarily or involuntarily, commonly experience debilitating symptoms. Alcohol and many other drugs can potentially be used to facilitate sexual assaults. The Drug Facilitated Crimes (DFC) Committee of the Society of Forensic Toxicologists (SOFT) developed a list of over 100 drugs and metabolites commonly encountered in a DFSA, which is organized into ten major categories [8]. Many of the drugs depress the central nervous system (CNS). Common symptoms of the CNS depressants include drowsiness, dizziness, loss of inhibition, loss of muscle control, loss of consciousness, sedation, slurred speech, and vomiting. These effects may exacerbate risk-taking behaviors, lead to vulnerability and inability to consent to sexual acts due to the severe impairment they can cause.

Despite the belief that drink-spiking is a common occurrence (proactive DFSA), a recent review determined that voluntary drug ingestion combined with alcohol intoxication (opportunistic DFSA) occurs more frequently. Anderson and colleagues published a systematic review to determine the global prevalence of DFSA reported in individuals at or above the age of consent (i.e., 16 years of age) [6]. The publication included results of eight studies from France, Canada, USA, Northern Ireland, Australia and the UK. In DFSA cases, alcohol remains the most commonly detected substance, often in combination with other substances. Cannabinoids and benzodiazepines are also frequently detected. While GHB (gamma hydroxybutyrate) and Rohypnol® (flunitrazepam) are perhaps the first drugs associated with DFSA, both drugs are rarely detected. In fact, flunitrazepam is not an available prescription drug in the United States.

Impact of Toxicological Evidence

Forensic toxicology can play an important role in the scientific investigation and the contribution of drugs in sexual assault cases in the following ways:

► **Helps to establish drug/toxicant concentration thresholds capable of impairing capacity to consent**—Toxicological evidence can be used to help understand gaps in the victim's recall of events that took place during or around the incident.

►**Helps corroborate or disprove a scenario**— Unlike most physical evidence types, toxicological evidence may be able to estimate a time window for drug exposure, which helps to support different accounts of the incident including behaviors.

Toxicology Evidence Analysis

While comprehensive toxicology screens are not recommended as standard protocol during a sexual assault examination, they are used when a DFSA is suspected [9]. A general procedure for toxicological evidence collection and analysis used to determine whether an individual was exposed to alcohol or other drugs before or during the time they were sexually assaulted is outlined in the workflow in Figure 1. Toxicological specimens, including urine and blood, are preferentially collected by a forensic nurse or other medical professional during a hospital examination. After collection, the samples are sent to a forensic laboratory, where they are analyzed.

Successful use of toxicological evidence in a sexual assault investigation [10]
In a case where a suspect used chloroform to incapacitate and sexually assault a victim, toxicological evidence was used to demonstrate that a DFSA had occurred. Chloroform was detected in the victim's blood in a concentration consistent with exposure by chemical inhalation, as well as on the scarf used to administer the chloroform. In addition to toxicological evidence, injuries such as scratches and bruising patterns on the victim's legs and wrists supported the claim that the victim was sexually assaulted. Read more about the case *here*.

There is not one procedure that can be used to test for all drugs and metabolites at once. In many cases, a specimen is analyzed several times using different techniques each specific for confirmation of a particular drug class or classes. Testing for drugs and other toxicants incorporates both presumptive and confirmatory testing: first, presumptive techniques are used to screen for the presence of substances. Screening techniques, which include immunoassays, liquid or gas chromatography-mass spectrometry (LC-MS or GC-MS) and sometimes colorimetric assays typically provide a quick, qualitative result that allows the toxicologist to determine if any substances are present [11]. However, less sensitive methods such as immunoassays are cautioned since drug concentrations in DFSA are often very low, in part, due to delayed reporting and would go undetected.

Drug screening is then followed by confirmation testing. The presence of a substance is confirmed and quantified by methods such as LC-MS and GC-MS, to determine the amount (i.e. concentration) present in the victim at the time of collection. Prior to analysis, analytes are extracted from biological samples using preparation techniques such as liquid-liquid extraction (LLE) and solid-phase extraction (SPE). Sample preparation is used to clean up and concentrate the analytes of interest prior to analysis. The toxicologist interprets the test results and reports the findings. Forensic toxicologists may also testify about their findings in court.

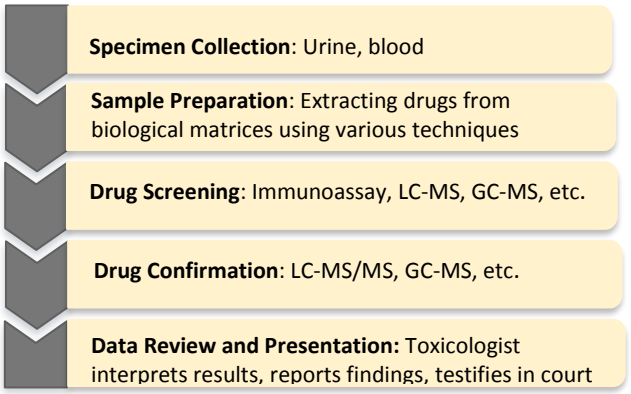

Figure 1: Workflow of toxicology evidence collection and analysis.

It should be noted that forensic toxicology testing and medical toxicology (i.e., testing performed in a hospital for clinical diagnosis and treatment) have different purposes. Because drug testing in a clinical setting is performed for immediate medical care of the patient to ensure health and safety, oftentimes it tests for drug classes in a qualitative manner (i.e., less sensitivity and selectivity)— drug classes are qualitatively detected at an administrative threshold.

Urine and blood are specimens of choice for toxicology in a suspected DFSA investigation. Both matrices can provide valuable information for a sexual assault case. Blood is typically recommended if collection occurs within 24 hours of the sexual assault; however, with more sensitive technologies and lower detection limits or slowed metabolism substances may be detected up to 48 hours [12, 13].

Urine

Urine is typically the preferred matrix for toxicological testing of suspected DFSA as it has several advantages over blood. First, the window of detection for parent drug and/or metabolites ranges from around 1.5-4 days (greater for cannabis) longer than that of blood. Second, urine typically contains a higher concentration of drugs and metabolites than blood [13]. Third, collection of urine from the sexual assault victim is non-invasive and can easily occur during medical treatment and saved for testing until legal consent is given. Finally, testing of urine is usually less expensive than testing other matrices, and it is typically easy to obtain a sufficient quantity of sample.

The primary disadvantage for urine is that the drug concentration cannot be accurately interpreted to determine impairment unlike the analysis of blood. For some individuals, providing a urine sample may be difficult for patients who are not able to produce enough urine at the point of medical treatment.

Recommendations for Urine collection [13]:

- collection within 120 hours of assault
- 100 mL of urine ideal
- specimen cup should contain preservative

There are numerous factors to consider for toxicological analysis of DFSA substances, including sample collection, preservation, and preparation; drug stability; analytical methodology, instrument sensitivity and specificity; and speed of analysis. Validated methods based on highly sensitive and selective analytical instrumentation are necessary for optimal identification and/or quantification. No matter when or where the evidence is collected, a chain-of-custody must be used to chronologically document the movement, location, and possession of the evidence up to and including the time it is presented in court and final disposition.

Blood

While blood is not always collected during a DFSA investigation, it can provide useful information that urine cannot. This matrix offers the best correlation between alcohol or drug concentration and performance impairment. It is important to note that the only well-established correlation between performance impairment of a drug and blood concentration is alcohol— there is no agreed upon limit for which impairment can be reliably demonstrated for other drugs.

As with urine, blood also has its disadvantages. Blood must be collected by a medical professional and drugs are generally detected for no more than one or two days since metabolites do not remain as long in the bloodstream. Collection of blood is an invasive process, but it is often used in medical treatments (e.g. collection of blood for HIV testing) following a sexual assault.

Recommendations for Blood collection [13]:

- collection within 24 hours, but not more than 48 hours
- 12 mL of blood ideal
- collect in a grey-top tube containing preservative

Hair as an Alternative Matrix

Although research into the use of hair as an alternative matrix to detect drugs and toxins has increased over the years, there are many considerations that need to be addressed before hair can be fully implemented. Drugs and their metabolite(s) may be present in hair samples at significantly lower concentrations compared to blood and urine and analytical advancements has further improved detection. Hair has a significantly longer window of detection – in some cases it could be 90 days or more [13]. Furthermore, segmental hair analysis can be used to establish timing of an event or repeated assaults over a long period of time [14]. Collection of hair involves non-invasive methods and the specimen can be easily handled and stored, allowing for retesting if necessary. Disadvantages of hair drug testing include the small number of forensic laboratories available to perform this specialized testing and the limited research and knowledge regarding the detection of the many classes of drugs occurring in DFSA cases.

Limitations of Toxicological Evidence

There are several issues that may limit the effective analysis of DFSA evidence. A delay in the investigation may limit the timely analysis of certain drugs or interpretation of the results. The absence of drugs or alcohol in a sexual assault victim does not indicate that a DFSA did not occur: DFSA victims commonly delay or do not report the crime due to amnesic effects of the drugs or the trauma they have experienced [15]. In addition, they may be hesitant to consent to a toxicology screen if they voluntarily consumed drugs around the time of the incident. Since many drugs, such as gamma-Hydroxybutyric acid (GHB) and ethanol, are quickly eliminated from the body, extended delays in sample collection lower the probability of detecting the drug or metabolites [15, 16]. Therefore, early reporting and subsequent sample collection

are very important. The complexity of testing for an unknown substance is challenging and requires a battery of technologies— given the small sample volume available and the expense, laboratories may only test for a limited list of substances unless they suspect a specific substance. Additionally, the preferred matrix may shift based on the time of victim reporting (e.g., if a victim presents greater than 1 week after the event, hair would be the only likely matrix to still contain the drug in a detectable amount). It is important that investigative and medical professionals assisting a sexual assault victim are aware of the drug detection windows of the various biological matrices that can be collected. Finally, validated methods specific to DFSA recommended minimum performance limits should be used by a laboratory to ensure accurate detection of drugs generally found at lower concentrations.

> It is important for victims, investigators, prosecutors and juries to understand that the failure to detect a drug in no way means the event was not a DFSA.

There are many confounding variables to consider in every case, and interpretation of results must be approached with caution. As stated previously, interpreting impairment based on a blood concentration alone is unreliable, and it can be difficult to determine the effect that multi-drug use had on the intoxication. For example, the detected drug concentrations of an individual who takes a prescribed medication would be interpreted differently than a person who does not take any prescribed medications. It is important to note that a negative result does not prove that no exposure occurred, especially in cases where specimens were collected past the drug's window of detection. Even in the absence of positive toxicology results, toxicologists can testify to the impact of drugs and alcohol on a victim's capacity to consent to sexual activity and suggest potential drug ingestion based on the victim's feelings and experiences during the incident.

In the past decade, the prevalence of more sensitive and selective technology has facilitated the detection of DFSA related substances; however, many laboratories are unable to upgrade their current instrumentation to support their caseload. Although some laboratories have personnel dedicated to method development and validation, this is typically not the case for most publicly funded laboratories. Limitations in funding or staff to dedicate to training further constrain method development and validation of new methods and instrumentation. This may result in the need for more staff to minimize delays in the judicial process. More research is needed for the development of highly discriminating, accurate, reliable, cost-effective, and rapid methods for the identification, analysis, and interpretation of drugs, including needs for:

- **Analysis of drugs in alternative matrices (e.g. hair, nails)**—Alternative matrices may provide a longer window of detection than urine and blood which could prove crucial in cases of delayed reporting.
- **Prevalence studies with current drug trends**—In order for toxicologists to know what drugs and metabolites to look for, it is important to know what substances are commonly in use.
- **Prevalence of negative results in suspected cases when toxicolgy is performed using mimimum performance limits**—Many times, a drug may not be detected even though it is suspected based on the investigation. Understanding how often this occurs can improve case interpretation.

Opportunities for Improvement in Toxicology Research

Forensic toxicology and DFSA is an active area of research, funded by the NIJ and other sources (**Table 1)**.

Table 1: Areas of current research in toxicology.

	Snapshot of Current NIJ Funded Research and Other Research Efforts	Potential Impact
Drug Prevalence in DFSA cases	**NIJ Research:** **Drug-facilitated, Incapacitated, and Forcible Rape: A National Study** **Awardee:** Medical University of South Carolina| **Award Number:** 2005-WG-BX-0006 **Research Goal:** Understand the incidence and key characteristics of drug-facilitated and forcible rapes. [17] **Estimate of the Incidence of Drug-Facilitated Sexual Assault in the U.S.** **Awardee:** University of Illinois at Chicago| **Award Number:** 2000-RB-CX-K003 **Research Goal:** Quantify the incidence of DFSA and understand the social aspects surrounding DFSA. [18] **Other Research:** A comprehensive literature review representing DFSA cases in all reporting countries performed to understand global trends in the rate and toxicology of reported DFSA in individuals at or above the age to consent to sexual activity (i.e., 16 years of age) [7]. A review of more than one-hundred and sixty international DFSA cases to document the differences in survivor, assailant, and assault characteristics between cases classified as predatory and non-predatory [19].	► Maintain a current account of drug trends in the toxicology findings in DFSA to inform testing protocols
Hair as an Alternative Matrix	**NIJ Research:** **Evaluating Analytical Parameters and Understanding Drug-Matrix Interactions in Forensic Hair Analysis** **Awardee:** Florida International University| **Award Number:** 2018-75-CX-0037 **Research Goal:** Understand drug interactions within the hair matrix, assess common methods to prepare and decontaminate hair samples. [20] **Optimization of Pretreatment Parameters in Hair Analysis for Drugs of Abuse and Understanding Protein-Drug Physiochemical Interactions** **Awardee:** Florida International University | **Award Number:** 2017-IJ-CX-005 **Research Goal:** Understanding the physiochemical interactions between the biological matrix and multiple chemical substances. [21] **Analysis of Cocaine Analytes in Human Hair II: Evaluation of Different Hair Color and Ethnicity Types** **Awardee:** RTI International | **Award Number:** 2018-DN-BX-K179 **Research Goal:** Evaluate cocaine analytes in hair of different color and ethnic origins. [22] **Analysis of Drugs of Abuse in Human Hair: Surface Contamination and Localization of Analysis** **Awardee:** RTI International | **Award Number:** 2013-DN-BX-K021 **Research Goal:** Evaluate surface contamination of human hair exposed to methamphetamine and heroin and its effects on drug tests. [23]	► Establish a timeline of drug exposure in a drug facilitated case for corroboration in court ► Improve methods to detect drugs in a hair specimen may help determine time of drug exposure ► Improve methods for hair testing of GHB by establishing when an individual has concentrations above known endogenous GHB concentrations

	Snapshot of Current NIJ Funded Research and Other Research Efforts (con t)	Potential Impact
Hair as an Alternative Matrix (continued)	**Other Research:** Evaluation of concentrations of drugs of abuse and their metabolites in hair, fingernails, and toenails. Results showed that nails can be considered as a useful alternative to hair for monitoring of long-term drug consumption. [24]. Analysis of hair samples from individuals with no known exposure to GHB to determine if a decision point can be established to differentiate between endogenous and exogenous GHB concentrations. Results showed overlap in concentrations between the two sources and suggested further research [25]. Evaluation of cocaine analyte concentrations in hair, comparing subjects with self-reported cocaine use against participants exposed to cocaine in controlled clinical settings [26].	► Establish a timeline of drug exposure in a drug facilitated case for corroboration in court ► Improve methods to detect drugs in a hair specimen may help determine time of drug exposure ► Improve methods for hair testing of GHB by establishing when an individual has concentrations above known endogenous GHB concentrations

Conclusion

Forensic toxicology plays a key role in understanding how drugs contributed to a sexual assault case. In cases of a drug-facilitated sexual assault, this evidence can provide a time window for an individual's drug exposure, account for gaps in a victim's recall of the incident and suggest inability to consent to sexual activity. Although toxicological evidence, along with other types of physical evidence, can help shed light on the events transpiring during the incident and bring justice to victims of sexual violence, it is important to remember that the absence of drugs or alcohol in a sexual assault victim does not indicate that a DFSA did not occur.

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Published: January 2019

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Suggested Citation

Ropero Miller, JD, and N. Bynum (2018). Forensic Technology Center of Excellence. *Beyond DNA: The Role of Toxicological Evidence in Sexual Assault Investigations*. U.S. Department of Justice, National Institute of Justice, Office of Investigative and Forensic Sciences.

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