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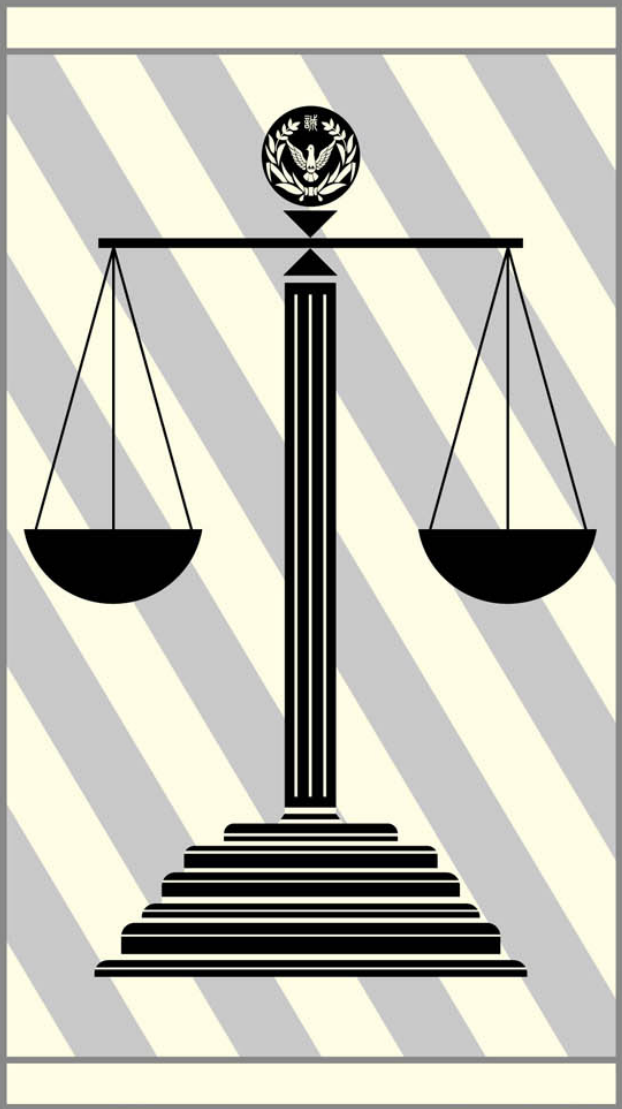

FORENSIC SCIENCE REVIEW

VOLUME TWENTY-TWO ■ NUMBER ONE ■ JAN. 2010

A Special Issue Devoted to the Topic on
Drug-Facilitated Sexual Assault

Objectives and Scope

The discipline of forensic science has nurtured many publications oriented toward research and case reports, also broad-based formal treatises. Rapid advances in forensic science have created a need for a review journal to bridge the gap between research-oriented journals and reference volumes.

The goal of *Forensic Science Review* is to fill this void and provide a base for authors to extrapolate state-of-the-art information and to synthesize and translate it into readable review articles. The addition of this journal extends the spectrum of forensic science publications.

Articles bring into focus various narrowly defined topics whose literature has been widely scattered. Articles are presented to stimulate further research on one hand and worthwhile technological applications on the other. The publisher's aim is to provide forensic scientists with a forum enabling them to accomplish this goal.

Technological applications based on basic research are emphasized. Articles address techniques now widely used in forensic science as well as innovations holding promise for the future.

A Special Issue Devoted to the Topic on

Drug-Facilitated Sexual Assault

Edited by

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FOREWORD

Awareness of drug-facilitated sexual assault (DFSA) over the past two decades has prompted many improvements in the reporting of these crimes by victims; evidence collection by sexual assault nurse examiners and law enforcement agents; toxicological analyses by laboratories; and adjudication by the legal system. This issue of *Forensic Science Review* is dedicated to the topic of DFSA and is intended to serve as a useful reference for those affected by these cases.

The articles in this issue cover a broad range of topics to include the numerous challenges that DFSA cases present, the difficulty of defining the frequency of DFSA, and how a laboratory can best manage these cases. Six articles are dedicated to some of the most commonly encountered drugs and drug classes in DFSA investigations. These reviews discuss the pharmacodynamics of the individual drugs so the reader can understand how these drugs may render a victim incapable of consenting or fighting off his or her attacker. The chemistry and pharmacokinetics are also discussed to emphasize the analytical difficulties associated with some of these drugs. A number of examples of documented cases using these drugs are presented. Discussions of useful analytical techniques capable of improving detection of the drugs or drug classes are also included.

It is our honor to have served as guest editors for this special issue of *Forensic Science Review*. We would like to thank the numerous authors who spent countless hours preparing these reviews. All serve on the Society of Forensic Toxicologists (SOFT) Drug-Facilitated Sexual Assault Committee and are dedicated to improving DFSA investigations. We are confident that their expert guidance presented in these pages will be of value to you.

Marc A. LeBeau
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Challenges of Drug-Facilitated Sexual Assault[a]

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Challenges of Drug-Facilitated Sexual Assault

REFERENCE: LeBeau MA, Montgomery MA: Challenges of drug-facilitated sexual assault; *Forensic Sci Rev* 22:1; 2010.

ABSTRACT: This article provides the reader with an understanding of the numerous challenges of drug-facilitated sexual assaults (DFSA). The challenges are categorized as follows: the drugs, reporting the crime, evidence collection, and laboratory analysis of specimens. The challenges associated with the drugs used to commit DFSA emphasizes the pharmacological effects of strong central nervous system depressants and how the pharmacokinetics and pharmacodynamics of these drugs create difficulties in an investigation. For example, while sexual assaults are generally considered to be a significantly underreported crime, the drug effects further complicate victims' reporting to law enforcement. Any delay in reporting decreases the ability of a laboratory to detect the presence of drugs or metabolites in useful evidentiary specimens such as blood and urine. Finally, differences in instrumentation and mission from one laboratory to the next will impact the ability to provide consistent identification of DFSA drugs or metabolites in these cases. Although the true prevalence of DFSAs will never be fully known, acknowledgment of the many challenges that come with these cases provides insight as to how to improve chances of successfully investigating DFSA allegations.

KEY WORDS: Drug-facilitated sexual assault, drug rape investigation.

INTRODUCTION

Typical DFSA Scenarios

Drug-facilitated sexual assault (DFSA) is a term used to describe crimes in which a person is subjected to a nonconsensual sexual act due to the incapacitating effects of alcohol and/or drugs [11]. The pharmacological effects of the drugs prevent the victim from being able to consent to the sexual act or fight off his/her attacker [21].

Although the media have portrayed this crime as occurring when the drug is secretly administered to a person through his/her food or drink, it is likely that many of these crimes occur after voluntary consumption of recreational drugs that have strong central nervous system (CNS) depressant effects [1]. Additionally, these cases may include prescription and/or over-the-counter (OTC) medications co-ingested with alcohol [9], resulting in incapacitation and nonconsensual sexual contact. The key is that the drugs assist, or facilitate, the crime.

The first successfully prosecuted DFSA crime on record in the United States [19] was the following case: On Oct. 18, 1900, a 17-year-old girl went to a local drugstore in Paterson, NJ, for some baby powder for an infant niece. At the front of the drugstore, she met two men, one of whom she had previously dated. The men persuaded the girl to accompany them into the bar across the street for a drink. Once inside, the girl consumed a cocktail, an absinthe drink, and two glasses of sparkling wine. The latter had been "spiked" with chloral hydrate by the bartender, and caused the girl to pass out at the bar. The men and the bartender moved her by carriage to a secluded area where they raped her. While she was being sexually assaulted, the girl died, presumably as a result of ingesting the chloral hydrate. The men dumped her body near the local river, but the body was found the next morning. At trial, one of the men, George J. Kerr, received 15 years for sexually assaulting the victim while she was incapacitated by the chloral hydrate. The other men were convicted of second-degree murder and received longer prison terms.

Prevalence of DFSA

The true prevalence of DFSAs is unlikely ever to be fully recognized [10]. Nonetheless, a number of studies have attempted to quantitate the incidence of DFSA. The first comprehensive study was supported by Roche Pharmaceuticals [4], the manufacturer of the benzodiazepine Rohypnol® (flunitrazepam). In this study, free toxicological analyses of urine specimens collected in suspected DFSA cases were performed by an independent forensic toxicology laboratory. Over the course of this study, 3,303 samples were analyzed; 73% of the samples were reported to have been collected within the first 24 h after the alleged drugging and over 61% of all samples were positive for one or more drugs, including ethanol. Critics of the study have noted that while the urine specimens were screened for Rohypnol® at a very low concentration, other benzodiazepines were not screened for at such low levels. Additionally, the study reported that 4% of the urine samples were positive for GHB; however, it is now known that the cutoff used in this study to differentiate between endogenous and exogenous GHB was too low [5,12], so GHB's prevalence was quite likely overestimated.

More recent studies in the United Kingdom have found that nearly 50% of 1,014 cases of alleged DFSA analyzed by the Forensic Science Service in London over a 2-year period were positive for ethanol and/or other

incapacitating drugs [22]. A follow-up study, entitled *Operation Mattise*, examined 120 cases of suspected DFSA over a 1-year period and agreed that ethanol continues to be the most common agent associated with DFSA [6]. More importantly, the report highlights the difficulties in investigating DFSA cases. Other studies on the rising incidence of DFSA cases have also been conducted in Australia [8], Canada [15], France [20], and Poland [2].

I. DRUG FACTORS

Investigations into DFSA allegations are fraught with a number of challenges [3]. The most important challenge centers around the drugs used to commit this crime [11]. The mainstream media have led the general public, including some investigators and prosecutors, to believe that there are only three or four drugs used to commit DFSA. In reality, there are more than 50 drugs known or suspected to have been used to commit DFSA [24]. Many of these drugs are well-known recreational drugs of abuse, prescription medications, or over-the-counter pharmaceuticals.

Although surreptitious administration does occur in many DFSA cases, it is important to recognize that in many cases the drugs are self-administered by the eventual victim. This conclusion is supported by a study, funded by the U.S. National Institute of Justice, which estimated that less than 5% of sexual assault cases involved a drug being surreptitiously administered to the victim, but when voluntary drug use is considered, over one-third of sexual assault cases may be facilitated by drugs [10].

Most drugs exist in a solid, tablet formulation, so surreptitious administration requires preparation on the part of the criminal, particularly to slip a drug into the drink of a victim without it being noticed. The pill or tablet may not immediately disappear if it is simply dropped into a drink. Instead it may fizz and bubble as it slowly begins to dissipate into the beverage. Furthermore, most tablets contain insoluble, cellulose-based fillers. These fillers will not completely dissolve into the drink and instead leave a grainy residue. For that reason, it is common for a perpetrator to dissolve the pill in small amounts of alcohol, filter the alcohol to remove the insoluble materials, and then transfer the drug/alcohol mixture into a small eyedropper bottle. Some drugs such as GHB, drugs found in gelatin capsules, and injectable drugs are already in a liquid form, thus minimizing the preparation that is required for surreptitious administration.

Many of the drugs used in DFSAs are fast-acting, strong CNS depressants that tend to mimic ethanol intoxication [23]. They can cause multiple pharmacological effects, including: relaxation, euphoria, decreased inhibitions, amnesia, impaired perceptions, difficulties in maintaining balance, impaired speech, drowsiness, complete loss of motor functions, vomiting, incontinence, unconsciousness, and even death. It is helpful to remember that nearly all of these drugs are capable of producing symptoms of general anesthetic agents—a common description used by many victims of DFSAs. Because the CNS depressant effects of these drugs are generally similar to one another, it is highly unlikely that one can determine the drug used in a DFSA case by symptoms alone.

To further complicate DFSA investigations, there are also significant variations in the pharmacokinetics (i.e., absorption, distribution, biotransformation, and elimination) of these drugs [11]. As a result, there is a variable window of time after ingestion that drugs are able to be detected in a sample provided by an alleged DFSA victim. For some, the presence of the drug may only be detectable in a urine sample collected just a few hours after ingestion [7]. For others, the drug is still detectable weeks after ingestion [18]. Of course, not knowing which of the 50 or more drugs that may have been used in a DFSA case makes it very difficult to interpret a negative toxicological finding.

II. REPORTING THE CRIME

It is well known that sexual assaults are significantly underreported; however, common sense suggests that the percentage of DFSA cases actually reported must be even lower than for other forms of sexual assault. In many cases, the victim suffers from memory impairment. In other instances, victims may be unclear as to the sequence of events and may delay reporting in order to try to piece together the information that they do remember [1].

Of course, delays in reporting usually mean specimens are not collected in a timely fashion, making it much more difficult for the toxicology laboratory to identify the incapacitating agents that may have been used [13]. Additionally, it is vital that the victim be forthright about any prescription, over-the-counter, or recreational drugs that were voluntarily ingested [11].

III. EVIDENCE COLLECTION

Another challenge facing investigators of DFSA is ensuring that the proper amounts of the most useful specimens are collected as quickly as possible and are properly preserved [13]. It is generally accepted that urine is the most useful specimen in typical DFSA investigations [12]. This is because drugs and their metabolites become concentrated in urine specimens prior to elimination from the body, and are therefore more readily detectable. This becomes most important when attempting to determine if there was a drug exposure 1 to 4 days before

the specimen was collected from the victim. Current recommendations by the Society of Forensic Toxicologists (SOFT) DFSA Committee are that urine specimens be collected as soon as possible after a DFSA, not to exceed 120 h after the suspected drug exposure [24]. If possible, 100 mL of urine should be collected to ensure that enough of the specimen is available for the laboratory to perform a sensitive and thorough analysis [11].

After ingestion, most drugs are below detectable levels in the blood within 24 h, limiting the usefulness of this specimen. In cases where blood can be collected a short time after drug ingestion, the combination of blood and urine specimens might provide a clear picture as to when exposure to the drug most likely occurred. Blood specimens should be placed into collection tubes containing sodium fluoride. In addition to urine, at least 7–10 mL of blood should be collected when it can be obtained within 24 h of the suspected drug exposure [11].

Many times a DFSA victim does not report the crime to medical and/or law enforcement personnel until days or weeks after the alleged crime [18]. At this point, it may no longer be possible to find evidence of a drug in blood or urine specimens, but hair specimens have shown some promise [16,17,25,26]. This is particularly true when the analytical laboratory is using newer analytical instrumentation with superior sensitivity.

Typically, head hair is sampled, although other body hair may also have some use. It is advisable to wait at least 1 month after the suspected drug exposure before collecting the hair sample. This allows the portion of hair that was exposed to the drug via the bloodstream to grow above the scalp or skin. With a hair clip, twist tie, string, aluminum foil, or rubber band, a section of hair about the diameter of a pencil can be secured and cut from the crown of the head as close to the scalp as possible. The cut hair should be clearly labeled to differentiate the cut end from the distal end. The cut hair should then be properly sealed and labeled in a paper envelope to ensure that a proper chain of custody is maintained.

Hair does present additional challenges compared to blood and urine specimens. There continue to be insufficient studies to fully evaluate whether all drugs that may potentially be used in DFSA will incorporate into the hair matrix. Without this information, a negative hair result may be misleading. Further, a positive hair result can also be challenging. Head hair grows at a rate of approximately 1 cm per month. Segmental analysis of hair is necessary to demonstrate that drug exposure happened around the period of the alleged DFSA and not at some other time. For example, if an alleged victim took a strong sedative a month before an alleged DFSA, the hair test must not confuse that previous ingestion with any drugs ingested the night of the DFSA. Only laboratory personnel skilled with handling hair specimens in this manner should be relied on to attempt segmental analyses to avoid inaccurate conclusions.

In some cases, another useful specimen may be vomit from the alleged victim [13]. Most drugs are capable of causing nausea and emesis to some degree. If a drug is not fully absorbed before vomiting occurs, the drug may be detected at relatively high amounts in a vomit stain.

IV. LABORATORY ANALYSIS OF DFSA SPECIMENS

As indicated earlier, many of the drugs used to facilitate sexual assault are potent CNS depressants. Because of their high potency, only small amounts of the drugs are required to achieve the intended pharmacological effect. This means that only very low concentrations of the drug are in the specimens to begin with [14]. Laboratories that perform these analyses must follow good laboratory practices and be able to adequately determine if any incapacitating substances exist in specimens collected hours, days, or weeks after ingestion.

Unfortunately, there have been many cases in which DFSA specimens were completely consumed or prematurely discarded because they were sent to laboratories that did not have appropriate methods or instrumentation to adequately carry out the toxicological analyses. In general, clinical laboratories are not able to detect subtherapeutic concentrations of these drugs, so they should not be relied on to determine if an individual was exposed to a drug when specimens are collected more than a few hours after ingestion. Many forensic toxicology laboratories may not be able to provide the needed analyses, unless they have taken the steps needed to improve the sensitivities of their methods.

To improve consistency in results provided by laboratories, the Society of Forensic Toxicologists (SOFT) DFSA Committee developed a chart of the most prevalent drugs associated with DFSAs and their recommended detection limits [24] when analyzing urine specimens (**Table 1**). These recommended detection limits are based on published analytical methods that utilized standard laboratory instrumentation. The committee's goal in developing this document was to encourage laboratories to evaluate their current capabilities and make improvements, as necessary. Furthermore, these guidelines provide a means of simplifying communication between analytical toxicologists and their customers (usually law enforcement personnel) by ensuring that common units and familiar street names for drugs are included in the chart.

Table 1. Society of Forensic Toxicologists (SOFT) DFSA recommended maximum detection limits (RMDL) for urine specimens

Target drug/metabolite	RMDL	Target drug/metabolite	RMDL	Target drug/metabolite	RMDL
Ethanol	10 mg/dL	*Antidepressants*		*Opiates and nonnarcotic analgesics*	
GHB and analogs	10 mg/L	Amitriptyline	10 μg/L	Codeine	10 μg/L
Benzodiazepines		Citalopram		hydrocodone	
Alprazolam	10 μg/L	Desipramine		hydromorphone	
Chlordiazepoxide		Desmethylcitalopram		Meperidine	
Diazepam		Desmethyldoxepin		Methadone	
Hydroxyalprazolam		Doxepin		Methadone metabolite (EDDP[a])	
Lorazepam		Fluoxedine		Morphine	
Nordiazepam		Imipramine		Normeperidine	
Oxazepam		Norsertraline		Norpropoxyphene	
Temazepam		Paroxetine		Oxycodone	
7-Aminoclonazepam	5 μg/L	Sertraline		Propoxyphene	
7-Aminoflunitrazepam		*Over-the-counter medications*		*Miscellaneous drugs*	
Clonazepam		Brompheniramine	10 μg/L	Carisoprodol	50 μg/L
Flunitrazepam		Chlorpheniramine		Meprobamate	
THCOOH[a]	10 μg/L	Desmethylbrompheniramine		Valproic acid	
Barbiturates		Desmethylchlorpheniramine		Cyclobenzaprine	10 μg/L
Amobarbital	25 μg/L	Desmethyldoxylamine		MDA[a]	
Butabital		Dextromethorphan		MDMA[a]	
Pentobarbital		Diphenhydramine		PCP[a]	
Phenobarbital		Doxylamine		Scopolamine	
Secobarbital	20 μg/L			Zolpidem	
				Clonidine	1 μg/L
				Ketamine	
				Norketamine	

[a] THCCOOH: 11-nor-9-carboxy-delta-9-tetrahydrocannabinol; EDDP: 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine; MDA: 3,4-methylenedioxyamphetamine; MDMA: 3,4-methylenedioxymethamphetamine; PCP: phencyclidine.

CONCLUSIONS

DFSA cases have occurred for hundreds of years; recent times have seen a concerted effort by law enforcement, medical professionals, the media, and toxicologists to raise public awareness of these crimes. Although the true prevalence of DFSAs may never be fully recognized, acknowledgment of the many challenges that come with these cases provides insight as to how to improve chances for successful investigation of DFSA allegations.

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The Frequency of Drug-Facilitated Sexual Assault Investigations[a]

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The Frequency of Drug-Facilitated Sexual Assault Investigations

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ABSTRACT: While there is a general belief throughout parts of the world that drug-facilitated sexual assault (DFSA) cases have dramatically increased in recent times, the true prevalence of DFSA will never be fully realized. This is due to the general underreporting of sexual assaults, the pharmacodynamics of the drugs used to commit these crimes, the challenges that delayed reporting can impose on the charges associated with these cases, and the lack of a uniform system of defining and statistically capturing data on sexual assaults that are facilitated by drugs. Over the years, a number of studies have attempted to quantitate the frequency of DFSA in various countries throughout the world. Unfortunately, no two studies have taken the same approach in their assessment of DFSA; therefore, it is difficult to combine their results to allow for a realistic evaluation of how prevalent DFSA really is. This manuscript reviews the studies that have attempted such an assessment of DFSA prevalence to compare and contrast their results.

KEY WORDS: Drug-facilitated sexual assault, frequency, prevalence.

INTRODUCTION

Recently, drug-facilitated sexual assault (DFSA) cases from throughout the world have been reported in the literature. This has led to the general belief that DFSA cases have dramatically increased in recent times. In reality, it is not clear whether the increased number of these cases actually results from a rise in the commission of the crime, from greater awareness, or from increased reporting. Nonetheless, the true prevalence of DFSA will never be fully realized. This is due to the general underreporting of sexual assaults, the pharmacodynamics of the drugs used to commit these crimes, the challenges that delayed reporting can have on the charges associated with these cases, and the lack of a uniform system of defining and statistically capturing data on DFSAs.

I. SEXUAL ASSAULT REPORTING

A recent study suggests that 18% of all women in the United States have been raped during their lifetimes, yet fewer than one in every six of those rapes were reported to law enforcement entities [16]. Other studies support these findings. For example, a 1987 study found the prevalence of forcible rape or rape following use of alcohol or drugs occurring since age 14 among a sample of female college students was more than 15% [18]. Another study of female college students reported a lifetime prevalence of forcible rape of 20% [2]. Others have determined that roughly one in seven U.S. women have been raped at least once in their lifetimes, but only 15-20% reported the rape to law enforcement [15,32]. Another study has determined that less than 5% of college-student rape victims report the crime to law enforcement [8].

A. Effects of Drug Pharmacodynamics on Reporting

Most drugs used to facilitate a sexual assault are central nervous system (CNS) depressants. Many are strong and fast-acting, with effects that mimic severe alcohol intoxication or general anesthetics. The result is a wide range of potential pharmacological effects that include relaxation, euphoria, decreased inhibitions, amnesia, impaired perceptions, difficulties in maintaining balance, impaired speech, drowsiness, loss of motor function, vomiting, incontinence, unconsciousness, and possible death. The nature of some of the more severe of these symptoms result in DFSA cases being less likely to be reported to law enforcement as compared to forcible rape [16, 24]. Furthermore, victims of DFSA have stated that they were "unclear if a crime was committed" or that they "didn't think the incident was serious enough" as reasons why they didn't report the crime [16].

B. Challenges Associated with Delayed Reporting

When the victim of a DFSA is unclear about the events leading up to the sexual assault because of the amnesiac effects of the drugs, it will lead to a delay in the reporting, if it is even reported at all. Often considerable time is spent trying to fill in memory gaps by calling friends or even the perpetrator, if he is known to the victim. Further complicating the reporting is the possibility that the victim is completely unconscious when the crime occurs and has no idea that she was assaulted. It is

unlikely that in these cases the victim will ever report, unless something arouses his or her suspicion.

Delays in reporting usually mean biological specimens are not collected in a timely manner. This makes it much more difficult for the toxicology laboratory to identify the incapacitating agents that may have been used [20].

C. Problems with Statistically Capturing DFSA

Lawmakers in the U.S. tend to rely on government statistics to assess the extent of sexual crimes, even though it is widely accepted that sexual assaults are underreported. Annual estimates of forcible rape are provided via the National Crime Victimization Survey (NCVS) and the Federal Bureau of Investigation's (FBI) Uniform Crime Reports (UCR). The FBI UCR is a measure of the number of forcible rapes or attempted rapes reported to police. Further, the FBI UCR definition of rape excludes forcible rapes involving oral sex, anal sex, or penetration with fingers or objects. It also excludes drug- or alcohol-facilitated sexual assaults when force and penile penetration are not both present [7].

The NCVS is an estimate of all forcible rape cases that happened to women and girls age 12 and older. These estimates are generated from rape screening questions that rely on the woman's personal acknowledgment of an incident as "rape" or "sexual assault". Yet, research has demonstrated that many women who experience an unwanted sexual event that qualifies as rape under the federal criminal code do not themselves label the incident as rape. Therefore, it has been suggested that the use of the FBI UCR and the NCVS as measures of rape produce substantial underestimates of the problem of rape in the U.S. [17,18].

Another problem is how laws from different jurisdictions define DFSA. For some, the drug must be surreptitiously administered to the victim for it to be considered a DFSA. Others identify specific drugs that must be used to facilitate the assault. Some laws eliminate ethanol as a DFSA drug, while others consider it a crime of DFSA whenever an individual takes advantage of the CNS-depressant effects of any drug in order to have nonconsensual sexual relations with another person.

The importance of a uniformly accepted definition of DFSA is vital in order to fully understand the subject. This is important for meaningful comparisons of research studies, but is also vital for public awareness and perception of DFSA.

Over the years, a number of studies have attempted to quantitate the frequency of DFSA in various countries throughout the world. Differences in how these studies have looked at the problem have resulted in vastly different perceptions of DFSA's prevalence. For example, some studies have focused on victim disclosures and symptoms. In many of these studies, the presence or absence of drugs and alcohol is secondary to the actual sexual assault. These studies generally do not attempt to distinguish between voluntary ingestion and surreptitious administration of drugs and alcohol.

In contrast, forensic studies tend to focus on the presence or absence of drugs and whether the ingestion was voluntary or involuntary. Unfortunately, even though it is well recognized that reporting delays decrease the likelihood of detecting drugs and alcohol in DFSA cases, the forensic-based studies tend to inadvertently tie the results (positive or negative) to the victim's credibility. Given this, and the disparity in how DFSA is legally defined, it is difficult to conduct meaningful surveys about the prevalence of the crime.

II. DFSA PREVALENCE STUDIES

DFSA is not a new crime. Throughout history, there are documented cases of alcohol, opioids, and other strong CNS depressants serving as a means of having sexual relations with a nonconsenting partner. In the United States, DFSA has been recognized as a serious problem, although other parts of the world seem to have been affected later or have been slower to recognize its presence.

A. North America

1. United States

ElSohly and Salamone conducted the first wide-scale attempt at quantifying the occurrence of DFSA in the United States [6]. This study was funded by Hoffmann-LaRoche, Inc., the pharmaceutical company that produces Rohypnol® (flunitrazepam). For a period of 26 months (1996 – 1999), law enforcement agencies, hospitals, and rape crisis centers from throughout the country were offered the opportunity to submit urine samples from alleged victims of DFSA. Unfortunately, at a time in which flunitrazepam was believed to be one of the most prevalent drugs used to commit DFSA, many were wary of sending samples to a laboratory for testing funded by Hoffmann-LaRoche. As a result, it is unclear how well this study's findings truly represent the number of DFSA investigations that occurred throughout the U.S. during this time period.

The samples were screened by traditional immunoassays for amphetamines, barbiturates, benzodiazepines, benzoylecgonine, cannabinoids, methaqualone, opiates, phencyclidine, and propoxyphene. Samples that screened

positive were confirmed by gas chromatography-mass spectrometry (GC-MS). Additionally, all samples were analyzed by GC-MS for flunitrazepam and gamma-hydroxybutyrate (GHB) and by gas chromatography-flame ionization detection (GC-FID) for ethanol.

Over the period of the study, 1,179 urine samples were analyzed from 49 states, Puerto Rico, and Washington, D.C. The number of submissions from each state was closely linked to the state's population. Therefore, the more populous states (i.e., California, Texas, New York, Florida, Pennsylvania, Minnesota, Illinois, Virginia, etc.) tended to have the most representation in the study.

Approximately 40% of the samples in this study were negative for all tested drugs, as well as alcohol. Alcohol and cannabinoids were detected in 38% and 18% of the samples, respectively. Additionally, samples from this study contained benzoylecgonine (8%), benzodiazepines (8%), amphetamines (4%), GHB (4%), opiates (2%), propoxyphene (1%), and barbiturates (1%). It should be noted that 35% of the positive findings contained more than one drug. Although a very sensitive technique was used to screen for flunitrazepam in these samples, very few had detectable amounts of this drug or its metabolite in them. These results suggested that Rohypnol® was not a prevalent drug used in commission of DFSA during the period of this study. Unfortunately, screening for most of the other drugs was at a cutoff concentration considerably higher than that used for flunitrazepam. Therefore, the negative urine results obtained for the other benzodiazepines, cannabinoids, benzoylecgonine, opiates, propoxyphene, and barbiturates may be misleading. It has also been recognized afterwards that the cutoff used in this study to differentiate between endogenous and exogenous GHB was too low [21], so GHB's prevalence was quite likely overestimated. The study did, however, demonstrate that DFSA involves a wide range of drugs, not just those that were popularized by the media.

In a similar study, Hindmarch and Brinkman reported on the results from 1,033 urine samples collected from individuals from the U.S. believed to have been sexually assaulted with the involvement of drugs [11]. In 2001, the authors of these first two studies combined their results and updated the database with an additional 1,091 samples [12]. As a result, they compiled a database containing 3,303 urine samples collected between the dates of June 1996 and February 2000. The combined results found that 61.3% of the urine samples were positive for one or more drug, with alcohol again representing the most common substance found (67% of all positive results). Cannabis was the second most prevalent drug, present in 30.3% of positive samples. Benzodiazepines (4.8%), cocaine (2.8%), amphetamines (1.9%), opiates (0.7%), barbiturates (0.5%), and propoxyphene (0.3%) composed the remaining positive drug findings. Interestingly, 73.0% of the samples in this study were collected within the first 24 hours of the sexual assault and 98.8% were collected within the first 72 hours after the incident. The authors recognized that even with the relatively short reporting and collection periods, alcohol and GHB were particularly susceptible to being underrepresented in these data due to their rapid rates of elimination.

Another 1999 study reported on the analysis of 2003 urine specimens from throughout the U.S. in an effort to identify specific DFSA drugs [31]. Almost two-thirds of the samples contained alcohol and/or drugs. Alcohol was present in 63% of the samples, while cannabis was detected in 30%. GHB and flunitrazepam were each detected in less than 3% of the positive samples.

A 2003 study funded by the Office of the Texas Attorney General conducted interviews of 1,200 adult Texans, both males and females, aged 18 and older [3]. Participants were asked detailed questions about their unwanted sexual experiences that occurred during three time periods: before the age of 14; between the ages of 14 and 17; and at age 18 or older. The results found that 18% of sexual assault victims reported being incapacitated and unable to consent to sex at the time of the assault due to alcohol and/or drugs.

A different approach to estimating the occurrence of DFSA cases in the U.S. can be found in a 2007 report [14]. For a period of January 2002 through March 2004, individuals who presented to four clinics (in Texas, California, Minnesota, and Washington) with complaints of being sexually assaulted were asked to voluntarily provide urine specimens; 144 cases were included in this study. These clinics were specifically established to examine and treat sexual assault victims and all were staffed by Sexual Assault Nurse Examiners (SANEs). In addition to the urine sample, the subjects were asked to answer a questionnaire about their personal drug and alcohol use, any suspected drugging, the time that elapsed since the assault, and their ages. The specimens were screened for 45 different drugs and ethanol.

The regions served by the clinics that participated in this study were not well defined, and sexual assault complainants in the area could have presented to another clinic or healthcare provider. Therefore, the researchers could not estimate the prevalence of DFSA, but they instead attempted to calculate the proportions of complainants with detectable amounts of drugs compared to (a) the number of enrollees in the study or (b) the total number of complainants seen at the clinic over the study period.

One of the goals of this study was to correlate self-reported drug use by the subjects with the analytical findings in their urine samples. Although the researchers interpreted their results as suggesting that there was considerable underreporting of personal drug use by the study's subjects, it is unclear how they differentiated self-administration from surreptitious administration for purposes of making this assessment.

With regard to DFSA, the authors classified cases as DFSA if (a) a drug was confirmed in urine that could have prevented the victim from giving consent and (b) the clinic visit occurred within 72 hours of the alleged sexual assault. It was recognized that these criteria would likely fail to capture alcohol-facilitated or GHB-facilitated sexual assaults. They also realized that other drugs may also drop below the limit of detection within the 72-hour window, so their assessment was that their calculations likely underestimated the true proportion of DFSA cases involved in the study. Using their definition, 43% of the subjects who participated in the study could be classified as DFSA cases. Compared to all the subjects who visited the clinics during the period as sexual assault victims, 7% of those were considered as DFSA. Of course, the relatively small number of participants in the study also calls into question the validity of any conclusions that can be drawn from these data.

A study conducted by the National Crime Victims Research and Treatment Center interviewed 5,001 U.S. women (ages 18–86) to attempt to obtain accurate information on the scope, nature, and consequences of rape in the United States [16]. Of the total sample, 3,001 represented all U.S. women and the other 2,000 represented women attending U.S. colleges and universities at the time of the study. Among the goals of the study was the attempt to identify the number of women in the U.S. and in college settings that had been raped while incapacitated by drugs or alcohol (both voluntary and involuntary impairment); identify the key characteristics of DFSA; examine the willingness of women to report the rape to law enforcement; and to compare DFSA to forcible rape.

Interviews were conducted for a five-month period between January and June of 2006. The 20-minute survey included assessment of basic demographic information, opinions and attitudes about reporting rape, questions specific to different types of rape (forcible or DFSA – whether it be self-induced or surreptitious administration), specific follow-up with women who suggested that they had experienced one of these types of rape, and assessment of mental health histories.

The results of the study found that nearly 1% of women in the general population were raped in the year prior to the study. Most of these rapes were forcible; however, nearly half experienced DFSA. In the college women, more than 5% had been raped the previous year and DFSA was more prevalent than forcible rape in these subjects. In fact, DFSA was nine times more prevalent among college women than among the general population women.

In terms of DFSA, the vast majority of cases involved alcohol (>96%), while the subjects reported that drugs alone (without any alcohol) were involved less than 5% of the time. Marijuana was the most prevalent drug reported in this study. It was estimated that alcohol combined with drugs facilitated the assault in 16–25% of the cases.

Another study, utilizing a Web-based survey, focused on undergraduate female students ($n = 5{,}446$) at two large public U.S. universities [19]. The results found that 19% of the respondents had experienced an attempted or completed sexual assault since entering college and over half of those assaults occurred while the victim was incapacitated due to the effects of alcohol or drugs. Although the majority of these were self-induced impairments, one in five were suspected to be surreptitious administration of the incapacitating substance.

2. Canada

A 2004 report took a retrospective look at a sudden increase in DFSA cases observed in a hospital-based sexual assault care referral service in Vancouver, British Columbia [23]. The results of this study found a 315% increase in reported DFSA cases from 1999–2002, as compared to the period of 1993–1998. Further, this study suggested women aged 15–19 experienced the highest incidence of DFSA.

Another Canadian study also attempted to estimate the prevalence of suspected DFSA cases [5]. During a 21-month period between 2005 and 2007, 882 sexual assault victims were evaluated for suspected drugging at seven hospital-based sexual assault treatment centers. Of these, 21% met the criteria for suspected DFSA, as defined in the study, thus emphasizing the need for specialized services to meet the needs of DFSA victims.

B. Europe

1. United Kingdom

A study published in 2005 reported on 1,014 DFSA investigations analyzed at the Forensic Science Service in London between January 2000 and December 2002 [29,30]. The laboratory analyzed blood and/or urine samples for alcohol, common drugs of abuse, and other strong CNS depressants. In 391 (39%) of the cases, the samples were collected within 12 hours of the alleged sexual assault. Alcohol was detected (either alone or in

combination with other drugs) in 46% of all cases and 81% of those cases in which samples were collected in the first 12 hours. Of the 391 cases in which samples were collected quickly, 233 (60%) had a back-calculated alcohol estimate that exceeded 0.15 g per 100 mL (0.15 g%).

Illicit drugs were detected in 34% of all cases, with cannabis being the most prevalent illicit drug (26%), followed by cocaine (11%). CNS depressants and other debilitating drugs were present in detectable amounts of just 2% of the cases and included MDMA, GHB, and benzodiazepines. Nine cases in this study involved individuals who were given or forced to ingest a tablet or illicit drug.

A 12-month study into the nature of DFSA in England for the period of November 2004 through October 2005 was entitled *Operation Matisse* [9]. Participants in this study were 120 individuals who reported to law enforcement that they suspected that they had been drugged and assaulted. Over 97% of the subjects were females and ranged between the ages of 13 and 52 (median 23 years old). Reporting had to occur within 72 hours of the sexual assault.

Of the 120 alleged victims, all but one reported consuming alcohol immediately prior to the assault; however, alcohol was detected in only 62 (52%) of the cases. Of these 62 alcohol-positive cases, it was estimated that 22 of these cases had blood alcohol concentrations >0.20 g% at the time of the assault.

Controlled or prescription medications were detected in 57 (48%) of the cases. Cannabis was the most commonly detected drug (20%), followed by cocaine (17%). Flunitrazepam was not detected in any of the cases and GHB was detected in two cases, despite its rapid elimination following ingestion.

In 2008, Hall et al. reported a 70% increase in DFSA cases between the years 1999 and 2005 in a jurisdiction in Northern Ireland [10]. During this period, positive findings in these cases increased by 12% while the average blood alcohol concentration remained fairly constant. Additionally, delays in specimen collection seemed to lessen during the period, although a considerable number of the specimens were collected >12 hours post incident. The identification of drugs in all cases doubled between 1999 and 2005, and included antidepressants, recreational drugs, benzodiazepines, and analgesics.

2. France

Numerous studies suggest that benzodiazepines are the most prevalent drug class used to facilitate crimes in France. Over a four-year period, there were 150 assaults reported in the Paris area in which the victim was under the influence of a CNS depressant [27]. Another study reported 196 cases of DFSA in Paris where the most predominant drug findings were the benzodiazepine class of drugs [22].

A retrospective study over the 1996–1997 time period found 23 benzodiazepine-facilitated sexual assault cases in eastern Paris [26]. More recently, a two-year study covering the period of Jan. 1, 2005, to Dec. 31, 2006, identified 52 cases of drug-facilitated crimes (including sexual assaults) in which 77% of the cases involved the use of benzodiazepines [28]. Antihistamines, neuroleptics, and GHB were also identified in the cases of this study.

The most comprehensive French study appears in a 2009 publication that analyzed 158 cases of alleged chemical submission between October 2003 and December 2007 [4]. Of these, benzodiazepines and related drugs were detected in 129 (82%) of the victims. Clonazepam and bromazepam were the most common of the benzodiazepines identified, while flunitrazepam (7%) and GHB (3%) were also detected in some cases. Zolpidem was also a common finding.

3. Poland

A 2005 published report described a 15-fold increase in DFSA cases in one laboratory in 2003 and 2004 compared to the three previous years [1]. The authors described the most common substances detected as amphetamine and cannabinoids, while alcohol, MDMA, benzodiazepines, propranolol, and lidocaine were detected in fewer cases.

C. Australia

Following an increase in the number of reports in New South Wales where the victim of a sexual assault appeared to have been drugged, statistics were collected on these cases [25]. This study found that over 21% of victims reporting for sexual assault services in this region in 2000, reported the use of drugs in their assault. The study was not able to distinguish between voluntary and involuntary ingestion.

A retrospective review in Melbourne found 17.5% of sexual assault cases during a 12-month period (from 2002 to 2003) could be classified as possible DFSAs [13]. Reporting delays were common, with 20 hours the median delay time. While 77% of the victims of these cases reported consuming alcohol in the hours prior to the assault, only 37% had detectable amounts of alcohol remaining in their samples. The average alcohol concentration in these samples was 0.11 g% at the time of analysis. Nearly one-half of the victims reported using prescription medications and more than one-fourth re-

ported voluntary recreational drug use prior to the assault. Approximately 20% of the case specimens contained detectable drugs and metabolites that the victim did not report voluntarily consuming. These drugs included cannabis, antidepressants, amphetamines, benzodiazepines, and opiates.

CONCLUSIONS

Although there is a general belief throughout parts of the world that DFSA cases have dramatically increased in recent times, the true prevalence of DFSA will never be fully known. This is due to the general underreporting of sexual assaults, the pharmacodynamics of the drugs used to commit these crimes, the challenges that delayed reporting can impose on the charges associated with these cases, and the lack of a uniform system of defining and statistically capturing data on sexual assaults that are facilitated by drugs. Over the years, a number of studies have attempted to quantitate the frequency of DFSA in various countries throughout the world. Unfortunately, no two studies have taken the same approach in their assessment of DFSA; therefore, it is difficult to combine their results to allow for a realistic evaluation of how prevalent DFSA really is.

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The Use of Alcohol to Facilitate Sexual Assault

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The Use of Alcohol to Facilitate Sexual Assault

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ABSTRACT: The presence of alcohol (ethanol) is a common toxicological finding in alleged cases of drug-facilitated sexual assault (DFSA). Alcohol was identified as the most frequently encountered drug in DFSAs more than a decade ago, and epidemiological studies to date confirm this initial finding. There is no single substance that is uniquely associated with DFSA. Alcohol has been used by humans for thousands of years and its effect on sexual behavior is well established. Despite the fact that alcohol has been the subject of scientific investigation for several hundred years, DFSA casework involving alcohol remains complex and poses numerous challenges. The prevalence of alcohol in DFSAs is reviewed within the context of toxicological findings and blood alcohol concentration (BAC). Pharmacological aspects are briefly presented, including pharmacokinetics and retrograde extrapolation. The effects of alcohol are discussed within the context of the pharmacodynamics of alcohol and the mechanistic issues associated with alcohol's disruption of memory. The amnesic effects of alcohol are reviewed, with particular focus on the two distinct types of alcohol-induced blackout: fragmentary and en bloc. The prevalence of and the BACs associated with this type of alcohol-mediated memory loss are described. Finally, biological specimens (blood, serum, and urine) are reviewed from a toxicological standpoint, and the associated methodology for quantitative alcohol determination is presented.

KEY WORDS: Alcohol, drug-facilitated sexual assault, ethanol, intoxication.

INTRODUCTION

Any substance that is given with the intent of lowering a victim's sexual inhibition and facilitating nonconsensual sexual contact is potentially a "date-rape drug." Despite the fact that other substances for this purpose have received widespread publicity, ethanol (alcohol) is by far the most frequently encountered drug in sexual assault. Alcohol and drugs are inextricably linked to sexual assault. Alcohol is certainly the most widely studied drug, and yet its occurrence and role in sexual assault is somewhat complicated. Because drug-facilitated sexual assault (DFSA) may occur in a variety of scenarios (surreptitious administration, fraudulent representation of a substance, voluntary consumption, or a combination of these), it is imperative that alcohol is given due toxicological and interpretive consideration. The potential for alcohol to be a causal agent in sexual assault is unquestionable, irrespective of the manner in which it is ingested. Alcohol is the prototypical central nervous system (CNS)-depressant drug, producing effects ranging from disinhibition to unconsciousness at low or high dose, respectively. In addition to the well-defined pharmacological effects of the drug, alcohol can profoundly influence sexual behavior. Alcohol is thought to enhance sexual behavior and aggressiveness in men [3] while women pay less attention to cues that would normally alert them to a dangerous or threatening situation [29]. Research indicates that perpetrators may seek out intoxicated individuals because they are easy targets [4].

Although many other drugs have gained notoriety due to their low dosages (e.g., flunitrazepam) and rapid onset of effects (e.g., gamma-hydroxybutyrate), alcohol may produce similar passivity, inability to resist, loss of inhibitions, muscle relaxation, and memory effects, all of which are sought-after effects for the perpetrator of sexual assault. Although statutory and legal approaches vary, a DFSA takes place when an individual is subjected to nonconsensual sexual acts while incapacitated or unconscious due to the effects of alcohol, drugs, or both. This state may render an individual unable to resist or consent to sex. Alcohol alone at sufficient dose can produce this state, and in combination with other depressant drugs, the potential for impairment is greatly increased.

It is not possible to determine the manner in which the drug was administered (i.e., voluntary or involuntary) from toxicological tests. It may, however, provide information that could help the court determine a person's ability to give consent to sexual activity, and this is the principal focus in DFSA cases, regardless of the manner in which the drugs were administered. In simple terms, a DFSA occurs when consent is absent or invalid due to the effects of alcohol, drugs, or a combination of both [30].

There is a growing perception that instances of DFSA are becoming more widespread. However, it is difficult to accurately gauge the issue because of increased awareness and effectiveness among the clinical and forensic communities. This is counterbalanced by underreporting, late specimen collection or lack of appropriate testing, or cases where the alleged victim decides not to pursue a criminal prosecution. In many instances, individuals may not inform the authorities or seek medical advice in a timely manner, if at all.

I. HISTORY

Archaeological evidence suggests that people have been consuming alcohol for many thousands of years. It is undisputed that alcohol by itself has the ability to incapacitate an individual, make them more vulnerable to sexual assault, and less able to recall the events that took place. Alcohol-mediated sexual assault has received widespread attention and study of late. The effect of alcohol on sexual behavior is not new, however. During the 1st century, Juvenal (Decimus Iunius Iuvenalis), a satirical Roman poet, noted: *"When she is drunk what matters to the Goddess of Love? She cannot tell her groin from her head."*

This notion persists into modern times in "Reflections on Ice-Breaking" by the U.S. poet and humorist Ogden Nash (1901–1972): *"Candy is dandy but liquor is quicker."*

In the Old Testament, Lot's daughters planned to intoxicate their father with alcohol to achieve pregnancy, believing that they were the only ones to escape the devastation of Sodom and Gomorrah.

A famous painting by Hendrik Goltzius (1558–1617), *"Lot and His Daughters,"* depicts Lot drinking wine from a bowl while his daughters are seducing him. Alcohol has been recognized as a means of obtaining illicit sex for thousands of years, and this notion persists today. It is estimated that alcohol intoxication is present in one-third to three-quarters of sexual assault cases [75] and involved in approximately one-half of all sexual assaults among college students [29].

II. EPIDEMIOLOGY

A. Prevalence and Blood Alcohol Concentration (BAC)

In a large nationally representative study of adolescent females in the United States, 11.8% reported some form of sexual assault and 2.1% reported some form of incapacitated sexual assault (either self-induced intoxication or the perpetrator's deliberate intoxication of the victim) [51].

Early epidemiological studies in the United States identified alcohol as the most common drug in cases of alleged sexual assault [17,27,72] **(Table 1)**. In fact, epidemiological studies to date suggest than no specific substance can be identified as *the* "date-rape drug" with the possible exception of alcohol [30]. The high prevalence of ethanol in sexual assault is perhaps not surprising since the majority of offenses are a form of acquaintance rape and involve a social setting or encounter in a bar, nightclub, party, or residence where alcohol is consumed. Of 1,179 specimens from alleged victims of sexual assault throughout the United States, 41% contained alcohol [17]. In a follow-up study involving 3,033 urine samples, alcohol was the most prevalent drug, accounting for 41% of the total samples, with 895 (30% of the all samples) containing alcohol alone [27].

More recent studies in Europe and the United States have confirmed this earlier observation, further suggesting that alcohol consumption increases vulnerability to sexual assault. In a 3-year study of 1,014 blood and urine samples in the United Kingdom (2000–2002), alcohol

Table 1. Prevalence of alcohol in epidemiological studies

Sampling	Specimens	Prevalence[a]	Ref.
n = 1014; majority (72%) collected within 24 h, some in excess of 48 h (U.K.)	Blood and urine	Alcohol (46%); cannabinoids; non-sedative therapeutic drugs; sedative therapeutic drugs; cocaine	[69]
n = 144; specimen collection up to 72 h (U.S.)	Urine	Cannabinoids (33%); cocaine; alcohol; amphetamines; opiates; benzodiazepines	[44]
n = 3303; majority (73%) collected within 24 h, some up to 72 h (U.S)	Urine	Alcohol (41%); cannabinoids; benzodiazepines; cocaine; amphetamines	[27]
n = 1179; majority collected within 24 h; 96% within 72 h; 468 negative for drugs and/or alcohol (U.S.)	Urine	Alcohol (41%); cannabinoids; cocaine; benzodiazepines; cocaine; benzodiazepines; amphetamines	[17]
n = 1806; collection time not known (typically 1–5 h; rarely >12 h) (Sweden)	Blood and urine	Alcohol alone (43%); alcohol and other drugs (12%)	[42]
n = 434; blood and urine if <24 h; urine only if >24 h; median delay 20 h (Australia)	Blood and urine	Alcohol (37%)	[30]
n = 169 (U.K.)	Plasma and urine	Alcohol (24%); amphetamines; canncbinoids; benzodiazepines; cocaine	[16]

[a] Prevalence is reported as the percentage of samples positive in the entire study sample (including negatives).

was the most frequently detected drug, representing 46% of the total samples [69]. As many as 81% of samples collected within 12 h (391 total), contained alcohol [68]. The authors reported estimated BACs of the alleged victims at the time of the incident based on an elimination rate of 0.018g/dL/h and a conversion rate of 1.33:1 to estimate BAC from urine alcohol concentration (discussed later). Of these, 23% contained no alcohol or unreliable data, 17% less than 0.15 g/dL, and 60% in excess of 0.15 g/dL. In a similar study conducted over 6 years in Northern Ireland, alcohol was present in 40–65% of cases. Average BACs during this time (1999–2005) were 0.160–0.218 g/dL and the range was 0.096–0.406 g/dL [24].

A recent 5-year (2003–2007) study of 1,806 alleged victims of DFSA from Sweden indicated that alcohol was present in 31% of cases and alcohol in combination with other drugs in an additional 12% [42]. The mean age of victims was 24 years and the median was 20 years. The majority of alleged victims (> 60%) were between 15 and 25 years of age at the time of the incident. The mean and median BACs were 0.124 and 0.119 g/dL, respectively. A reporting limit of 0.01 g/dL was employed and the range of BACs at the time of sampling was 0.01–0.37 g/dL. Quantitative BACs were reported for the time of sampling, rather than the time of the alleged assault. From a quantitative standpoint this study is advantageous because 1,431 of the total 1,806 specimens were blood. Assuming a mean BAC of 0.124 g/dL and an average elimination rate of 0.015 g/dL/h, the authors estimated the BAC at the time of the alleged incident to be 0.199 g/dL. Assuming a range of elimination rates (0.01–0.025 g/dL/h), the average reported range was 0.174–0.249 g/dL. The victims' age and BAC were positively correlated, with BAC increasing steadily with age. Blood and urine alcohol concentrations were highly correlated (N = 703). In this retrospective data set, information concerning the elapsed time between the incident and sample collection was not always known, but the authors indicate that samples were typically collected within 5 h.

The majority of published studies involve retrospective data collection. A recent multicenter study in the Unites States highlights some of the many challenges [44]. Participation rates among alleged victims of DFSA are sometimes inherently low. Specimen collection times for the majority of studies span 12–72 h, and this has the potential to skew the epidemiological data. Drugs with short half-lives or fast elimination rates are more likely to be underrepresented and an inverse relationship between prevalence and collection time is expected. In an early U.S. study [17], the distribution of specific drug-positive samples with collection time was investigated. If the overall prevalence is higher than the prevalence for a specific (i.e., later) time interval, one might conclude that the prevalence is underestimated. This phenomenon was particularly evident for alcohol due to the rapid elimination.

Analytical limitations are also a major consideration. In a Canadian study of 172 alleged DFSA victims receiving treatment in the emergency room, toxicology results were located in only 20 cases and alcohol was detected in only four instances [52]. This highlights the importance of testing in a forensic rather than clinical setting. This same study reported a doubling in the rate of suspected DFSA in 1999 compared with the preceding 6 years. It is hard to evaluate this type of data because of increased awareness and observer bias on the part of health care providers in general, as well as improvements in analytical methodology. Several studies have addressed DFSA from a clinical perspective [49,52,53,77]. In one study, women 15–19 years of age were reported to have the highest incidence of DFSA [53]. One could argue that clinical studies are advantageous because they do not exclude cases that are not pursued forensically (as a result of a criminal investigation). This approach may provide a more complete picture of the overall problem. However, many clinical studies define DFSA very differently from those in a medicolegal or forensic setting.

In a yearlong Australian study involving 434 cases of adult sexual assault, alcohol was detected in 37% of cases, with an average BAC of 0.11g/dL at the time of the alleged offense [30]. As many as 77% of individuals reported alcohol consumption in the hours prior to the incident. The median delay in sample collection was 20 h, which explains the discrepancy between the self-reported rate (77%) and detection rate (37%). Ninety-five percent of the subjects were female and the average age was 26 years. In only 15% of cases, the alleged victim had a "clear and concise" recall of events; 59% were "unclear" or "patchy," and 24% had "no recollection." The mean and median collection times were 23 and 20 h, respectively. Cases were most commonly reported between Thursdays and Mondays. As many as 71% of the 77% of alleged victims who self-reported alcohol consumption had ingested four or more standard drinks. The average BAC at the time of testing was 0.11g/dL and the average delay between the alleged incident and sampling was 11 h. Assuming no alcohol was consumed post assault, the authors conclude the average BAC was 0.22–0.33 g/dL at the time of the incident, lending weight to the suggestion that DFSAs often occur in the setting of heavy alcohol consumption [30]. BACs in this range clearly have a significant impact on a person's level of consciousness, ability to provide consent, and accurately recall details of the event. In the Australian study, many subjects were unable to provide a clear account of

events, in part due to the amnesic effect of alcohol at such high BACs [30]. Poor recall of events provides a number of challenges from the standpoint of medical management, investigation, and possible prosecution.

In a British study, the number of DFSA-related toxicology requests in a clinical setting increased by 77% from mid-year 2002–2003 to 2003–2004 [16]. The number of requests peaked in December of each year, which the authors attribute to increased socialization during the holiday season. In contrast, a Swedish study over 5 years evaluated seasonal variations and identified an increased number of cases over the warmer months (May–August) [42]. Interestingly, the British study included an increased proportion of male victims (34%) compared with female (66%) and the mean age was 25 years (range 11–73). Alcohol concentrations in urine were 0–0.303 g/dL and correspondingly 0–0.247 g/dL in plasma.

It is difficult to evaluate the proportion of DFSAs involving clandestinely administered drug, as opposed to voluntary administration (or both). Analytical tests cannot differentiate the two, and discrimination of voluntary and involuntary drug use largely depends on the case history, investigation, and self-reporting. Self-reported data are not necessarily reliable, as alleged victims tend to underreport drug use, and even analytical limitations are also prone to underreporting due to delays in specimen collection and detection limits of the methodology. Due to the social setting frequently encountered in alcohol-related DFSAs, it is reasonable to conclude that a larger proportion of alcohol-related DFSAs involve voluntary administration, compared with other drugs. Surreptitious administration of alcohol is still possible and can be readily accomplished by adding concentrated liquor to a drink that the alleged victim is ingesting.

Some epidemiological studies have also attempted to address a subset of cases where an alleged victim is covertly administered a substance for the purpose of incapacitation. Contrary to media reports and popular belief, data suggest that deliberate "spiking" is not the typical DFSA scenario and that clandestinely administered drug represents a small percentage of cases, perhaps as low was 2% [69] to 4% [44]. In a European review of individuals receiving clinical treatment following sexual assault, 53% knew the assailant, 20% involved an allegation of violence, and 24% involved a suspicion of surreptitious administration (or spiked drink). Only 11% of victims reported alcohol intoxication at the time of the offense [77]. The authors infer that the high proportion of victims who believe they were covertly drugged suggests a reluctance to acknowledge (to themselves and others) that they had too much to drink. The loss of responsibility and control associated with acute alcohol intoxication is perceived as a factor leading up to the assault, resulting in feelings of self-blame [77].

Studies also show that alleged victims tend to underreport their drug use. In a study from the United Kingdom, approximately one-quarter to one-third of alleged victims admitted to the use of an illicit drug [69]. Similar rates of admission were reported in a U.S. study [44]. In the latter study, however, alcohol was admitted in almost 46% of the cases, but confirmed in only 10%. This finding highlights two important factors: First, alleged victims are more willing to admit to alcohol use than other drugs; second, there is a relationship between drug prevalence (detectability) and collection interval. Low rates of alcohol confirmation are to be expected in studies where specimen collection is delayed and may occur up to 72 h after the alleged incident.

Alcohol-mediated sexual assault has been identified as a significant public health issue among young people and adolescents [51]. Almost 30% of high school students report drinking prior to age 13 [11]. The prevalence of DFSA was greatest among 15–17-year-olds in a study of adolescents [51] with most victims identifying a known adolescent perpetrator. The prevalence of alcohol-mediated DFSA among adolescents and young adults is certainly not without pharmacological consequence.

B. Caveats

One must bear in mind that prevalence data in any study must be viewed within the context of the methodology that is used. Criteria for data inclusion varies between publications, as do the limitations of the methodology. Some studies perform targeted confirmatory analyses for drugs of interest in the absence of positive screening tests, while others do not; detection limits vary with analytical methodology, and detection times are specimen dependent for most drugs. Although analytical variables might be less important for alcohol compared with other drugs, some studies use reporting limits or administrative cutoffs for alcohol, whereby samples are reported as negative below a specified amount (e.g., 0.01 g/dL). Given the extended interval between the alleged assault and specimen collection in some cases (24–72 h), no detectable alcohol may remain. Using a conservative elimination rate of 0.015 g/dL/h, a BAC of 0.18 g/dL could be undetectable at 12 h or as much as 0.36 g/dL can be eliminated in 24 h. In contrast, drugs that are detectable in urine for several days to weeks (e.g., cannabinoid metabolites) may have a tendency to be overrepresented, since it is also possible that the drug was used long enough before the alleged assault to have negligible pharmacological consequence, yet still be detected.

III. ALCOHOL AND SEXUAL BEHAVIOR

A. Risk and Perception

DFSA can be characterized further into two types: proactive DFSA, the covert or forcible administration of an incapacitating or disinhibiting substance by an assailant for the purposes of sexual assault; and opportunistic DFSA, sexual activity between an assailant and an individual who is profoundly intoxicated by his or her own actions to the point of near or actual unconsciousness [5].

Alcohol-induced disinhibition leads to greater sexual risk-taking behavior [25]. In casual relationships, assailants may seek out intoxicated women because they are perceived as easy targets [29,46], whereas in close relationships the effects of alcohol may be perceived as a signal for sexual intimacy [1,2]. The relationship between alcohol and sex, and the perceptions and vulnerability of those engaged in sexual encounters have been studied. One study showed that alcohol consumption in young women (aged 21–29) created a positive perception of the male behavior and increased the perception of benefit over risk and subsequent sexual vulnerability [74]. Another study involving male and female college students indicated that intoxication created the perception of sexual arousal in the female and the perception of appropriate behavior by the male [2]. Among college-aged women, the odds of experiencing sexual aggression were nine times higher on days with heavy alcohol consumption, compared with days without alcohol [59]. A linear relationship is said to exist between quantity of alcohol consumed and the severity of sexual assault [2].

Although the victim's state of sobriety or intoxication appears to be more significant than that of the offender [29], alcohol consumption in the latter has also been studied. Most research indicates a positive correlation between the perpetrators' and victims' alcohol consumption [25]. The perpetrators' alcohol consumption during the sexual assault had a curvilinear relationship with their aggressiveness and subsequent injuries to the victim [2]. The highest levels of aggression, resistance, and injury occurred when the perpetrator did not drink at all, or when they consumed the largest quantities (resulting in a U-shaped relationship). In victims, there was a negative linear correlation between victim alcohol consumption and outcome: The more the victim drank, the lower the perpetrators' aggressiveness, victims' resistance, and victims' injuries. These observations have been confirmed in other studies, showing that the offender is less likely to physically restrain the victim in cases where the victim is intoxicated. Force may not be necessary when the victim is intoxicated and subsequently less able to resist. Experimental studies have shown that alcohol may reduce intentions to resist sexual advances from an acquaintance, while increasing intentions to pursue relationship-enhancing behaviors [76].

A composite of surveys addressing rape and intoxication among 24,000 women in 1997, 1999, and 2001 was recently published [55]. Of the 4.7% of respondents who indicated they had been raped, 72% experienced rape while intoxicated. Using data from police reported rape over a 5-year period (1999–2004), the state of sobriety or inebriation of the victim appeared more significant than the assailant [29]. Alcohol use that occurs within the context of the date can lead to the misinterpretation of friendly cues as sexual invitations, diminished coping responses, and the female's inability to ward off a potential attack [65]. It has been suggested that although alcohol sometimes mitigates responsibility for the perpetrator, it generally tends to lower perception of the victims' credibility [31].

B. Scenario

The issue of self-induced intoxication versus deliberate and covert intoxication by a perpetrator remains a contentious issue, to the extent that some authors believe that sexual assaults involving voluntary consumption of incapacitating substances should be removed from DFSA datasets [9]. There are several DFSA misconceptions that are evident in the literature: First, that DFSA exclusively involves surreptitious administration of the drug; and second, that a victim reporting unexpected effects is evidence of *covert* drug administration. The former is not in line with statutory approaches in most jurisdictions, and the latter is a significant hindrance, because victims may tend to grossly underestimate the impairing effects of alcohol for a variety of reasons (embarrassment, guilt, or naivety, particularly in young or novice drinkers).

The notion that these cases introduce "bias" is not supported by widespread legal or statutory definitions of DFSA in developed countries that largely address issues of incapacitation, intoxication, and ability to provide consent, rather than the manner in which an intoxicating substance was administered. Failure to recognize the increased risks associated with voluntary consumption of drugs like alcohol is a public health concern. This is of particular importance given that covert administration of drugs in the setting of adult sexual assault is encountered less frequently.

It is not surprising that sexual assaults occur within the context of social interactions such as dates, nightclubs, or parties, where shared alcohol consumption is commonplace. There is greater vulnerability for the victim when alcohol provides the means, social occasion, and/or the opportunity for the offense to occur [29]. There is a strong

association between alcohol consumption and sexual violence [30]. Alcohol consumption by either party precedes up to 50% of sexual assaults [22,78]. In a British study of cases reported to the police, alcohol and/or drug-assisted rape was compared with a control group whereby alcohol/drug use was not implicated for either the victim or offender. The common locations for assaults where intoxication was suspected were private homes, hotels, and nightclubs. By comparison, the assault was most likely to occur in the victim's home if they were sober. Victims who were intoxicated were more likely to have moved from one location to another [29].

Data concerning the prevalence of alcohol-mediated sexual assault in social settings is compelling and cause for concern. Increased awareness of the risks associated with excessive alcohol use and sexual assault are greatly needed. More recently, public health and advisory campaigns focusing on DFSA are highlighting the dangers of excessive alcohol consumption instead of covert or surreptitious administration of a substance, which is markedly less common [15]. It is imperative that potential victims are encouraged to protect themselves accordingly, and emphasis placed on changing offenders' attitudes that intoxicated victims present an opportunity for a sexual encounter [29].

Research has shown that attributions vary significantly when alcohol is involved in sexual assaults. More blame and responsibility may be attributed to the perpetrator for taking advantage of the victim. More commonly, however, more blame and responsibility are attributed to victims who voluntarily ingest alcohol or drugs [19]. These factors have the potential to influence outcome from a criminal justice standpoint.

IV. PHARMACOLOGY

Alcohol can produce a wide range of effects, from confusion and dizziness to coma and death **(Table 2)**. The effects and degree of intoxication depends on many factors, including prior experience (frequency and dose) of alcohol. Inexperienced drinkers are more susceptible to the intoxicating effects compared with social or heavy drinkers who may have developed considerable tolerance. Tolerance is typically lost during periods of abstinence, however, and it is difficult to predict the degree to which a "tolerant" individual can disguise the intoxicating effects of the drug. Rapid increases in BAC, such as those experienced on an empty stomach or when alcohol is consumed at a rapid rate, also play a role. Concurrent use of other depressant drugs increases the potential for intoxication or adverse consequences. Alcohol impairs cognitive and motor functions and can impair a person's ability to engage in higher-order cognitive processes such as abstraction and problem solving [63].

Table 2. Clinical effects of alcohol

Confusion
Dizziness
Memory loss
Impaired judgment
Behavioral changes
Cognitive impairment
Reduced inhibitions
Drowsiness
Lack of muscle coordination
Nausea
Vomiting
Loss of consciousness
Coma
Death

A. Pharmacokinetics

The pharmacokinetics of alcohol were studied and reported by Erik Widmark (1889–1945) during the early decades of the 20th century [84]. Since that time, numerous studies have confirmed Widmark's early findings and further expanded our knowledge and understanding of the pharmacokinetic issues. After the peak BAC is reached, alcohol is eliminated in a linear fashion, following zero-order kinetics. According to Widmark, following moderate doses of alcohol (0.6–0.9 g/kg) on an empty stomach the mean zero-order elimination rate constant was 0.015 g/dL/h. Linear elimination kinetics persisted until low BACs were reached. Apparent volumes of distribution (Widmark's r-factor) in women (0.52 L/kg) and men (0.64 L/kg) reflect the differences in water content between the sexes. Small gender differences in elimination rate were documented (slightly higher for women), but differences in the apparent volume of distribution (Vd) has a significant impact from a medicolegal standpoint.

Following oral ingestion, alcohol is absorbed from the mucosal surfaces of the gastrointestinal tract by simple diffusion. The absorption rate is proportional to the concentration gradient across the membrane. Absorption is influenced by many factors, including the surface area available for absorption, blood flow, concentration of alcohol, and the length of time the alcohol remains in contact with the membranes. Alcohol contact in the mouth and esophagus occurs briefly, compared to the stomach, where alcohol may be present for a significant period and there is efficient blood flow and a large surface available for absorption. The small intestine, however, at a length of 2.8 m and a surface area of 300 m^2, is even more efficient for the purposes of absorption. Although interindividual differences vary considerably due to differences in gastric and intestinal motility, gastric emptying time, and regional blood flow, approximately 20–25% of absorption

takes place in the stomach, compared to 75–80% in the small intestine [7]. Peak BACs are lower and occur later when alcohol is consumed with or after a meal. The reduction in area under the curve (AUC) due to food intake is due to the reduced absorption efficiency (due to prolonged gastric emptying time), and Michaelis-Menten elimination kinetics (whereby small amounts of alcohol are metabolized at proportionately higher rates). Fasting subjects typically achieve peak BACs within 0.5–2.0 h (average 0.75–1.35 h), depending on dose and time of last food intake. In contrast, nonfasting subjects may peak within 1.0–6.0 h (average 1.06–2.12 h). The size and time of the meal have the greatest effect on BAC but macronutrients have also been shown to influence absorption rate and peak BAC to a lesser extent. In social drinking situations where large doses of alcohol were ingested (3.0–5.7 g/kg) over an extended period (5–10 h), peak BACs occurred within a few minutes of the end of drinking [43,88], or even preceded the last drink. Significant interindividual variation was observed, however, and this variability should be considered.

Alcohol is absorbed most efficiently when the BAC is at 10–30% (20–60 proof). More dilute alcoholic beverages decrease the concentration gradient across the membrane, reducing the efficiency of absorption (Fick's Law) and may delay gastric emptying due to the ingestion of large volumes. Conversely, highly concentrated beverages may irritate the gastric mucosa and pyloric sphincter, causing increased mucous secretion and delayed gastric emptying. The rate of absorption of alcohol from the gut depends on the time of day, drinking pattern, dosage form, concentration of alcohol, and most importantly, the fed or fasted state of the individual.

Alcohol is a small and hydrophilic drug with negligible protein binding or solubility in fat. Following absorption, it distributes into the total body water and this is the basis for the quantitative relationship that exists between dose and concentration described by Widmark. Distribution of alcohol into the total body water contributes to the gender- and age-related differences in disposition. Volumes of distribution among adult males average 0.62–0.79 L/kg (range 0.59–0.90) and for adult females, 0.55–0.66 L/kg (range 0.46–0.86) [70]. Alcohol is largely excluded from adipose tissue due to the low fat-water partition coefficient (0.018). Therefore, individuals of the same sex and body weight may have different volumes of distribution due to differences in adiposity. Age is another important factor, whereby total body water and Vd of alcohol decreases with increasing age.

The Widmark equation expresses the quantitative relationship that exists between the blood concentration (C) in g/L, the dose (A) in g, the volume of distribution (r) in L/kg and the body weight (p) in kg:

$$C = A / r\,p$$

The Widmark equation allows the theoretical estimate of dose to be calculated from the BAC, or vice versa. However, sufficient time for absorption and distribution should be allowed before attempting to use the equation [40,84]. The pharmacokinetics of alcohol has been extensively reviewed in the literature [28,33,85].

The majority of alcohol is oxidized (95–98%) and the remainder is excreted unchanged in the breath, urine, and sweat. Oxidation occurs primarily in the liver and takes place in three stages: transformation of ethanol to acetaldehyde, with subsequent formation of acetate, and finally, carbon dioxide and water. There are two principal enzyme systems involved in the metabolism of alcohol, alcohol dehydrogenase (ADH), and microsomal enzymes within the family of cytochrome P_{450}. The rate-limiting oxidation of alcohol to acetaldehyde is governed by ADH, which has a low Michaelis-Menten constant (Km) of 0.05–0.1 g/L. Differences between individuals are also dependent on the isoenzymes of ADH that are inherited. Class I AHD exists in multiple molecular forms and exhibits genetic polymorphisms among racial and ethnic populations [13,58]. When moderate doses are ingested, zero-order elimination applies since the ADH quickly becomes saturated. Liver microsomes are also capable of oxidizing alcohol and this secondary metabolic pathway involves CYP2E1. The microsomal enzymes have a higher Km (0.5–0.8 g/L) and are inducible, operating more efficiently after repeated and prolonged exposure. These also contribute to faster elimination rates among heavy as opposed to occasional drinkers. Variability of elimination rates have been reviewed, and rates as high as 0.036 g/dL/h are reported [34]. The oxidative enzymes become saturated and operate at full capacity after the first few drinks. In social situations where moderate BACs are reached, elimination is adequately described by zero-order kinetics. However, at very high (0.3 g/dL or more) or very low concentrations (0.02 g/dL or less) first-order elimination kinetics may apply. Variability in ethanol pharmacokinetics has been reviewed elsewhere [58].

B. Retrograde Extrapolation

Alcohol is the most widely studied drug and the pharmacokinetics have been well understood for decades. Elimination is the sum of all processes that remove alcohol from the systemic circulation, including metabolism and excretion. Zero-order elimination takes place in the postabsorptive state following moderate doses (> 0.5g/kg) of alcohol, producing a constant rate of ethanol loss per unit time [34]. This allows the BAC at the time of the alleged assault to be estimated based on the measured BAC at the time of sampling [33].

This is the basis of retrograde extrapolation, which has been used by some authors to back-calculate BAC at the time of the alleged incident, based on the sampling interval [42,69]. These calculations require a number of assumptions including no alcohol consumption after the incident, the peak BAC had been reached prior to the incident and sampling, and elimination of ethanol from the blood using zero-order kinetics [34]. Typically an average and a range of alcohol elimination rates are used; average 0.015 g/dL/h or a range 0.01–0.025 g/dL/h [34,36]. Elimination rates are dependent on a number of variables, including the tolerance or past use of alcohol. Elimination rates as high as 0.036 g/dL/h have been reported in alcoholics [34]. Since the majority of DFSA cases involve young adults, some with limited drinking history, it seems reasonable to assume an average elimination rate of 0.015 g/dL/h in the absence of other information [42]. Retrograde extrapolation is utilized with greater frequency in alcohol-impaired driving. By comparison however, many of these subjects are heavy or experienced drinkers with average elimination rates closer to 0.019 g/dL/h [38]. This highlights the importance of investigative information, including past alcohol use, from an interpretive standpoint.

If retrograde extrapolation is used, the assumptions should be clearly stated. It should be recognized that the actual elimination rate is unknown, but rather an average or range of elimination rates is used based on population-based studies. Retrograde extrapolation should not be performed if BACs are very low (0.02 g/dL or less) since elimination may not be zero order because alcohol-metabolizing enzymes are not fully saturated. Caution must be used because chronic users may metabolize alcohol considerably faster than naïve or social drinkers. In addition to metabolic tolerance, phenotypic differences in ADH discussed earlier may also influence elimination rate.

The time to reach the maximum or peak BAC after the end of drinking depends largely on the drinking pattern, consumption of food and the alcohol content. In the absence of a heavy meal, peak BACs are reached typically within 30–60 min of the last drink [37,41]. Witness statements and other investigative documents such as restaurant or bar receipts may also provide additional information. Interpretations should be provided within the context of the drinking conditions, fed or fasted state, duration and frequency of drinks. Theoretical estimates of BAC, dose, or extrapolative data should be used conservatively. Regardless of the type of calculation that is performed, the magnitude of uncertainty should be emphasized.

C. Pharmacodynamics

Alcohol is a dose-dependent depressant drug. It has the ability to depress CNS functions in a manner similar to sedatives, hypnotics, opioids, and other therapeutic and abused drugs. From the standpoint of DFSA, alcohol has the potential to produce impairment and alter behavior.

The most commonly reported symptoms in DFSA are confusion, dizziness, drowsiness, impaired judgment, anterograde amnesia, loss of muscle control, reduced inhibitions, nausea, hypotension, bradycardia, and loss of consciousness [49]. Upon restoration of consciousness or sobriety, alleged victims of sexual assault may continue to report multiple symptoms such as drowsiness, confusion, dizziness, deficits in memory and psychomotor control, weakness, unsteadiness, and impaired judgment. Alcohol alone may increase the potential for aggressive behavior and disinhibitory reactions [49]. Behavioral changes, lethargy, ataxia, impaired memory, and cognitive function are associated with acute intoxication, and concentrations of 0.3 and 0.4 g/dL are associated with respiratory depression and coma. Much lower alcohol concentrations may incapacitate or increase susceptibility to sexual assault when used in combination with other CNS depressants.

The clinical effects of alcohol are both dose- and time-dependent. Alcohol produces both cognitive and psychomotor impairment. It can impair the individuals' ability to provide consent and recall events. It may be particularly difficult for individuals to evaluate their alcohol consumption when consuming cocktails or mixed drinks that contain a variety of mixed spirits. Fortification or "spiking" of an alcoholic or nonalcoholic drink with high-potency alcohol can have severe and unintended consequences for the alleged victim.

The effects of alcohol vary depending on the quantity and concentration of alcohol consumed, the speed of drinking, and the tolerance of the individual. Rapid consumption of concentrated liquor in a young person with little or no exposure to alcohol could produce profound CNS-depressant effects sufficient to produce incapacitation, helplessness, or unconsciousness. In contrast, a more experienced drinker might consume a similar quantity of alcohol and experience less severe effects. Alcohol is a dose-dependent depressant drug and classifications of clinical signs and symptoms with BAC are widely used **(Table 3)**. It is important to note that these classification systems are characterized by overlapping ranges that exist due to the continuum of effects produced by alcohol, rather than discrete effects at any particular BAC. Inter- and intraindividual variations must also be considered. Impairment is more pronounced during the ascent of the BAC rather than the BAC on the descending slope due to acute tolerance or the Mellanby effect [45,50].

The effects of alcohol are mediated through actions at a variety of neurotransmitters, involving a complex interplay between excitatory and inhibitory systems. These interactions are responsible for alcohol's diverse effects and substantial interactions with other drugs [54]. Alcohol increases dopamine in the nucleus accumbens, producing pleasurable effects via the reward pathway of the mesolimbic system. Release of noradrenaline contributes to the enlivening effects of alcohol and its popularity as a social lubricant. Analgesia, pleasure, and stress-relieving effects may be mediated through the actions of the endogenous opioids. Importantly, alcohol can potentiate gamma-aminobutyric acid (GABA) through certain subunits of the $GABA_A$ receptor. This accounts for its anxiolytic and ataxic actions and in part for its amnesic and sedative effects. It also blocks excitatory *N*-methyl-D-aspartate (NMDA) causing amnesia and other CNS-depressant effects. The pleasurable and mood-altering effects of alcohol are also associated with its interaction with serotonin or 5-hydroxytryptamine (5-HT) and the stimulation of the 5-HT(3) receptor is responsible for alcohol-induced nausea. These complex interactions contribute to the euphoric, sedative, and impairing effects of ethanol. Despite the fact that alcohol is the most widely used drug, mechanistic aspects are still the subject of research.

The effects of alcohol can also be described in terms of their sites of action. Frontal lobes are sensitive to low concentrations of alcohol, altering thought and mood. Interactions at this site may result in loss of inhibitions, loss of self control, euphoria, increased confidence, altered judgment, and impaired attention. At low to moderate doses, alcohol may have a stimulating effect due to the depression of inhibitory central mechanisms. For this reason, while in the intoxicated state, behavior or actions may be uncharacteristic in nature. As the BAC increases (0.1 g/dL or more), deficits in memory, cognition, vision

Table 3. Stages of acute alcohol influence/intoxication (copyright by K.M. Dubowski, 2006; used with permission)

Blood-alcohol conc. (g/dL)	Stage of alcoholic influence	Clinical signs/symptoms
0.01–0.05	Subclinical	Influence/effects not apparent or obvious Behavior nearly normal by ordinary observation Impairment detectable by special tests
0.03–0.12	Euphoria	Mild euphoria, sociability, talkativeness Increased self-confidence; decreased inhibitions Diminution of attention, judgment, and control Beginning of sensory-motor impairment Slowed information processing Loss of efficiency in critical performance tests
0.09–0.25	Excitement	Emotional instability; loss of critical judgment Impairment of perception, memory, and comprehension Decreased sensitory response; increased reaction time Reduced visual acuity, peripheral vision, and glare recovery Sensory-motor uncoordination; impaired balance; slurred speech; vomiting; drowsiness
0.18–0.30	Confusion	Disorientation, mental confusion; vertigo; dysphoria Exaggerated emotional states (fear, rage, grief, etc.) Disturbances of vision (diplopia, etc.) and of perception of color, form, motion, dimensions Increased pain threshold Increased muscular uncoordination; staggering gait; ataxia Apathy, lethargy
0.25–0.40	Stupor	General inertia; approaching loss of motor functions Markedly decreased response to stimuli Marked muscular uncoordination; inability to stand or walk Vomiting; incontinence of urine and feces Impaired consciousness; sleep or stupor
0.35–0.50	Coma	Complete unconsciousness; coma; anesthesia Depressed or abolished reflexes Subnormal temperature Impairment of circulation and respiration Possible death
0.45+	Death	Death from respiratory arrest

(occipital lobe), and coordination (cerebellum) are possible. Impairment is progressive and at BACs of 0.4 g/dL or more, autonomic functions (medulla) of the CNS may be affected, resulting in decreased respiration, peripheral collapse, coma, or death.

Tolerance is a complex issue, involving pharmacological, environmental, and behavioral factors [73]. Genetic factors also play a role in the overall effects of alcohol, tolerance, and dependence. Not to be confused with chronic tolerance, acute tolerance is a decrease in a response to alcohol that occurs over time within a single exposure. This is sometimes termed the Mellanby effect (discussed earlier) and is characterized by an increased response to alcohol on the ascending rather than descending limb of the BAC curve.

V. ALCOHOL AND MEMORY

Three distinct types of memories exist: Primary memory (short-term information storage necessary for the thought process and understanding); secondary memory (including explicit and implicit memory); and tertiary memory (or long-term memory). Subsequent memory loss, or amnesia, can be classified as anterograde amnesia (characterized by impaired information acquisition, consolidation, or storage) or retrograde amnesia (involving loss of early memories). The latter is principally associated with cerebral injuries or disease states rather than consumption of alcohol or drugs. Alcohol-mediated amnesia is anterograde rather than retrograde [32]. More typically, alcohol or drugs produce the former, likely due to the effect of alcohol on the GABA-receptor complex. The hippocampus and amygdala play an important role in memory function. Disruption or damage of these structures inhibits the conversion of information into memory, although prior events and memories might not be affected [21].

When alcohol is involved, victims are less likely to remember an alleged assault and the events afterwards [29]. Recent estimates suggest that nearly 50% of students in the U.S. regularly consume more than 4 or 5 drinks per occasion at least once every 2 weeks, a level of consumption often referred to as *binge* or *episodic drinking* [61]. It is well established that heavy episodic drinking significantly increases the individual's risk of experiencing alcohol-induced memory impairment.

It is reported that at BACs less than 0.15 g/dL memory impairments tend to be small to moderate [81]. In early studies these were termed "cocktail party memory deficits" [67]. However, as the dose increases, memory effects become more profound and blackouts, or periods of time where an individual cannot recall events that occurred while they were intoxicated, become more likely.

A. Blackouts

An alcohol-induced blackout is defined as memory loss or amnesia that occurs during any part of a drinking episode, without loss of consciousness [32]. This should not be confused with "passing out." During the blackout the individual is unable to recall entire events that occurred while intoxicated. Alcohol-induced blackouts have been studied since the 1940s.

As many as 51% of college students in the Unites States who consume alcohol report that they have experienced a blackout [82]. Among those reporting blackouts, the median number of blackouts was 3 and the average was 6.6. More than half (55.5%) engaged in a variety of risky behaviors during the elapsed time; as many as 24.8% report at least one blackout during sexual activity [82]. In a later study of college-aged students who had experienced blackouts, 10% reported intercourse with someone they knew; 42% reported sexual activity besides intercourse with someone they knew; and 12% reported sexual activity besides intercourse with someone they did not know [83].

Alcohol may produce partial or complete blackouts for events that occurred while the person was drinking and the period afterwards [81]. Alcohol consumption at sufficient dose may induce a so-called dissociative state. Dissociation is defined as a disruption of the normally integrated functions of consciousness, memory, identity, and motor behavior that does not necessarily cloud one's consciousness. During the blackout, an individual may be awake and conscious, engaged in activity or conversation, and may appear to be oriented [45]. Although memory loss may be extensive, it is not necessarily irreversible and individuals may recall information later, sometimes spontaneously.

There are two biological mechanisms underlying alcohol-induced blackout; the first involves an encoding deficit; the second, a state-dependent retrieval deficit [79]. In the former, alcohol temporarily inhibits biochemical processes in the brain that are necessary to form new memories. In the latter, information stored into memory during an intoxicated state is inaccessible when the individual is sober. It has been suggested that the level of alcohol intake associated with blackouts has a greater effect on the input, acquisition, or processing of new information, rather than the retrieval [32].

In one study involving time-dependent memory loss [20], intoxicated subjects could recall information 2 min after the event occurred, but not 30 min or 24 h later. This would support the idea that blackouts disrupt the transfer of information from short-term to long-term storage. The average peak BAC in this study was 0.28 g/dL. Early studies suggest that blackouts often began at BACs of 0.2

g/dL, but as low as 0.14 g/dL [66]. Although early studies of alcohol-mediated memory effects involved heavy drinkers or alcoholics, there is clear evidence that blackouts occur in social drinkers [81]. In a study of American college students who had experienced an alcohol-induced blackout, the average number of drinks consumed prior to the blackout was estimated to be 11.5 [81]. This highlights the difficulty associated with estimated BACs or self-reported number of drinks, and the associated memory effect; these data must be interpreted with caution due to the considerable interindividual differences and reliability of self-reported data while a subject is intoxicated.

B. Fragmentary and En Bloc Blackouts

Two distinct types of alcohol-induced blackout are described: en bloc (complete) or fragmentary (sometimes called "grayout"). En bloc blackouts begin and end at definitive points with full permanent amnesia for interim events. They are characterized by a sense of lost time. They are not state dependent, which means that memory losses cannot be recalled and are likely to be permanent [32]. These require high BACs that disrupt the limbic system to prevent lasting memories to be encoded. Fragmentary blackouts involve a more transient and forgetful memory loss, whereby memory traces form but are difficult to access. These are thought to result not from acute limbic system damage, but from retrieval-based difficulties associated with frontal lobe functions [26]. Fragmentary blackouts are characterized by memories that are often recalled when a subject is told about it later or otherwise prompted. Fragmentary blackouts occur more often and are experienced over a wider range of BACs. The likelihood on an en bloc blackout increased significantly at BACs of 0.24 g/dL, whereas rates of fragmentary blackouts rose more steadily over moderate to high BAC. The incidence of fragmentary blackouts rose from more than 10% at BACs of 0.13–0.18 g/dL, to more than 60% at 0.31–0.42 g/dL [26]. In a more recent study of subjects reporting complete (en bloc) or fragmentary blackouts, the mean reported BACs were 0.22 and 0.23 g/dL, respectively [62]. These authors suggest that BACs of 0.310 g/dL or more have a 0.50 or greater probability of having an alcoholic blackout. Fragmentary blackouts occur with greater frequency and in one study accounted for 78% of all alcohol-induced blackouts [26,83]. The greater prevalence of this type of memory loss, together with the greater dispersion across moderate to high BACs, would suggest that this type of memory impairment poses a significant challenge in DFSA from an investigative standpoint.

It is unquestionable that the occurrence of blackouts is positively correlated with dose. Individuals who report heavy drinking (consuming 5 or more drinks per occasion for more than 4 days in the past month), suffer amnesia to a far greater extent than infrequent, occasional, or more moderate drinkers [32]. The authors conclude that there is empirical evidence that susceptibility to alcohol-mediated memory loss is a predictable outcome of relatively frequent heavy drinking. However, these memory effects are not to be confused with more acute cognitive disruptions of memory that are related to long-term alcohol abuse. It has also been suggested that blackouts may be more likely when alcohol is consumed rapidly, on an empty stomach, or under conditions that cause a rapid rise in the BAC [32,62,81,82]. In a study of alcohol-induced blackouts among American college students [82], women were just as likely to experience a blackout, despite the fact that their alcohol consumption per occasion was approximately half that of the male counterparts. Some researchers have suggested that women are more susceptible to alcohol-induced memory impairments compared to men when given comparable doses of alcohol [56] and that this may be the result of well-established pharmacological differences between the sexes. However, in a national longitudinal study in the U.S. involving 12,686 young adults [32], males were more than twice as likely to experience blackouts compared with females.

C. Mechanism of Action

The neuropharmacological mechanisms underlying blackouts have been investigated. Alcohol disrupts information processing in various parts of the brain, including the hippocampus, which plays an important role in the formation of memories and events. Alcohol disrupts activity in the hippocampus directly (through effects on circuitry) and indirectly (by interfering with interactions between the hippocampus and other brain regions) [81]. Alcohol also impairs the performance of frontal-lobe mediated tasks, like those required for planning, decision-making, and impulse control [10,80]. High doses of alcohol adversely affect functions associated with the prefrontal and temporal lobes, including verbal fluency, memory, and complex motor control [63].

A study that examined the genetic epidemiology of alcohol-induced blackouts suggested a substantial genetic contribution [57]. The genetic contribution to blackout risk may arise from genes whose products mediate alcohol's effects on neurotransmission. Blackouts are reported to occur as a result of two actions: potentiation of $GABA_A$-mediated inhibition, and antagonism at excitatory NMDA glutamate receptors. Genes whose products have indirect or direct effects on $GABA_A$-mediated transmission could contribute to the genetic risk of alcoholic blackout. Likewise, it was proposed that polymorphisms that affect the structure of NMDA receptors, subunit composition, or the

degree to which various receptors are expressed, could also contribute genetic risk specific to blackout [57].

VI. ALCOHOL AND OTHER DRUGS

As many as 49% of alleged sexual assault victims report the use of prescription drugs [30]. Of these, 73% were taking two or more, and 32% were taking three or more drugs. Despite the fact that alcohol is clearly the most widely reported substance, multiple drug use is commonplace in DFSA. In one of the earliest and most representative epidemiological studies in the United States, the most common drugs in combination with alcohol were cannabinoids, cocaine, and benzodiazepines [17].

Alcohol-drug interactions can occur in two ways: First, alcohol can influence the pharmacokinetics of other drugs by altering gastric emptying or liver metabolism (e.g., induction of CYP450 2E1). Alternatively, drugs may influence the pharmacokinetics of alcohol by inhibiting gastric ADH or altering gastric emptying. Pharmacodynamic interactions exist between alcohol and a wide variety of over-the-counter, prescription, and illicit drugs. Alcohol may have an additive or synergistic effect with other CNS depressants including some anxiolytics, anticonvulsants, antihistamines, antidepressants, analgesics, hypnotics, and sedatives. These interactions can be pronounced even at low BACs. Although alcohol may also have an antagonistic effect with some drugs, the enhanced effects are encountered with greater frequency, and these are highly significant because they may produce a degree of impairment that is much greater than anticipated.

Beverages with very high alcohol concentrations may delay gastric emptying and this may affect the absorption of some drugs (e.g., propranolol). Enzyme induction of CYP2E1 has the potential to influence a wide variety of drugs that make use of this isoenzyme. Benzodiapeines that undergo *N*-demethylation (e.g., diazepam) or hydroxylation (e.g., alprazolam), may have reduced clearance compared with those undergoing simple conjugation (e.g., lorazepam) Decreased metabolism of carbamazepine has also been observed in alcohol-dependent individuals. Drugs that increase the rate of gastric emptying (e.g., erythromycin) increase the bioavailability of alcohol. Rates of gastric emptying influence alcohol absorption because it governs alcohol's access to the small intestine, the major absorptive surface. There is ongoing debate over the possible interaction between alcohol and H_2 receptor agonists [18].

Benzodiazepines are encountered in sexual assault casework with some degree of frequency because they are widely prescribed as anticonvulsants, anxiolytics, tranquilizers, and sedatives. Alcohol enhances the effect of benzodiazepines [71]. Increased sedation, psychomotor impairment, dizziness, mental clouding, lethargy, and other depressant effects are expected when alcohol is used in combination with benzodiazepines or other drugs that interact with GABA receptors (e.g., zolpidem). Barbiturates also produce additive effects with alcohol due to their involvement with GABA. First-generation antihistamines such as chlorpheniramine and diphenhydramine are competitive agonists of histamine, and these too potentiate the effects of alcohol. Tricyclic antidepressants, gamma-hydroxybutyrate (GHB), and other CNS depressants enhance alcohol's effects. In addition to the predictable pharmacodynamic effect of combined use of CNS depressants, other interactions that influence mood and other behaviors are possible. Tricyclic antidepressants have also been reported to produce unexpected behaviors when combined with alcohol.

Drugs that are not characterized as CNS depressants per se, but produce CNS-depressant effects (e.g., narcotic analgesics and cannabinoids), also have the potential to increase impairment. This has been well understood and well studied from the standpoint of impaired driving for many years. The combined effects of these drugs are highly significant in sexual assault cases, most notably from the standpoint of prevalence. In numerous epidemiological DFSA studies, cannabinoids were the second most frequently reported drug, after alcohol [17,27,42,69] (Table 1). It is well established that the combined use of alcohol and cannabinoids produces additive effects in terms of both mental and psychomotor function. It has been suggested that full and irretrievable memory loss associated with en block blackouts occurs more often when other drugs are used in combination with alcohol [26] and the combination of alcohol and marijuana produces greater memory effects than when either drug is used alone [14].

VII. BIOLOGICAL SPECIMENS

Low reporting rates, delays in reporting, and subsequent delays in specimen collection are commonplace in DFSA cases. A U.S. study estimated that as many as 84% of women do not report sexual assault to the police [8,47]. This is due to psychological barriers (shame, embarrassment), fear of stigmatization, poor recollection of events, and overall confidence in the criminal justice system. In cases where no drugs are detected, an important consideration is the delay in reporting and subsequent sample collection. This is particularly important for alcohol given its rapid elimination from the body. Amnesic effects further complicate the situation, since individuals who are unclear of the events that took place prior to the alleged assault are more likely to delay reporting.

A. Blood

Blood should be collected as soon as possible by venipuncture. Evacuated blood collection tubes containing sodium fluoride (1%) as preservative and potassium oxalate (0.25%) as anticoagulant are preferred (i.e., grey-top tubes). All samples should be refrigerated prior to analysis.

Guidelines for specimen collection vary by jurisdiction, but blood and urine should be collected as soon as possible following a sexual assault. Blood is particularly important if the alleged incident took place within the past 12 h. Specimens should be stored under refrigerated conditions. Microorganisms have the potential to produce and degrade alcohol in situ, influencing the concentration at the time of analysis.

Considerable time delays between the incident and reporting are not uncommon, and these have toxicological consequences. Despite the fact that alcohol is consumed in a much larger dose (grams) compared with many other depressant drugs (milligrams), an individual can eliminate a significant quantity of alcohol during the interval between the alleged incident and specimen collection. Assuming a conservative burnoff rate of 0.015 g/dL/h, an individual with a BAC of 0.18 g/dL at the time of the incident may produce a negative BAC result 12 h later. The detection time in urine is longer but more prone to interpretive issues, which are discussed later.

B. Serum

In a clinical setting, a serum alcohol concentration (SAC) or plasma alcohol concentration (PAC) is often reported instead of whole blood. Alcohol concentrations in these specimens are nonequivalent due to the relative water content of the biofluids. SAC/BAC ratios reported over decades are typically in the range 1.03–1.26, but PAC/BAC ratios as high as 1.35 and 1.59 have been reported [60,64]. Most studies report a normal distribution of both PAC/BAC and SAC/BAC ratios, which means that using a conversion factor higher than the average will decrease BAC and increase the proportion of underestimated BACs. PAC/BAC ratios averaging 1.10:1 (range 1.03–1.24) were reported by Jones et al. [39] and mean SAC/BAC ratios of 1.14 (range 1.04–1.26) and 1.15 (range 1.10–1.25) were reported by Charlebois and Hak, respectively [12,23]. Both intra- and interindividual differences in the percentage of whole blood volume occupied by the red blood cells (hematocrit) make it challenging to apply a uniform correction factor when converting plasma or serum to whole blood. Serum and plasma samples are reported to have the same alcohol concentrations [86]. Serum and plasma alcohol concentration ratios among 50 subjects averaged 1.00 (range, 0.98–1.04), which would suggest that serum alcohol concentrations are applicable to plasma. Variations in the plasma or serum alcohol concentrations relative to whole blood are expected, and these may result from sample preparation techniques, analytical procedure, data analysis, and subject condition.

C. Urine

Urine should be collected as soon as possible after an alleged assault. It should be preserved with sodium fluoride (1%) and refrigerated to prevent degradation or production of alcohol in situ. If blood is not available, urine is a viable alternative for alcohol determination. The relationship between blood and urine alcohol concentration (UAC) has been investigated over many years and has been extensively reviewed in terms of its utility in forensic casework [35,37]. The UAC and BAC time curves are usually shifted in time, with the BAC decreasing prior to the UAC. Typical UAC/BAC ratios are less than one in the early absorption phase, and greater than one in the postabsorptive or distribution phase. This relationship is the basis of evidentiary alcohol testing of urine using the UAC/BAC ratio, typically 1.3. This is utilized in impaired driving cases whereby two specimens are collected (a void and the "sample"). However, in DFSA cases, only one urine sample is typically collected. In the Jones study of alleged sexual assault victims [42], a significant number of cases (n = 703) contained paired blood and urine samples. Linear regression among this population indicated UAC = 0.047 + 1.12 BAC (units in g/dL). Despite the fact that a second urinary void was not collected 30–60 min after the first void, the results were highly correlated (r = 0.93). Although the relationship between BAC and UAC has been studied, the elimination kinetics of alcohol from urine requires further study. For this reason, retrograde extrapolation from urine in the absence of blood is not recommended [43].

D. Other Specimens

Although biological specimens are the most frequently encountered evidential items in alleged DFSA cases, alcohol determination in other specimens or in nonbiological evidence is sometimes necessary. Beverages, glasses, residues, and the like are sometimes submitted for toxicological testing. In the case of alcohol, the low boiling point and subsequent volatility of the analyte in question must be considered when determining the appropriateness of testing and the subsequent interpretation of the results.

VIII. ANALYTICAL METHODOLOGY

A significant number of DFSA cases originate in a clinical setting and are subjected to a battery of clinical toxicology tests. Enzymatic assays that are frequently used in hospital settings to determine alcohol concentrations should be confirmed using more robust analytical methodology.

A. Clinical Assays

In a clinical setting an enzymatic assay to determine the concentration of alcohol in serum is commonly used. One of the most commonly used methods uses the alcohol dehydrogenase (ADH) catalyzed reaction of ethanol with nicotinamide adenine dinucleotide (NAD). Ethanol in the presence of the cofactor NAD is converted to acetaldehyde and NADH. A trapping agent such as hydrazine or semicarbazide reacts with the acetaldehyde, forming a stable derivative. Given the reversible nature of the reaction, the removal of acetaldehyde shifts the reaction to the right. NADH is measured spectrophotometrically at 340 nm. Modifications of this method have been developed for highly automated analyzers. In the Abbot X assay, NADH is measured based on its reaction with thiazoyl blue dye to form a chromagen. The technique, called radiative energy attenuation, is based on the principle that the fluorescence of a solution containing a fluorophore (fluoroscein) and a chromagen is related to the absorbance in the solution.

Rapid automated assays that determine alcohol in serum, urine, and treated whole blood are ideal for hospital use where toxicology results must be available within the timeframe that allows for effective clinical management. In a forensic setting, whole blood is the preferred specimen and more robust methodology is preferred. Enzymatic alcohol assays can provide useful information but are not without medicolegal limitations. Ethanol concentrations may be elevated in postmortem serum samples or those with elevated lactate or lactate dehydrogenase levels. However, this interference is reported to be assay specific and more common with earlier enzymatic assays [87]. The rapid nature of these tests is offset by their moderate specificity. Furthermore, the assays may not be run in duplicate and forensic handling and chain of custody issues need to be considered.

Despite the limitations, clinical assays are encountered with some degree of frequency. Although enzymatic assays are certainly not the preferred technique for alcohol determination, they can provide useful information if interpreted cautiously. Hospital laboratory serum and whole-blood alcohol concentrations were compared in 212 consecutive patients in a recent study [6]. Although a wide range of serum/whole-blood alcohol ratios were observed, the authors suggest that a linear regression model could be used to predict BACs from SACs obtained by enzymatic means, within a desired confidence level.

B. Forensic Methods

Due to its volatility, the most common technique for alcohol analysis involves gas chromatography with flame ionization detection (GC-FID). Chromatographic methods of analysis are preferred for forensic uses and the methodology receives widespread acceptance throughout the courts. Specimens are frequently analyzed in duplicate and the use of two columns (dual headspace GC) is becoming more common. *N*-propoanol or *t*-butanol are commonly used as internal standards. Both direct injection and headspace sampling procedures are used. The volatility of the alcohol relative to the aqueous biological specimen makes headspace analysis an attractive technique and reduces instrument maintenance. Chromatographic assays provide the necessary sensitivity and specificity for forensic alcohol analysis. Detection limits or cutoff concentrations of 0.01 g/dL are commonly reported and these are appropriate for DFSA purposes [16,42]. Quantitative analysis of alcohol using chromatographic procedures are routinely used in crime laboratories or medical examiner's offices. The methodology is well established and has been described elsewhere [48].

CONCLUSIONS

Alcohol is a dose-dependent CNS depressant that has the potential to impair and incapacitate an individual in a comparable fashion to many other drugs, some of which have received far greater publicity and notoriety. Alcohol is eliminated from the body rapidly and is susceptible to delays in specimen collection that complicate its toxicological interpretation. DFSA occurs whenever an individual is unable to consent to sexual contact due to some form of incapacitation, regardless of the manner in which the substance was administered. Covert administration of a substance (or proactive DFSA) is less common than voluntary ingestion of impairing substances, whereby the sexual assault is more opportunistic in nature. This is commonly the case for alcohol, which is consumed in social settings with widespread acceptance. It is unquestionable that alcohol influences sexual behavior and alters perceptions of both the alleged victim and the perpetrator, but voluntary consumption may also influence perceptions of those reviewing a case, with major consequence from a criminal justice standpoint. Alcohol produces an array of well-characterized mental and physical impair-

ment. These effects have been extensively studied, largely from the standpoint of traffic safety and impaired driving. The effect of alcohol on memory is particularly important in DFSA investigation, since alcohol-induced amnesia may delay reporting due to patchy or incomplete recall of events and poses a number of investigative challenges.

Despite the fact that alcohol is perhaps the best described of all drugs in terms of its pharmacological, toxicological, and analytical aspects, it remains one of the most challenging substances in DFSA cases. The gap that exists between public perception of DFSA and the evidence from epidemiological studies is a matter of concern. Prevention and sexual assault education initiatives would do well to focus on the potential risks associated with alcohol, in addition to other more potent, but less frequently encountered sedatives.

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The Use of Benzodiazepines to Facilitate Sexual Assault[a]

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The Use of Benzodiazepines to Facilitate Sexual Assault

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ABSTRACT: Benzodiazepines are one of the classes of drugs most commonly associated with drug-facilitated sexual assault. As a widely prescribed class of medications and abused drugs, benzodiazepines are extensively available. Their sedating and amnesic effects make them effective candidates for use in drug-facilitated assaults. Detection methods for benzodiazepines and their metabolites in biological fluids are plentiful, but methods must be tailored to the low concentrations of drugs and metabolites expected to be encountered in these cases.

KEY WORDS: Benzodiazepines, drug-facilitated sexual assault.

INTRODUCTION

Benzodiazepines are one of the most widely prescribed drug classes in the world. They are also commonly abused. Benzodiazepines are used clinically for their muscle-relaxant properties, as well as their anxiolytic, amnesic, and hypnotic effects. They may be abused alone or in combination with other drugs.

Because of their effects, benzodiazepines have become commonly associated with drug-facilitated sexual assaults (DFSA). The combination of their sedative effects and their ability to cause amnesia make them desirable to a potential perpetrator of a DFSA. Since benzodiazepines may be present in urine for days after a single exposure, their detection in DFSA cases is more likely than drugs that are more rapidly metabolized and excreted (e.g., gamma-hydroxybutyrate and ethanol). Care should be taken in the analysis of urine samples for benzodiazepines and metabolites in DFSA cases to ensure that methods are as sensitive as possible. Sensitivity may be increased by hydrolyzing urine samples to cleave glucuronide conjugates or by derivatizing sample extracts to achieve low detection limits. Benzodiazepines have also been detected after limited dosing in less traditional biological specimens such as hair and fingernails.

I. CHEMISTRY

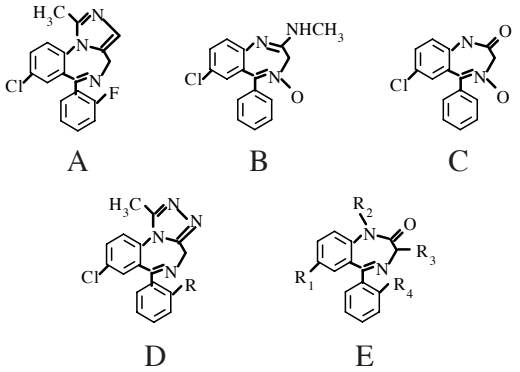
Benzodiazepines are lipophilic acids, with a fairly large pKa range; 2.4 for alprazolam and 6.2 for midazolam [2]. Structurally, they consist of a benzene ring fused to a seven-membered 1,4-diazepine ring with a 5-aryl substituent (**Structure 1**). A chlorine atom, a fluorine atom, or a nitro group is often attached to the benzene ring. Fused triazolo rings are attached to the diazepine ring in alprazolam, estazolam, and triazolam, while midazolam contains an imadazo ring attached to the diazepine ring [8].

Chlordiazepoxide, the first benzodiazepine, was introduced by Hoffman LaRoche in 1960. Since that time, many benzodiazepines have been developed and marketed around the world. Many references cite benzodiazepines as one of the most commonly prescribed classes of drugs [10]. The following benzodiazepines are currently available by prescription in the United States: alprazolam, chlordiazepoxide, clonazepam, clorazepate, diazepam, estazolam, flurazepam, lorazepam, midazolam, oxazepam, quazepam, temazepam, and triazolam [20]. Flunitrazepam, tetrazepam, and prazepam are legally available in other countries.

II. PHARMACOLOGY

A. Administration

Benzodiazepines are used as anxiolytics, hypnotics, anticonvulsants, muscle relaxants, and adjuncts to anesthesia, as well as to treat alcohol withdrawal. They may be classified as short-acting, intermediate, or long-acting. Short-acting benzodiazepines may be used to treat sleeplessness, while long-acting benzodiazepines may be used to treat generalized anxiety. Oral doses of benzodiazepines range widely, depending on the drug's potency. For example, triazolam may be administered in 0.5- or 1-



Structure 1. Chemical structures and core structures of benzodiazepines.

mg doses, while chlordiazepoxide may be administered in 20- to 50-mg doses. Some benzodiazepines are available in extended-release formulations, and some are available in formulations designed for quick oral disintegration. Benzodiazepines may also be administered intravenously.

B. Abuse Potential

Benzodiazepines are known to have abuse potential. They are commonly abused in conjunction with opioids and/or ethanol since they ease symptoms of withdrawal. They are also used by individuals who abuse cocaine and other stimulants to ease the "crash" experienced after the stimulants' effects are gone [10,34]. Short-acting benzodiazepines may have more abuse potential than longer-acting benzodiazepines [6]. In the early 1990s, concern grew in the U.S. over flunitrazepam as a drug of abuse. Flunitrazepam was not legally available in the U.S. at that time, but was available in Mexico, where it was marketed as Rohypnol by Hoffman La Roche. Interestingly, Calhoun and associates interviewed abusers who identified tablets of clonazepam as "the new Roche dos" and complained that this new drug was not very good, which indicated that perhaps clonazepam was being sold on the streets as flunitrazepam, and that the drug abuser preferred flunitrazepam to clonazepam [7].

C. Pharmacokinetics

The pharmacokinetic properties of benzodiazepines are very important in cases of suspected DFSA, because the analyst must know what analytes to look for in each matrix. For example, when diazepam is consumed, the parent drug and its primary metabolite, nordiazepam, are all the focus in blood and hair, but oxazepam, temazepam, and nordiazepam glucuronides are the analytes expected in urine samples.

Absorption rates of benzodiazepines vary widely. The rate may be affected by the solubility of the drug, the particle size of the drug, and by the presence or absence of food in the stomach [17]. When surreptitious administration of a benzodiazepine in a beverage is considered, the time to peak plasma concentration may be reduced due to the fact that the drug is already in solution, and absorption may be quicker than when a tablet is consumed under ordinary conditions.

Lipid solubility of benzodiazepines is important when considering intravenous administration. A lipid-soluble benzodiazepine such as diazepam will pass through the blood-brain barrier more rapidly than a drug such as lorazepam, which has less lipid solubility [17].

Most benzodiazepines are extensively metabolized to both active and inactive metabolites (**Table 1**). Common metabolic processes for benzodiazepines include

Table 1. Major benzodiazepines and their metabolites

Benzodiazepine	Structure designation	Functional group	Major metabolites
Midazolam	A[a]	N/A	Hydroxymidazolam, hydroxymidazolam glucuronide
Chlordiazepoxide	B[a]	N/A	Norchlordiazepoxide, demoxepam, nordiazepam, oxazepam, oxazepam glucuronide
Demoxepam	C[a]	N/A	Nordiazepam, oxazepam, oxazepam glucuronide
Alprazolam	D[a]	R: H	α-Hydroxyalprazolam, α-hydroxyalprazolam glucuronide
Triazolam	D[a]	R: Cl	Hydroxymethyltriazolam, hydroxytriazolam, hydroxymethyltriazolam glucuronide, hydroxytriazolam glucuronide
Diazepam	E[a]	R_1: Cl; R_2: CH_3; R_3: H; R_4: H	Nordiazepam, oxazepam, temazepam, oxazepam glucuronide
Flurazepam	E[a]	R_1: Cl; R_2: CH_2CH_2-$N(C_2H_5)_2$; R_3: H; R_4: F	Desalkylflurazepam, hydroxyethylflurazepam, hydroxyethylflurazepam glucuronide
Nitrazepam	E[a]	R_1: N_2O; R_2: H; R_3: H; R_4: H	7-Aminonitrazepam, 7-acetamidonitrazepam, 7-aminonitrazepam glucuronide, 7-acetamidonitrazepam glucuronide
Clonazepam	E[a]	R_1: N_2O; R_2: H; R_3: H; R_4: Cl	7-Aminoclonazepam, 7-acetamidoclonazepam, clonazepam glucuronide, 7-aminoclonazepam glucuronide, 7-acetamidoclonazepam glucuronide
Flunitrazepam	E[a]	R_1: N_2O; R_2: CH_3; R_3: H; R_4: F	7-Aminoflunitrazepam, desmethylflunitrazepam
Temazepam	E[a]	R_1: Cl; R_2: CH_3; R_3: OH; R_4: H	Temazepam glucuronide, oxazepam, oxazepam glucuronide

[a] *See* Structure 1 for structure (or core structure) designations.

hydroxylation, desmethylation, and glucuronidation. For example, alprazolam is hydroxylated to form hydroxyalprazolam, which is further metabolized via glucuronidation. Diazepam is demethylated to form nordiazepam, which is then hydroxylated to form temazepam and oxazepam. These metabolites may be excreted unchanged, or after glucuronidation. Lorazepam is solely metabolized via glucuronidation. Most oxidative benzodiazepine metabolism is performed by cytochrome P450 subtype 3A4 [28].

Plasma concentrations of low-dose benzodiazepines may not reach more than a few nanograms per milliliter following therapeutic administration. Two-milligram doses of flunitrazepam administered daily to volunteers for 28 days resulted in peak plasma concentrations of flunitrazepam within 3 hr of dosing, and remained under 10 ng/mL for most of the duration of the study [44]. In a separate study, whole blood was collected from subjects after receiving a 1.0-mg dose of flunitrazepam, but the blood concentrations could not be accurately determined with a 0.2-ng/mL detection limit [4]. This illustrates why urine is the best specimen for most suspected DFSA cases.

Benzodiazepines and metabolites are excreted in the urine. Oxazepam and nordiazepam are common metabolites of multiple benzodiazepine preparations. Therefore, the identification of a benzodiazepine metabolite in a urine sample does not always conclusively define which benzodiazepine was administered.

Several studies have been performed in which single doses of benzodiazepines have been administered and urine has been analyzed for several days to estimate detection times in urine. After administration of 3 mg of clonazepam to 10 volunteers, 7-aminoclonazepam, the primary urinary metabolite of clonazepam, was measured in urine samples for 28 days [32]. The 7-aminoclonazepam was detected in urine samples from all 10 volunteers up to 14 days after the administration of the drug, with a 50-pg/mg detection limit. The metabolite was still detectable in 1 subject 28 days after the administration of the single 3-mg dose.

A similar study was performed in which 2 mg of flunitrazepam was administered to 10 volunteers [33]. Here, 7-aminoflunitrazepam was detected in urine from 3 volunteers throughout the 28-day urine collection period with a detection limit of 3 pg/mg. Five days after administration, flunitrazepam was detected in only 1 subject.

Tetrazepam (50 mg) was administered to 3 volunteers [23]. Urine from all 3 volunteers was positive for tetrazepam for approximately 11.5 days (280 hr) after administration with a 10-ng/mL detection limit. When 10 mg diazepam was administered to 3 subjects, temazepam and oxazepam were found to be the most abundant metabolites over the 280-hr urine-collection period [23]. Diazepam itself was detected only 8 hr after administration, and in just a single subject.

Benzodiazepines are excreted to some degree in the hair and sweat. Low levels (pg/mg) of 7-aminoflunitrazepam have been identified in the hair of subjects who were dosed with 2 mg of flunitrazepam [33]. Likewise, low levels of tetrazepam or diazepam were identified in the hair of volunteers who were dosed with these benzodiazepines [23]. Alprazolam, clobazam, diazepam, nordiazepam, oxazepam, temazepam, and triazolam have been identified in nail samples from individuals taking benzodiazepines chronically [21]. Alprazolam, 7-aminoflunitrazepam, 7-aminoclonazepam, lorazepam, and bromazepam have all been identified in hair samples from alleged victims of DFSA [42].

D. Pharmacodynamics

Benzodiazepines bind to $GABA_A$ (gamma-aminobutyric acid) receptors in the central nervous system, facilitating binding of GABA, and thereby increasing chloride conductance [40]. Because GABA is an inhibitory neurotransmitter, binding of GABA leads to sedation and decreased excitability. Other drugs such as zolpidem and zopiclone, while not chemically considered to be benzodiazepines due to differing ring structures, also bind at the $GABA_A$ receptor, and act similarly.

Effects and side effects of benzodiazepines are similar to that of most central nervous system depressants and include: drowsiness, lethargy, light-headedness, sedation, ataxia, reduced anxiety, reduced concentration, reduced cognition, confusion, reduced alertness, and anesthesia. One effect of benzodiazepines that is not seen with all CNS depressants is anterograde amnesia. Anterograde amnesia refers to the ability of benzodiazepines to prevent memory formation while the individual is under the influence of the drug. Because of this effect, benzodiazepines are often prescribed in preoperative situations.

Paradoxical reactions to benzodiazepines such as flunitrazepam have included restlessness, aggression, and agitation. Bramness and colleagues reported these paradoxical reactions in approximately 6% of 415 drivers found to be under the influence of flunitrazepam [5]. They also reported that these paradoxical reactions were not dependent on blood concentrations of flunitrazepam.

E. Drug Interactions

The sedative effects of benzodiazepines are enhanced when taken in conjunction with other central nervous

system depressants, including ethanol, barbiturates, and zolpidem. The depressant effects of some antihistamines can be enhanced when taken in conjunction with benzodiazepines.

Alprazolam may affect the absorption of other drugs taken orally since it relaxes muscles associated with the gastrointestinal system [28]. Ethanol inhibits the formation of flunitrazepam metabolites by about 10-40% [28].

Some antidepressants—specifically fluvoxamine, fluoxetine, and nefazadone—may result in elevated benzodiazepine blood concentrations due to interactions with benzodiazepine metabolism via oxidation [39]. Propoxyphene, oral contraceptives, grapefruit juice, and other CYP3A4 inhibitors may also increase the depressant effects of benzodiazepines.

F. Benzodiazepines and DFSA

Since primary effects of benzodiazepines include sedation and amnesia, it is no surprise that this class of drugs is often associated with DFSA.

In 1999, the first large-scale study of urine samples from alleged sexual assault victims was published [15]. Of a total of 1,179 urine samples, benzodiazepines were identified in 97 samples (8.2%). Whether these positive results were from therapeutic use or surreptitious administration is unknown. Although this testing was very sensitive for flunitrazepam and metabolites, with a detection limit of 1 ng/mL, screening for other benzodiazepines was by immunoassay with enzymatic hydrolysis and a cutoff of 50 ng/mL. Urine samples that screened positive for benzodiazepines by immunoassay in this study but could not be confirmed by gas chromatography/mass spectrometry (GC/MS) were reanalyzed using a more sensitive liquid chromatography/time-of-flight mass spectrometry method with detection limits in the range of 0.5–3 ng/mL [13]. Benzodiazepines and metabolites were identified in 13 urine samples from alleged sexual assault victims that previously had been undetermined. Specific analytes identified in these cases were 7-aminoclonazepam, alprazolam, alpha-hydroxyalprazolam, triazolam, alpha-hydroxytriazolam, and nitrazepam. A total of 156 urine samples that had screened positive for benzodiazepines in an expansion of this study were later retested with more sensitive methodology [14]. Oxazepam, nordiazepam, and temazepam were identified with the most prevalence in these 156 urine samples.

Hair testing in cases of suspected DFSA has been recommended when so much time has elapsed as to make collection of blood and urine fruitless, or when exposure to chemical agents is suspected to have occurred over time [24]. In one example, hair was analyzed from a victim who alleged being drugged and sexually assaulted over a period of years [23]. Benzodiazepines including 7-aminoclonazepam, clonazepam, bromazepam, clobazam, oxazepam, nordiazepam, lorazepam, lormetazepam, tetrazepam, and loprazolam were identified throughout the 33-cm length of her hair. Alprazolam has also been identified in the hair of a juvenile who allegedly was drugged and abused by her father for a period of years [43].

III. METHODS OF ANALYSIS

A. Extraction Techniques

Numerous methods for the extraction of benzodiazepines and their metabolites from urine, blood, and other biological matrices have been published. In order to lower detection limits, enzymatic or acidic hydrolysis of glucuronide conjugates is critical before the extraction of urine samples for most benzodiazepines [29]. Liquid-liquid extraction has been used successfully for benzodiazepines and their metabolites [13,23,41]. Samples are typically alkalinized to an approximate pH of 9 before extraction into organic solvents such as chloroform/isopropanol, chlorobutane, or butyl acetate. Solid-phase extraction (SPE) has also been used for benzodiazepine extraction [22,29,35,37]. On-line SPE has been performed successfully as well [16]. Methods typically start with 1-2 mL of urine or blood, but detection limits as low as 1-2 ng/mL have been achieved using less than 1 mL of sample [23].

B. Instrumental Methods

Many laboratories use GC/MS to identify benzodiazepines and their metabolites in biological samples [18,26, 41]. Chemical derivatization may be employed to improve sensitivity and chromatographic performance of benzodiazepines and metabolites [11,26,41]. Negative ion chemical ionization has also been used for benzodiazepine detection [18,26,32,33].

As liquid chromatography/mass spectrometry (LC/MS) has gained popularity in forensic toxicology laboratories in recent years, benzodiazepines have been a popular class of drugs for this analysis. In 2008, a review of liquid chromatography/tandem MS (LC/MS/MS) methods included 34 references of published methods for benzodiazepine analysis [30]. Many methods use multiple reaction monitoring of one or more transitions for each analyte [22,23,29,35]. Full-scan MS/MS has also been used [25]. Alternatively, a combination of MS and MS/MS can be employed [38]. Time-of-flight MS has

also been used to identify benzodiazepines and their metabolites in biological fluids [14]. Although not as specific, methods using LC with ultraviolet/visible detection have also been published in recent years [37].

One research group dosed volunteers with single doses of either lorazepam (2.5 mg), bromazepam (6 mg), flunitrazepam (1 mg), clonazepam (2 mg), zolpidem (10 mg), or zopiclone (7.5 mg), and collected urine from the volunteers for 144 hours for testing using different analytical methodologies [9]. They demonstrated that LC/MS/MS was preferred over immunoassay, LC with diode array detection, GC/MS, or LC/MS, due to LC/MS/MS's higher sensitivity allowing detection of all substances 144 hours after dosing.

C. Immunoassay

Immunoassay is frequently employed in forensic toxicology laboratories as a front-line screen for common drug classes. However, this may not be the best way to screen blood and urine samples for benzodiazepines in cases of suspected DFSA, since typical cutoff concentrations for benzodiazepines may not be low enough for application in these cases. For example, in one study comparing two different immunoassay techniques for common drugs of abuse, a cutoff concentration for benzodiazepines of 300 ng/mL was chosen [27]. This is much higher than the detection limits suggested by the Society of Forensic Toxicology's (SOFT) DFSA Committee, which range from 5–10 ng/mL [19].

Another drawback with immunoassay screening for benzodiazepines in DFSA cases is that kits may not target the optimal urinary metabolites. Further, cross-reactivities for different benzodiazepines vary widely for different kits. Elian examined several enzyme-linked immunosorbent assay (ELISA) kits for clonazepam and determined that the cross-reactivities of the kits for 7-aminoclonazepam, the major urinary metabolite of clonazepam, were all less than 10% [12]. This would pose a serious drawback in the detection of a single dose of clonazepam in a DFSA case.

Enzymatic hydrolysis of urine samples before immunoassay testing has been proposed to increase sensitivity [3,31,36]. However, these articles have focused on clinical detection of benzodiazepines, and the detection limits have not approached the levels suggested by the SOFT DFSA Committee. Laboratories relying solely on immunoassay screening for the detection of benzodiazepines in suspected DFSA cases should be aware of the detection limits for the different benzodiazepines and metabolites.

CONCLUSIONS

Benzodiazepines are one of the most commonly encountered classes of drugs in DFSA investigations. Reasons for this phenomenon may include the relatively long half-lives of many of the benzodiazepines and their metabolites, which allow them to be detected in urine samples for several days after administration. Additionally, the sedating and amnesic effects of the benzodiazepines make them attractive to a perpetrator desiring these effects in prospective victims. Benzodiazepines are also widely available, as they are a commonly prescribed drug, and they are also a drug class that is commonly abused. In order to increase the likelihood of detecting benzodiazepines in DFSA cases following limited dosing, forensic toxicology laboratories may choose to increase the sensitivity of their methods by using targeted methods and by hydrolyzing urine samples before analysis.

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The Use of GHB and Analogs to Facilitate Sexual Assault[a]

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The Use of GHB and Analogs to Facilitate Sexual Assault

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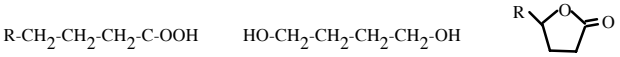
ABSTRACT: γ-Hydroxybutyric acid (GHB) and its metabolic precursors, γ-butyrolactone (GBL) and 1,4-butanediol (1,4-BD), may be among the most favored drugs used to commit drug-facilitated sexual assault (DFSA). In fact, federal legislation was enacted in the form of the Hillory J. Farias and Samantha Reid Date-Rape Drug Prohibition Act of 2000 to control and penalize use and distribution of GHB, GBL, and 1,4 BD. Unfortunately, solid proof of their use in many cases is difficult to obtain because GHB, GBL, and 1,4-BD have strong sedative and memory-impairing effects and are rapidly eliminated after ingestion. To further complicate the matter, GHB is a metabolite of γ-aminobutyric acid (GABA), a naturally occurring neurotransmitter in humans. This review focuses on the chemistry and pharmacology of these drugs and their use in DFSA. An overview of analytical techniques used to identify their presence is provided, as well as guidance on the toxicological interpretation of findings of GHB in biological specimens.

KEY WORDS: 1,4-BD, 1,4-butanediol, γ-butyrolactone, drug-facilitated sexual assault, GBL, GHB, γ-hydroxybutyrate.

INTRODUCTION

Although γ-hydroxybutyric acid (GHB) has been studied clinically for many decades, its popularity as a recreational drug of abuse is still relatively new. GHB first became popular in the late 1970s after a paper introduced GHB as a "steroid" that increased growth hormone release. However, once the body-building community started using GHB, it was also discovered that it had euphoric and relaxing properties similar to ethanol, but GHB was more potent. This fueled GHB's popularity since it could be used to get "drunk" with much less volume and calories compared to ethanol. As GHB's use as a recreational drug of abuse increased, the dangers of the drug were discovered. By the 1990s, GHB's dangerous side was becoming more prevalent and steps to control its use were initiated. Federal legislation was enacted in 2000 to control and penalize use and distribution of GHB. This forced GHB production to move underground and increased the popularity of GHB's metabolic precursors, γ-butyrolactone (GBL) and 1,4-butanediol (1,4-BD). Even today the search continues for legal alternatives to GHB, such as γ-valerolactone (GVL). Chemical structures of GHB and related compounds included in this review are shown in **Structure 1**.



A **B** **C**

Structure 1. Chemical structures of γ-hydroxybutyric acid (**A**, R: OH); γ-aminobutyric acid (**A**, R: NH_2); 1,4-butanediol (**B**); γ-butyrolactone (**C**, R: H); and γ-valerolactone (**C**, R: CH_3).

GHB

Clinical Use of GHB in Humans. Early pharmacological use of GHB in humans was as an anesthetic adjuvant. Initial GHB studies focused on its use as an experimental γ-aminobutyric acid (GABA) analog in the treatment of seizure disorders [85]. The authors hypothesized that since GHB could readily cross the blood-brain barrier, it could facilitate the synthesis of GABA in the brain. Although GHB did not produce elevated GABA synthesis, the research revealed that GHB had pharmacologic properties that rendered it useful as an anesthetic adjuvant. Blumenfeld et al. listed nine qualities observed from their use of GHB for human anesthesia: mimics natural sleep, causes negligible reduction in respiratory volume, has cardiotonic effects, produces relaxation for ease of intubation, potentiates other central nervous system (CNS) depressants, does not change oxygen consumption, permits easy control of respiration, provides very stable vital signs, and permits slow induction of anesthesia [10].

GHB (sodium oxybate) is approved for medical use under the brand name Xyrem and is marketed in the United States (Jazz Pharmaceuticals), Canada (Valeant Pharmaceuticals International), and Europe (UCB). It is approved for the treatment of cataplexy associated with narcolepsy [77]. It is also used in Germany as an anesthetic agent under the brand name Somsanit. It is approved as well for use in the treatment of opioid and alcohol withdrawal under the brand name Alcover in Italy and Austria [72]. The latter use is not surprising since ethanol and GHB are similar compounds, both in structure and pharmacology. Cross-tolerance between ethanol and subanesthetic doses of GHB has been observed in rats, which may explain why alcoholics being treated with GHB do not experience sedation at doses that would

sedate a non-alcohol-tolerant individual [24]. Adverse effects have been mild except for occasional replacement of alcohol addiction with GHB addiction, resulting in some subjects self-medicating with additional GHB to enhance its effects [2,8]. Treatment with GHB has also been investigated for opiate withdrawal syndrome [59], cocaine addiction [45], fibromyalgia [138,142], and essential tremor [57].

In 2000, GHB became a Schedule I controlled substance in the U.S. [124] and a Schedule IV substance under the 1971 Convention on Psychotropic Substances, resulting in GHB control in all member states of the European Union. Canada lists GHB and all salts as Schedule III substances.

History of Illicit Use of GHB. In 1977, a study was published that would permanently change the relative obscurity of GHB and its analogs. Takahara et al. administered GHB to six healthy adult males and showed an approximate 10-fold increase in plasma growth hormone concentration that peaked 45 min after administration [161]. This effect persisted for about 15 min before the growth hormone concentration declined toward pretreatment level. Growth hormone concentration at 120 min post dose was still above baseline but two-thirds below the peak concentration. Based on this report, some bodybuilders postulated that GHB would increase growth hormone concentration, thereby optimizing their muscle-building potential. A more recent study by Van Cauter et al. showed that the increase in growth hormone secretion was correlated with the enhancement of slow-wave sleep [163]. Growth hormone release did not occur prior to sleep onset. The growth hormone stimulating effect of a 2–3 g dose of GHB was seen during the first 2 h of sleep as an increase in amplitude and duration of the normal growth hormone secretory pulse associated with sleep onset, as opposed to an increase in the total number of growth hormone release pulses.

The use of GHB by bodybuilders seemed harmless until emergency room reports associated with GHB toxicity began accumulating [121]. Users soon discovered that GHB had a mood-elevating quality and introduced GHB into the party drug scene. GHB is known by numerous street names (**Table 1**, Column 1). Many of these slang names feature the letters "G", "H", and "B" (e.g., "Georgia Home Boy" or "Great Hormones at Bedtime"). GHB's street name "Liquid Ecstasy" results in it often being confused with methylenedioxymethamphetamine (MDMA), which is better known as Ecstasy. In 1990, the FDA warned consumers of the dangers of GHB, but the incidence of poisonings continued to rise [53]. GHB, as well as products containing GHB, were removed from the market and GHB sales moved underground. Users soon discovered that GHB could easily be synthesized from readily available precursors. For example, the industrial solvent GBL, when made alkaline with lye and heated,

Table 1. Slang names and industrial and commercial synonyms for GHB, GBL, and 1,4-BD

Slang name	Industrial synonym	Commercial synonym
Cherry Meth	α-Butyrolacton	Butane-1,4-diol
Easy Lay	BLO	1,4-Butylene glycol
G	BLON	1,4-Dihydroxybutane
G-Caps	1,2-Butanolide	Diol 1,4B
Gamma OH	1,4-Butanolide	Polymeg
Georgia Home Boy	4-Butanolide	Poly(tetrahydrofuran)
Great Hormones at Bedtime	Butyric acid lactone	Sucol B
GHBA	Butryl lactone	Tetramethylene-1,4-diol
Grievous Bodily Harm	Butyrolactone	1,4-Tetramethylene glycol
Liquid Ecstasy	4-Butyrolactone	
Liquid X	C-1070	
Natural Sleep 500	4-Deoxytetronic acid	
Oxy-Sleep	γ-6480	
Salt Water	γ-BL	
Soap	γ-Hydroxybutyric acid cyclic ester	
Sodium Oxybate	γ-Hydroxybutryic acid lactone	
Scoops	4-Hydroxybutyric acid-γ-lactone	
Vita G	4-Hydroxy-γ-lactone	
	1,4-Lactone	
	NCI-C55875	
	Tetrahydro-2-furanone	

yields GHB. With addition of an acid, such as vinegar, to adjust the pH, the solution of GHB was ready for consumption Illicit GHB is especially dangerous because its concentration is unknown and can vary greatly from batch to batch. Coupled with the fact that it has a very steep dose-response curve [67], it is very easy to accidentally overdose on GHB. Additionally, the clandestine manufacturing process may introduce contaminants that have additional health consequences.

GBL and 1,4-BD

Research on GHB was expanded to include compounds that were analogs or metabolic precursors of the drug: GBL and 1,4-BD. Sprince et al. investigated the potential anesthetic properties of GBL and 1,4-BD [157]. They found that, compared to GHB, sleep induction time was faster with GBL and slower with 1,4-BD. This was an early clue to the metabolic relationship among these three compounds. Additional studies demonstrated that GBL was simply a prodrug for GHB [133].

History of Illicit Use of GBL and 1,4-BD. While GHB was placed as a Schedule I drug of the U.S. Federal Controlled Substances Act in 2000, GBL was instead identified as a List I chemical and a controlled-substance analog. Additionally, 1,4-BD was also listed under the controlled-substance analog section [124]. Some European countries have also placed controls on GBL and 1,4-BD [72]. The international restrictions on the production and sale of GHB are thought to have shifted recreational use from GHB toward its precursors [33,16,123,166,168].

Unfortunately, scheduling has not curbed the illicit use of this trio. With the placement of GHB in Federal Schedule I, there appears to be more interest on the part of the illicit manufacturers in producing a "GHB product" that will stay in the lactone form and not spontaneously convert to GHB due to the relatively less-severe penalties associated with GBL. In fact, a seizure of solid GBL was reported in California; liquid GBL had been adsorbed onto silicon dioxide powder and then placed into clear capsules [28]. This increases the drug's danger because the lactone form, based on its physical characteristics and its increased solubility in lipids, has been shown in animal studies to be absorbed by the gut more efficiently than GHB [96]. In 1999, the FDA requested removal of health supplement products containing GBL [54], but this was only a fraction of the products that contained this compound. The most common use of GBL is as an industrial solvent, with U.S. production of approximately 80,000 tons per year. Due to its wide use as a safe, effective, safe and effective biodegradable degreaser, it is difficult to replace. Some manufacturers of diet-aid products containing GBL have masked the presence of this ingredient by using one of the many chemical synonyms for GBL in the list of ingredients on the product label (Table 1, Column 2).

GBL has been detected in low concentrations in alcoholic beverages, tobacco smoke, coffee, tomatoes, cooked meats, and several foodstuffs [62]. Because it is rapidly converted to GHB in the body, it produces nearly the identical pharmacological effects of GHB. At equimolar doses, GBL produced a more prolonged hypnotic effect in rats compared to GHB [62].

To a lesser extent, 1,4-BD has followed the same path as GBL, and has gained popularity in recent years. Like GHB and GBL, 1,4-BD has been associated with adverse events, including death [169]. The major use for 1,4-BD in the U.S. is as an industrial compound. However, unlike GBL, 1,4-BD is not typically used to manufacture illicit GHB. The conversion of 1,4-BD to GHB is an industrial process and cannot be accomplished in a household setting. The pharmacological effects of 1,4-BD are ultimately those of GHB, the metabolic product of 1,4-BD. With the increased attention on GHB toxicity, the FDA requested that products containing 1,4-BD be removed from the market [52]. As with GBL, this action could also lead manufacturers to increase their use of one of the many chemical synonyms of 1,4-BD to disguise its presence in the product (Table 1, Column 3).

Other Analogs

In addition to GBL and 1,4 BD, the recent emergence of diet-aid products containing GVL has caused some concern as to the pharmacological effects of this compound and its safety for human consumption. GVL is an FDA-approved food additive and adjuvant that is safe for human consumption at 0.9322 μg/kg per day per person. One currently available diet-aid product, Tranquili-G, recommends a dose of GVL around 3 g—a dose much larger than the safe amount approved by the FDA. In rats and rabbits, administration of GVL produced marked muscular weakness, mild anesthesia, and an increase in the rate of respiration. The oral LD_{50} for GVL was 8.8 g/kg and 2.5 g/kg for rats and rabbits, respectively [30]. Past research has documented that GVL is quickly metabolized by the lactonase enzyme to 4-methyl-GHB (4-Me-GHB) in human blood and rat liver microsomes [51], similar to the way GBL is metabolized by that enzyme to GHB; 4-Me-GHB is a structural analog of GHB, but GVL is not. It has been demonstrated in the rat model that GVL has similar pharmacological properties to GHB mediated through the 4-Me-GHB metabolite [111]. There are no reports of the detection of GVL or 4-Me-GHB in DFSA cases.

I. CHEMISTRY

GHB ($C_4H_8O_3$) is a simple hydroxylated short-chain fatty acid with a molecular weight of 104.1 g/mol. The first report of its synthesis was in 1874 [141]. It may be encountered as the colorless, free acid liquid form, or supplied in the sodium salt form (sodium oxybate) with a molecular weight of 126.1 g/mol. It may also appear in the potassium salt form (mw: 142.2 g/mol). The salt forms are typically white or off-white in color and are readily soluble in water.

GBL ($C_4H_6O_2$) is a hygroscopic colorless oily liquid that is miscible in water. It has a molecular weight of 86.1 g/mol and a density of 1.129 g/mL. It is a lactone (Structure 1) that is hydrolyzed under alkaline conditions into GHB.

In solution, GHB coexists in a state of equilibrium with its lactone, GBL. The conversion of GHB to GBL is dependent on the matrix, pH, and temperature. Because GHB has a pKa of 4.72, it will predominate when the pH of the matrix is greater than 4.72, while GBL will predominate if the pH is lower than the pKa. Additionally, the presence of the plasma enzyme lactonase will affect the equilibrium since it also converts GBL to GHB.

The interconversion between GHB and GBL is often exploited during analysis to avoid the need for derivatization in gas chromatographic analyses. In an environment of a concentrated, dehydrating acid solution (e.g., sulfuric acid), complete conversion of GHB to GBL will take place. This reaction progresses more rapidly as the temperature is increased [23].

The analog 1,4-BD ($C_4H_{10}O_2$) is an aliphatic alcohol (Structure 1) that exists as a colorless, viscous liquid. It has a molecular weight of 90.1 g/mol and a density of 1.01 g/mL.

II. PHARMACOLOGY

A. Administration

The FDA-approved formulation of GHB (Xyrem) is taken at bedtime and again 2.5–4 h later. The dose of Xyrem is to be titrated to effect; the recommended starting dose is 4.5 g/night divided into two equal doses of 2.25 g. The starting dosage can then be increased to a maximum of 9 g/night in increments of 1.5 g/night (0.75 g per dose). The dosage range for the average person is 6 to 9 g/night. The efficacy and safety at doses higher than 9 g/night have not been investigated [76].

Illicit GHB, GBL, and 1,4-BD are also nearly always taken orally. As with other drugs, illicit formulations vary widely in the amount of active drug they contain. The cap of a water bottle is often used as the measuring device to deliver a recreational dose of GHB. Depending on the size of the cap and the concentration of the solution, the recreational user may receive approximately 5 g of GHB in one capful [6,117].

B. Pharmacokinetics

After ingestion, GHB is rapidly absorbed with blood concentrations peaking within 15–60 min [11,12,47,50,70, 122,143]. Research suggests that this absorption is capacity-limited [4,50,95,122], so increased doses result in longer times to peak concentration. Absorption may be enhanced when GHB is consumed on an empty stomach and slowed by consuming a high-fat meal [11].

In rats, the oral bioavailability of GHB is 59–65% [95,96]. In humans, GHB has been shown to exhibit first-pass metabolism when given orally with about 25–65% bioavailability when compared to an equivalent intravenous dose [12,48,50,96].

In clinical studies on GHB, the average peak plasma concentrations (first and second peak) following administration of a 9-g daily dose divided into two equivalent doses given 4 h apart were 78 and 142 mg/mL, respectively [77]. The initial clinical effects are noted 15–20 min after oral administration and peak clinical effects occur 30–60 min post ingestion [48,70,108,115,122].

The lipid-soluble nature of GHB allows it to readily cross the blood-brain barrier to exert its primary effect in the CNS [86,115]. No appreciable plasma protein binding occurs [122]. Distribution to target tissues (e.g., brain, liver, and kidneys) occurs rapidly and follows a two-compartment model with a volume of distribution (V_D) of 0.4–0.6 L/kg [108]. The concentration of GHB in the brain of a dog reaches its peak after approximately 10 min [149]. GHB crosses the placental barrier at a similar rate to that in the blood-brain barrier [125].

The distribution of GHB into the CSF appears to lag behind that in blood or brain. After a 500-mg/kg intravenous dose of GHB was administered to dogs, plasma concentration peaked within 5 min; brain concentration peaked within 10 min but it was 170 min before CSF concentrations reached their maximum [149]. This suggests a passive diffusion of GHB from serum or brain into the CSF. In alcohol-dependent patients, GHB did not accumulate in the body with repeated dosing. The mean peak plasma concentrations of therapeutic oral doses of 25 and 50 mg/kg of GHB per day given to 50 alcohol withdrawal syndrome patients were 55 mg/L (range = 24–88) and 90 mg/L (range = 51–158), respectively [48,153].

The primary pathway for GHB metabolism involves conversion to succinic semialdehyde and then to succinic

acid via GHB dehydrogenase and succinic semialdehyde dehydrogenase (**Figure 1**). After succinic acid enters the Krebs cycle, it is ultimately expired as carbon dioxide and water [105,132,162]. Only a fraction (< 1–5%) of GHB is eliminated unchanged in the urine [47,48,50,122,143].

GHB exhibits zero-order elimination kinetics after an intravenous dose and, therefore, has no true half-life. A daily therapeutic dose of 25 mg/kg has an apparent half-life of about 30 min in humans, as determined in alcohol-dependent patients under GHB treatment [50]. In contrast, an apparent half-life of 1–2 h was observed in dogs when they were given high intravenous doses of GHB [149].

Roth and Giarman determined that a lactonase enzyme in blood and liver rapidly catalyzed the hydrolysis of GBL to GHB (**Figure 2**) [133]. Administration of GHB and GBL intracisternally (i.e., directly into the CNS) provided no opportunity for biotransformation to occur, as the lactonase enzyme does not display any substantial activity in brain or cerebrospinal fluid (CSF).

As discussed earlier, GBL and 1,4-BD both metabolize to GHB (Figure 2). These analogs are converted to GHB rapidly and can be complete within 10 min of ingestion. Thus, GBL and 1,4-BD closely mimic the pharmacokinetics and pharmacodynamics of GHB [74,126,127,130], but there are some differences. The absorption of GBL has been documented to occur faster than that of GHB [96].

It has been proposed that GBL may also distribute differently than does GHB. An early study comparing the distribution of equimolar doses of GHB and GBL in rats found that although peak plasma concentrations were higher with GHB, they remained elevated longer with GBL. In addition, concentrations of GBL in the lean muscle mass of the rat were always elevated compared to concentrations of GHB [130]. This suggests sequestration of GBL into lean muscle prior to its conversion to GHB. Because lean muscle does not contain lactonase, it is conceivable that this could occur and the GBL may redistribute into the blood to be converted to GHB by the blood or liver lactonases. This may explain the prolonged elevation of GHB blood concentrations that occur when GBL is given. Neither GHB nor GBL are sequestered in fat.

The analog 1,4-BD requires a two-step enzymatic conversion to GHB that results in a slightly longer time to peak GHB plasma concentration and an extended time of elevated GHB concentration. The conversion process of 1,4-BD to GHB can be slowed or inhibited by co-administration of ethanol, pyrazole, or disulfiram [74].

C. Pharmacodynamics

Although nearly every organ system is affected by GHB, its primary effects are in the CNS. After 1- to 5-g doses, GHB levels in the CNS increase 100- to 500-fold [18,107].

Although it has been shown to mediate sleep cycles, temperature regulation, cerebral glucose metabolism, blood flow, memory, and emotional control [97], GHB's most important effect is strong CNS depression as a result of its influence on a variety of neurotransmitter systems.

Several studies of GHB in the 1970s revealed that anesthetic doses of GHB cause an increase in dopamine concentration in the brain by blocking impulse flow in central dopaminergic neurons [15,119,136,137]. The net effect of blocking the impulse flow is to cause a buildup of dopamine in the dopaminergic nerve terminals. Sethy et al. determined that GHB may have a similar effect on brain concentration of acetylcholine, increasing acetylcholine concentration by decreasing impulse flow in cholinergic neurons [148]. The reason GHB is abused is probably not attributable to an increased concentration of brain dopamine due to inhibition of its release [104]. Paradoxically, at subanesthetic doses of GHB, an excitation of dopamine neurons was observed [31,131]. Many drugs of abuse cause an increase in dopamine in the synapse via

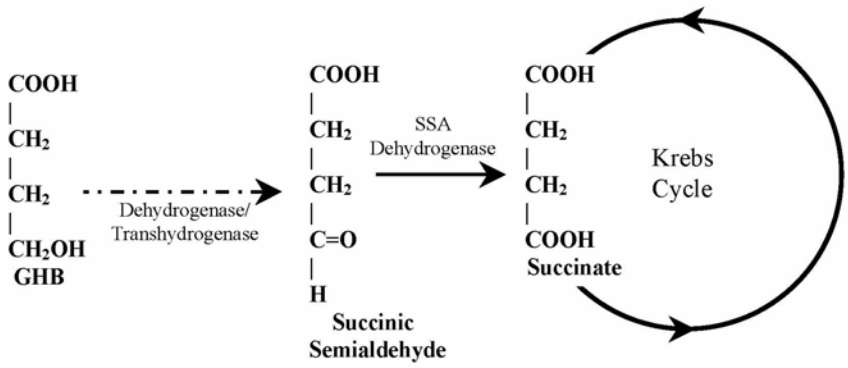


Figure 1. Normal GHB metabolism.

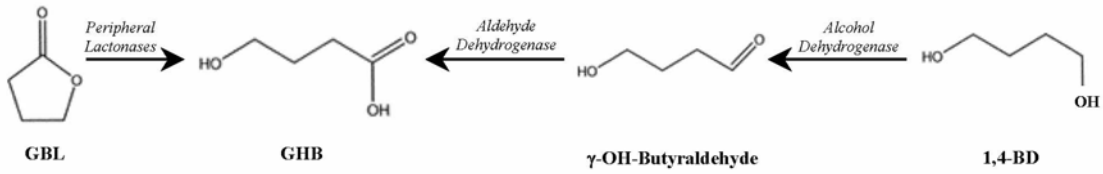


Figure 2. Metabolism of 1,4-BD and GBL.

various mechanisms. Subanesthetic doses of GHB cause an initial stimulation of dopamine neurons producing elevations of synaptic dopamine that may play a part in the reinforcing effect of GHB.

Research has demonstrated that GHB appears to have a distinct receptor site in the brain with both high- and low-affinity components. Current research suggests that this receptor appears to be a G protein-coupled presynaptic receptor that is distinct from the $GABA_B$ receptor [154]. In addition, there is also evidence that GHB is a weak agonist at the $GABA_B$ receptor [19,100,106,151,153]. However, the mechanism of action of GHB is still not resolved. Researchers have postulated that GHB has some capacity as a neurotransmitter and/or neuromodulator, and investigation continues in this area.

Behavioral effects are observed in subjects who have ingested GHB. Low doses of GHB (approximately 0.5–1.5 g) cause induction of a state of relaxation and tranquillity, placidity, sensuality, mild euphoria, a tendency to verbalize, emotional warmth, and drowsiness. Higher doses, like those involved in drug-facilitated crimes (1.5 g or more), can induce more obvious clinical manifestations and adverse effects including confusion, dizziness and drowsiness, nausea and vomiting, agitation, nystagmus, loss of peripheral vision, hallucinations, suppression of the gag reflex, confusion, agitation, anterograde amnesia, hypothermia, somnolence, uncontrollable shaking or seizures, clonic muscle movements, bradycardia, respiratory depression, apnea, coma, and death [11,20–22,34,36,60,98,99,156]. Blood concentrations exceeding 260 μg/mL have been associated with deep sleep; 156–260 μg/mL with moderate sleep; 52–156 μg/mL with light sleep; and less than 52 μg/mL with wakefulness [70]. In animal experiments, the median lethal dose is 5–15 times the coma-inducing dose [164].

Although there have been some reports of seizures associated with GHB intoxication, there is no evidence of true seizure activity as measured by EEG in humans [44]; however, only GHB doses consistent with safe anesthesia have been evaluated in these EEG studies. Clonic muscle movements and severe parasympathomimetic activity including profuse salvation, defecation, and urination have been documented in dogs treated with toxic and lethal doses of GHB [103]. The clonic muscle movement was so prominent that a barbiturate was also administered to effect convenient anesthesia.

Another complicating factor is that GHB used outside clinical settings is frequently used in combination with other drugs. This could affect the pharmacology of GHB in many ways depending on the type and dose of co-ingested drug. By far the most common drug taken in combination with GHB is ethanol [102,118]. This combination is especially dangerous because ethanol potentiates GHB's CNS-depressant effects as demonstrated by depression of the startle response (a measure of sensory responsiveness) in rats [110]. GHB has been implicated in fatalities both when administered alone [112] and when used in combination with other drugs [49].

The most likely negative outcome of chronic GHB use is addiction, with a GHB withdrawal syndrome having been documented with such use [26,36,72]. The clinical presentation of GHB withdrawal ranges from mild clinical anxiety, agitation, tremors, and insomnia to profound disorientation, increasing paranoia with auditory and visual hallucinations, tachycardia, elevated blood pressure, and extraocular motor impairment. Symptoms, which can be severe, generally resolve without sequelae after various withdrawal periods, although one documented death has occurred [169]. Treatment with benzodiazepines has been successful for symptoms of a mild withdrawal syndrome.

D. Drug Interactions

In vitro studies with pooled human liver microsomes indicate that GHB does not significantly inhibit the activities of CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A up to the concentration of 378 mg/mL [77]. The strong CNS depressant effect of GHB, GBL, and 1,4-BD contraindicates its use with other CNS depressants such as alcohol, benzodiazepines, barbiturates, sedative antidepressants and antihistamines, narcotics, and hypnotics such as zolpidem and zopiclone. A potentially life-threatening drug interaction has been reported between GHB and HIV-1 protease inhibitors [69].

III. ENDOGENOUS GHB

The endogenous nature of GHB in the human body results, in part, from the normal metabolism of GABA in the CNS (**Figure 3**) and from its production outside the CNS. In the CNS, GABA is converted into succinic semialdehyde (SSA) via GABA aminotransferase. Most of the formed SSA is oxidized to succinic acid (SA) via SSA dehydrogenase where it enters the Krebs cycle and is converted to water and carbon dioxide. However, a small amount of the SSA is reduced to GHB via SSA reductase. GHB is typically oxidized back to SSA via GHB ketoacid transhydrogenase and then is converted to SA before entering the Krebs cycle, but a small amount of GHB may instead undergo beta-oxidation to 3,4-dihydroxybutyric acid and 3-keto-4-hydroxybutyric acid [162].

There is evidence that there are sources of endogenous GHB in the body other than from GABA. For example, GHB is present in extraneural sites (i.e., heart, lung, liver, skeletal muscle, kidney, and hair) that have either no or very little amounts of GABA present [121,168], yet the concentrations of GHB in these tissues are 5–10 times higher than the GHB concentrations found in the brain [134,135]. It has also been shown that 1,4-BD is an endogenous product from fatty acids and may be a source of GHB in peripheral tissues [7].

A genetic disorder called GHB aciduria occurs when there is a deficiency of succinic semialdehyde dehydrogenase. Persons with this disorder have elevated concentrations of GHB in their blood, spinal fluid, and urine [64]. The clinical manifestations of the increased GHB concentration can range from mild oculomotor problems and ataxia to severe psychomotor retardation, but it is most commonly characterized by mental, motor, and language delays accompanied by hypotonia [64].

IV. GHB AND DFSA

DFSAs occur after a victim is rendered unconscious or otherwise incapable of consenting to a sexual act following the voluntary or involuntary use of drugs [42,43,75,87,146,165]. As a result of its strong sedative and memory-impairing effects, GHB has been implicated in a number of DFSA cases [1,39,42,66,78,83,91,97,116, 120,160]. Of all the drugs used to commit this crime, GHB and its analogs may be among the most favored by rapists, although statistically it is difficult to prove [46]. This is partly due to the rapid onset of strong sedative and amnesiac symptoms, but also because these compounds tend to be colorless and odorless, making them easier to secretly administer in a drink.

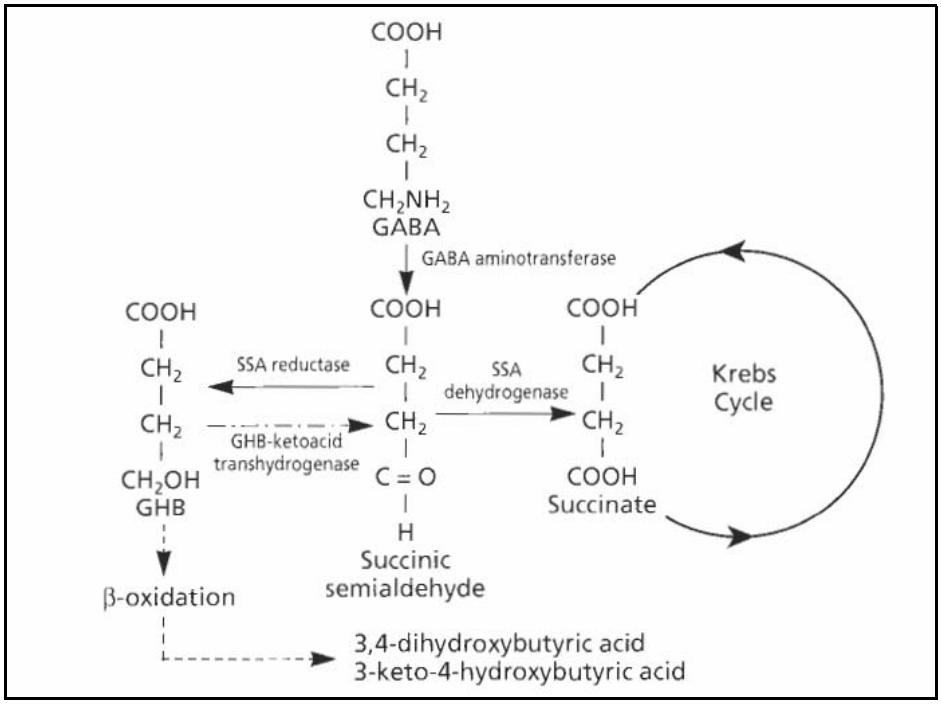


Figure 3. Normal pathway to GHB production from GABA.

Another factor that makes GHB, GBL, and 1,4-BD attractive for use by rapists is that these chemicals are readily available for use. Besides GHB being simple to synthesize, these drugs are also easy to purchase on the Internet, on the street, in fitness facilities, and in dance clubs.

The strong sedative effect of GHB and its related products also have some characteristics that are unlike other drugs used to commit DFSA. In particular, GHB, GBL, and 1,4-BD may cause the user to pass from a completely alert state to deep unconsciousness within 10–15 min after ingestion [61]. Additionally, GHB demonstrates an amnesic effect when someone is under its influence. GHB-assisted sleep generally lasts only 3–4 h, after which the user awakes feeling unusually refreshed [60]. This latter effect is likely due to GHB's rapid clearance from the body.

A bystander who sees an individual under the influence of GHB is likely to assume the individual has consumed too much alcohol. To a rapist, this is another attractive characteristic of these drugs, as witnesses may claim the victim was intoxicated; a factor that many juries weigh when deliberating on a DFSA case [80,101,128,144,145].

The popularity of GHB in DFSA is also due to the complications that arise because of the natural presence of GHB in the body. After ingestion, the rapid elimination of GHB results in only low exogenous concentrations remaining in the body. These low concentrations often cannot be readily differentiated from endogenous concentrations.

Exogenous GHB may only be detected for up to 8 h in blood and up to 12 h in urine following ingestion [11–14,50,68]. As is often the case in DFSA, victims may not report the crime or provide evidentiary samples until much later than this, thereby preventing toxicological detection of the drug.

Finally, because GHB, GBL, and 1,4-BD have become such popular recreational drugs, the rapist may not need to slip the drug into the victim's drink to incapacitate him or her. Many users voluntarily consume these products for their euphoric effects and then become victims of sexual assault. For example, in surveys of illicit GHB use in the United States and Australia, over half of the respondents reported some degree of unintentional loss of consciousness as a result of their GHB use [29,117].

It should be noted that the rapid biotransformation of GBL and 1,4-BD to GHB prevents their detection in most DFSA cases. In these cases, it has been suggested that the analytical toxicologist should focus only on finding GHB [46].

A number of DFSA cases involving GHB have been reported in the literature. For example, Stillwell reported on a GHB-linked DFSA of a 48-year-old female [160]. The victim reported unconsciousness for approximately 4 h. A urine sample was collected after the 4 h of unconsciousness. GHB was identified in the urine at concentration of 26.9 mg/L. Amitriptyline and nortriptyline were also identified in the sample.

In 2000, Couper and Logan reported on a sexual-assault case in which GHB was identified in the victim's blood at 3.2 mg/L [25]. In this case, a 38-year-old woman presented extremely confused, hyperactive, with slurred speech, and appeared to be hallucinating and having some convulsions. She thought she had been slipped a drug and raped. The blood was taken as part of a sexual-assault evidence-collection kit within 8 h of the incident. Other CNS depressants were also identified in the blood sample.

GHB was identified in the urine of a sexual-assault victim who reported attending a house party, consuming one 6-oz beer, beginning to feel ill, and then going into a bathroom. Her next recollection was of a male assaulting her [94].

In 2009, a case was reported that involved a 24-year-old female who was repeatedly dosed with GHB and morphine, prior to being sexually assaulted. The victim was unaware of the assaults until she later received homemade video clips of the crimes [129].

Another case that began as a potential sexual assault ended in the death of the teenage victim. The victim was slipped GHB in a can of soda and later became unconscious. Her attackers realized something was very wrong and took her to a hospital. The analysis of an antemortem blood and urine specimen collected at admission (about 6 h after GHB ingestion) showed GHB levels of 510 and 2,300 μg/mL, respectively. The girl died 14 h later. At autopsy the heart blood had a GHB concentration of 15 μg/mL. This level is consistent with levels seen in cases with postmortem GHB production with no exogenous GHB use. However, the case history and other specimens helped document the GHB toxicity with a postmortem urine level of 150 μg/mL and a vitreous humor GHB level of 128 μg/mL [112].

V. METHODS OF ANALYSIS

GHB can be directly analyzed following liquid-liquid or solid-phase extraction techniques. These extracts tend to be nonspecific and contain many other endogenous carboxylates and polar molecules.

One approach to overcome the polar, nonvolatile nature of GHB has involved acid conversion of GHB to its lactone form prior to extraction into an organic solvent. GBL is more amenable to conventional methods of extraction and instrumental analysis because it is

considerably less polar and more volatile than GHB, allowing for direct injection of the extract into a gas chromatograph (GC) or gas chromatograph-mass spectrometer (GC-MS) [32,149]. A drawback of this approach is that it increases column and injection port maintenance because the procedure does not include a back-extraction step to remove unwanted contaminants and interferences [149]. Further, if the strong mineral acid used to convert the GHB to GBL is accidentally carried into the injection solvent, the acid will cause rapid degradation of the GC column's stationary phase. Some authors have utilized headspace GC-FID or GC-MS after lactone conversion of GHB to overcome these pitfalls [91].

Another variation to this method incorporates the use of methane chemical ionization GC-MS for the identification of the lactonized GHB [55]. Chemical ionization mass spectrometry is a softer ionization technique that results in less fragmentation. This usually allows for improved method sensitivity compared to the more traditional electron impact ionization mass spectrometry; however, the low molecular weight of GBL (86.1 Da) prevents this technique from being as valuable as it is with higher-molecular-weight compounds.

To avoid the need to convert GHB into GBL for analysis, many authors have derivatized GHB to increase its thermal stability, improve its volatility, and increase its molecular weight. The most popular approach has involved use of trimethylsilyl (TMS) to silylate the hydroxyl and carboxylate moieties of GHB [25,27,37,63,113]. The advantage of such a procedure is that it allows for detection of higher-molecular-weight fragments that are more diagnostic of the target analyte.

One such method employed a copolymeric solid-phase extraction procedure and a solvent cleanup prior to derivatization with *N,*O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and 1% trimethylchlorosilane (TMCS) [84]. The derivative was analyzed with selected ion monitoring (SIM) GC-MS. A limitation of this procedure is that urea also forms a silyl derivative with some fragments common with GHB-diTMS. Furthermore, the monitored SIM ions are the $(M-15)^+$ ion of the GHB-diTMS (*m/z* 233) and two naturally occurring isotopes of the diTMS derivative (*m/z* 234 and 235). To overcome this, it has been suggested that chemical ionization GC-MS be employed to these extracts [82].

Another, more unique approach for the analysis of biological fluids for GHB involves headspace solid-phase microextraction (SPME) [9,55,58,116]. The use of SPME allows for cleaner samples to be introduced into the GC, thus avoiding some of the instrument maintenance problems discussed above.

More recently the use of gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS) has been explored to discriminate between endogenous and exogenous GHB concentrations [139,140]. Further, a direct analysis in real time (DART) ion source coupled to a time-of-flight mass spectrometer has been used to perform rapid quantitative analysis on urine specimens for GHB [79].

The use of liquid chromatography-mass spectrometry [167], capillary electrophoresis [13], and capillary electrophoresis-mass spectrometry [165] for the analysis of blood and urine for GHB has also been reported.

The analytical method used to measure GHB can have an effect on the quantitative results that are obtained. Elliott reported significant differences in the measured concentrations of GHB in the same urine specimens, using different quantitative analytical methods [40]. In some of the urine specimens, there was over a 10-fold difference between the measured concentrations in the same sample (e.g., 5 μg/mL vs. 82 μg/mL and 6 μg/mL vs. 75 μg/mL). The most consistent results were with those methods that used GC/MS techniques and a deuterated GHB analog as the internal standard [40].

VI. TOXICOLOGICAL INTERPRETATION OF GHB FINDINGS

The most difficult aspect of toxicological analyses of GHB is interpretation of the numbers that are generated. Helrich et al. correlated blood concentrations of GHB with state of consciousness in 16 adult human patients [70]. This study revealed that GHB blood concentrations as high as 99 mg/L could be achieved with the patient still displaying an "awake" state. A light sleep state was characterized by the subject spontaneously coming in and out of consciousness. Subjects in the medium sleep state were clearly asleep but were able to be roused. At the highest concentrations studied, GHB produced a deep sleep characterized by response to stimuli with a reflex movement only. It is clear from these data that blood concentrations of GHB display a large overlap across the four states of consciousness described. For example, a subject with a blood concentration of 250 mg/L could be in a light, medium, or deep sleep state. The smallest dose given, 50 mg/kg, produced peak plasma concentrations no greater than 182 mg/L and the largest dose given, 165 mg/kg, produced peak plasma concentrations greater than 416 mg/L. Fourteen patients received doses of 100 mg/kg resulting in peak blood concentrations ranging from 234–520 mg/L. Twelve of the 16 patients required intubation, but the need for intubation did not necessarily correlate to those patients that received the higher doses of GHB.

Metcalf et al. observed electroencephalographical (EEG) changes in 20 humans given oral doses of GHB in the range of 35–63 mg/kg [115]. The EEG pattern was similar to that seen in natural slow-wave sleep. Profound coma was observed at approximately 30–40 min post dose in subjects given oral GHB doses greater than 50 mg/kg.

There have been a number of published reports of urinary GHB concentrations following ingestion of GHB or one of its metabolic precursors. In one study, a driver was found asleep in his car and was unable to stand unassisted. He had a urinary GHB concentration of 1,975 mg/L approximately 2 h post ingestion [158]. Another study found GHB in the urine of two impaired drivers at concentrations of 1,086 and 1,041 mg/L, respectively [56].

A third case reported on three comatose emergency room patients that arrived simultaneously after ingesting GHB [35]. All three patients had Glascow Coma Scores (GCS) of 3 upon admission, but regained consciousness within 3.5 h of drug ingestion. The urinary GHB concentrations were 521, 1,857, and 141,000 mg/L 1 h after ingestion of ethanol and GHB. Noteworthy is that GHB was detected in only one of the patients serum at a concentration of 101 mg/mL. Five hours after the GHB was ingested, all three patients were discharged. Their GHB concentrations in urine at discharge were 286, 571, and 857 mg/L, respectively.

In another report, a female patient who ingested 4.5 g of a 1,4-BD product was taken to the hospital [169]. Upon arrival, the patient had a GCS of 11 and had a GHB concentration of 716 mg/L in her urine, while her serum contained 317 mg/L of GHB. Sometime later, the same patient was again hospitalized after consumption of more 1,4-BD. In this incident, the patient had to be intubated and received mechanical ventilation for 3 days. Her admission urine sample contained 5,140 mg/L of GHB.

Zvosec and colleagues also reported on a 22-year-old male that overdosed on a 1,4-BD product [169]. In this case, the patient had a GCS of 3, was intubated, and received mechanical ventilation. After 4 h, the patient was extubated and GHB was detected in his urine at a concentration of 415 mg/L.

In a report of 27 cases of nonfatal intoxication of GHB and GBL, urine concentrations of GHB ranged from 5–5,581 mg/L in these patients [41]. The mean GHB concentration in urine was 1,732 mg/L, compared to the mean of 245 mg/L for plasma.

It must be emphasized that most of the subjects in the above reports were still under the influence of GHB when their specimens were collected. This is not likely to be the case when dealing with instances of DFSA. In one such case, a 27-year-old female was invited to a male friend's home for dinner and to watch a movie. After dinner, she agreed to have a cocktail but did not remember any of the events that followed. She awoke a couple of hours later, confused and completely nude in the man's bed. She left his house and got immediate medical attention. Approximately 4 h after consuming the cocktail, she provided blood and urine specimens for testing. The results identified GHB in both the blood and urine specimens at concentrations of 47 and 308 mg/L, respectively [91].

In vitro production of GHB further complicates toxicological interpretation. Animal and human studies have demonstrated that endogenous GHB concentrations can rise postmortem and under inappropriate specimen storage conditions. A 2001 study found that endogenous GHB concentrations increased in urine samples, even when specimens were stored frozen at -20 ºC [89]. Kerrigan et al. reported *in vitro* production of GHB in urine specimens stored up to 8 months at 21, 4, and -20 ºC [81]. Significant *in vitro* production of GHB in urine was also observed in 31 urine samples repeatedly analyzed over a 189-day period [93].

Antemortem blood buffered with citrate (yellow top tube) has been shown to display an increase in GHB concentration over time [90]. Ten antemortem citrate buffered whole blood specimens were analyzed for GHB after various storage periods from 6 to 36 months at -20 ºC. Although no exogenous GHB use was suspected, all of the specimens had concentrations of GHB ranging from 4–13 mg/L with a mean of 9 mg/L.

Antemortem blood and urine endogenous levels of GHB have been documented to be less than 1 mg/L and less than 10 mg/L, respectively. Anderson and Kuwahara analyzed 50 antemortem blood specimens from individuals with no evidence of GHB use [3]. No detectable amounts of GHB were observed in any of the blood specimens, using a limit of detection of 0.5 mg/L. Similarly, endogenous GHB concentrations were measured in 192 blood specimens from living subjects thought to be non-GHB users [25]. All measurable blood GHB concentrations were below 1 mg/L, using a detection limit of 0.5 mg/L.

LeBeau and co-workers investigated urinary GHB levels to differentiate between endogenous and exogenous concentrations [88]. Every urine void produced by five non–GHB-using male subjects over a 1-week period was individually collected and analyzed for the presence of endogenous GHB, using a limit of detection of 0.19 mg/L. Overall, 129 urine specimens were analyzed and the mean endogenous GHB concentration detected was 1.59 ± 1.42 mg/L (range 0.00–6.63 mg/L). Urine specimens from three females were also analyzed with an endogenous

GHB range of 0.00–1.70 mg/ and a mean concentration of 0.31 ± 0.25 mg/L. While there were significant intra- and interindividual variations in the urinary levels of endogenous GHB, the concentrations did not fluctuate to levels that were higher than the laboratory's reporting level for urinary GHB of 10 mg/L.

A follow-up study examined endogenous concentrations of GHB in single urine samples collected from 207 individuals [92]. Endogenous urinary GHB concentrations in males (n = 130) ranged from 0.0–2.7 mg/L with a mean concentration of 0.27 mg/L, while females (n = 77) ranged from 0.00–0.98 mg/L with a mean concentration of 0.29 mg/L. This difference of endogenous GHB concentrations in urine between the sexes was determined to be insignificant.

Race was also evaluated in regard to endogenous GHB. Caucasians (n = 186) ranged from 0.00–2.70 mg/L, with an average endogenous GHB concentration of 0.28 mg/L and a median concentration of 0.22 mg/L. African Americans (n = 11) ranged from 0.00–0.52 mg/L with an average of 0.21 mg/L (median = 0.24 mg/L). Similarly, Hispanics (n = 7) ranged from 0.12–0.49 mg/L with an average concentration of 0.29 mg/L and a median concentration of 0.28 mg/L. Only two individuals of Asian ethnicity participated in the study; their endogenous GHB concentrations were 0.00 and 0.35 mg/L. These data suggest that race does not appear to have an effect on endogenous GHB concentrations. Other variables considered for their effect on endogenous GHB concentrations in urine included age, select medical conditions, and ingestion of medications. Other authors have considered the effects of drugs such as 5-fluorouracil [168] or chemicals such as tetrahydrofuran [17] on GHB concentrations.

The real problem this presents is in the interpretation of exogenous GHB use, GHB toxicity, or GHB overdose resulting in a fatality. Since GHB is rapidly cleared from the body, even at elevated doses, if there is any survival time, the blood concentrations can easily fall into the range of postmortem production. Therefore, a urine specimen should be collected in addition to blood in suspected GHB cases. In postmortem cases, if urine is not available, vitreous fluid or CSF is useful. GHB concentrations in postmortem eye fluid and urine from decedents with no exogenous GHB exposure have been documented to be less than 10 mg/L [111]. It is suggested that all collected specimens be preserved with at least 2% sodium fluoride and stored at refrigerator temperature or frozen and that citrate containing tubes be avoided. The cutoff concentration for reporting exogenous GHB consumption in a specimen must be set above the suspected postmortem production or antemortem endogenous GHB concentration.

Doherty et al. observed an increase in the GHB concentrations in brain specimens after 6 h with a further increase if the specimens were left at room temperature [32]. Snead et al. also observed an increase in GHB concentrations in CSF after 12 h of storage at room temperature [152]. It was subsequently discovered that if animals were killed using microwave irradiation, postmortem GHB accumulation was blocked [38]. This suggests some type of enzymatic conversion from a GHB precursor.

One source of the postmortem GHB increase is the metabolism of previously sequestered GABA that is being released from storage vesicles as the natural decomposition process occurs. Excess GABA would be exposed to the GABA transaminase enzyme, which could convert it to succinic semialdehyde that could in turn be converted to GHB in addition to proceeding on to succinic acid (Figure 3).

Another source of postmortem GHB production is 1,4-butanediamine (putrescine), a biogenic polyamine initially detected in decaying animal tissues, but now known to be present in all eukaryotic and prokaryotic cells, where it is important for cell proliferation and differentiation [114]. Research on polyamine metabolism by Seiler demonstrated the formation of GABA from putrescine both in visceral organs and in the CNS of vertebrates [147]. This is an enzymatic process in the polyamine metabolic pathway that involves diamine oxidase (DAO) and aldehyde dehydrogenase to form GABA. In addition, Snead et al. observed an 80–100% increase in GHB concentrations in rat brain after intracerebroventricular administration of putrescine [154]. All of these theories are consistent with the observation that microwave irradiation prevents postmortem accumulation of GHB as the radiation denatures the enzymes. This is also supported by the fact that excessive GHB production is not seen in blood specimens that have an enzyme inhibitor added [159].

Regardless of the source of the increased concentration of GHB postmortem, it can be a significant problem in determination of a cause of death due to GHB toxicity. Postmortem production of GHB can result in blood concentrations of GHB that would produce significant effects in a living person. Anderson and Kuwahara analyzed heart blood, femoral blood, and urine from 96 postmortem cases with no suspected exogenous GHB use and 50 antemortem blood specimens also with no evidence of GHB use [3]. The specimens were stored at 4 ºC with sodium fluoride added to the blood as a preservative. They obtained the following results in the postmortem specimens: heart blood 1.6–36 mg/L, femoral blood 1.7–48 mg/L, and urine 0–14 mg/L and no detectable amount of GHB in any of the antemortem blood specimens with

a limit of detection of 0.5 mg/L. The upper end of the postmortem blood range overlaps the range detected in blood during therapeutic application of a 25 mg/kg per day dose of GHB, 24–88 mg/L. The minor difference between GHB concentrations in heart and femoral blood suggests more than a postmortem redistribution issue.

CONCLUSIONS

GHB and its prodrugs, GBL and 1,4-BD, have become popular recreational drugs of abuse. As such, there has been an increase in the number of reports of GHB-facilitated sexual assaults. This is a critical observation given that GHB and its analogs are among the most challenging DFSA drugs to detect, given their rapid elimination from the body and GHB's endogenous nature in biological samples. Their primary clinical effect of strong CNS depression may lead to memory impairment and unconsciousness, making it difficult, if not impossible, for victims to defend themselves from their attacker or remember who the attacker(s) may have been. Added difficulties for interpretation of positive GHB findings include *in vitro* production of GHB in blood and urine specimens.

The real problem this presents is in the interpretation of exogenous GHB use, GHB toxicity, or GHB overdose resulting in a fatality. Because GHB is rapidly cleared from the body, even at elevated doses, if there is enough time between drugging and specimen collection (or any survival time in a postmortem case) then the blood concentrations can easily fall into the range of normal endogenous concentration or postmortem production. Therefore, a urine specimen should be collected in addition to blood in suspected GHB cases. When urine is not available in postmortem cases, eye fluid or CSF is indicated. It is advised that all specimens be preserved with at least 2% sodium fluoride and stored at refrigerator temperature or frozen. Citrate-containing tubes must be avoided. The cutoff concentration for reporting exogenous GHB consumption in a specimen must be set above the suspected postmortem production or antemortem endogenous GHB concentration.

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Laureen Marinetti received her B.S. (1983) in forensic science and her M.S. (1991) in criminal justice with a concentration in forensic science under Professor Jay Siegel at Michigan State University (East Lansing, MI). She then completed her Ph.D. at Wayne State University (Detroit, MI) in pharmaceutical sciences with a concentration in physiology under Dr. Randall Commissaris's supervision. Dr. Marinetti is currently the chief forensic toxicologist of the Montgomery County Coroner's Office and Miami Valley Regional Crime Laboratory (Dayton, OH), overseeing the daily operation of a full-service analytical toxicology laboratory that processes approximately 2,500 toxicology test requests per year in both postmortem forensic toxicology and human performance forensic toxicology.

While at Michigan State, Dr. Marinetti did a graduate assistantship that included teaching undergraduate forensic science students both in analytical methods and theory. In 1987, she began her career with the Michigan State Police Crime Laboratory in the Toxicology Subunit. There she worked in forensic toxicology in the areas of human performance and postmortem drug testing as well as method development and court testimony. In 1998, Dr. Marinetti took a deferred retirement from the Michigan State Police to pursue her doctorate at Wayne State in the Department of Pharmaceutical Sciences in the College of Pharmacy and Allied Health Profession. Also in 1998, Dr. Marinetti was awarded a Forensic Toxicology Fellowship from the Wayne County Medical Examiner's Office (Detroit, MI), working in the board-certified postmortem forensic toxicology laboratory. Under the guidance of Dr. Bradford Hepler and Dr. Dan Isenschmid, she consulted and apprenticed in postmortem forensic toxicology in the areas of method development, interpretation, quality assurance, and drug-abuse demographics.

Dr. Marinetti's research interests include all aspects of gamma hydroxybutyric acid (GHB) and its pharmacological analogs, gammabutyrolactone (GBL), gammavalerolactone (GVL), and 1,4-butanediol(1,4-BD). She has made several presentations on various toxicology topics concerning both postmortem and human performance toxicology and drug-facilitated sexual assault. Dr. Marinetti also has several publications in peer-reviewed journals and textbooks in the field of toxicology.

Dr. Marinetti is a member of the Society of Forensic Toxicologists (SOFT) and of SOFT's Drug-Facilitated Sexual Assault Committee. She is also a fellow of the Toxicology Section of the American Academy of Forensic Sciences (AAFS) and past president of the Midwestern Association of Toxicology and Therapeutic Drug Monitoring as well as a member of the International Association of Forensic Toxicologists and the California Association of Toxicologists. In 2007, Dr. Marinetti fulfilled the requirements to achieve diplomate status with the American Board of Forensic Toxicology. She has been qualified as an expert witness in analytical and interpretive forensic toxicology and pharmacology in excess of 175 times in federal, district, municipal, common pleas, and circuit courts in Michigan, Ohio, Missouri, and Florida.

In 1999 and 2000, Dr. Marinetti received the Substance Abuse Educator Award from Wayne State for her presentations to various organizations on GHB and its analogs. Additionally, she was awarded the Education Research Award by SOFT for her research on GHB and GVL in both 2000 and 2002. Also in 2002, she received a certificate of appreciation from the Drug Enforcement Administration and the Irving Sunshine Young Scientist Award from the Toxicology Section of the AAFS in 2005.

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In 2004, Dr. LeBeau won the FBI Director's Award for Outstanding Scientific Advancement and in 2008 he was the recipient of the End Violence Against Women (EVAW) International Visionary Award.

The Use of "Z-Drugs" to Facilitate Sexual Assault

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The Use of "Z-Drugs" to Facilitate Sexual Assault

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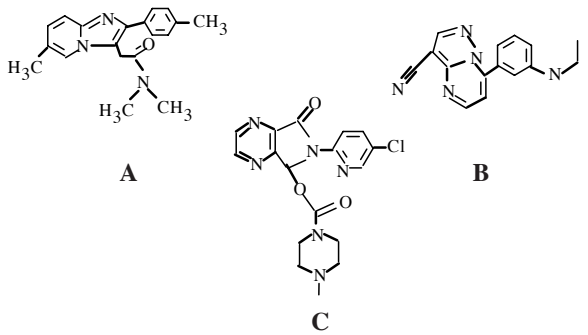
ABSTRACT: Zolpidem, zopiclone, and zaleplon are commonly referred to as the "Z-drugs." The Z-drugs are non-benzodiazepine hypnotics indicated for the short-term treatment of insomnia. Since becoming widely prescribed as sleep aids in the United States, they are increasingly being detected in a variety of forensic specimens. We present a comprehensive overview of the basic chemistry, pharmacodynamics, and pharmacokinetics of zolpidem, zopiclone, and zaleplon, including their interaction with other prescription drugs and ethanol, findings in drug-facilitated sexual assault (DFSA) casework, and methods of analysis.

KEY WORDS: Drug-facilitated sexual assault, zaleplon, zolpidem, zopiclone.

INTRODUCTION

Zolpidem (Ambien®, Stilnox, Zolnod), zopiclone (Imovane®, Zimovane) or eszopiclone (Lunesta®), and zaleplon (Sonata®, Starnoc) (**Structure 1**) are relatively new non-benzodiazepine sedative/hypnotics used for short-term treatment of insomnia. They are commonly referred to as the "Z-drugs." These agents may be preferred over benzodiazepines because they are as efficacious but are less likely to cause significant rebound insomnia and tolerance. Due to the pharmacodynamic and pharmacokinetic properties of these drugs, they are being encountered more frequently in a variety of forensic cases, especially drug-facilitated sexual assault (DFSA).

Zolpidem is currently the most widely prescribed sleep aid in the United States [30]. Zolpidem was introduced for clinical use in Europe in 1986 and has been in use in the United States since 1993 [61]. It is available in 5- and 10-mg tablets. Ambien CR® (zolpidem tartrate extended release) is available in 6.25- and 12.5-mg tablets. In December 2008, the Food and Drug Administration (FDA) approved Zolpimist™, a 5- and 10-mg oral spray of zolpidem. In March 2009, the FDA approved 5- and 10-mg sublingual tablets of zolpidem (Edluar®; formerly known as Sublinox®).



Structure 1. Chemical structures of zolpidem (**A**), zaleplon (**B**), and zopiclone (**C**).

Zopiclone has been used clinically as a hypnotic agent since 1994 [2]. Racemic zopiclone is available in 3.75- and 7.5-mg tablets. Eszopiclone is the "S" isomer of racemic zopiclone. Eszopiclone is available in 1-, 2-, and 3-mg tablets.

The FDA approved Sonata® (zaleplon) in 1999 for the short-term treatment of insomnia in adults [81]. Zaleplon is available in 5- and 10-mg tablets.

I. CHEMISTRY

A. Zolpidem

Zolpidem (Structure 1A) is an imidazopyridine derivative with the chemical name *N,N*-6-trimethyl-2-(4-methylphenyl)imidazo[1,2-]pyridine-3-acetamide [48]. The free base has a molecular weight of 307.4, with the salt form, zolpidem hemitartrate, having a formula weight of 764.9. The salt has an appearance of a white to off-white powder, with a melting point from 193–197 ºC. The tartrate salt is slightly soluble in water (23 g/L at 20 ºC), sparingly soluble in methanol, and practically insoluble in methylene chloride. Zolpidem tartrate has a pKa of 6.2 and its octanol/water coefficient is 3.85. The ultraviolet spectrum of zolpidem in 0.1 N HCl exhibits a lambda max at 294 nm (A1/1 = 564) [59].

B. Zopiclone/Eszopiclone

Zopiclone (Structure 1B) is a racemic mixture of two stereoisomers. Eszolpiclone has a single chiral center with the (*S*)-configuration [43].

Zopiclone is a cyclopyrrolone derivative with the chemical name 4-methyl-1-piperazine-carboxylic acid 6-(5-chloro-2-pyridnyl)-6,7-dihydro-7-oxo-5H-pyrrolo[3,4-b]pyrazin-5-yl-ester [48]. It is a white or slightly yellow powder in appearance, with a melting point of 178 ºC and

a molecular weight of 388.8. Zopiclone is practically insoluble in water and alcohol, sparingly soluble in acetone, and freely soluble in methylene chloride. The ultraviolet spectrum of zolpiclone in 0.1 N HCl exhibits a lambda max at 303 nm.

C. Zaleplon

Zaleplon (Structure 1C) is a pyrazolopyrimidine derivative with the chemical formula $C_{17}H_{15}N_5O$ and chemical name *N*-[3-(3-cyanopyrazolo[1,5-a]pyrimidin-7-yl)phenyl]-*N*-ethylacetamide. The molecular weight of zaleplon is 305.3. Its solid state is a white to off-white powder that is practically insoluble in water and sparingly soluble in alcohol or propylene glycol. Its partition coefficient in octanol/water is constant (log PC = 1.23) over the pH range of 1–7 [52].

II. PHARMACOLOGY

Zolpidem, zopiclone, and zaleplon are non-benzodiazepine hypnotics used for the short-term treatment of insomnia. They share similar pharmacodynamic and pharmacokinetic properties and are similar to the benzodiazepines in some regards. However, due to variation in binding to the γ-aminobutyric acid (GABA) receptor subunits, these three drugs show subtle differences in their effect on sleep stages and as antiepileptics, anxiolytics, and amnesiacs.

Zolpidem is a $GABA_A$ receptor agonist and shares some of the pharmacological properties of benzodiazepines. The major modulatory site of the $GABA_A$ receptor complex is located on its alpha (α) subunit and is referred to as the benzodiazepine or omega (ω) receptor site. At least three subtypes of the receptor have been identified. In contrast to the benzodiazepines, which nonselectively bind to and activate all ω receptors, zolpidem preferentially binds to the benzodiazepine ω_1 receptor site [67]. This subtype is thought to be the one most associated with sleep [62]. Although selective binding of zolpidem on the ω_1 receptor is not absolute, it may explain the relative absence of myorelaxant and anticonvulsant properties.

The precise mechanism of action of eszopiclone as a hypnotic is unknown. However, it is believed to result from its interaction with GABA-receptor complexes located close to or coupled to benzodiazepine receptors [67]. Chouinard et al. reported that zopiclone binds to specific benzodiazepine receptor subtypes with greater affinity for ω_1 than for ω_2 receptors [6].

Zaleplon also selectively binds to the benzodiazepine ω_1 site on the ω_2 receptor subunit located on the $GABA_A$ complex to produce its therapeutic sedative properties [34]. It has lower affinity for the α_2 and α_3 subtypes. It selectively enhances the action of GABA similar to benzodiazepines.

A. Administration

1. Zolpidem

The recommended dose of zolpidem is 10 mg in adults, decreasing to 5 mg in elderly or debilitated patients and those with liver failure [41]. Zolpimist™ (zolpidem tartrate) offers the potential benefit of a faster rise in drug blood concentrations, potentially leading to a faster onset of action. In a clinical trial, assessing the relationship between speed of absorption and attainment of therapeutic drug concentrations showed that 79% of the subjects using 10-mg Zolpimist™ reached therapeutic concentra°fl-tions at 15 min post dosing, while 26% of the subjects using 10-mg tablets reached therapeutic concentrations at that time point. An additional study found that 5-mg dosing in elderly patients demonstrated similar results (65% vs. 19%) [80].

2. Zopiclone/Eszopiclon

The recommended dose of zopiclone is 7.5 mg in adults, which should be decreased to 3.75 mg in elderly patients and those with chronic respiratory failure or impaired renal or liver function [15]. The dosing for eszopiclone is 1–3 mg [23, 52]. It is recommended that the starting dose be 2 mg for non-elderly patients immediately before bedtime. Dosing can be initiated at or increased to 3 mg if clinically indicated, since this dose is more effective for sleep maintenance. A 1-mg starting dose is recommended for elderly patients and/or those with severe hepatic impairment.

3. Zaleplon

The recommended dose of zaleplon for non-elderly patients is 10 mg, although for lower weight individuals 5 mg may be sufficient. Elderly patients appear more sensitive to effects of hypnotics so the recommended dose for that patient population is 5 mg [52]. Twenty mg of zaleplon is comparable to 10 mg of zolpidem [20].

B. Pharmacodynamics

The Z-drugs all share a short half-life and duration of action as compared to the benzodiazepines. Drover compared their pharmacokinetics and pharmacodynamics [9]. The author concluded that zolpidem and zopiclone have a more delayed elimination than zaleplon, so may have prolonged drug effects, resulting in residual sedation and side effects.

1. Zolpidem

Zolpidem reduces the time to onset and increases the duration of sleep in healthy volunteers when administered in doses as low as 5–7.5 mg at bedtime. Dose increases tended to occur early in therapy (within 30 days) and were usually the result of initial poor efficacy [52]. The efficacy of static dosages of zolpidem appeared to increase during the first 4 weeks of therapy. Zolpidem given at the recommended dose does not appear to cause rebound insomnia or other withdrawal reactions after abrupt discontinuation of administration. Zolpidem does not possess significant anticonvulsant or myorelaxant properties [62]. Several clinical trials conducted to assess the efficacy of zolpidem failed to demonstrate tolerance after multiple dosing [60]. However, studies conducted with nonhuman primates did suggest tolerance. Ware et al. found in a clinical trial comparing zolpidem and triazolam that tolerance to sedation may develop [79].

Individuals taking zolpidem may experience amnesia shortly after ingestion and into their sleep cycle. Canaday reported that two patients experienced amnesia after taking zolpidem; they could not recall telephone conversations that took place within an hour of taking the drug [4]. However, Frattola et al. reported no amnesia in patients taking zolpidem for bedtime sedation [14]; however, this inference appears to be based on patient assessments made in the morning on awaking, not immediately after ingestion [49]. Healthy volunteers did not appear to experience amnesia. They were tested the morning after dosing, by being asked to recall events occurring only 10 min after a single 10-mg dose of zolpidem. Praplan-Pahud reported that 15% of patients experienced anterograde amnesia after zolpidem administration [55]. Other studies suggest that the anterograde amnesia is dose-related [51,64]. One study found that anterograde amnesia was associated with 20-mg zolpidem doses and not 10-mg doses when given as an oral pre-anesthetic medication [5]. The post-ingestion sedation induced by zolpidem correlates with the coincident anterograde amnesia effects [28].

A limited number of case reports have suggested zolpidem-associated hallucinations. The precise mechanism of zolpidem-associated hallucinations is unknown; however, it may be related to serotonin reuptake inhibition. Elko reported on five patients who experienced visual hallucinations, lasting 1–7 h, shortly after taking zolpidem [12].

Other side effects include indigestion, headache, nausea, and allergy [52]. Less common effects (e.g., lack of bladder control, lack of coordination, ringing inside the ears, inflammation of the sinus, speech problems, lethargy, light-headedness, loss of appetite, abdominal pain, vision problems, nightmares, amnesia, anxiety, arthritis, sensitivity to light, sleepwalking, and speech difficulties) have also been reported. Zolpidem did not appear to produce adverse respiratory effects in patients with obstructive sleep apnea or chronic obstructive pulmonary disease [17].

2. Zopiclone/Eszopiclone

Zopiclone shares many of the same pharmacological properties with the benzodiazepines and the other Z-drugs. It can induce hypnotic, tranquilizing, anticonvulsant, and sedative effects; however, it has a lesser effect on memory functions [67]. It also shares some characteristics with barbiturates, such as anticonvulsant, myorelaxant, and anoxiolytic properties [31].

3. Zaleplon

Zaleplon is used for the short-term treatment of insomnia where difficulty in falling asleep is the primary problem. Unlike many other hypnotic drugs, zaleplon does not interfere with sleep architecture and can be administered up to 5 weeks without the risk of dependence or rebound insomnia when discontinued [8,77]. It is also effective in the treatment of middle-of-the-night insomnia without causing residual hangover effects [71,77]. It has been shown to be active in a number of anticonvulsant models, and studies in anxiolytic models suggest weak anxiolytic activity. It possesses a reduced risk of tolerance compared to triazolam, is less likely to potentiate the effects of ethanol, and is unlikely to produce amnesic effects [27].

As expected, sedation and psychomotor function impairment were experienced when zaleplon was administered at single fixed doses of 10 mg and 20 mg at 1 h post dosing, the time of peak exposure for both doses. Consistent with the rapid clearance of zaleplon, impairment of psychomotor function was no longer present as early as 2 h post dosing in one study and none of the studies after 3–4 h. Short-term memory impairment followed a similar time sequence with the tendency of this effect greater after 20 mg [34]. Since zaleplon has a rapid onset of action, it should be taken immediately prior to going to bed or after going to bed and experiencing difficulty falling asleep. Caution should be exercised while operating a motor vehicle or machinery.

Two clinical findings reported to occur after several weeks of nightly use of a rapidly eliminated sedative/hypnotic are increased wakefulness during the last quarter of the night and the appearance of increased signs of daytime anxiety. When studied, wakefulness was not significantly longer with zaleplon than with placebo during the last quarter of the night on nights 20 and 30 in a 35-

night sleep study. Similarly, no daytime anxiety was reported. Rebound insomnia may be dose-dependent, showing some objective and subjective evidence after 20 mg but not at 5 mg or 10 mg. At all doses, the rebound effect appeared to resolve by the second night after discontinuation. There were no instances of withdrawal delirium, withdrawal-associated hallucinations, or any other manifestations of severe sedative/hypnotic withdrawal [34].

Stillwell reported a case of impaired driving and zaleplon use [66]. The symptoms reported were those of central nervous system (CNS) depression and included slow movements and reactions, poor coordination, and lack of balance. The zaleplon concentration was determined to be 0.13 ug/mL. He concluded that blood concentrations with doses exceeding therapeutic concentrations have the potential to cause impairment of psychomotor function and driving ability.

"Sleep driving," described as driving while not fully awake after ingestion of a sedative/hypnotic, with amnesia for the event has been reported with zaleplon alone at therapeutic doses. These events can occur in naïve as well as experienced users. The use of ethanol or other CNS depressants appears to increase the risk of such behaviors, as does use of dosages beyond the maximum recommended dose.

Zaleplon has an abuse potential similar to benzodiazepines and benzodiazepine-like hypnotics [34]. Other than a mild rebound insomnia, which resolved by the second night, zaleplon does not seem to produce a physical dependence and a subsequent withdrawal syndrome [34].

Other adverse effects reported as frequent by the manufacturer include back pain, chest pain, fever, migraine, constipation, dry mouth, anxiety, depression, nervousness, difficulty concentrating, bronchitis, rash, conjunctivitis, and taste perversion.

Zolpidem and zaleplon have been studied to determine their effects on driving ability, memory, and psychomotor performance [72]. Memory and psychomotor test performance was unaffected after both doses of zaleplon and zolpidem (10 mg). In contrast, zolpidem (20 mg) did impair performance on psychomotor and memory tests. The authors concluded that zaleplon (10 and 20 mg) is a safe hypnotic devoid of next-morning residual impairment when used in the middle of the night.

Drover el al. [10] compared the pharmacokinetic and pharmacodynamic profiles of zaleplon with those of zolpidem. The pharmacodynamic characteristics measured sedation, mood, mental and motor speed, and recent and remote recall. Zaleplon was eliminated more rapidly, produced no memory loss, and caused less sedation than zolpidem at the same doses. The sedation scores of the zaleplon groups returned to baseline in less time than those of the zolpidem groups (4 vs. 8 h; $P < 0.05$). Zaleplon had no effect on recent or remote recall, whereas zolpidem had a significant effect on both measures ($P < 0.05$) [10].

Vermeeren et al. [70] compared residual effects of zaleplon (10 mg), zopiclone (7.5 mg), and placebo to a social dose of ethanol on car driving, memory, and psychomotor performance. Tests included a highway driving test, word learning, critical tracking, and divided attention, as well as subjective assessments of sleep, mood, and effects of treatments on driving. Plasma concentrations of 0.03 g% ethanol-impaired performance on all tests. Zaleplon's residual effects did not differ significantly from those of placebo in any test. In contrast, zopiclone had significant effects on driving, divided attention, and memory. The magnitude of impairment in the driving test observed the morning after zopiclone 7.5 mg was twice that observed with ethanol.

C. Pharmacokinetics

1. Zolpidem

Zolpidem has a bioavailability of approximately 70%, has a short elimination half-life ($t_{1/2}$), and is rapidly absorbed, usually within 20–40 min. Zolpidem has a limited volume of distribution, approximately 0.54 to 0.68 L/kg [48]. A single-dose crossover study in which 45 healthy subjects received 5- and 10-mg zolpidem tartrate tablets, determined the mean zolpidem Cp_{max} to be 59 ng/mL (29–113 ng/mL) and 121 ng/mL (58–272 ng/mL), respectively, occurring at a mean time (t_{max}) of 1.6 h for both doses [63]. The mean $t_{1/2}$ was 2.6 h (1.4–4.5) and 2.5 h (1.4–3.8) for the 5- and 10-mg tablets, respectively [48, 63].

The effect of food on the pharmacokinetics of zolpidem was studied in 30 healthy male volunteers. The authors compared the pharmacokinetics of a 10-mg zolpidem dose when administered while fasting or 20 min after a meal. The results demonstrated that with food, mean AUC and Cp_{max} were decreased by 15% and 25%, respectively, while mean t_{max} was prolonged by 60% (from 1.4–2.2 h). The $t_{1/2}$ of zolpidem remained unchanged.

Zolpidem is extensively metabolized by the liver into inactive compounds with a significant first-pass effect and less than 1% unchanged drug is detected in urine samples [1,48]. Zolpidem biotransformation is mediated by cytochrome P450 (CYP), with CYP3A4 being a predominant isozyme [53]. There is evidence to suggest a contributory role of CYP1A2, 2C9, 2C19, and 2D6, but limited, if any, involvement of CYP2A6, 2B6, 2E1, and 2C8 [74,75]. Potential inhibitors of these isozymes could decrease the rate of zolpidem metabolism, if administered

concurrently, while potential inducers could increase the rate of metabolism. Zolpidem metabolites are pharmacologically inactive, and are mainly excreted through renal and fecal elimination. The primary metabolites have been identified [39,53] and are a result of oxidation of each of the three methyl groups and the imidazopyridine ring of zolpidem.

Parent zolpidem can be detected following a single-dose (10 mg) administration. Urine and hair were collected from three volunteers who were administered 10 mg of zolpidem tartrate [73]. Urine samples were collected every 12 h. Zolpidem was detected for up to 60 h, with peak concentrations (ranging from approximately 5 to 25 ng/mL) reached at 12 h. After 36 h, zolpidem concentrations were found to be less than 2 ng/mL. Hair samples were also collected 3–5 weeks post dosing. Zolpidem was detected in the proximal (root) end of the hair. The concentrations ranged from 1.8–9.8 pg/mg.

2. Zopiclone/Eszopiclon

The pharmacokinetics of zopiclone has been studied in healthy subjects (adult and elderly) and patients with hepatic or renal disease. The pharmacology of zopiclone is characterized by a short $t_{1/2}$ (4–5 h) and high bioavailability (80%) [67]. Zopiclone is extensively metabolized by CYP 3A4 and CYP 2E1 via oxidation and demethylation. The predominant metabolites are the inactive *N*-dimethyl metabolite and the N-oxide metabolite, which demonstrates some activity, albeit less than the parent drug. A considerable amount of these metabolites are excreted in the urine, and about 50% of an oral dose is decarboxylated and excreted via the lungs [6].

Eszopiclone is rapidly absorbed, with a time to peak (t_{max}) of approximately 1 h and a half-life of approximately 6 h [52]. The primary metabolites of eszopiclone are (S)-zopiclone-*N*-oxide and (S)-*N*-desmethylzopiclone.

3. Zaleplon

Zaleplon is rapidly and almost completely absorbed following oral administration with peak plasma concentrations achieved within approximately 1 h. Although well absorbed, its bioavailability is approximately 30% because it undergoes significant presystemic metabolism [34]. Greenblatt et al. studied the relationship of dose, plasma concentration, and time to the effects of zaleplon and zolpidem after single oral doses [20]. Kinetics of each was not significantly related to dose. Zaleplon has a more rapid elimination (apparent elimination half-life ($t_{1/2}$ of 1 h) and higher apparent oral clearance (approximately 4,300 mL/min) than zolpidem ($t_{1/2}$ of 2.0–2.5 h; apparent oral clearance 340–380 mL/min). There are fewer residual side effects after taking a single dose at bedtime. By comparison, zolpidem and zopiclone have a more delayed elimination, so there may be a prolonged drug effect. This can result in residual sedation and side effects but be useful for sustained treatment of insomnia with less waking during the night.

Zaleplon is a lipophilic compound with a volume of distribution of approximately 1.4 L/kg following intravenous administration, indicating substantial distribution into extravascular tissues. The in vitro plasma protein binding is approximately 60±15% and is independent of zaleplon concentration over the range of 10–1,000 ng/mL. This suggests that zaleplon disposition should not be sensitive to alterations in protein binding. The blood-to-plasma ratio for zaleplon is approximately 1:1, indicating that it is uniformly distributed throughout the blood with no extensive distribution into red blood cells [34].

Zaleplon is primarily metabolized by aldehyde oxidase into 5-oxo-zaleplon and to a lesser extent by cytochrome CYP3A4 to form desethylzaleplon, which is probably quickly converted by aldehyde oxidase to 5-oxo-desethylzaleplon. These metabolites are converted to glucuronides and eliminated with less than 1% of unchanged drug intact in urine. All of zaleplon's metabolites are pharmacologically inactive [34]. After either oral or IV administration, zaleplon is rapidly eliminated with a mean $t_{1/2}$ of approximately 1 h. The oral-dose plasma clearance of zaleplon is about 3 L/h/kg and the IV zaleplon plasma clearance is approximately 1 L/h/kg [34].

D. Drug-Drug Interactions

Hesse et al. found the addition of ethanol to treatment with the Z-drugs resulted in additive sedative effects without altering the pharmacokinetic parameters of the drugs [26]. Zolpidem, like other sedative drugs, has a CNS-depressant effect and caution should be used when administered with other CNS depressants. An additive effect on psychomotor performance between alcohol and zolpidem has been demonstrated [52].

Clinical trials have studied potential interactions of the Z-drugs with the following type drugs: cytochrome P450 inducers (rifampicin), CYP inhibitors (azoles, ritonavir, and erythromycin), histamine H_2 receptor antagonists (cimetidine and ranitidine), antidepressants, antipsychotics, antagonists of benzodiazepines, and drugs causing sedation. Rifampicin significantly induced the metabolism of the Z-drugs and decreased their sedative effects, indicating that a dose increase of these agents may be necessary when they are administered with rifampicin. Ketoconzole, erythromycin, and cimetidine inhibited the

metabolism of the Z-drugs and enhanced their sedative effects, suggesting that a dose reduction may be required. Coadministration of eszopiclone (3 mg) to subjects receiving ketoconazole (400 mg), a potent inhibitor of CYP3A4, resulted in a 2.2-fold increase in exposure to eszopiclone [43].

III. DRUG-FACILITATED SEXUAL ASSAULT

The Z-drugs are efficacious agents for DFSA due to their ubiquitous availability, low dose, and various dosage forms facilitating clandestine administration. Their basic pharmacology — such as rapid onset of action, effectiveness to induce and maintain sleep/unconsciousness, and amnesic properties — also makes them efficacious DFSA agents. Goulle et al. have shown that anterograde amnesia clearly occurs with many benzodiazepines and with hypnotic drugs structurally unrelated to the benzodiazepines but sharing some of their properties, such as zolpidem [19]. Anterograde amnesia has been demonstrated to be dose-dependent, and the effect is associated with hypnotic drugs; however, the receptors are different. Zolpidem was found to be the most frequently encountered compound in drug-facilitated crimes investigated by the *Institut de Medicine Legale*, Strasbourg, France. Villain et al. report two cases of zolpidem-facilitated sexual assault [73]. Their first case involved a young female patient who was admitted to the hospital for "gastric disorders." She was offered a cup of coffee by a male nurse and became unconscious. Upon awakening, she sensed that she had been sexually assaulted; however, she did not initially make a report. Approximately 6 days post assault, she reported the incident and hair was subsequently collected 15 days after the alleged assault. The proximal 2-cm portion of hair was tested by LC/MS/MS and was found to contain 4.4 pg/mg of zolpidem. Two other distal segments were tested and zolpidem was not detected. The second case involved a 37-year-old female who was assaulted by a friend, following a drink. A blood sample was collected 9 h post assault and was found to contain 390 ng/mL of zolpidem. A hair sample was collected 4 weeks post assault and a segmental analysis, root to 8 cm, revealed concentrations of 22, 47, 67, and 9 pg/mg, respectively. These results documented prior exposure to or use of the drug. The victim did not challenge the result when interviewed.

The use of chemical agents such as the Z-drugs has also been found in the abuse of the elderly. Hair is a valuable specimen especially in the instance of delayed reporting [35]. Kintz et al. reported a case in which a 12-year-old victim was repeatedly sexually assaulted by a male partner of her babysitter [36]. She had no clear memory of the assaults; however, she did indicate "severe mental confusion" after each occurrence. A scene investigation revealed numerous boxes of zolpidem at the babysitter's home. Hair was collected approximately 2 weeks after the last alleged assault. Toxicological analysis revealed the presence of zolpidem at a concentration of 6.7 ng/mg. The suspect did not challenge the results once confronted.

The authors are not aware of any published reports of DFSA involving zopiclone/eszopiclone or zaleplon.

IV. METHODS OF ANALYSIS

There are a number of analytical tools available to detect targeted compounds used in DFSA cases. The immunologic assays will target specific compounds, which may or may not include metabolites. Often the specimen is collected hours or days post dosing and thus the assay should focus on metabolites to detect drug exposure. The challenge for toxicologists is that in many instances an analytical standard for the metabolite does not exist.

A. Extraction Techniques

The Z-drugs are easily extracted from biological matrixes by using either liquid-liquid extraction or solid-phase extraction techniques. Keller et al. described a simple extraction technique of zolpidem from alkalinized body fluids with ethyl acetate [32]. Meeker et al. extracted zolpidem from body fluids and tissues with chlorobutane containing 1.5% isopentanyl alcohol [46]. Villain et al. extracted zolpidem from hair utilizing methylene chloride/diethyl ether (80:20) [73]. Hempel and Blaschke extracted zopiclone from alkalinized urine with chloroform/isopropanol (9:1) [25]. Gaillard et al. utilized a C-18 solid-phase extraction column to extract zopiclone from plasma, eluting with methanol-chloroform (9:1) [15]. Gupta also used a C-18 solid-phase column to extract zopiclone and two major metabolites, eluting the compounds with an acidified methanol [22]. Zhang et al. validated an LC/MS method that extracted zaleplon from alkalinized plasma with ethyl acetate [82].

B. Immunoassays

In 1997, the lack of cross-reactivity of zolpidem with drugs in standard drug screens (Syva EMIT II or Abbott ADx) was reported by Piergies et al. [54]. They concluded that zolpidem does not cross-react with either the EMIT or ADx assays for benzodiazepines, opiates, barbiturates, cocaine, cannabinoids, or amphetamines.

In the same year, De Clerck and Daenens developed a specific and sensitive radioimmunoassay (RIA) for zolpidem and its metabolites in urine and serum, using rabbits as the test species [7]. The limit of quantitation of their assay was 0.1 mg/L. Using the assay, drug concentrations in urine following a single oral ingestion of the drug reached C_{max} at 3 h after dosing (994 mg/L), and could still be easily detected in the urine after 48 h (6.3 mg/L). In serum, the highest concentration was detected 2 h after dosing (122 mg/L), decreasing to 1.2 mg/L after 48 h.

In 2004, a direct ELISA microplate assay for zolpidem became commercially available [83]. The assay can be used with human serum, whole blood, or urine. Following a single oral dose of zolpidem (5 mg), positive results were obtained from urine samples 8 h after dosing, at levels above the high calibrator of 25 ng/mL.

In 2008, Reidy et al. evaluated the use of the commercially available ELISA kit and GC/MS to screen blood and urine specimens for zolpidem [57]. As part of this study it was determined that the ELISA kit demonstrated no cross-reactivity to zaleplon or zopiclone at a spiked urine concentration of 1,000 ng/mL.

Mannaert and Daenens have described a radioimmunoassay for the determination of *N*-desmethylzopiclone [44]. These authors have also described a fluorescence polarization immunoassay for this zopiclone metabolite [45]. However, immunoassay reagents for the detection of zopiclone or its metabolites are not yet commercially available.

The authors are unaware of any commercially available immunoassays for the detection of zaleplon or its metabolites.

C. Gas Chromatography

In 1995, Meeker et al. analyzed postmortem specimens for zolpidem using both GC/NPD and GC/MS technology [46]. The following year, Stanke et al. published a method for the determination of zolpidem and zopiclone in plasma using GC/NPD [65]. The detection limit for zolpidem was 1 ng/mL and the method was reproducible over a wide concentration range.

Lichtenwalner and Tully used a GC/MS method to determine zolpidem in a postmortem case [42]. Quantitative analysis by GC/MS determined zolpidem in blood and urine, as well as a total of 7 mg unabsorbed zolpidem in the gastric contents.

GC/MS is routinely used in forensic laboratories for the detection of zolpidem in postmortem cases. Keller et al. reported a case of deliberate poisoning with zolpidem, where a simple clean-up step and GC/MS analysis was adequate for quantitative results [32].

Gunnar et al. evaluated the most common commercially available silylating reagents to achieve optimal derivatization condition for analyzing 14 benzodiazepines and two hydroxy metabolites, as well as zolpidem and zaleplon using whole blood [21]. Tert-butyldimethylsilyl (TBDMS) derivatives proved to be more stable, reproducible, and sensitive than corresponding trimethylsilyl (TMS) derivatives for the GC/MS method used.

One of the first GC assays for zopiclone was reported by Kennel et al. [33]. Boniface et al. suggested that the compound purported to be zopiclone by Kennel was most likely a decomposition product [3]. They demonstrated that zopiclone may thermally decompose under certain GC conditions. These authors also reported that zopiclone is very unstable in nucleophilic solvents such as ethanol or methanol. They suggested a slightly enhanced stability in acetonitrile, isopropanol, and toluene. Gaillard et al. proposed a GC method in which zopiclone was totally converted to its decomposition product during solid-phase extraction and subsequent GC ECD analysis [15].

D. Liquid Chromatography With Fluorescence Detection

Zolpidem has been detected in plasma, using a simple fluorescence detection procedure, without the need for more expensive mass spectrometric detectors. Ring and Bostick reported an 8-min run time for the high-performance liquid chromatography (HPLC) analysis of zolpidem, with a linear range of 1–400 ng/mL of human plasma [58]. The described LC assay lent itself easily to automation.

Durol and Greenblatt analyzed plasma samples taken following a single administration of zolpidem, using HPLC with fluorescence detection [11]. The excitation and emission wavelengths were 254 and 390 nm, respectively, and the lower limit of quantitation reported was 1–2.5 ng/mL. This sensitive method was used to study pharmacokinetic profiles in humans.

In 1997, Ptacek et al. reported a simple and reproducible method for the determination of zolpidem in human plasma [56]. The method involved protein precipitation with methanol and reverse-phase chromatography with fluorescence detection. The excitation and emission wavelengths were 244 nm and 388 nm, respectively. The limit of quantitation was 1.5 ng/mL and the assay was linear up to 400 ng/mL.

Miller et al. described a simple and sensitive method for the analysis of zopiclone in plasma using reverse-phase HPLC and spectrofluorometry [47]. The method had a lower limit of detection of 2 ng/mL in plasma.

In 1996, Gupta described a reverse-phase HPLC procedure with fluorescence detection for the simultaneous extraction of zopiclone and its main metabolites, *N*-desmethylzopiclone and zopiclone-*N*-oxide, in serum, blood, and urine [22]. Zopiclone and its metabolites were quantitated to 2 ng/mL in serum and 10 ng/mL in urine.

E. Liquid Chromatography With Ultraviolet and Diode Array Detection

In 1999, Wang et al. published a simple procedure for the analysis of zolpidem in serum microsamples using HPLC with UV detection [78]. The limit of detection was 3 ng/mL using a detection wavelength of 240 nm. They applied their method to study pharmacokinetics of zolpidem in rats.

Tracqui et al. used a diode array detection system to analyze specimens for zolpidem and other hypnotic drugs [68]. The extraction procedure was a single-step liquid-liquid solvent extraction method using chloroform-2-propanol-n-heptane (60:14:26, v/v), at a pH of 9.5. The analytical method was rapid, simple, and had a run time of 15 min. The UV data were collected over the range 200–400 nm.

Boniface developed a reverse-phase HPLC method for the determination of zopiclone [4]. The method utilized a liquid extraction, with UV detection. The limit of detection was determined to be 4 ng/mL in whole blood.

F. Liquid Chromatography With Mass Spectral Detection

Kratzsch et al. also published a wide-range screening procedure for the analysis of drugs in plasma; it included zolpidem, using liquid chromatography with mass spectrometric detection (LC/MS) [40]. Their method isolated the drugs from the plasma using liquid-liquid extraction before injection into the LC/MS system. The assay was linear over the therapeutic range.

A method for the detection of zolpidem in whole blood was recently reported by Giroud et al. [18]. After a single-step extraction, the drugs were separated by gradient-elution using an ammonium formate buffer/acetonitrile eluent and an Inertsil ODS-3 column. Methaqualone was used as internal standard. The recovery of zolpidem was more than 70% and the assay was applied routinely to forensic cases.

Kintz et al. presented a procedure using LC/MS/MS for the analysis of hair from sexual assault victims [37]. The authors reported a limit of quantitation of 0.5 pg/mg of hair. Vajta et al. reported the use of LC/MS/MS to determine the metabolism of zolpidem [69]. When compared to other chromatographic/mass spectrometric-based techniques, reverse-phase high-performance liquid chromatography coupled with thermospray LC/MS/MS seemed to be an excellent method for the elucidation of unknown metabolic structures, since it allowed identification by direct injection of concentrated urine. However, it was noted during the thermospray process that loss of formaldehyde from a hydroxymethyl amide metabolite occurred. This degradation was not observed when this metabolite was analyzed by GC/MS following trimethylsilylation.

Feng et al. presented a sensitive and rapid chromatographic procedure using electrospray ionization-mass spectrometry in selected-ion monitoring mode in combination with a simple and efficient sample preparation for determination of zaleplon in human plasma. The separation of zaleplon, internal standard, and possible endogenous compounds is accomplished on a phenomenex Luna 5-μ C8(2) column with methanol water (75:25, v/v) as the mobile phase. Analysis takes less than 6 min. The calibration curve of zaleplon in the range of 0.1–60.0 ng/mL in plasma is linear with a correlation coefficient of > 0.9992 and the detection limit is 0.1 ng/mL.

A sensitive and specific liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry method has been developed by Zhang et al. [82] and validated for the identification and quantification of zaleplon in human plasma using estazolam as an internal standard. Plasma samples were extracted with ethyl acetate and evaporated to dryness. The reconstituted solution of the residue was injected onto a prepacked Shim-pack VP-ODS C18 column and chromatographed with a mobile phase of methanol:water (70:30). Detection was performed on a single-quadrupole mass spectrometer by selected ion monitoring mode via atmospheric pressure chemical ionization source. The mean standard curve was linear over the concentration range of 0.2–100 ng/mL. The limit of detection was 0.1 ng/mL.

Kintz et al. were able to screen 17 benzodiazepines and hypnotics including the Z-drugs in oral fluid by LC/MS/MS [36]. The method involves extraction of 0.5 mL of oral fluid treated with phosphate buffer in the presence of diazepam used as internal standard with diethyl ether/methylene chloride (50/50) and separation using LC/MS/MS. The limits of quantification for all benzodiazepines and hypnotics range from 0.1–0.2 ng/mL. Linearity was observed from the limit of quantification of each compound to 20 ng/mL. Coefficients of variation at 2 ng/mL range from 4–8% for all drugs except zopiclone (34%). Extraction recovery measured at the same concentration was higher than 90%. Ion suppression was evaluated for each compound and was lower than 10% for all drugs except zopiclone (93%). These results were found suitable

to screen for 17 benzodiazepines in oral fluid and detect them other than at very low concentrations, making this method suitable for monitoring subjects under the influence. For this reason, this method would probably also be suitable for DFSA casework.

Nordgren et al. used direct injection of urine and LC/MS/MS with rapid chromatography and atmospheric pressure chemical ionization as a screening method that included zopiclone and metabolites, zolpidem and metabolites, and zaleplon [50].

Giroud et al. developed an atmospheric pressure ionization LC/MS method for the determination of zolpidem and zaleplon in whole blood and successfully applied it to forensic cases [18].

G. Capillary Electrophoresis

A less common method of analysis for the Z-drugs is capillary electrophoresis. Hempel and Blaschke reported two methods in 1996 for the analysis of zolpidem by capillary electrophoresis [24,25]. One of these reports included an enantioselective capillary electrophoretic method of the analysis of zopiclone and its metabolites in urine [25]. The method involved a liquid-liquid extraction and UV laser-induced fluorescence detection. The method was also applicable to saliva samples. The authors reported that their methods for the analysis of zolpidem and zopiclone were fast and simple as compared to HPLC.

Horstkotter et al. developed a CE method using laser-induced fluorescence (LIF) for the determination of zaleplon and its metabolites in urine [29]. The fluorescence intensities of the metabolites differ so that the 5-oxo metabolites fluoresced 10-fold lower than the unchanged drug and the *N*-de-ethylated metabolite resulting in limits of quantification, including a 10-fold preconcentration step by solid-phase extractions, of 10 ng/mL for zaleplon and *N*-de-ethylzaleplon and 100 ng/mL for 5-oxozaleplon and 5-oxo-*N*-deethylzaleplon.

SUMMARY

The Z-drugs (zolpidem, zopiclone/eszopiclone, and zaleplon) are widely prescribed sedative/hypnotics that share similar pharmacology with the benzodiazepines. This review has included an overview of the basic chemistry, pharmacodynamic, and pharmacokinetic properties of these drugs, as well as drug interactions and methods of analysis. Specifically discussed are dose administration, receptor-activity relationships, therapeutic effects, adverse reactions, interactions with ethanol and other drugs, and the role or potential role of Z-drugs in DFSA.

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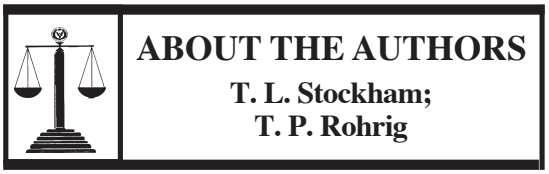

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The Use of Over-The-Counter Medications to Facilitate Sexual Assault

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The Use of Over-The-Counter Medications to Facilitate Sexual Assault

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ABSTRACT: Over-the-counter drugs are medications that are available without the requirement of a prescription. They are considered relatively safe and well-tolerated when taken in accordance with the dosing instructions on the package label. However, when taken alone or in combination with other drugs, they possess pharmacological properties that have the potential to facilitate sexual assault. This chapter reviews the chemistry and pharmacology of these drugs. Additionally, a brief overview of analytical methodology is presented.

KEY WORDS: "Date-rape" drugs, drug-facilitated sexual assault, over-the-counter drugs, toxicology

INTRODUCTION

Over-the-counter (OTC) drugs are medications that are available without the requirement of a prescription. The U.S. Food and Drug Administration (FDA) regulates the manufacture and sale of OTC drugs in the United States. They determine whether the drugs have medical benefits to treat conditions that do not necessitate the direct supervision of a physician. OTC drugs are considered relatively safe and well-tolerated when taken in accordance with the dosing instructions on the package label. However, some OTC drugs possess pharmacological properties that, alone or in combination with other drugs (including ethanol), have potential to facilitate sexual assault [2]. Thus, any drug that diminishes the mental or physical capacity of a potential victim may be used to commit a drug-facilitated sexual assault (DFSA).

A sexual assault may be facilitated by covertly or forcibly administering a drug to an individual. In an alternative scenario, sexual activity occurs with a victim who is intoxicated by self-medication to the point of near or actual unconsciousness. The ingestion of OTC drugs discussed in this chapter may result in pharmacological effects that alter states of consciousness and/or lower inhibitions.

I. CHEMISTRY

The OTC medications reviewed in this chapter represent three classes of drugs: antihistamines, antitussive agents, and decongestants.

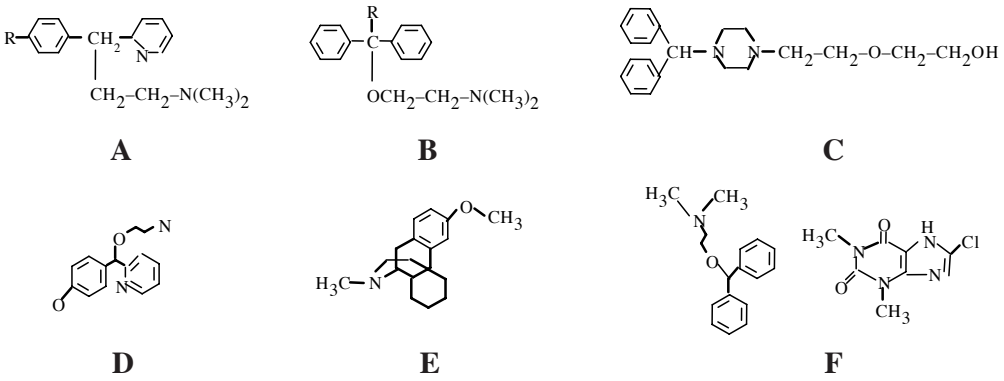
The first-generation antihistamines were discovered approximately 60 years ago and have been available for at least the last 40 years [20]. Several classes of antihistamines are recognized; the first-generation have significant sedating abilities but the second-generation are generally considered to be nonsedating antihistamines. Due to this adverse effect, the first-generation antihistamines present the greatest potential for use in DFSAs. The compounds are divided into several chemical classes, including ethylenediamines, ethanolamines, alkylamines, phenothiazines, piperazines, and piperidines, with a range of pKa of approximately 7–9.3 [22]. The general structure (**Structure 1**) of these antihistamines consists of a substituted ethylamine backbone ($X–CH_2–CH_2–N$) with two terminal aromatic rings. Each of the other five chemical classes is a variation of this structure. The compounds in this review are categorized as follows: brompheniramine and chlorpheniramine are alkylamines; dimenhydrinate, diphenhydramine, and doxylamine are ethanolamines, and hydroxyzine is a piperazine.

Dextromethorphan (the d-isomer of 3-methoxy-*N*-methymorphinan) is an antitussive agent and is a nonopioid synthetic analogue of codeine, with an approximate pKa of 8.3. Tetrahydrozoline is an imidazole decongestant ($C_{13}H_{16}N_2$), (2-[1,2,3,4-tetrahydro-1-naphthyl]-imidazoline) widely available in topical ocular and nasal formulations with a pKa of approximately 9.6.

II. PHARMACOLOGY

A. Administration

Antihistamines are compounds that antagonize the effects of histamine by binding to histaminergic receptors. Four subclasses of receptors have been identified, H1–H4 [4]. These receptors are located in smooth muscle, cardiac muscle, gastric parietal cells, the central nervous system (CNS), endothelial cells, and hematopoietic cells. First-generation antihistamines primarily act on the H1 receptor



Structure 1. Chemical structures of: brompheniramine (**A**, R: Br); chlorpheniramine (**A**, R: Cl); doxylamine (**B**, R: CH_3); diphenhydramine (**B**, R: H); hydroxyzine (**C**); carbinoxamine (**D**); dextromethorphan (**E**); and dimenhydrinate (**F**).

subtype and include brompheniramine, chlorpheniramine, diphenhydramine, doxylamine, and hydroxyzine. Second-generation antihistamines, such as cimetidine and ranitidine, are referred to as nonsedating antihistamines, and will not be discussed further.

Brompheniramine is available for oral administration in tablet or liquid form with recommended doses of 2–4 mg every 4–6 h. Pediatric (ages 2–12 years) doses may be half the adult dose depending on the formulation. The d-isomer is more potent than the racemate, and both are marketed as the maleate salt. The drug is commonly available in mixtures, as "cold and allergy elixirs" or chewable tablets in combination with phenylephrine, pseudoephedrine, acetaminophen, or dextromethorphan [30].

Chlorpheniramine (an analogue, carbinoxamine, is available by prescription) is the *p*-chloro analogue of brompheniramine. The maleate salt is marketed as a racemic mixture for oral administration in the form of caplets, syrup, liquid, or chewable tablet. Single oral doses are typically 0.5–4 mg every 4–6 h. Drug combinations include phenylephrine, pseudoephedrine, acetaminophen, and dextromethorphan.

Dimenhydrinate, the 8-chlorotheophyllinate salt of diphenhydramine, is an antihistamine administered to prevent and treat the symptoms of motion sickness, such as nausea and dizziness. It is available in tablet form. Recommended dose for adults is 50–100 mg every 4–6 h, with 50% and 25% adult doses every 6–8 h for children aged 6–12 and 2–5 years, respectively [29].

Diphenhydramine is available for oral administration in chewable tablets, caplet, gel capsules, or liquid form. Common salt forms are hydrochloride and citrate. Recommended doses are 25–50 mg every 4–6 h for adults and 50% adult dose for children aged 6–12 years. Diphenhydramine is available in combination with acetaminophen, phenylephrine, and pseudoephedrine. This drug is also available OTC for dermal application in the form of cream, spray, gel, and stick in combination with zinc acetate, calamine, and benzenthonium chloride.

Doxylamine is available for oral administration in liquid, tablet, or capsule form as the succinate salt. The recommended dose is 12–25 mg every 4–6 h depending on dose and formulation. Doxylamine is typically marketed in combination with dextromethorphan, acetaminophen, phenylephrine, or pseudoephedrine.

Hydroxyzine is available as the hydrochloride or pamoate salt in capsule or tablet form for oral administration. Recommended doses of this antihistamine are 50–100 mg every 6 h for adults and 50 mg for children less than 6 years. This drug is also used as an antiemetic, anti-anxiety, and preoperative sedative, and is available non-OTC for IM and IV injection. The recommended dose for each application varies [28].

Antitussive medications are utilized to suppress the cough reflex. The cough reflex involves both the central and peripheral nervous systems and the smooth muscle of the bronchial tree [4]. These medications may be available OTC, such as dextromethorphan, or by prescription, such as codeine. Unlike codeine, which acts at opioid receptors, dextromethorphan acts centrally to raise the cough threshold.

Dextromethorphan ((+)-3-methoxy-*N*-methyl morphinan) is available for oral use as the hydrobromide salt in the form of syrup, caplets, drops, liquid, or chewable tablets. The recommended dose is 15–30 mg every 4–8 h depending on the formulation. Typical drug combinations include phenylephrine, pseudoephedrine, acetaminophen, and chlorpheniramine, and, less commonly, brompheniramine and pheniramine.

Tetrahydrozoline is classified as a sympathomimetic amine used in OTC eye and nasal preparations for the treatment of eye redness/dryness, irritation, and congestion. Therefore, it is the only compound discussed in this chapter that is not available for oral administration. The

recommended dose for adults is 2–4 drops of 0.1% solution/3–4 squirts of 0.1% spray in each nostril every 4–8 h for nasal congestion and 1–2 drops of a 0.05% solution to each eye up to four times daily. Several of these drugs, such as diphenhydramine and dimenhydrinate, are also available by prescription for intravenous and intramuscular administration.

B. Pharmacokinetics

These OTC drugs are well absorbed after oral administration. Liquid formulations are generally absorbed faster, based on time to peak concentration, than tablet or gel-cap forms. **Table 1** illustrates several pharmacokinetic parameters for each compound by the oral route. Plasma protein binding is generally >70% where known. These drugs are widely distributed with apparent volumes of distribution exceeding 3 L/kg in adults [1,4,23]. The cytochrome P450 system produces multiple metabolites, which may be pharmacologically active (e.g., cetirizine). Specific isozymes have not been identified for several drugs, but the demethylation reactions of diphenhydramine, doxylamine, hydroxyzine, and dextromethorphan are mediated by CYP2D6 and that of chlorpheniramine is mediated by CYP3A4. Serum elimination half-lives (h) range from single digits to more than 24 h. There is little renal metabolism.

C. Pharmacodynamics

HI antagonists reverse the effects of histamine release such as vasoconstriction, increased capillary permeability, and edema, in addition to blocking flare and itching. The CNS effects of these drugs may be stimulatory or depressive at therapeutic doses. The major adverse effects of these compounds are sedation and impairment of cognitive function and psychomotor performance [33]. Other effects include anticholinergic actions such as confusion, blurred vision, dry mouth, and light-headedness. Dimenhydrinate diminishes vestibular stimulation and depression of labyrinth function. This drug may also act on the medullary chemoreceptor trigger zone.

Dextromethorphan suppresses the cough reflex by acting directly on the central cough in the medulla. Dextromethorphan is an NMDA receptor antagonist and also an agonist at sigma receptors. Sigma receptors mediate central excitation, which may result in dystonia and delusions [10].

Alpha 2 adrenergic agonists reduce the sympathetic outflow from the CNS. They decrease arterial blood pressure by modulating cardiac output and peripheral resistance. Therefore, they are used primarily for the treatment of hypertension. In addition, alpha 2 agonists reduce intraocular pressure by reducing the production of aqueous humor [4]. Adverse effects of these compounds

Table 1. Pharmacokinetic and pharmacodynamic parameters of drug ingested by oral route

Drug	Pharmacologic category	Absorption	Time to onset of action (min)	Tmax (h)	Duration of action (h)	Therapeutic serum conc. (ng/mL)	Metabolism metabolites	Elimination T1/2 (h)	Excretion
Brompheniramine	Ist Generation Antihistamine H1 Antagoinst	Rapid	15–60	2–5	6–8	15–25	LIVER Norbrompheniramine Dinorbrompheniramine PPA[a]	20–25	Urine
Chlorpheniramine	Ist Generation Antihistamine H1 Antagoinst	Rapid	15–60	2–6	6–8	10–25	LIVER Norchlorpheniramine Dinorchlorpheniramine	14–25	Urine
Dimenhydrinate	Anticholinergic	Rapid	15–30	2–3	3–6	50–110	LIVER Diphenhydramine	6–9	Urine
Diphenhydramine	Ist Generation Antihistamine H1 Antagoinst	Rapid	15–60	1-4	6–8	25–100	LIVER Dinordiphenhydramine Diphenylmethoxy-acetic acid	3–10	Urine
Doxylamine	Ist Generation Antihistamine H1 Antagoinst	Rapid	15–60	1-4	6–8	50–150	LIVER Nordoxylamine Dinordoxylamine	7–13	Urine
Hydroxyzine	Ist Generation Antihistamine H1 Antagoinst	Rapid	15–30	2–3	4–6	50–100	LIVER Norhydroxyzine Cetirizine	14–25	Urine
Dextromethorphan	Antitussive	Rapid	15–30	2–2.5	EM 4–6	EM[a] 3–8 PM[a] 25–40	LIVER Dextrorphan	EM 3–4	Urine
Tetrahydrozoline	α_2-Adrenergic agonist	Rapid	15–30	UNK[a]	12–24	UNK	UNK	2–4	UNK.

[a] PPA: Beta bromophenyl-2-pyridine propionic acid and glycine conjugate; EM: etensive metabolizer; PM: poor metabolizer; UNK: unknown.

include sedation, sleep disturbances, and postural hypotension.

D. Drug Interactions

Interactions of these compounds with other drugs have been described. The interactions may be pharmacokinetic—e.g., increasing blood drug concentrations—or pharmacodynamic, e.g., increased CNS depression resulting in sedation and dizziness. The compounds discussed in this chapter have at least additive pharmacodynamic effects when coadministered with other CNS depressants such as ethanol, opioids, muscle relaxants, benzodiazepines, and barbiturates. Patients ingesting hydroxyzine are warned to avoid valerian, St. John's wort, kava kava, and gotu kola because CNS depression may increase [23]. In addition, several specific interactions have been documented although the precise mechanisms have not been elucidated in many instances. Brompheniramine, chlorpheniramine, hydroxyzine, dimenhydrinate, and diphenhydramine may antagonize the actions of donepezil, galantamine, and rivastigmine [11–17]. Chlorpheniramine increases the effect of ethotoin, fosphenytoin, mephenytoin, and phenytoin.

Diphenhydramine coingested with thioridazine and mesoridazine results in increased risk of cardiotoxicity and arrhythmias. Inhibition of CYP 2D6 results in increased concentrations of atomoxetine. Anticholinergic effects may be increased with coingestion of tricyclic antidepressants.

Coingestion of dextromethorphan and antidepressants such as fluoxetine and paroxetine, and the antiparkinsonism drug, selegiline, may result in serotoninergic syndrome. Selegiline is an MAO-type B inhibitor, and concomitant use of monoamine oxidase inhibitors with antihistamines and dextromethorphan will increase the anticholinergic and CNS-depressant effects of these drugs. Moclobemide is contraindicated with dextromethorphan. Quinine and quinidine may increase the toxicity of dextromethorphan.

CYP P450 inhibitors will increase serum concentrations of these drugs, increasing the potential for toxicity. These include erythromycin, fluconazole, itraconazole, ketonazole, and metronidazole [11–17].

Warnings posted of possible drug interactions with tetrahydrozoline include MAO inhibitors (selegiline, phenelzine) and beta blockers (atenolol, metoprolol). In addition, treatment with tetrahydrozoline may mask symptoms of ototoxicity due to aminoglycoside antibiotics.

E. OTC Drugs and DFSA

Even though the OTC drugs discussed in this chapter have the potential to facilitate sexual assault, due to their sedating effects, there are few reports of detection in the literature. In a prospective study of the prevalence and risk factors associated with DFSA, DuMont et al. reported approximately 21% of victims of alleged sexual assault met the criteria for DFSA [6]. In addition, these individuals were more likely than other victims to have ingested not only OTC drugs, but ethanol and street drugs prior to the alleged incident. According to self-reported data, the OTC medications admitted by the participants were analgesics and antiemetics. In another study of sexual assault complainants in four locales in the United States, 7% (N = 859) of cases were determined to be DFSA based on toxicological analysis of urine and hair for 45 drugs [19]. OTC compounds included in this study were diphenhydramine, chlorpheniramine, doxylamine, and dextromethorphan (LODs = 5–25 ng/mL) [18]. Hall et al. reported that the number of positive drug cases in alleged sexual assault instances doubled between 1999 and 2005 [9]. The most common drugs included ethanol, antidepressants, recreational drugs, benzodiazepines, and analgesics. The study did report the detection of the OTC drugs acetaminophen [n = 26], salicylate [n = 1] and ephedrine [n = 1] from 282 cases. However, the article did not provide comprehensive information concerning all drugs that could be detected in the analytical protocol used in the study. Scott-Ham and Burton reported the results of toxicological testing in 1,014 suspected cases of DFSA in London, England, during 2000–2002 [25]. Ethanol was the drug most frequently detected (46%), followed by illicit drugs (34%). Other drugs detected were benzodiazepines, GHB, and MDMA. These articles demonstrate that laboratories may not be looking for OTC drugs or may not be reporting them if present, or that the prevalence of OTC compounds is low in these cases.

Tetrahydrozoline has been implicated in a case of sexual assault [26]. Recently, Stillwell et al. reported an alleged DFSA linked to the use of tetrahydrozoline [27]. In this case the female victim voluntarily ingested alcoholic beverages surreptitiously spiked with tetrahydrozoline. This compound was identified in a urine specimen collected approximately 7 h post ingestion and analyzed by gas chromatography/mass spectrometry (GC/MS). A urine ethanol concentration of 0.15 g/dL was also reported.

III. METHODS OF ANALYSIS

An efficient and comprehensive drug-screening procedure is essential to either exclude the involvement of drugs in a DFSA case, or to detect such substances if they are present. The OTC drugs discussed in this review have been analyzed in a variety of biological specimens. Suitable sample preparation is an important prerequisite. This involves isolation and, if necessary, cleavage of conjugates and/or derivitization of the drugs and metabolites. Isolation is usually performed by liquid-liquid extraction at a pH at which the analyte is unionized, or by solid-phase extraction preceded or followed by clean-up steps with subsequent detection by liquid or gas chromatography [3,7,8,18,21,24,27,34]. However, because some of the compounds are typically used in a single low dose, awareness of the strengths and the limitations of each procedure are of critical importance in the systematic analysis of specimens for the presence of drugs in any DFSA case.

A. Extraction Techniques

Chromatographic techniques require some form of isolation procedure to separate the drugs from biological matrix. The typical procedures can be separated into liquid-liquid extraction (LLE) and solid-phase extraction (SPE).

SPE is well suited to isolate drugs of abuse as well as prescription and OTC drugs in biological matrices such as blood and urine [5,35]. Wingert et al. identified brompheniramine, carbinoxamine, chlorpheniramine, dextromethorphan, and doxylamine utilizing a procedure for basic drugs using 2 mL of blood by SPE using CSDAU303 columns and a modified procedure from United Chemical Technologies Inc. (31) followed by GC/MS analysis [34].

LLE remains the most common sample preparation procedure. These compounds are extracted from blood and urine into organic solvents at alkaline pH. They are readily soluble in solvents such as chloroform and n-butyl chloride and extracted in most common protocols designed to isolate alkaloidal and basic drugs. They are also readily back-extracted into acid, and then into organic solvents, without significant loss [3,7,24,27].

Marinetti et al. performed quantitations of dextromethorphan, diphenhydramine, chlorpheniramine, and brompheniramine using LLE designed to isolate alkaloidal and basic drugs [21]. This was accomplished by extraction into n-butyl chloride from pH 8.9, 1.2M Tris buffered biological specimens. Saturated sodium chloride was added to the specimen to make the extraction more uniform and to aid in the phase separation. A back-extraction into acid was performed followed by re-extraction into methylene chloride after alkalinization. The samples were then subjected to gas chromatography. Additionally, the detection of carbinoxamine was performed by a GC/MS drug screen of an LLE of the basic fraction [21].

Stillwell et al. used a modified LLE procedure for alkaloidal and basic drugs for the detection and identification of tetrahydrozoline from a previously published method described elsewhere [7] and analyzed samples using gas chromatography with a flame ionization detector (FID) and GC/MS [27].

The majority of analytical procedures are designed to detect the parent compound and since the parent does not form glucuronide or sulfate conjugates, hydrolysis is not required.

B. Instrumental Methods

1. Immunoassay

Although immunoassay screens may be helpful in detecting sedative-hypnotics (barbiturates and benzodiazepines) and opioid analgesics, there are no commercially available immunoassay kits for the detection of OTC drugs such as brompheniramine, chlorpheniramine, dextromethorphan, dimenhydrinate, diphenhydramine, doxylamine, hydroxyzine, and tetrahydrozoline. As a result, more comprehensive, broader-based procedures are necessary for the detection of these drugs. The wide variety of compounds detected by these comprehensive screening procedures emphasizes the importance of their use.

2. Liquid Chromatography

The application of high-performance liquid chromatography (HPLC) methodology allows for the simultaneous analysis of various antihistamines. Gergov et al. reported the simultaneous screening and quantitation of 18 antihistamine drugs [8]. This included the detection of brompheniramine (20 ng/mL), carbinoxamine (8 ng/mL), chlorpheniramine (40 ng/mL), diphenhydramine (1000 ng/mL), and hydroxyzine (90 ng/mL) in blood. This method utilized a TRIS-buffer butyl acetate basic extraction. Dibenzepin was used as the internal standard. The extracts were assayed by HPLC on C_{18} reversed-phase columns using acetonitrile–ammonium acetate mobile phase at pH 3.2. The mass spectrometric analysis was performed with a triple-stage quadrupole mass analyzer. The reported identification and quantitation of these antihistamines were well within therapeutic concentrations.

3. Gas Chromatography

Gas chromatography (GC) is the separation technique most frequently used for the analysis of these compounds. For GC separation, fused silica capillaries with polymethyl siloxane or methyl phenyl siloxane phases have been used. These phases produce good chromatographic results. Subsequent detection by GC with nitrogen-phosphorus detection (NPD) and FID has been reported [3,7,21,24,27,32,34].

Due to the sensitivity and specificity, GC-MS, especially in the full-scan electron impact ionization (EI) mode, has been the preferred method for the identification of these OTC drugs in forensic cases.

CONCLUSIONS

OTC drugs such as antihistamines and decongestants are widely available. They are frequently self-administered, especially during the allergy and influenza seasons. In addition, they have side effects that render them eligible to incapacitate an individual and, therefore, facilitate sexual assault. The CNS-depressant and anticholinergic effects of many of these medications result in the potential for multiple drug interactions that may have additive if not potentiating results. To date, the detection rate of OTC drugs in alleged cases of DFSA has been low compared with other compounds. However, laboratories performing toxicological analysis in these cases should include sedating OTC medications in their testing menus.

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The Use of Miscellaneous Prescription Medications to Facilitate Sexual Assault

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The Use of Miscellaneous Prescription Medications to Facilitate Sexual Assault

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ABSTRACT: Drugs used to facilitate sexual assaults are typically those that rapidly render the potential victim unconscious or sedated, and produce memory loss or amnesia. Many of these drugs are difficult to detect due to a delay in biological specimen collection. Detection is further hampered as the drugs are often administered in single low doses and are rapidly and extensively metabolized, resulting in low concentrations in biological specimens. Miscellaneous prescription drugs such as the barbiturates, antipsychotics, opioids, tricyclic antidepressants, ketamine, and chloral hydrate have the potential to produce varying degrees of sedation; however, they are not frequently detected in drug-facilitated sexual assault cases. A review of the literature shows that these drugs are often knowingly taken by the victim before or subsequent to the assault, and therefore may contribute to the sedation or unconsciousness experienced by the victim when ethanol or other central nervous system drugs are co-administered. Most barbiturates, opioids, and tricyclic antidepressants are routinely screened for in hospitals and forensic toxicology laboratories, and may be detectable in a urine specimen for several days. Antipsychotics, particularly the atypical class, ketamine, and chloral hydrate, generally require more targeted analyses. This review provides an overview of the pharmacodynamics, pharmacokinetics, and common analytical methods for the barbiturates, antipsychotics, opioids, tricyclic antidepressants, ketamine, and chloral hydrate.

KEY WORDS: Amitriptyline, antipsychotics, barbiturates, chloral hydrate, chlorpromazine, drug-facilitated sexual assault, ketamine, morphine, narcotic analgesics, neuroleptics, opioids, phenobarbital, prescription medication, sedative-hypnotics, tricyclic antidepressants.

INTRODUCTION

A drug-facilitated sexual assault (DFSA) can occur when there is a covert or forcible administration of a substance to a victim for the purpose of committing a sexual assault. The intent is to cause sedation, incapacitation, disinhibition, and/or memory loss. Another form of DFSA can occur when a potential victim, who is cognitively and/or physically impaired by his/her own administration of alcohol (ethanol) or drugs, is "taken advantage of" and sexually assaulted. A range of illicit, prescription, and even over-the-counter medications are capable of causing varying degrees of sedation, especially when taken with ethanol.

For the purposes of a DFSA, archetypal drugs would include those substances that act rapidly, produce significant sedative or hypnotic effects, block the victim's memory of events, are eliminated from the body quickly, result in low concentrations in biological specimens, and are difficult to detect analytically. Ideal physical characteristics of the drug would include being colorless, odorless, and tasteless, and being easy to obtain and administer to a potential victim.

A myriad of substances have been reported or associated with DFSA cases including ethanol, benzodiazepines, sedative-hypnotics, muscle relaxants, antidepressants, cannabinoids, cocaine, amphetamines, 3,4-methylenedioxy-methamphetamine (MDMA, Ecstasy), opioids, ketamine, chloral hydrate, and γ-hydroxybutyrate (GHB). Most of these substances have central nervous system (CNS)-depressant activities and are capable of causing some degree of sedation or incapacitation, while others could potentially render a prospective victim susceptible to an assault. DFSA drugs can be bought illegally, be prescribed to the perpetrator or someone they know, or are available through the perpetrator's place of employment (e.g., in a medical field).

Many aspects need to be considered for successful toxicological investigations of DFSA cases, including the timeliness of specimen collection, the use of sensitive and specific analytical techniques, and a thorough investigation. Additionally, knowledge of the pharmacological properties of DFSA drugs is particularly useful. This review covers a number of these factors for several drug classes that are less frequently encountered in DFSA cases—barbiturates, antipsychotics, opioids, tricyclic antidepressants (TCAs), ketamine, and chloral hydrate. These are generally not the classically thought-of DFSA drugs that are "spiked" into a victim's drink, although there have been a handful of alleged instances of surreptitious administration. Instead, potential victims often take these drugs themselves, either via prescription or recreationally, and are taken advantage of. This may especially occur when such drugs are administered with ethanol or another sedating drug. An overview of the chemistry, pharmacology, and methods of analysis for these drugs is given, with a focus on single oral doses more so than chronic use of the drug or intravenous/intramuscular administration.

Background on Use in DFSA

Reported incidents of barbiturate, antipsychotic, TCA, ketamine, chloral hydrate, and/or opioid use in suspected DFSA cases are infrequent compared to other substances such as ethanol and benzodiazepines. If detected in biological specimens, these miscellaneous drugs are often assumed to have been taken voluntarily by the victim either before or subsequent to the sexual assault; however, there have been a few individual case reports describing the deliberate use of these drugs to facilitate the sexual assault. The six sections below cover both suspected voluntary use and deliberate use of barbiturates, antipsychotics, opiods, TCAs, ketamine, and chloral hydrate.

Elsohly and Salamone [26] analyzed urine specimens from 1,179 suspected DFSA cases collected from across the United States over a 26-month period. In addition to being specifically tested for ethanol, GHB, and flunitrazepam metabolites, the urine specimens were screened by immunoassay for amphetamines, barbiturates, benzodiazepines, cocaine metabolites, cannabinoids, methaqualone, opioids, phencyclidine, and propoxyphene. Positive immunoassay results were confirmed by gas chromatography-mass spectrometry (GC-MS). Overall, opioids were detected in 25 cases (10 of which were also positive for ethanol); 17 cases were positive for propoxyphene (8 of which were also positive for ethanol); and 12 cases were positive for barbiturates (4 of which were also positive for ethanol). TCAs and ketamine were not tested.

Scott-Ham and Burton [111] analyzed blood and/or urine specimens from 1,014 cases of alleged DFSA cases in London over a 3-year period (2000–2002). Specimens were screened by immunoassay for amphetamine, barbiturates, benzodiazepines, cannabinoids, cocaine metabolites, methadone, methamphetamines, and opioids, with all positives confirmed by either GC-MS or high-performance liquid chromatography (HPLC). Specimens were additionally analyzed for ethanol, low-dose benzodiazepines including flunitrazepam, GHB and related compounds, ketamine, and trichlorinated compounds including chloral hydrate. Overall, ethanol and/or drugs were identified in 659 cases. Opiate analgesics (codeine and/or morphine, propoxyphene, dihydrocodeine, and methadone) were detected in 103 cases; heroin was detected in 12 cases, typically in combination with cocaine, cannabis, and/or ethanol; thioridazine was detected in 3 cases; ketamine was detected in 3 cases; olanzapine, chlorpromazine, and tramadol were also detected. The presence of amitriptyline was found in 5 cases, none of which was attributed to the "deliberate spiking" of the victim's beverage; however, one of those cases involved the victim being "forced" to consume amitriptyline and diazepam tablets. The authors did not attribute any of the other aforementioned substances to forced ingestions or deliberate spiking, and instead believed these drugs were most likely consumed voluntarily before or subsequent to the alleged assault.

Hurley et al. reported on 76 cases of suspected DFSA over a 12-month period in Melbourne, Australia [54]. Blood and/or urine specimens were screened for amphetamines, benzodiazepines, cannabinoids, cocaine metabolites, and opioids by immunoassay. Specimens were also analyzed for ethanol, GHB, and basic and neutral drugs by GC-MS. Positive drug results were confirmed using MS. Drugs not reportedly consumed by the victim were detected in 15 cases, raising the possibility of covert drug administration. Of these 15 cases, oxycodone and tramadol were detected in 1 case each; codeine and nordiazepam were detected in 1 case; and codeine and clozapine were detected in another case. TCA and ketamine were not found.

Hall et al. performed a retrospective study of 314 alleged DFSA cases in Northern Ireland over a 7-year period (1999–2005) where toxicology results were available [43]. A total of 90 cases were positive for drugs, either alone or in combination with ethanol. One or more opioids (including heroin, codeine, morphine, dihydrocodeine, tramadol) were detected in 28 cases either alone or, more commonly, with ethanol or other analgesic, prescription, or illicit drugs. The authors assumed that such drugs were consumed voluntarily, possibly post assault, and had not been used surreptitiously for the purposes of a sexual assault.

Jones et al. reported on the toxicological analysis of blood and urine from 1,806 cases of alleged DFSA in Sweden during 2003–2007 [59]. Blood and urine were analyzed specifically for ethanol and GHB, and were screened by immunoassay for amphetamines, benzodiazepines, cannabinoids, cocaine metabolites, and opioids; and for basic and neutral drugs by GC. Positive drug results were confirmed using GC-MS. Codeine was detected in 29 cases, and tramadol was detected in 18 cases. Buprenorphine, propoxyphene, ethyl morphine, morphine, and methadone were also detected.

Frison et al. reported on a 61-year-old woman who had been sexually assaulted during hospitalization for a minor surgical operation [34]. During her operation, the woman received the local analgesics mepivacaine and ropivacaine, as well as the general anesthetic propofol. Despite being fully awake post-operatively, the woman reached her ward in an unconscious state. Blood and urine specimens were not collected at the time; however, head and pubic hair samples were collected approximately 1

month later after analysis of residual fluid from the infusion set revealed the presence of the barbiturate thiopental, which had not been prescribed. Thiopental and its metabolite, pentobarbital, were detected in the hair samples. The thiopental had presumably been administered via the intravenous line that was still in place in the woman's arm while she was in an elevator being transferred back to the ward.

Gaulier et al. reported on a 29-year-old woman who was sexually assaulted in France after being forced to drink an unknown beverage [39]. The woman was found in a drowsy state approximately 3 h after the drugging. Two weeks later, a suspect admitted to the assault and to preparing a drink containing acepromazine and forcing the woman to drink it. Acepromazine, a veterinary and human tranquilizer/sedative drug common in Europe, was detected in the residue of a glass. No drugs, including acepromazine, were detected in the victim's blood despite the blood being collected only 8 h post assault. However, acepromazine was detected in scalp hair collected 1.5 months after the assault.

Rossi et al. reported on a 24-year-old woman who was allegedly sexually assaulted when she was studying abroad for a 1-year period [104]. The woman recalled waking up with a strong headache and nausea. She also noticed small abrasions on her arm which were possible puncture wounds. After returning home, the woman received sexual videos of herself from her time abroad, and she was convinced she had been sexually assaulted while under the influence of drugs. Segments of her hair, corresponding approximately to the period of time before and after the alleged assault, revealed the presence of GHB and morphine.

Chloral hydrate is not routinely screened for in laboratories and can easily be overlooked. This substance and its metabolites must be targeted for the necessary testing. Unfortunately, chloral hydrate must be suspected in DFSA cases for the laboratory to perform the necessary analyses.

I. BARBITURATES

Barbiturates are nonselective CNS depressants that have been available for use since the early 1900s. They were originally used as general sedatives and hypnotics, as tranquilizers, and to treat various psychiatric conditions. However, their use has declined over the years due to their significant CNS-depressant effects, and they have largely been replaced for such indications by the benzodiazepines, phenothiazines, and antipsychotics that have a greater margin of safety. Nowadays, barbiturates are primarily indicated for use as daytime sedatives, nighttime hypnotics, anticonvulsants, in migraine therapy, and for reduction of cerebral edema secondary to head injury. Chemical structures of barbiturates included in this review are shown in **Structure 1**.

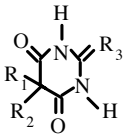
Phenobarbital has been available since 1912 and is the most widely used anticonvulsant worldwide (*see* **Table 1**). It is a long-acting barbiturate used in the treatment of most types of seizures and is the first-line choice for the treatment of neonatal seizures. It is also indicated as a daytime sedative to treat anxiety and to relieve intracranial pressure in head trauma cases. Pentobarbital is a short-acting barbiturate and has been available since 1930. It is still occasionally used to decrease brain pressure resulting from acute brain injury, and as a daytime sedative and a short-term nighttime hypnotic. It can also be used as a preoperative sedative and in the treatment of seizures.

Amobarbital is an intermediate-acting barbiturate, available since 1924. It is occasionally used as a daytime sedative and nighttime hypnotic, and is also used to control seizures. Secobarbital is a short-acting barbiturate, available since 1928. It is used occasionally as a sedative and hypnotic, and also as a preoperative sedative. Butalbital is a short-acting barbiturate prescribed for tension headaches. It is only found in combination with other drugs such as acetaminophen, aspirin, caffeine, codeine, and phenacetin. Butabarbital is a short- to intermediate-acting barbiturate used as a sedative and hypnotic.

Thiopental, thiamylal, and methohexital are ultrashort-acting barbiturates used as induction agents in general anesthesia. Thiopental can also be used to lower intracranial pressure and reduce cerebral oxygen demand in instances of cranial injury.

A. Chemistry

Barbiturates are substituted pyrimidine derivatives and the basic structure common to this class of drugs is barbituric acid. The chemistry of the barbiturates is very similar and the details for only phenobarbital, pentobarbital, secobarbital, and thiopental are covered here.



Structure 1. Chemical structures of barbiturates: amobarbital (R_1: C_2H_5; R_2: $CH_2CH_2CH(CH_3)_2$; R_3: O); phenobarbital (R_1: C_2H_5; R_2: C_6H_6; R_3: O); pentobarbital (R_1: C_2H5; R_2: $CH_2CH_2CH_2CH(CH_3)$; R_3: O); secobarbital (R_1: $CH{=}CHCH_3$; R_2: $CH_2CH_2CH_2CH(CH_3)$; R_3: O); thiopental (R_1: C_2H_5; R_2: $CH_2CH_2CH_2CH(CH3)$; R_3: S).

Table 1. The duration of action, time of onset, and half-life for select barbiturates

Barbiturate	Trade name	Duration of action	Onset (min)[a]	Half-life (h)
Amobarbital	Amytal®	Intermediate	30–60	15–40
Pentobarbital	Nembutal®	Short	10–15	15–48
Phenobarbital	Phenob®, Donnatal® Quadrinal®, Luminal®	Long	60	48–120
Secobarbital	Seconal®	Short	10–15	15–40
Thiopental	Pentothal®	Ultrashort	Immediate [b]	6–46

[a] Onset following oral administration unless otherwise indicated.
[b] Intravenous administration.

Phenobarbital is a barbiturate derivative formally known as 5-ethyl-5-phenylbarbituric acid [85]. It has the empirical formula $C_{12}H_{12}N_2O_3$ and a molecular weight of 232.2. In its pure form it is a colorless crystalline solid or a white crystalline powder. It is a weak acid with a pK_a of 7.4 and is soluble in ethanol. The sodium salt is a white, hygroscopic powder, granules, or flakes, and is soluble in water and ethanol.

Pentobarbital is formally known as 5-ethyl-5-(1-methylbutyl)barbituric acid [85]. It has the empirical formula $C_{11}H_{18}N_2O_3$ and a molecular weight of 226.3. In is pure form it is a white crystalline powder or colorless crystals. It is a weak acid with a pK_a of 8.0 and is soluble in ethanol. The sodium salt is a white, hygroscopic crystalline powder or granule and is freely soluble in water and ethanol.

Secobarbital is formally known as 5-allyl-5-(1-methylbutyl)barbituric acid [85]. It has the empirical formula $C_{12}H_{18}N_2O_3$ and a molecular weight of 238.3. In its pure form it is a white amorphous or crystalline powder. It is a weak acid with a pK_a of 7.9 and is freely soluble in ethanol and slightly soluble in water. The sodium salt is a white, hygroscopic powder, and is soluble in water and ethanol.

Thiopental is formally known as 5-ethyl-5-(1-methylbutyl)-2-thiobarbituric acid [85]. It has the empirical formula $C_{11}H_{18}N_2O_2S$ and a molecular weight of 242.3. The sodium salt is a white to yellowish-white to pale green, hygroscopic powder and is soluble in water and partly soluble in ethanol. It is a weak acid with a pK_a of 7.6.

B. Pharmacology

1. Administration

Phenobarbital is available as either the free acid or sodium salt. It is available in 15-, 30-, or 100-mg tablets; a 20-mg/5-mL elixir for oral administration; and solutions of 65 or 130 mg/mL for parenteral injection. For daytime sedation in adults, typical doses are 30–120 mg daily in two or three divided doses, while doses ranging from 100–300 mg are given as a night-time hypnotic. As an anticonvulsant, 50–100 mg is administered two or three times daily.

Pentobarbital is supplied as the racemic mixture in the form of both the free acid and the sodium salt. It is available in 50- or 100-mg tablets and a 50-mg/mL solution for parenteral injections. Adult doses are normally administered once daily within a range of 50–200 mg.

Secobarbital is available as the free acid or sodium salt, either alone or in combination with other sedative-hypnotic drugs. It can be administered orally, rectally, and parenterally. As a sedative-hypnotic, typical doses are 100 mg at bedtime, or 200–300 mg administered preoperatively.

Amobarbital is available as the free acid or sodium salt. It is available by itself in 100- and 200-mg capsules; or in combination with secobarbital in capsules containing 25, 50 or 100 mg of each drug. As a sedative-hypnotic, typical doses range from 15–200 mg at bedtime, and doses of 65–500 mg are administered intravenously or intramuscularly for the control of seizures.

Thiopental is supplied as the sodium salt in 500–2,500-mg vials for reconstitution as a 2–50-mg/mL solution. It is typically administered intravenously to adults in 100–250-mg initial doses.

2. Pharmacokinetics

Barbiturates are absorbed well orally, with the sodium salts being more rapidly absorbed than the free acids. Onset of action varies typically from 20–60 min following oral administration, slightly faster following intramuscular injection, and within 1–5 min with intravenous injection. The barbiturates are rapidly distributed to all tissues and fluids throughout the body, with high concentrations found in the brain, liver, and kidney. Following the oral administration of most barbiturates, peak therapeutic blood, plasma, or serum concentrations are less than 5 mg/L (*see* **Table 2**) and they are mostly detectable for at

least 12–24 h. Barbiturates are typically extensively metabolized in the liver, and except for phenobarbital, relatively little of the unchanged drug appears in the urine.

a. Phenobarbital

The oral bioavailability of phenobarbital ranges from 70–90%. It is a long-acting barbiturate with an onset of action of about 60 min following oral administration and a reported half-life of anywhere from 48–120 h. The therapeutic plasma range is 2–30 mg/L. A single oral 30-mg dose given to 3 subjects resulted in an average peak serum concentration of 0.7 mg/L [122]. The same dose repeated for 21 days resulted in an average peak concentration of 8.1 mg/L. A single oral 100-mg dose given to 6 subjects resulted in peak serum concentrations of 2.1–3.8 mg/L at 0.5–4 h (average time of 1.5 h) [127]. Biotransformation of phenobarbital occurs by *N*-glucoside formation to *N*-glucosyl-phenobarbital, and by oxidation to p-hydroxyphenobarbital, which is consequently conjugated with glucuronic acid. Dihydrodiol is a minor metabolite. Following a single labeled dose, 78–87% was excreted in the urine within 16 days as unchanged drug (25–33%), *N*-glucosyl-phenobarbital (24–30%) and free or conjugated p-hydroxyphenobarbital (18–19%) [118].

b. Pentobarbital

The oral bioavailability of pentobarbital is 100%. It is a short-acting barbiturate with an onset of action of approximately 10–15 min following oral administration and a dose-dependent half-life of 15–48 h. The therapeutic plasma range is 1–10 mg/L. A single oral 100-mg dose of pentobarbital given to 7 subjects resulted in peak serum concentrations of 1.2–3.1 mg/L at 0.5–2.0 h [115]. These concentrations diminished slowly and were on average 0.3 mg/L at 48 h. A single oral 50-mg dose given to 5 subjects resulted in peak plasma concentrations of 0.62–0.88 mg/L at 1 h [85]. The main metabolites of pentobarbital are 3'-hydroxypentobarbital, *N*-hydroxypentobarbital, and 3'-carboxypentobarbital. As much as 86% of a labeled dose is excreted in the urine within 6 days, mostly as 3'-hydroxypentobarbital [118]. Only approximately 1% is excreted as unchanged drug.

c. Secobarbital

This is a short-acting barbiturate with an onset of action of approximately 10–15 min and a reported half-life of 15–40 h. A single 231-mg/70-kg oral dose of secobarbital resulted in a peak blood concentration of 2.0 mg/L at 3 h. Secobarbital was still detected at 1.3 mg/L following 20 h [18]. The main metabolites of secobarbital are secodiol, 3'-hydroxysecobarbital, and 5-(1-methylbutyl)barbituric acid. Only about 5% of the drug is excreted unchanged in the urine within 2 days [123].

Table 2. Peak concentrations observed following the administration of a single dose of select barbiturates

Barbiturate	Single oral dose (mg)	Cmax [a] (mg/L)	Tmax [b] (h)	Specimen
Amobarbital	120	1.8	2.0	Serum
Pentobarbital	50	0.6–0.9	1.0	Plasma
	100	1.2–3.1	0.5–2.0	Serum
Phenobarbital	30	0.7	—	Serum
	100	2.1–3.8	0.5–4.0	Serum
	600	18	4.5	Blood
Secobarbital	231[c]	2.0	3.0	Blood
Thiopental	400[d]	28	< 1 min	Plasma

[a] Maximum concentration. [b] Time to maximum concentration.
[c] mg/70 kg. [d] Intravenous administration.

d. Amobarbital:

This is an intermediate-acting barbiturate with an onset of action of approximately 30–60 min and a half-life of 15–40 h. A single 120-mg oral dose of amobarbital resulted in a peak serum concentration of 1.8 mg at 2 h [55]. The metabolite 3'-hydroxy-amobarbital has approximately one-third the pharmacological activity of amobarbital and peak concentrations of this metabolite are seen at 26 h. Other metabolites are *N*-glucosylamobarbital and 3'-carboxyamobarbital. Following a single labeled dose, up to 92% was excreted in the urine within 6 days as unchanged drug (1–3%), free 3'-hydroxy-amobarbital (30–50%), *N*-glucosylamobarbital (29%), and 3'-carboxyamobarbital (5%).

e. Thiopental

This is an ultrashort-acting barbiturate with an almost immediate onset of action following intravenous administration. Its half-life is 6–46 h, which is dose-dependent. When 400 mg was administered to a patient intravenously over 2 min, the peak plasma concentration was 28 mg/L almost immediately [14]. This declined to 7 mg/L after 15 min and 3 mg/L at 90 min. Concentrations of its metabolite pentobarbital usually average approximately 10% of the thiopental level. Only about 0.3% of an administered thiopental dose is excreted unchanged in the urine over a 48-h period.

3. Pharmacodynamics

Barbiturates are general CNS depressants and the primary manifestation of drug usage is sedation. Depending on the dose, barbiturates are capable of producing effects from mild sedation to hypnosis, to anesthesia and deep coma. Barbiturates primarily enhance the response of gamma-amino butyric acid (GABA), the primary inhibitory neurotransmitter in the CNS, at $GABA_A$

receptors. This decreases neuronal excitability, interferes with the transmission of impulses, decreases motor activity, and impairs cognitive function, producing drowsiness, sedation, and hypnosis.

Due to their relatively long half-lives, barbiturates can have a "hangover" effect due to their slow excretion. Barbiturates also have a narrow therapeutic index and severe intoxication can cause unconsciousness, respiratory depression, hypotension, nausea and aspiration, renal failure, cardiac arrhythmias, and death.

4. Ethanol-Drug Interaction

Concurrent use of ethanol and other CNS depressants is likely to cause enhancements of the sedative-hypnotic effects of the barbiturates. As with most CNS depressants, the toxicity of barbiturates is potentiated by ethanol.

C. Methods of Analysis

The detection of barbiturates, like most drugs or drug classes, often depends on the dose of the drug, the time elapsed after administration, and the capabilities of the analytical method utilized. However, various screening and confirmatory methods are available that simultaneously detect multiple common barbiturates in whole blood, plasma, and urine. Moreover, both clinical laboratories and forensic laboratories are capable of detecting barbiturates, particularly in urine specimens. As with most alleged DFSA cases, urine is the specimen of choice for analysis although most barbiturates are detectable in blood for at least 12–24 h, even if single oral doses are administered.

1. Extraction Techniques

The extraction of barbiturates is typically performed using acidic-neutral conditions (e.g., pH of approximately 5–7) and generally does not require extensive procedures. Both liquid-liquid and solid-phase extraction techniques are commonly employed, and specimen extraction volumes of 1 mL or less are usually adequate for the detection of barbiturates in the low mg/L range. Although most reported methods are targeted toward the parent barbiturate drug, detection of the hydroxylated metabolites in urine may extend the detection time for several h to days.

2. Instrumental Methods

Several commercial immunoassays are available for the detection of barbiturates in plasma and urine, but these methods cross-react with most barbiturate derivatives and the results must be confirmed for forensic purposes using qualitative testing. The types of immunoassay available include enzyme multiplied immunoassay technique (EMIT), enzyme-linked immunosorbent assay (ELISA), and radioimmunoassay (RIA). Most immunoassays are targeted to detect secobarbital at a cutoff concentration of 0.1–0.2 mg/L, with sufficient cross-reactivity with most other barbiturates at similar concentrations.

GC is a common method for separating and analyzing multiple barbiturates and several metabolites. It is highly sensitive and specific, particularly when used in combination with MS. Limits of detection are typically in the range of 0.05–1 mg/L or lower. Numerous techniques have been described including flame-ionization detection (FID) or nitrogen-selective detection of the underivatized drugs [95,108]. Sufficient resolution and chromatography is attained without derivatization; however, chromatography can be improved with derivatization—e.g., FID of the methyl or ethyl derivatives; and electron-capture detection (ECD) of the 2-chloroethyl or pentafluorobenzyl derivatives [80,96].

HPLC has commonly been employed for years and typical detection limits of less than 1 mg/L are attained. HPLC using a C_8 or C_{18} reverse-phase analytical column can provide separation using a variety of simple mobile phases such as acetonitrile:phosphate buffer [117]. However, interference from endogenous components can be an issue when using the low wavelength typically used for barbiturates. When used in combination with MS (LC-MS) or tandem-MS (LC-MS-MS), detection limits as low as 0.001–0.05 mg/L are often observed [29,84].

Specific properties of several barbiturates, relevant to their detection, are shown in **Table 3**.

II. ANTIPSYCHOTICS

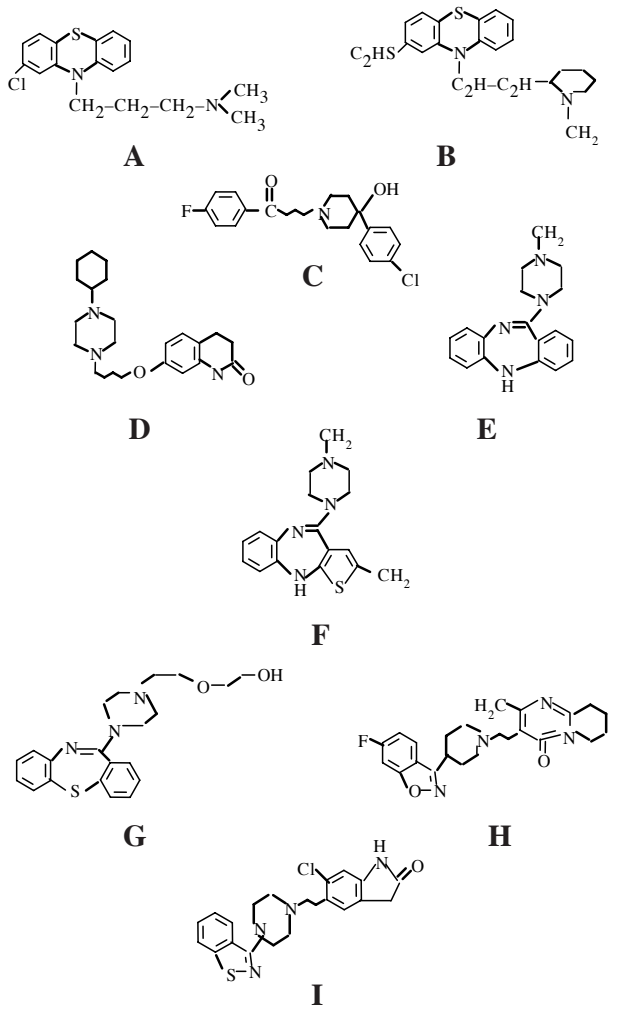
Chemical structures of antipsychotics included in this review are shown in **Structure 2**. Pharmacologically, the older "typical" antipsychotics such as chlorpromazine (available since 1952), thioridazine (1959) and haloperidol (1967) used to be referred to as "major tranquilizers" as they were the first choice for calming or sedating psychotic patients. More recently, "atypical" antipsychotics have been developed and include clozapine (1980s), risperidone (1990), ziprasidone (1992), olanzapine (1996), quetiapine (1997), and aripiprazole (2002) [*see* **Table 4**]. Atypical antipsychotics are named as such as their binding profile to dopamine receptors and effects on various dopamine-mediated behaviors differs from the typical antipsychotic drugs.

Antipsychotics, now also referred to as neuroleptics, are CNS depressants that are primarily indicated for the treatment of schizophrenia and the control of other psychotic disorders. The primary manifestation of drug

Table 3. Some specific properties of barbiturates

Compound	UV absorption (nm)	Principal ion (*m/z*)
Phenobarbital	239 (pH 9.2); 254 (pH 13)	204, 117, 146, 161, 77, 103, 115, 118
Pentobarbital	239 (pH 9.2); 255 (pH 13)	141, 156, 43, 41, 157, 55, 39, 98
Secobarbital	239 (pH 9.2); 254 (pH 13)	167, 168, 41, 43, 97, 124, 39, 55
Thiopental	255 (pH 9.2); 303 (pH 13)	172, 157, 173, 4, 41, 55, 69, 71

use is sedation, and adverse reactions to antipsychotic therapy include sedation, drowsiness, and dizziness, particularly at the beginning of treatment. Thioridazine is usually indicated for the management of schizophrenic patients who have not been responsive to, or cannot tolerate, other antipsychotics; clozapine is used to treat severely ill schizophrenic patients who do not respond adequately to standard antipsychotic treatment; and aripiprazole is additionally approved for the treatment of acute manic and mixed episodes associated with bipolar disorder, and as an adjunct for the treatment of major depressive disorder. The active metabolite of risperidone, 9-hydroxyrisperidone, is also now available for use as an antipsychotic under the trade name of Invega®.



Structure 2. Chemical structures of antipsychotics: chlorpromazine (**A**); thioridazine(**B**); haioperidol (**C**); aripiprazole (**D**); clozapine (**E**); olanzapine (**F**); Quetiapine (**G**); risperidone (**H**); ziprasidone (**I**).

A. Chemistry

Chlorpromazine is a phenothiazine derivative formally known as 3-(2-chlorophenothiazin-10-yl)-*NN*-dimethylpropylamine [85]. It has the empirical formula of $C_{17}H_{19}ClN_2S$ and a molecular weight of 318.9. In its pure form it is a white to creamy-white powder or waxy solid. It has a pK_a of 9.3, is soluble in ethanol but practically insoluble in water. The hydrochloride salt is readily soluble in both water and ethanol.

Thioridazine is a phenothiazine derivative formally known as 10-[2-(1-methyl-2-piperidyl)ethyl]-2-methylthiophenothiazine [85]. It has the empirical formula of $C_{21}H_{26}N_2S_2$ and a molecular weight of 370.6. In its pure form it is a white or slightly yellow crystalline powder that darkens on exposure to light. It has a pK_a of 9.5 and is soluble in ethanol but practically insoluble in water. The hydrochloride salt is readily soluble in both water and ethanol.

Haloperidol is a butyrophenone derivative formally known as 4-[4-(4-chlorophenyl)-4-hydroxypiperidino]-4'-fluorobutyrophenone [85]. It has the empirical formula $C_{21}H_{23}ClFNO_2$ and a molecular weight of 375.9. It is a white to faintly yellowish, amorphous or microcrystalline powder. It has a pK_a of 8.3, and is somewhat soluble in ethanol and practically insoluble in water.

Aripiprazole is a quinolinone derivative formally known as 7-[4-[4-(2,3-dichlorophenyl)-1-piperazinyl]-butoxy]-3,4-dihydrocarbostyril [97]. It has the empirical formula of $C_{23}H_{27}Cl_2\ N_3O_2$ and a molecular weight of 448.4. It is a white crystalline powder and is practically insoluble in water (pH dependent), and has a pK_a of 7.3.

Clozapine is a tricyclic bibenzodiazepine derivative formally known as 8-chloro-11-(4-methylpiperazin-1-yl)-5*H*-dibenzo[*b,e*][1,4]diazepine [85]. It has the empirical formula of $C_{18}H_{19}ClN_4$ and a molecular weight of 326.8. In its pure form it is a yellow crystal.

Table 4. Peak concentrations observed following the administration of a single dose of common antipsychotics

Antipsychotic (Trade Name)	Half-life (h)	Single oral dose (mg)	Cmax [a] (ng/mL)	Tmax [b] (h)	Specimen
Typical					
Chlorpromazine (Largactil®, Thorazine®)	15–30	25, 150	1, 18	2.8, 3	Plasma
Thioridazine (Mellaril®)	26–36	25, 100	50, 240	4, 1.7	Serum
Haloperidol (Haldol®)	14–41	10	3	5	Serum
Atypical					
Aripiprazole (Abilify®)	60-90	10	39	5	Plasma
Clozapine (Clozaril®, Leponex®)	4–12	100	140	1.5	Plasma
Olanzapine (Zyprexa®)	21–54	5, 5	6.8, 7.1	5.1, 4.4	Plasma
Quetiapine (Seroquel®)	3–9	25	45	1.3	Plasma
Risperidone (Risperdal®)	3–20	1, 2, 4	7.9, 16, 27	0.8, 1.5, 1.5	Plasma
Ziprasidone (Geoden®)	2–8	20	51	3–6	Plasma

[a] Average maximum concentration.
[b] Time to maximum concentration.

Olanzapine is a tetracyclic thienobenzodiazepine derivative formally known as 2-methyl-4-(4-methyl-1-piperazinyl)-10*H*-thieno[2,3-b][1,5]benzodiazepine [85]. It has the empirical formula of $C_{17}H_{20}N_4S$ and a molecular weight of 312.4. It is a yellow crystalline solid, practically insoluble in water. It has a pK_a of 5.0 and 7.4.

Quetiapine is a dibenzothiazine derivative formally known as 2-[2-(4-dibenzo [*b*,*f*][1,4]thiazepin-11-yl-1-piperazinyl)ethoxy] [71]. It has the empirical formula of $C_{42}H_{50}N_6O_4S_2$ and a molecular weight of 767.0. It is a white to off-white crystalline powder with moderate solubility in water. It has a pK_a of 3.3 and 6.8.

Risperidone is a benzisoxazole derivative formally known as 3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-2-methyl-4H-pyrido[1,2-a]pyrimidin-4-one [71]. It has the empirical formula of $C_{23}H_{27}FN_4O_2$ and a molecular weight of 410.5. It is a white or almost white powder that is practically insoluble in water and sparingly soluble in ethanol. It has a pK_a of 3.1 and 8.2.

Ziprasidone is a chemically related to risperidone and is formally known as 5-[2-[4-(1,2-benzisothiazol-3-yl)-1-piperazinyl]ethyl]-6-chloro-1,3-dihydro-2*H*-indol-2-one [97]. It has the empirical formula of $C_{21}H_{21}ClN_4OS$ and a molecular weight of 412.9. It is a white to off-white powder, with a pK_a of 8.2 and 13.3.

B. Pharmacology

1. Administration

Chlorpromazine is available as the hydrochloride salt. It is available in 10-, 25-, 50-, 100- and 200-mg normal-release tablets; 30- and 150-mg sustained-release capsules and a 10-mg/5-mL syrup for oral administration; and 25-mg/mL ampules for injection. Single doses for adults are usually in the range of 25–100 mg for acute disturbances, and up to 2,400 mg per day for chronic therapy.

Thioridazine is available as the hydrochloride salt. It is supplied as 10-, 15-, 25-, 50-, 100-, 150- and 200-mg tablets; a 25- or 100-mg/5-mL syrup; and a 30- or 100-mg/mL concentrate for oral administration. Daily adult doses are typically in the range of 100–800 mg, given in divided doses.

Haloperidol is available as the lactate salt and is supplied in tablets of 0.5–20 mg strength; a 1–2 mg/mL concentrate for oral administration; and a 5-mg/mL concentrate for intramuscular injection. Daily adult doses are often in the range of 0.5–5 mg, given two or three times a day. Total daily doses typically do not exceed 100 mg.

Clozapine is available as the free base in 25- and 100-mg tablets for oral administration. At the beginning of treatment, starting doses are 12.5 mg given once or twice a day. This is gradually increased to maintenance doses in range of 300–450 mg, given in two or three divided doses.

Olanzapine is available as the free base in 2.5-, 5-, 7.5-, and 10-mg tablets and as a 5-mg/mL solution for intramuscular injection. Oral doses are typically in the range of 10–20 mg, given once a day.

Quetiapine is available as the fumarate salt in 25-, 50-, 100-, 200-, 300-, and 400-mg tablets. Daily oral doses are usually in the range of 150–800 mg, given in two or three divided doses.

Risperidone is available as the free base in 0.25-, 0.5-, 1-, 2-, 3-, and 4-mg tablets; in 0.5-, 1-, 2-, 3-, and 4-mg oral disintegrating tablets; and a 1-mg/mL solution for oral administration. Daily doses range from 2–6 mg.

Ziprasidone is available as hydrochloride or mesylate salt. It is supplied in 5–80-mg tablets or capsules for oral administration, and a 10–20-mg/mL solution for intramuscular injection. Daily doses are typically in the range of 10–120 mg.

Aripiprazole is available as the free base. It is supplied in 2-, 5-, 10-, 15-, 20-, and 30-mg standard tablets; a 10- or 15-mg disintegrating tablet; a 1-mg/mL oral solution; and a 7.5-mg/mL solution for intramuscular injection. The recommended starting and target dose is 10–15 mg once a day (adults) and 10 mg/day for adolescents. Up to 30 mg/day can be prescribed.

2. Pharmacokinetics

The pharmacokinetics of antipsychotics is complex and these drugs are extensively metabolized. Blood concentrations can vary from one patient to another and can be relatively low following single therapeutic doses, requiring targeted analysis for their detection [see Table 4].

a. Chlorpromazine

Its oral bioavailability varies due to considerable first-pass metabolism by the liver. The onset of action is usually 30–60 min following oral administration, and the average half-life is 15–30 h (reported range of 7–120 h). A single oral dose of 25 mg given to 4 subjects resulted in an average peak plasma concentration of 1 ng/mL at 2.8 h [71]. A single oral dose of 150 mg resulted in an average peak plasma concentration of 18 ng/mL at 3 h, declining to 13 ng/mL by 6 h [50]. Chlorpromazine is extensively metabolized and at least 20 different metabolites have been isolated. Major biotransformation pathways include sulfoxidation, *N*-demethylation, *N*-oxidation, phenolic hydroxylation, and dehalogenation. The metabolites norchlorpromazine and 7-hydroxychlorpromazine are active. Anywhere from 20–70% of an oral dose is excreted in the urine, with less than 1% unchanged drug.

b. Thioridazine

The average systemic bioavailability is approximately 60%. It is rapidly absorbed and has a half-life of 26–36 h. Maximum plasma concentrations are reached within 2–4 h following oral ingestion. A single 25-mg oral dose resulted in an average serum concentration of 50 ng/mL at 4 h. The concentrations of the primary active metabolites were 170 ng/mL for mesoridazine and 50 ng/mL for sulforidazine [19]. A single 100-mg oral dose given to 5 subjects resulted in an average peak serum thioridazine concentration of 240 ng/mL at 1.7 h [5]. Anywhere from 2.5–17% of a daily dose is excreted in the 24-h urine, with only 0.5% as unchanged drug. Over time, approximately 35% of a dose is excreted in urine, with 30% appearing as the metabolites and less than 4% as unchanged drug.

c. Haloperidol

Readily absorbed after oral administration, with a bioavailability of about 65%, it is rapidly taken up in the brain and has an elimination half-life of 14–41 h. Peak concentrations are generally reached within 2–6 h following oral administration. A single 10-mg oral dose to 7 subjects resulted in an average peak serum concentration of 3 ng/mL at 5 h [32]. It is extensively biotransformed to inactive metabolites, namely 4-fluorobenzoylpropionic acid and 4-fluorophenylaceturic acid. Approximately 40% of a dose is eliminated in the urine within 5 days, with only about 1% as unchanged drug.

d. Aripiprazole

This is well absorbed orally and its bioavailability is 87%. Peak plasma concentrations are achieved 3–5 h after oral administration. It has an elimination half-life of approximately 75 h (range 60–90 h), which can almost double in poor metabolizers. Single 10-mg-per-day oral doses given to 6 subjects for a 2-week period resulted in a peak plasma concentration of 39 ng/mL at 5 h after the initial dose, and 163 ng/mL at 2.8 h following the last dose [72]. Aripiprazole undergoes extensive hepatic metabolism including dehydrogenation to the active metabolite dehydro-aripiprazole, and also hydroxylation and *N*-dealkylation. Dehydro-aripiprazole can accumulate to approximately 40% of the aripiprazole concentration. Following a single oral labeled dose, 25% of the dose is eliminated in the urine, with less than 1% as unchanged drug.

e. Clozapine

The oral bioavailability is 60–70%. The elimination half-life is 8 h (range 4–12 h) after a single dose, and is longer with chronic dosing. A single 100-mg oral dose given to 12 subjects resulted in an average peak plasma concentration of 140 ng/mL after 1.5 h [1]. Clozapine is almost completely metabolized and the two main metabolites are norclozapine and clozapine-*N*-oxide. Approximately 80% of an administered dose is excreted in the urine, with only trace amounts as the unchanged drug.

f. Olanzapine

Is well absorbed and has an elimination half-life of 21–54 h (average 30 h). A single 5-mg oral dose resulted in an average peak plasma concentration of 6.8 ng/mL at an average of 5.1 h [73]. Similarly, a single 5-mg oral dose to 10 subjects resulted in an average peak plasma concentration of 7.1 ng/mL at 4.4 h [74]. Olanzapine is extensively biotransformed to inactive metabolites, with

the major metabolites being the 10-*N*-glucuronide and 42-*N*-desmethylolanzapine. Following a single oral labeled dose, 57% of the drug was eliminated in the urine over a 4-day period, with approximately 7% as the unchanged drug.

g. Quetiapine

The oral bioavailability is 100% and it has a half-life 2.7–9.3 h (average 6 h). A single 25-mg oral dose given to 12 subjects resulted in an average peak plasma concentration of 45 ng/mL at 1.3 h [42]. Quetiapine is biotransformed into approximately 20 inactive metabolites, although the 7-hydroxyquetiapine is active. Following a single labeled oral dose, 73% of the dose is eliminated in the urine over 4-day period, with less than 1% as unchanged drug.

h. Risperidon

The oral bioavailability is approximately 70%, and the elimination half-life is as low as 3 h for extensive metabolizers and 20 h for poor metabolizers. A single 1-mg oral dose given to 9 subjects (extensive metabolizes) resulted in an average peak plasma concentration of 7.9 ng/mL at 0.8 h [52]. A single 2-mg oral dose given to 22 subjects resulted in an average peak plasma concentration of 16 ng/mL at 1.5 h [133], and a single 4-mg oral dose given to 24 subjects resulted in an average peak plasma concentration of 27 ng/mL at 1.5 h [11]. Risperidone is extensively metabolized in the liver, producing 9-hydroxyrisperidone through hydroxylation as its major active metabolite. A minor metabolic pathway is through *N*-dealkylation. Following a single labeled oral dose, 70% of the dose is eliminated in the urine over a 7-week period; in extensive metabolizers, approximately 4% is excreted as unchanged drug and 32% as 9-hydroxyrisperidone, while in poor metabolizers approximately 30% is excreted as the unchanged drug and 8% as 9-hydroxyrisperidone.

i. Ziprasidone

The oral bioavailability is approximately 60%, and the half-life is 2–8 h. A single 20-mg oral dose given to 9 subjects resulted in an average peak plasma concentration of 51 ng/mL at 3–6 h [44]. Ziprasidone is extensively metabolized to at least 12 inactive metabolites. Following a single oral labeled dose, 20% of the drug is excreted in the urine over an 11-day period, with less than 5% as the unchanged drug.

3. Pharmacodynamics

When used for the treatment of psychiatric disorders, the range of dosages used can differ dramatically from one patient to another; however, tolerance to very high doses is usually attained over an extended period of time. Single doses of most antipsychotics can produce subjective sedation; however, after chronic dosing, patients usually become tolerant to the sedative effects. Other adverse effects may include drowsiness, confusion, tremor, muscle rigidity, and ataxia. For those antipsychotics with additional anticholinergic effects, symptoms such as dry mouth, blurred vision, and urinary retention can be experienced. This anticholinergic action may also produce a degree of amnesia or memory loss.

The proposed mechanism of action of antipsychotics is unknown, but they are thought to act primarily by inhibition of the neurotransmitter dopamine. For the typical antipsychotics, this is primarily mediated by antagonism at dopamine D_2 receptors. Most atypical antipsychotics are thought to have a different mechanism of action, mediated through a combination of primarily dopamine D_2 and serotonin 5-HT_2 antagonism. Specific receptor affinities are detailed below.

Chlorpromazine has a high binding affinity for dopamine D_2 and serotonin 5-HT_2 receptors. It also has a high affinity at dopamine D_1, D_3, D_5, and serotonin 5-HT_1 receptors, and acts as an antagonist at histamine H_1 receptors, adrenergic a_1 and a_2 receptors, and muscarinic M_1 and M_2 receptors.

Aripiprazole's mechanism of action is thought to be mediated through a combination of partial agonist action at dopamine D_2 receptors and serotonin 5-HT_{1A} receptors, and antagonism at serotonin 5-HT_{2A} receptors. It also has high binding affinity with dopamine D_3 receptors, and moderate affinity at dopamine D_4 receptors, serotonin 5-HT_{2C} and 5HT_7 receptors, adrenergic a_1 receptors, and histamine H_1 receptors.

Clozapine has a high binding affinity for dopamine D_4 receptors, and moderate affinity at dopamine D_1, D_2, D_3, and D_5 receptors. It also acts as an antagonist at adrenergic, cholinergic, histaminergic, and serotonergic receptors.

Olanzapine is a serotonin receptor (5-HT_{2A}, 5-HT_{2C}, 5-HT_6) and dopamine receptor (D_{1-4}) antagonist, with additional anticholinergic properties. It also has high affinity for histamine H_1 and adrenergic a_1 receptors, and moderate affinity for serotonin 5-HT_3 and muscarinic M_{1-5}.

Quetiapine is an antagonist at serotonin 5-HT_{1A} and 5-HT_2 receptors, dopamine D_1 and D_2 receptors, histamine H_1 receptors, and adrenergic a_1 and a_2 receptors.

Risperidone is a serotonin and dopamine receptor antagonist, with high affinity for serotonin 5-HT_2 receptors, dopamine D_2 receptors, adrenergic a_1 and a_2 receptors, and histamine H_1 receptors. It also has a low-to-moderate affinity for serotonin 5-HT_{1C}, 5-HT_{1D} and 5-HT_{1A}, and a weak affinity for dopamine D_1 receptors.

Ziprasidone functions as an antagonist at dopamine D_2 receptors, serotonin 5-HT_{2A} and 5-HT_{1D} receptors,

and an agonist at 5-HT_{1A}. It also has a high affinity for dopamine D_3 receptors, serotonin 5-HT_{2A}, 5-HT_{2C}, 5-$HT_{1A,}$ and 5-HT_{1D} receptors, and adrenergic a_1 receptors; and a moderate affinity for histamine H_1 receptors.

4. Ethanol-Drug Interaction

Overall, concurrent use of ethanol and other CNS depressants is likely to cause enhancements of the sedative effects of the antipsychotics. However, ethanol has not been shown to enhance the decremental effects of haloperidol, aripiprazole, quetiapine, or ziprasidone. Ethanol may decrease the plasma concentration of olanzapine.

C. Methods of Analysis

The detection of antipsychotics relies heavily on the dose of the specific drug, the time elapsed after administration, and the capabilities of the analytical method utilized. As with most alleged DFSA cases, urine is the specimen of choice for analysis. The higher-dose typical antipsychotics are readily detected in the urine by suitable techniques (e.g., common basic and neutral drug screens), and are detectable for up to several days after an alleged assault. However, many of the lower-dose atypical antipsychotics can be extremely difficult to detect, even with targeted analyses. Furthermore, little if any of the parent drug is evident in the urine, so the detection of the metabolites would potentially extend the detection time for several h to days. Olanzapine causes additional concern analytically by undergoing significant oxidation in vitro.

1. Extraction Techniques

Antipsychotics are readily extracted from biological specimens by liquid-liquid extraction following alkalinization, or by solid-phase extraction. With suitable instrumentation, specimen extraction volumes of 1 mL are usually adequate for the detection of antipsychotics, even in the low mg/L concentration ranges.

2. Instrumental Methods

Commercial immunoassays tests for the antipsychotics either do not exist or are not readily available. Instead, chromatographic methods such as HPLC and GC are necessary. HPLC, typically coupled with MS or tandem-MS, is preferable for the analysis of many of the atypical antipsychotic drugs.

GC is a common method for separating and analyzing antipsychotics [51,60,129]. It can be highly sensitive and specific, particularly when used in combination with MS, and detection limits of 1–10 ng/mL can be attained. Most antipsychotics drugs contain a secondary or tertiary amine; subsequently nitrogen-phosphate detection is a good choice. However, derivatization is often needed to improve the chromatography for some of the antipsychotics (e.g., haloperidol). Thermal degradation of risperidone precludes its determination using GC.

Many recently published methods for the analysis of a range of antipsychotics employ HPLC, LC-MS, or LC-MS-MS [60,65,133]. Reverse-phase C_8 and C_{18} analytical columns are commonly used. Peak tailing and resolution can often be a problem when analyzing antipsychotics, and this can be suppressed by silanization of the reversed-phase support, using ion-pair chromatography, and by adding alkylamines such as triethylamine to the mobile phase. Detection limits are often in the range of 0.1–5 ng/mL.

Specific properties of the antipsychotics, relevant to their detection, are summarized in **Table 5**.

III. OPIOIDS

The opioids are a class of substances that bind to opioid receptors (e.g., delta, kappa, mu) to produce analgesia. They include natural, semisynthetic, and synthetic alkaloidal substances and are obtained via prescription or through illegal sources. The principal indication for most opioids is the relief of moderate to severe pain in both acute and chronic pain management.

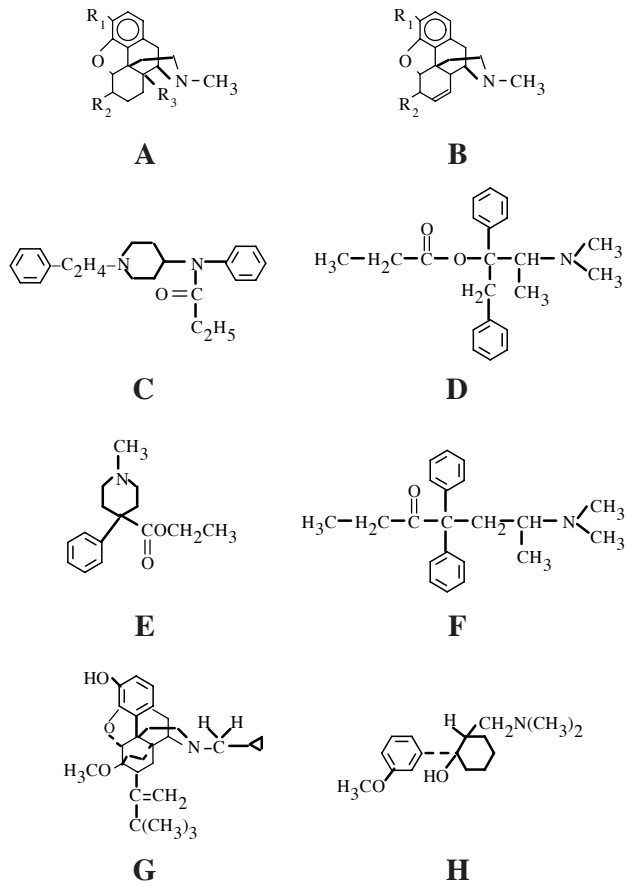
Table 5. Some specific properties of antipsychotics

Compound	UV absorption (nm)	Principal ion (*m/z*)
Chlorpromazine	255 (acidic)	58, 86, 318, 85, 320, 272, 319, 273
Thioridazine	262 (acidic); 275 (alkaline)	98, 370, 126, 99, 40, 70, 371, 258
Mesoridazine	—	98, 70, 99, 42, 386, 126, 55, 41
Haloperidol	245 (acidic)	224, 42, 237, 226, 123, 206, 239, 56
Clozapine	245, 297 (acidic); 238, 261, 297 (akaline)	243, 256, 70, 245, 192, 227, 258, 326
Olanzapine	—	242, 229, 213, 42, 56, 43, 198, 312
Quetiapine	—	21, 144, 239, 45, 321, 209, 95, 211
Risperidone	237, 275 (acidic)	220, 191, 204, 178, 192, 221, 233, 410

Other indications include their use as an antitussive, anti-diarrheal agent, preoperative analgesia, and maintenance therapy for narcotic addicts. The primary manifestation of drug usage is sedation; other common side effects following opioid use includes drowsiness, confusion, weakness, lethargy, ataxia, visual disturbances, respiratory depression, and constipation.

Naturally occurring opioids are those obtained by extraction from the opium poppy, *Papaver somniferum*. The milky resin that seeps from incisions made in the unripe seedpod is dried and powdered to make opium, which contains a number of alkaloids including morphine, codeine, and thebaine. While opium itself has been used historically for hundreds of years, morphine was not isolated until 1805. Semisynthetic opioids derived from morphine include heroin, codeine, and hydromorphone. Opioids derived from codeine include dihydrocodeine and hydrocodone, while buprenorphine, oxycodone, and oxymorphone are synthesized from thebaine. Synthetic opioids have chemical structures dissimilar to morphine and include meperidine, methadone, propoxyphene, and tramadol.

Chemical structures of opioids included in this review are shown in **Structure 3**.



Structure 3. Chemical structures of opioids: morphine (**A**, R_1: OH; R_2: OH; R_3: H); hydromophone (**A**, R_1: O; R_2: OH; R_3: H); oxymophone (**A**, R1: O; R_2: OH; R_3: OH); codeine (**A**, R_1: OH; R_2: OCH_3; R_3: H); hydrocodone (**A**, R_1: O; R_2: OCH_3; R_3: H); xycodone (**A**, R_1: O; R_2: OCH_3; R_3: OH); heroin (**B**, R_1: $COOCH_3$; R_2: $COOCH_3$); dihydrocodeine (**B**, R_1: OH; R_2: OCH_3); phentanyl (**C**); propoxyphene (**D**); meperidine (**E**); methadone (**F**); buprenorphine (**G**); tramadol (**H**).

A. Chemistry

Morphine is the principal alkaloid of opium with a chemical name of (5a,6a)-7,8-didehydro-4,5-epoxy-17-methylmorphinan-3,6-diol monohydrate [85]. It has the empirical formula of $C_{17}H_{19}NO_3.H_2O$ and a molecular weight of 303.4. In its pure form it is a white crystalline powder, or colorless or white acicular crystals. It has a pK_a of 8.0 and 9.9, and is slightly soluble in ethanol and water. The sulfate salt is readily soluble in water and soluble in ethanol.

Buprenorphine is a synthetic thebaine derivative with a chemical name of [5a,7a(S)]-17-(cyclopropylmethyl)-a-(1,1-dimethylethyl)-4,5-epoxy-18,19-dihydro-3-hydroxy-6-methoxy-a-methyl-6,14-ethenomorphinan-7-methanol [97]. It has the empirical formula of $C_{29}H_{41}NO_4$ and a molecular weight of 467.6. The pure form and the hydrochloride salt are a white or almost white crystalline powder. It has a pK_a of 8.5 and 10.0. The hydrochloride salt is soluble in ethanol and sparingly soluble in water.

Codeine is an alkaloid obtained from opium but is typically prepared by 3-O-methylation of morphine. Its chemical name is (5a,6a)-7,8-didehydro-4,5-epoxy-3-methoxy-17-methylmorphinan-6-ol monohydrate. It has the empirical formula of $C_{18}H_{21}NO_3.H_2O$ and a molecular weight of 317.4. In its pure form it is a colorless crystal or a white crystalline powder. It has a pK_a of 8.2, and is soluble in ethanol and water. The phosphate and sulfate salts are soluble in water and slightly less soluble in ethanol.

Dihydrocodeine is prepared by the hydrogenation of codeine and has the chemical name of (5a,6a)-4,5-epoxy-3-methoxy-17-methylmorphinan-6-ol [85]. It has the empirical formula of $C_{18}H_{23}NO_3$ and a molecular weight of 301.4. It has a pK_a of 8.8. The tartrate salt is a colorless crystal or white crystalline powder that is soluble in water and sparingly soluble in ethanol.

Fentanyl has the empirical formula of $C_{22}H_{28}N_2O$ and a molecular weight of 336.5. The chemical name is *N*-phenyl-*N*-[1-(2-phenylethyl)-4-piperidinyl]propanamide [85]. It has a pK_a of 8.4 and is sparingly soluble in water; however, the citrate salt is soluble in ethanol and water. The citrate salt is a white granule or a white glistening crystalline powder.

Hydrocodone is prepared from codeine and has the empirical formula of $C_{18}H_{21}NO_3$ and a molecular weight of 299.4 [85]. The chemical name is 4,5-epoxy-3-methoxy-17-methylmorphinan-6-one. It has a pK_a of 8.3, is soluble in ethanol but practically insoluble in water. The tartrate salt is a white crystal or crystalline powder and is soluble in water and ethanol.

Hydromorphone is formally known as 4,5-epoxy-3-hydroxy-17-methylmorphinan-6-one [85]. It has an empirical formula of $C_{17}H_{19}NO_3$ and a molecular weight of 285.3. It has a pK_a of 8.2, is freely soluble in ethanol and slightly soluble in water. The hydrochloride salt is a white crystalline powder and is soluble in water and ethanol.

Meperidine, also known as pethidine, is formally known as 1-methyl-4-phenyl-4-piperidinecarboxylic acid ethyl ester [85]. It has an empirical formula of $C_{15}H_{21}NO_2$ and a molecular weight of 247.3. It is an oily liquid that slowly crystallizes and has a pK_a of 8.7. The hydrochloride salt is a white crystalline powder that is soluble in water and slightly soluble in ethanol.

Methadone has the empirical formula of $C_{21}H_{27}NO$ and a molecular weight of 309.5 [85]. Its chemical name is 6-dimethylamino-4,4-diphenyl-3-heptanone. It has a pK_a of 8.3, and the hydrochloride salt is soluble in ethanol and water. The hydrochloride salt is a colorless crystal or white crystalline powder.

Oxycodone is prepared from thebaine and has the empirical formula of $C_{18}H_{21}NO_4$ and a molecular weight of 315.4 [85]. Its chemical name is (5a)-4,5-epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one. It has a pK_a of 8.9, is soluble in ethanol but practically insoluble in water. The hydrochloride salt is a white crystalline powder that is soluble in water and ethanol.

Oxymorphone has the empirical formula of $C_{17}H_{19}NO_4$ and a molecular weight of 301.3 [85]. Its chemical name is (5a)-4,5-epoxy-3,14-dihydroxy-17-methylmorphinan-6-one. It has a pK_a of 8.5 and 9.3. The hydrochloride salt is a white powder that is soluble in water and ethanol.

Propoxyphene has the empirical formula of $C_{22}H_{29}NO_2$ and a molecular weight of 339.5 [85]. Its chemical name is (+)-(1*S*,2*R*)-1-benzyl-3-dimethylamino-2-methyl-1-phenyl-propyl propionate. It has a pK_a of 6.3 and is very slightly soluble in water. The hydrochloride salt is a white or slightly yellow powder that is soluble in water and ethanol. The napsylate salt is a white powder that is soluble in ethanol but practically insoluble in water.

Tramadol has the empirical formula of $C_{16}H_{25}NO_2$ and a molecular weight of 263.4 [85]. Its chemical name is (±)-*trans*-2-dimethylaminomethyl-1-(3-methoxy-phenyl)-cyclohexanol. It has a pK_a of 9.4 and the hydrochloride salt is readily soluble in water.

B. Pharmacology

1. Administration

Morphine is available as the sulfate salt in solutions of 0.5–25 mg/mL for subcutaneous, intramuscular, intravenous, epidural, and intrathecal administration. It is also available as normal release 15–30-mg tablets or capsules, a 2–4-mg/mL solution and a 20-mg/mL concentrate for oral administration. Sustained-release tablets or capsules contain 15–200 mg. Typical oral doses of morphine can range from 30–120 mg daily in divided doses, or up to 400 mg daily in opioid-tolerant patients.

Buprenorphine is available as the hydrochloride salt in 0.2- and 0.4-mg sublingual tablets, and 0.3-mg/mL ampules. Typical doses are 0.2–0.4 mg sublingually or 0.3–0.6 mg parenterally, every 6 to 8 h. High-dose tablets of 2 and 8 mg are also available for maintenance therapy of opiate addicts, in doses from 2–16 mg daily.

Codeine is available as the phosphate or sulfate salt, ether alone or in combination with other drugs such as nonnarcotic analgesics and antihistamines. It is available in 15-, 30-, and 60-mg tablets. For pain relief, typical doses range from 15–60 mg orally or subcutaneously every 4 h, with a total daily dose of 60–240 mg.

Dihydrocodeine is supplied as the bitartrate salt in 16-mg tablets or capsules for oral administration. Typical doses of 16–32 mg may be taken every 4 h.

Fentanyl is available as the citrate salt in 50 mg/mL solutions for intravenous, intramuscular, and epidural administration. Single doses of 25–100 mg are administered via these routes as needed. Transdermal patches are 2.5, 5, 7.5, or 10 mg. Transdermal fentanyl patches are available containing 2.5–10 mg of the drug, providing a dose of 25–100 mg/h for 72 h. An oral transmucosal preparation containing 200–1,600 mg has also been developed and is to be consumed within 15 min, up to four times a day.

Heroin is illegally available and can be smoked, snorted, and administered intravenously and subcutaneously. Black tar heroin is typically dissolved, diluted, and injected, while higher purity heroin is often snorted or smoked. Recreationally, daily heroin doses of 5–1,500 mg have been reported, with an average daily dose of 300–500 mg. Heroin may be cut with inert or toxic adulterants such as sugars, starch, powdered milk, quinine, and ketamine.

Hydrocodone is available as the bitartrate salt in tablets, capsules, and cough syrup in strengths of 2.5–10 mg, either by itself or in combination with acetaminophen or ibuprofen. Oral doses may be taken every 4–6 h, with a daily maximum recommended doses of 45 mg.

Hydromorphone is supplied in 2-, 4-, and 8-mg tablets, and 1- and 5-mg/5-mL syrups for oral administration. Typical oral doses range from 1–4 mg, every 4–6 h. It is also available for parenteral and rectal administration.

Meperidine is available as the hydrochloride salt in 50- or 100-mg tablets, and a 50-mg/5-mL solution for oral administration. Solutions of 25–100 mg/mL are available for parenteral injection. Typical single doses range from 25–150 mg, with a maximum recommended daily dosage totaling 1,200 mg.

Methadone is available as the hydrochloride salt in 5-, 10-, and 40-mg tablets or diskets, and 1-, 2-, or 10-mg/mL solutions for oral administration. Solutions of 10 mg/mL are also available for parenteral use. Single doses in nontolerant adults typically range from 5–10 mg, whereas tolerant individuals may ingest daily doses of 20–200 mg for narcotic maintenance therapy.

Oxycodone is supplied as the hydrochloride or terephthalate salt. It is available as normal-release tablets in strengths of 2.5 and 5 mg, sustained-release tablets in strengths of 10, 20, 40, and 80 mg, and solutions of 1 and 20 mg/L for oral administration. Single oral doses of the normal-release tablets in nontolerant adults typically range from 2.5–5 mg every 6 h, whereas chronic pain patients may ingest daily doses of 20–160 mg.

Oxymorphone is available as the hydrochloride salt in 1- and 1.5-mg/mL ampules for intramuscular or subcutaneous administration, and in a 5-mg suppository for rectal administration.

Propoxyphene is available as the hydrochloride salt or the napsylate salt, either alone or in combination with aspirin and acetaminophen. It is supplied in tablets and capsules in strengths of 32, 50, 65, and 100 mg. Typically, doses of 128–390 mg for the hydrochloride salt and 200–600 mg for the napsylate salt are administered daily.

Tramadol is available as the hydrochloride salt in 37.5- and 50-mg strength tablets or capsules for oral administration, and a 50-mg/mL strength solution for parenteral injection. Typical doses are 50–100 mg every 4–6 h, for a daily maximum of 400 mg.

2. Pharmacokinetics

a. Morphine

The oral bioavailability of morphine ranges from 20–40% due to first-pass metabolism, and it has a half-life of 1.3–7 h. Peak plasma morphine concentrations usually occur within an hour of oral administration, and within 5 min following intravenous injection. A single 30-mg oral dose of a normal-release tablet resulted in an average peak plasma concentration of 24 ng/mL at 0.8 h (*see* **Table 5**); while a single 60-mg oral dose of a sustained-release capsule resulted in an average peak plasma concentration of 16 ng/mL at 7.9 h [10]. Oral doses of 10–80 mg resulted in peak serum morphine concentrations of 50–260 ng/mL. Morphine is metabolized by conjugation with glucuronic acid to morphine-3-glucuronide and morphine-6-glucuronide. A small amount of morphine is also demethylated to normorphine, followed by conjugation. Following oral administration, approximately 60% of the dose is excreted in the urine within the first 24 h. Almost 90% of a single morphine dose is eliminated within 72 h, with about 75% as morphine-3-glucuronide and less than 10% as unchanged drug. Morphine itself is also a metabolite of codeine, ethylmorphine, heroin, and pholcodine.

b. Buprenorphine

The half-life of buprenorphine is 2-4 h. A single 2-mg sublingual dose given to 6 subjects resulted in an average peak plasma concentration of 1.6 ng/mL at 1.3 h [28], whereas a single 4-mg sublingual dose given to 6 subjects yielded an average peak plasma concentration of 3.3 ng/mL at 0.8 h [67]. A single 7.7-mg sublingual dose and an 8-mg oral dose were given to 6 subjects 1 week apart – average peak plasma concentrations of 7.1 ng/mL at 0.9 h and 2.9 ng/mL at 1.2 h were attained for the sublingual and oral dosages, respectively [89]. Biotransformation of buprenorphine occurs primarily by *N*-dealkylation and conjugation to norbuprenorphine, which is pharmacologically active. Following a single labeled intramuscular dose, 27% is excreted in the urine within 144 h.

c. Codeine

Codeine is well absorbed orally and has a half-life of approximately 2-4 h. A single 15-mg oral dose given to 2 subjects resulted in an average peak serum concentration of 30 ng/mL at 2 h [110]. A single 30-mg oral dose given to 17 subjects resulted in an average peak plasma concentration of 38 ng/mL at 0.5 h, declining to 18 ng/mL by 4 h [92]. A single 60-mg oral dose given to 20 subjects resulted in an average peak plasma concentration of 134 ng/mL at 1 h [30]. A single 120-mg oral dose resulted in an average peak plasma concentration of 470 ng/mL at 1.2 h [64]. Codeine is biotransformed via *O*-demethylation to morphine and via *N*-demethylation to norcodeine. Over 95% of a single dose is excreted in the urine within 48 h, mostly as free or conjugated codeine, and the conjugated forms of morphine and norcodeine.

d. Dihydrocodeine

The half-life is 3.4–4.5 h. A single 30- or 60-mg oral dose given to 7 subjects resulted in average peak plasma

Table 5. Peak concentrations observed following the administration of a single dose of select opioids

Opioid	Half-life (h)	Single oral	Cmax [a] dose (mg)	Tmax [b] (ng/mL)	Specimen (h)
Buprenorphine	2–4	2[c], 4[c], 7.7[c]	1.6, 3.3, 7.1	1.3, 0.8, 0.9	Plasma
		8	2.9	1.2	Plasma
Codeine	2-4	15, 30	30, 38	2, 0.5	Serum, plasma
		60, 120	134, 470	1, 1.2	Plasma
Dihydrocodeine	3-5	30, 60	70, 150	1.6, 1.8	Plasma
		60	205	1.3	Plasma
Fentanyl	3–12	800 ug [d]	2.1	0.4	Plasma
Hydrocodone	3–9	5, 10	11, 24	1.5, 1.5	Serum
		10	2.5–2.9 mg/L	4.3–6.7	Urine
Hydromorphone	1-4	4	22	0.8–1.5	Plasma
		8	4.7	-	Plasma
		8[e], 16[e], 32[e]	0.7, 1.5, 2.4	9–13.5	Plasma
Meperidine	2–5	50, 100	140, 170	2, 1.3	Serum, plasma
Methadone	15–55	15	75	4	Plasma
Morphine	1–7	30	24	0.8	Plasma
		60 [e]	16	7.9	Plasma
Oxycodone	4–6	10	30	0.8-2.5	Plasma
		20 [e]	23	3.2	Plasma
		40 [e], 80 [e]	39, 99	—	Plasma
Oxymorphone	—	1.5	1.2–1.9 mg/L4		Urine
Propoxyphene	8–24	130	230	2	Plasma
Tramadol	4–7	100	280	2	Serum

[a] average maximum concentration. [b] time to maximum concentration. [c] sublingual administration. [d] oral transmucosal administration. [e] sustained-release preparation.

concentrations of 70 ng/mL at 1.6 h and 150 ng/mL at 1.8 h, respectively [105]. A single 60-mg oral dose given to 14 subjects resulted in an average peak plasma concentration of 205 ng/mL at 1.3 h [35]. Biotransformation is most likely via *N*-demethylation to nordihydrocodeine and via *O*-demethylation to dihydromorphine, followed by conjugation. Approximately 90% of a single oral dose is eliminated in the 24-h urine, mostly as free and conjugated dihydrocodeine, and lesser amounts of the free and conjugated nordihydrocodeine. Dihydrocodeine itself is also a metabolite of hydrocodone.

e. Fentanyl

The oral bioavailability following transmucosal administration is approximately 50% and the half-life is 3–12 h. A single 140-mg/70-kg intravenous dose given to 4 subjects resulted in an average peak serum concentration of 11 ng/mL, declining to approximately 1 ng/mL at 1 h [37]. Following the application of 25-, 50-, 75-, and 100-mg/h transdermal patches, serum fentanyl concentrations of 0.3–1.2 ng/mL, 0.6–1.8 ng/mL, 1.1–2.6 ng/mL, and 1.9–3.8 ng/mL were reached within 24 h, respectively [97]. An 800-mg transmucosal dose given to 12 subjects over 15 min resulted in an average peak plasma concentration of 2.1 ng/mL at 0.4 h [25]. Up to 85% of a labeled intravenous dose is excreted in the urine over a 3–4 day period, with 0.4–6% as the unchanged drug and 26–55% as norfentanyl. Other metabolites include hydroxyfentanyl, hydroxynorfentanyl, and despropionylfentanyl.

f. Hydrocodone

The half-life is 3.4–8.8 h. A single 5-mg oral dose to 1 subject resulted in a peak serum concentration of 11 ng/mL at 1.5 h [47]. A single 10-mg oral dose given to 5 subjects resulted in an average peak serum concentration of 24 ng/mL at 1.5 h, declining to 7 ng/mL by 8 h [7]. A single 10-mg oral dose given to 2 subjects resulted in peak urine concentrations of 2.5 and 2.9 mg/L for hydrocodone and 0.2–0.6 mg/L for total hydromorphone at 4.3–6.7 h [112]. Biotransformation occurs via *O*- and *N*-demethylation to hydromorphone and norhydrocodone; reduction of the 6-keto group to 6-a-hydrocodol and 6-a-hydromorphol; followed by conjugation. Approximately 26% of a dose is excreted in the urine within 72 h, with about 12% as the unchanged drug, 5% as norhydrocodone,

and 4% as conjugated hydromorphone. The metabolite 6-a-hydrocodol is also known as dihydrocodeine, while hydrocodone itself is also a metabolite of codeine.

g. Hydromorphone

The oral bioavailability is 51% and the half-life is 1.5–3.8 h. A single 4-mg oral dose given to 6 subjects resulted in an average peak plasma concentration of 22 ng/mL at 0.8–1.5 h [120]. A single 8-mg oral dose of the normal-release tablet given to 12 subjects resulted in an average peak plasma concentration of 4.7 ng/mL at 0.8–1 h [3]. In the same study, a single 8-, 16-, or 32-mg sustained-release oral dose given to 12 subjects resulted in average peak plasma concentrations of 0.7 ng/mL, 1.5 ng/mL, and 2.4 ng/mL, respectively, at 9–13.5 h. A single 4-mg intramuscular dose given to 2 subjects resulted in peak urinary total hydromorphone concentrations of 3.9–4.3 mg/L at 6.4–7.0 h [120]. Hydromorphone is metabolized to the 6a- and 6b-hydroxy metabolites (6-hydromorphol), followed by conjugation. Approximately 6% of a dose is excreted as free hydromorphone in the 24-h urine, and 30% as conjugated hydromorphone. Hydromorphone itself is a metabolite of hydrocodone.

h. Meperidine

Undergoes considerable first-pass metabolism and the oral bioavailability is 50–60%. It has a half-life of 2–5 h. A single 100-mg oral dose given to 4 subjects resulted in an average peak plasma concentration of 170 ng/mL at 1.3 h [77]. A single 50-mg oral dose given to 6 subjects resulted in an average peak serum concentration of 140 ng/mL at 2 h [114]. Meperidine is metabolized to normeperidine, and both are then de-esterified to meperidinic acid and normeperidinic acid. Approximately 70% of a dose is excreted in the urine in 24 h, with up to 10% as unchanged drug and 10–20% as normeperidine. Urinary excretion of meperidine and normeperidine may both be enhanced to about 30% if the urine is acidic, compared to less than 5% when the urine is alkaline.

i. Methadone

The oral bioavailability averages 79% and the half-life is 15–55 h. A single 15-mg oral dose given to 15 subjects resulted in an average peak plasma methadone concentration of 75 ng/mL at 4 h, declining to 30 ng/mL at 24 h [56]. Methadone is metabolized by mono- and di-*N*-demethylation, with spontaneous cyclization of the resulting unstable metabolites to 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrroline (EDDP) and 2-ethyl—methyl-3,3-diphenyl-1-pyrroline (EMDP). This is followed by conjugation with glucuronide. Small amounts of methadol and normethadol are also produced. Following a 5-mg oral dose, methadone and EDDP accounted for 5% of the dose each in the 24-h urine. Acidification of the urine resulted in excretion of 22% of the dose as unchanged methadone and only 2% as EDDP.

j. Oxycodone

The oral bioavailability averages 42% and the half-life is 4–6 h. A single 10-mg oral dose to 12 subjects resulted in an average peak plasma concentration of 30 ng/mL at 0.8–2.5 h [68]. A single 20-mg controlled-release dose given to 28 adults resulted in an average peak plasma concentration of 23.2 ng/mL at 3.2 h [61]. Subjects receiving single 40- or 80-mg controlled-release tablets achieved average peak plasma concentrations of 39 and 99 mg/L, respectively [97]. Oxycodone is metabolized by *O*-demethylation to oxymorphone and by *N*-demethylation to noroxycodone. Approximately 30–60% of a single dose is excreted in the 24-h urine as free (13–19%) and conjugated oxycodone (7–29%), and conjugated oxymorphone (13–14%). Following a single 10-mg oral dose, concentrations of total oxycodone and total oxymorphone in 2 subjects did not exceed 2.5 mg/L [120].

k. Oxymorphone

Concentrations in plasma, serum, and blood following therapeutic administration are not readily available. Oxymorphone is extensively metabolized to the 6a- and 6b-hydroxy metabolites (6-oxymorphol), followed by conjugation. About 50% of an oral dose is excreted in the urine in 5 days, mostly in the first 24 h, mainly as conjugate oxymorphone (44%) and only about 2% as unchanged drug. Peak urinary concentrations of total oxymorphone in 2 subjects receiving a single 1.5-mg oral dose ranged from 1.2–1.9 mg/L and occurred at 4 h post dose [120]. Oxymorphone itself is also a metabolite of oxycodone.

l. Propoxyphene

Undergoes considerable first-pass metabolism and the oral bioavailability is about 40%. It has a half-life of 8–24 h. A single 130-mg oral dose given to 6 subjects resulted in an average peak plasma concentration of 230 ng/mL at 2 h [121]. Propoxyphene is biotransformed via *N*-demethylation to norpropoxyphene and dinorpropoxyphene. Approximately 35% of a dose is excreted in the 24-h urine, with about 13% as norpropoxyphene and up to 5% as unchanged drug. A total of 60–70% of a dose is excreted in urine in about 5 days.

m. Tramadol

Oral bioavailability is about 65% and the half-life is 4.3–6.7 h. A single 100-mg dose given to 10 subjects resulted in an average peak serum concentration of 280 ng/mL at 2 h [69]. Biotransformation is via *N*- and *O*-

demethylation, followed by conjugation with glucuronide acid and sulfate. The major metabolites formed are *N*-desmethyltramadol, *N*,*O*-didesmethyltramadol, and *O*-desmethyltramadol, with the latter having greater analgesic activity than the parent drug. Approximately 90% of an oral dose is excreted in the urine within 3 days, with about 30% as the unchanged drug and 20% as the free and conjugated *O*-desmethyltramadol.

3. Pharmacodynamics

The pharmacological effects of the opioids are due to their interaction with several opioid receptors in the CNS and gastrointestinal tract, namely the mu (μ), kappa (κ), and delta (δ) receptors (*see* **Table 6**). The opioids bind to the receptors with differing affinity resulting in the varying responses observed with individual opioids. Furthermore, the effects depend heavily on the dose of opioid, the route of administration, and previous exposure/tolerance.

Interactions at the m_1-receptors result in CNS depression, analgesia, pain modulation, respiratory depression, miosis, euphoria, and decreased GI motility; while the m_2-receptors are involved in respiratory depression, drowsiness, nausea/vomiting, and mental clouding. m-receptors are also involved in changes in body temperature, tolerance, and increased addiction potential. Kappa receptor interactions produce analgesia (spinal), diuresis, sedation, dysphoria, miosis, and mild respiratory depression. Delta receptors are involved in analgesia, dysphoria, and delusions.

4. Ethanol-Drug Interaction

Concurrent use of ethanol and other CNS depressants may cause enhancements of the sedative and drowsiness effects of the opioids. As with most CNS depressants, the toxicity of opioids is potentiated by ethanol and there is a higher risk of respiratory depression and profound sedation as dose increases. However, ethanol has not been shown to significantly enhance any deleterious effects of codeine or propoxyphene.

C. Methods of Analysis

1. Extraction Techniques

Most of the polar opioids and their metabolites undergo conjugation prior to elimination; therefore, hydrolysis of the sample is often necessary to determine the total amount of the drug in a specimen. Acid hydrolysis (hydrochloric acid) and enzyme hydrolysis (b-glucuronidase or sulfatase) can be used to cleave off the conjugated group. Acid hydrolysis is rapid and complete; however, its disadvantage is that it converts heroin and 6-acetylmorphine to morphine.

Opioids are readily extracted from biological specimens by liquid-liquid extraction (pH range 8–10), or by solid-phase extraction. Liquid-liquid extraction is efficient and cost-effective for analyzing a small number of chemically similar opioids, whereas solid phase extraction appears to be preferred when analyzing multiple opioids.

Table 6. Receptor binding of common natural, semisynthetic and synthetic opioids

Opioid	Common trade names	Receptor binding
Natural		
Morphine	Astramorph®, Duramorph®, MSContin®, Roxanol®	Strong μ agonist; weak κ, δ agonist
Codeine	Nucofed®	Weak μ agonist, weak δ agonist
Semi-synthetic		
Buprenorphine	Buprenex®, Subutex®	Partial μ agonist, κ antagonist
Dihydrocodeine	Synalgos-DC®	μ agonist
Heroin	—	μ agonist (acts as a prodrug)
Hydrocodone	Lortab®, Vicodin®	μ agonist
Hydromorphone	Dilaudid®	Strong μ agonist
Oxycodone	Oxycontin®, Percocet®, Percodan®	μ agonist
Oxymorphone	Numorphan®	Strong μ agonist
Synthetic		
Fentanyl	Actiq®, Duragesic®, Sublimaze®	Strong μ agonist
Meperidine	Demerol®	Strong μ agonist
Methadone	Dolophine®	Strong μ agonist
Propoxyphene	Darvocet®, Darvon®	μ agonist
Tramadol	Ultram®	μ agonist

2. Instrumental Methods

The most commonly used screening technique for the detection of opioids is immunoassay. Commercial immunoassays are readily available for the opioids, and specific immunoassays are also available for buprenorphine, fentanyl, methadone, and propoxyphene. For the opiate immunoassay, morphine is typically used as the target molecule and cross-reactivity toward structurally related morphine-like compounds is common. However, it is advised that laboratories be fully aware of the capabilities and limitations of their immunoassay systems as several opioids do not cross-react sufficiently with morphine [77]. The degree to which an individual opioid will cross-react with morphine is noted on the package insert provided by the manufacturer.

Many opioids and their metabolites chromatograph well without derivatization. Consequently, GC coupled with FID, nitrogen-phosphorus detection, or MS is often used as a comprehensive drug screen or confirmatory method for opioids such as codeine, hydrocodone, meperidine, methadone, oxycodone, propoxyphene, and tramadol. To improve the chromatographic characteristics of other opioids, a derivatizing reagent may be added. Polar groups on the opioids or their metabolites may be derivatized with trimethylsilyl-, perfluoroester-, heptafluorobutyryl-, or trifluoroacetyl-agents [15,50,78, 81,125]. Detection limits of between 1–50 ng/mL are typically observed.

LC, LC-MS, and LC-MS-MS negate the need for derivatization, and the opioids and their glucuronide metabolites can be detected in concentrations as low as 0.1–5 ng/mL [16,20,40]. LC methods typically use reverse-phase C_{18} columns and the mobile phases are relatively simple (e.g., acetonitrile/ammonium acetate or acetonitrile/water/trifluoroacetic acid).

Specific properties of the opioids, relevant to their detection, are summarized in **Table 7**.

IV. TRICYCLIC ANTIDEPRESSANTS

The use of TCAs in cases of DFSA is a rarely reported occurrence. This class of drugs depresses the CNS but usually has a slow onset of action unless there is an overwhelming dose. Furthermore, although these drugs are still used in antidepressant therapy, they drugs have been superseded by other antidepressant drugs with fewer adverse side effects, and are therefore are less readily available. The newer antidepressants include the selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs). Many side effects of TCAs relate to antimuscarinic properties such as dry mouth, dry nose, blurry vision, lowered gastrointestinal motility or constipation, urinary retention, cognitive and/or memory impairment, and increased body temperature. In the event of an overdose, clinical symptoms would be expected to be displayed within 1 h. These TCAs are not commonly abused and thus availability may somewhat limited to individuals being treated for depression or pain. Overdoses of these drugs may be life-threatening. Delayed adverse effects may be expected in a naïve individual with a dose of 300–700 mg. Delayed effects may include hypotension, confusion, concentration difficulties, stupor, drowsiness, muscle rigidity, vomiting, hypothermia, or hyperpyrexia.

However, since the TCAs are CNS-depressant drugs, they could be effective in sedating a potential victim, especially in a naïve individual and when consumed in combination with ethanol and/or other CNS-depressant drugs.

Table 7. Some specific properties of opioids

Compound	UV absorption (nm)	Principal ion (*m/z*)
Morphine	285 (acidic); 298 (alkaline)	285, 162, 42, 215, 286, 124, 44, 284
Buprenorphine	286 (acidic); 300 (alkaline)	55, 378, 43, 29, 57, 410, 379, 84
Codeine	285 (acidic)	299, 42, 162, 124, 229, 59, 300, 69
Dihydrocodeine	283 (acidic)	301, 44, 42, 59, 164, 70, 302, 242
Fentanyl	251, 257, 263 (acidic)	245, 146, 42, 189, 44, 105, 29, 43
Hydrocodone	280 (acidic)	299, 242, 59, 243, 42, 96, 70, 214
Hydromorphone	280 (acidic); 290 (alkaline)	285, 96, 229, 228, 70, 214, 115, 200
Meperidine	251, 257, 263 (acidic)	220, 191, 204, 178, 192, 221, 233, 410
Methadone	253, 259, 264, 292 (acidic)	72, 73, 91, 293, 223, 165, 85, 71
Oxycodone	280 (acidic)	315, 230, 31, 70, 44, 42, 258, 140
Oxymorphone	281 (acidic); 292 (alkaline)	301, 216, 44, 42, 70, 302, 203, 57
Propoxyphene	252, 257, 263 (acidic)	58, 117, 208, 115, 193, 91, 179, 130
Tramadol	272 (acidic)	58, 263, 135, 77, 150, 188, 218.

Chemical structures of TCAs included in this review are shown in **Structure 4**.

A. Chemistry

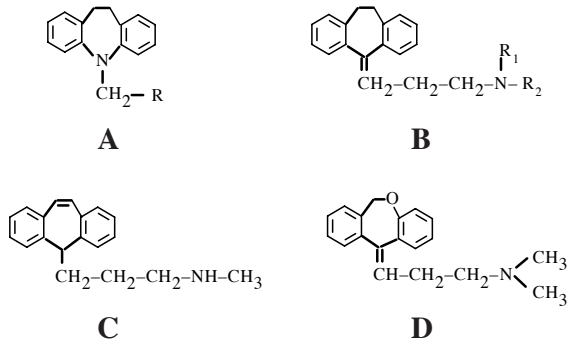
The TCA drugs are so named because structurally, they consist of an iminodibenzyl or dibenzocycloheptane "tricyclic" nucleus with position 5 of the chemical structure occupied by a dialkylamino function. A nonscientist can look at the chemical structure and see three cyclic portions to each chemical structure, thus, tricyclic. The TCAs resemble phenothiazines in structure and cause mood elevation in depressed patients rather than acting as tranquilizers. The classic prototype medications comprising this class of drugs include imipramine, desipramine, amitriptyline, nortriptyline, doxepine, protriptyline, and trimipramine. Doxepin and amitriptyline have more sedating effects than desipramine and protriptyline. These drugs are also being used to treat pain and other illnesses (e.g., migrane headache). The differences in the structures primarily occur at position number 5, and the group attached to position 5 is usually a primary or secondary amine.

Desipramine has a molecular formula of $C_{18}H_{22}N_2$ with a molecular weight of 266.4 and is a white powder. Nortriptyline has a molecular formula of $C_{19}H_{21}N$ with molecular weight of 263.4, and is a white to off-white powder. Amitriptyline is a white powder with a molecular formula of $C_{20}H_{23}N$ and molecular weight of 277.41. Protriptyline has a molecular formula of $C_{19}H_{21}N$ and has a molecular weight of 263.4.

B. Pharmacology

1. Administration

Amitriptyline and desipramine hydrochloride tablets are produced in a variety of sizes: 10, 25, 50, 75, 100, 150 mg; and an injectable solution of 10 mg/mL. Imipramine hydrochloride is produced in 10-, 25-, and -mg tablets and a 25-mg/2-mL injection. Protriptyline is produced as 5- or 10-mg tablets. Dosage ranges differ for each of the TCAs, and also differ for an adult versus an elderly patient. For adults the normal daily dosage (mg/day) ranges from 30–60 for protriptyline; 50–150 for nortriptyline; 75–300 for imipramine and desipramine; 150–300 for amitriptyline, and 200–400 for doxepin [102]. The dose ingested, however, is not a good predictor of the subsequent clinical outcome. Doses of less than 20 mg/kg are unlikely to be fatal or cause severe complications but individual variation in age, absorption, protein binding, and metabolism make it difficult to predict the outcome [21,62,113]. Putting this in more specific DFSA terms, if approximately 5 to 15



Structure 4. Chemical structures of tricyclic antidepressants: desipramine (**A**, R: $CH_2CH_2NHCH_3$); imipramine (**A**, R: $CH_2CH_2NHCH_3$); trimipramine (**A**, R: $CH(CH_3)CH_2N(CH_3)_2$; amitriptyline (**B**, R_1: CH_3; R_2: CH_3); nortriptyline (**B**, R_1: H; R_2: CH_3); protriptyline (**C**); doxepine (**D**).

tablets were ground up and placed into someone's beverage, the presence of the white binding material may or may not be detected by the victim. The TCA will, however, become dissolved in the solution (i.e., the beverage).

2. Pharmacokinetics

TCAs are rapidly absorbed from the gastrointestinal tract after ingestion, and undergo first-pass metabolism. They are highly protein bound and have a large volume of distribution. TCAs or their metabolites are to some extent excreted through the gastric mucosa and reabsorbed from the gastrointestinal tract. Active metabolites are formed by N-demethylation (e.g., amitriptyline to nortriptyline and imipramine to desipramine). Most TCAs are further metabolized in the liver, forming oxidative (-OH) metabolites along the rings, and many of those have pharmacologic activity. Approximately 70% of the dose is excreted in the urine in 6 to 8 days [86]. The rate of metabolism of TCAs varies widely from individual to individual, chiefly on a genetically determined basis, and that is a primary reason to perform therapeutic drug monitoring in patients receiving the medication. For example, up to a 36-fold difference in plasma level may be noted among individuals taking the same oral dose of desipramine. Certain drugs, particularly the psychostimulants and the phenothiazines, increase plasma levels of TCAs by competition for the same metabolic enzyme systems, when administered at the same time.

The half-life is relatively prolonged in most individuals (15–30 h). Eight human volunteers were followed for 96 h following one 75-mg amitriptyline oral dose, and the amiyriptyline and nortriptline half-lives were 22 and 26 h, respectively [38]. No adverse effects were reported at the administered dose. The relatively prolonged half-lives of TCAs would enable a longer window of detectability in

both blood and urine in a suspected DFSA victim. If the victim happens to be taking cimetidine, there may be significant increases in the plasma concentrations of the TCAs, even with one dose. Other substances, particularly barbiturates, ethanol, and/or tobacco products can induce liver enzyme activity and thereby reduce TCA plasma concentrations. The therapeutic range in patients undergoing daily medication ranges from 50–300 ng/mL, depending on the dose, the drug, the genetics of the user, and compliance [48,49,93].

3. Pharmacodynamics

Therapeutically, TCAs are administered orally, usually beginning at a low dose and increasing over days. For example, healthy adult patients are typically started at 50–75 mg/day increasing by approximately 25 mg/day every 2 or 3 days until ~150 mg/day is reached [38]. Concentrations of the drug in the plasma typically reach c max about 4 to 8 h after a dose. This "delay" factor would not make this class of drugs optimal in DFSA incidents. Literature suggests that the mechanism of some depressions have a biochemical basis in the form of a relative deficiency of neurotransmitters such as norepinephrine and serotonin, and the TCAs increase those brain transmitters.

The clinical features of TCA usage can be grouped according to the effects on the peripheral autonomic system (anticholinergic effects), the cardiovascular system and the central nervous system. Anticholinergic features are common and may aid diagnosis in a DFSA victim. Generally, anticholinergic effects may not cause serious clinical effect. Sweating is reduced and thus heat dissipation is reduced causing an increase in body temperature. Other cholinergic effects include dry mouth, blurred vision, urinary retention, and myoclonic twitching [45,79,103].

The most common cardiovascular effect is a sinus tachycardia. However, the most important toxic effect of TCAs is the slowing of depolarization of the cardiac action potential by inhibition of the sodium current, and this delays propagation of depolarization through both myocardium and conducting tissue [13]. This results in prolongation of the QRS interval and the PR/QT intervals with a predisposition to cardiac arrhythmias. This inhibition of sodium flux into myocardial cells can occur to such an extent that depressed contractility can result [75,119]. Fortunately, the overall incidence of serious cardiovascular arrhythmias is low. Hypotension is more common with an incidence of 14–51% having been reported [36]. Depressed contractility also occurs and, coupled with the reduction in peripheral resistance, contributes to hypotension.

The CNS effects of TCAs can include confusion, disturbed concentration, transient visual hallucinations, dilated pupils, agitation, hyperactive reflexes, stupor, drowsiness, muscle rigidity, and/ or vomiting [9,53,97]. Seizures and even coma may result if the blood concentration reaches a high level [70].

3. Ethanol-Drug Interaction

Obviously, prescription drugs should not be combined with ethanol since the potential effect(s) of combining the two is frequently unpredictable. This would especially be accurate in a DFSA case where the victim is unsuspecting or unprepared for what may happen. One significant study involved five healthy volunteers who received just 25 mg of (oral) amitriptyline, where volunteers were drinking for 1 h and followed for 8 h by oral ethanol (or juice), dosed to achieve and maintain blood ethanol concentrations of 0.08%. In the presence of ethanol, the amitriptyline free plasma concentrations were increased by a logarithmic mean of 204%, 186%, and 127% at 1.5, 2, and 2.5 h, respectively. The time of peak amitriptyline plasma concentrations, mean postural sway was increased over baseline by 92% with, and 2% without, ethanol; likewise, mean short-term memory (word recall) was decreased over baseline by 71% with, and 37% without, ethanol. Ethanol increases free amitriptyline plasma concentrations during the period of drug absorption; this is due to a decrease in amitriptyline hepatic clearance, resulting in decreased first-pass extraction. Together with the pharmacodynamic interaction, the kinetic changes provide a rationale for the toxicity of this combination and its deleterious effects on psychomotor skills [23].

Another report suggests that a slowing of metabolism occurs [116]. The author goes on to say that since many TCAs are metabolized by CYP2D6, it is conceivable that the combined use of these drugs results in a mutual decline in metabolic clearance, thereby elevating the TCA concentration in blood.

C. Methods of Analysis

1. Extraction Techniques

In order to achieve the proper "forensic" identification in DFSA cases, an extraction must be conducted to separate the biological matrix from the TCAs, and this separation must be followed with instrumental analysis. If the unknown blood concentration of the TCA is determined with GC and NP detection, for example, then mass spectrometry must at least be used to qualitatively confirm the presence of the tricyclic. Furthermore, if the blood specimen is collected too late after the incident, then urine will provide a longer window of detectability.

Numerous methods exist for the extraction and separation of TCAs from biological fluids. Usually this is achieved with the utilization of liquid-liquid extraction (LLE) or solid-phase extraction (SPE). Similar interesting modifications of LLE or SPE are published. For example, separation and preconcentration were achieved using a liquid-phase microextraction (LPME) method using a micropipette with a disposable tip holding a microliter volume of solvent and placing it into a stirring system to concentrate the nortriptyline. The solvent was then analyzed using atmospheric pressure MALDI-MS (AP-MALDI-MS) plasma. The authors named this process as micropipette extraction (MPE) [130]. Drugs can be extracted from alkalinized urine into solvent and derivatized with MSTFA/ammonium iodide/ethanethiol reagent and GC-MS [100]. Solid-phase microextraction coupled to liquid chromatography and mass spectrometry (SPME-LC-MS) has been used to analyze for the TCAs desipramine, imipramine, nortriptyline, amitriptyline, and clomipramine (internal standard) in plasma samples. In this method drugs are extracted onto a fiber material, and the drug desorption is carried out by exposing the fiber to the liquid chromatography mobile phase for 20 min [2].

2. Instrumental Methods

Numerous analytical techniques can be used for the quantitation of the TCAs, but specific identification requires MS. Typically, GC-MS or LC-MS methods are used. The method of Wu [130] uses AP-MALDI-MS after extraction as outlined above. Other methods use LC-MS [2], GC-MS-EI [88,107,109], and GC-MS-CI [58,128]. Some of the derivatization reagents used for the secondary amine TCAs include heptafluorobutyric anhydride, heptafluorobutyryl imidazole, trifluoroacetic anhydride, and 4-carboxyhexafluorobutyryl chloride.

The principal MS ions for the TCAs are summarized in **Table 8**.

Table 8. Some specific properties of tricyclic antidepressants

Compound	Principal ion (*m/z*)
Desipramine	235, 195, 208, 44, 234, 193, 194, 71
Nortriptyline	44, 202, 45, 220, 218, 215, 911
Amitriptyline	58, 59, 202, 42, 203, 214, 217
Protriptyline	192, 70, 44, 165

V. KETAMINE

Ketamine (**Structure 5**) has over the past few years been thought of as a "club drug," which connotates drugs that are encountered at nightclubs and "raves." It is marketed as a dissociative general anesthetic for human and veterinary use, with the only known source of ketamine being diversion of pharmaceutical products. There are reports that a significant number of veterinary clinics are being robbed specifically for their ketamine supplies. The DEA report that a major source of ketamine in the United States is a product diverted from pharmacies in Mexico. Because this is an illicit drug it is often suspected to be found in circumstances alleging DFSA, and the rapid onset of action (approximately 20 min) would make this drug suspected [24].

A. Chemistry

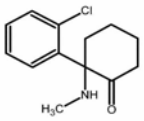
Ketamine is chemically 2-(2-chlorophenyl)-2-(methylamino)-cyclohexanone with a CAS number of 6740-88-1 and a molecular weight of 237.7. The chemical structure is a derivative of phencyclidine and is represented in Structure 5.

The drug has been in clinical use since mid-1960. Ketamine is partially water soluble at pH 7.4 (pK_a 7.5), and 5 to 10 times more lipid soluble than thiopental. The relatively high lipid solubility produces a large volume of distribution, which results in extensive distribution to peripheral sites and CNS sites. The drug is usually in solution in the hydrochloride form. The structure (2-(*O*-chlorophenyl)-2-methylamino cyclohexanone) contains a chiral centre at the C-2 carbon and two enantiomers of the ketamine exist: S-(+)- and R-(-)-ketamine. Commercially available ketamine preparations contain equal concentrations of the two enantiomers.

B. Pharmacology

1. Administration

The commercial preparation, Ketalarâ, is a racemic hydrochloride mixture of (SR)-ketamine in sterile solution at pH 3.5 to 5.5. Concentrations of ketamine are 10, 50, and 100 mg/mL, with benzethonium chloride added as a preservative for intravenous or intramuscular use. Some chronic pain patients are prescribed large doses to be taken orally. Children undergoing outpatient dental surgery are often sedated with oral ketamine at 4–10 mg/kg [41,76].



Structure 5. Chemical structure of ketamine.

2. Pharmacokinetics

Bioavailability following an intramuscular dose is 93%, intranasal dose 25–50%, and oral dose 20 ± 7%. Much of the oral dose is metabolized in the first-pass effect following oral consumption. Ketamine is rapidly distributed into brain and other highly perfused tissues, and is 12% bound in plasma. The plasma half-life is 2.3 ± 0.5 h. Oral administration produces lower peak concentrations of ketamine, but an increased amount of the metabolite norketamine and dehydronorketamine. Ketamine and its metabolites undergo hydroxylation and conjugation. Norketamine is pharmacologically active and produces effects similar to those of ketamine. There are minor differences between the pharmacokinetic properties of the S-(+) and R-(-)-isomers [131].

Ketamine analgesia is associated with a plasma concentration of 150 ng/mL following intramuscular administration, but much lower (40 ng/mL) following oral administration. Higher norketamine concentration is found following oral administration (probably from first-pass metabolism). Awakening from ketamine anaesthesia takes place at plasma concentrations of 640–1,120 ng/mL [41,101]. Although ketamine is structurally similar to PCP, it is 10–50 times less potent in blocking the NMDA effects.

3. Pharmacodynamics

The onset of effects occurs within seconds if smoked, 1–5 min if injected, 5–10 min if snorted, and within 15–20 min after oral administration. Effects generally last 30–45 min if injected, 45–60 min if snorted, and 1–2 h following oral ingestion. Clinically, ketamine is often readministered due to its relatively short duration of action. Blood concentrations higher than 600 ng/mL are associated with general anesthesia.

The clinical effects involve analgesia, anesthesia, and sympathomimetic effects that are mediated by different sites of action. Noncompetitive NMDA receptor antagonism is associated with the analgesic effects; opiate receptors may contribute to analgesia and dysphoric reactions; and sympathomimetic properties may result from enhanced central and peripheral monoaminergic transmission. Ketamine blocks dopamine uptake and therefore elevates synaptic dopamine levels. Inhibition of central and peripheral cholinergic transmission could contribute to induction of the anesthetic state and hallucinations.

Users have likened the physical effects of ketamine to those of PCP, and the visual effects to LSD. Use in surgery has produced reports of emergence reactions such as disorientation, dream-like experiences, vivid imagery, hallucinations, and delirium among the most important adverse reactions [41].

Psychological properties include awareness of general environment, sedation, dream-like state, vivid dreams, feelings of decreased invulnerability, increased distractibility, and disorientation; subjects are generally uncommunicative (i.e., cataplexy or a trancelike state with loss of voluntary motion and failure to react to stimuli). Intense hallucinations, impaired thought processes, out-of-body experiences, and changes in perception about body, surroundings, time, and sounds can occur. Delirium and hallucinations can be experienced after awakening from anesthesia. There is a high incidence of adverse effects, including amnesia, anxiety, chest pain, palpitations, agitation, rhabdomyolysis, flashbacks, delirium, dystonia, psychosis, schizophrenic-like symptoms, dizziness, vomiting, seizures, and paranoia. Some of the beneficial therapeutic effects include anesthesia and/or insensitivity to pain.

Some subjects have experienced drug-related dreams 24 h later. Larger doses of ketamine may produce what users refer to as a "K-hole" when the user is on the brink of being fully sedated and is likened to an out-of-body or near-death experience. High doses of ketamine may result in severe respiratory depression, muscle twitches, dizziness, slurred speech, nausea, and vomiting. One of the most dangerous effects of ketamine is the helpless and/or confused state the user may be put into after use of the drug. This causes the user to have difficulty with balance, combined with numbness, muscle weakness, and impaired vision. The combined effects can leave the user vulnerable to particular forms of crime, especially "date rape" [57].

4. Ethanol-Drug Interaction

The expected effect(s) of ketamine combined with ethanol is not very predictable, and little data exists concerning their interaction. Both ketamine and ethanol affect the brain, and in particular the *N*-methyl-D-aspartate (NMDA) receptors. Ketamine blocks the calcium-channel–associated NMDA glutamate receptors. Ethanol is an antagonist of NMDA receptors. Both ketamine and ethanol have CNS-depressant effects.

C. Methods of Analysis

1. Extraction Techniques

The ability to detect ketamine in biological specimens is related to the dose of the drug, the time elapsed after administration, and the capabilities of the analytical laboratory. As with most alleged DFSA cases, urine is the specimen of choice for analysis. For screening, ketamine immunoassay kits are marketed with a cutoff at approximately 100 ng/mL. More specific analysis

techniques (e.g., common alkaline and neutral drug screens) readily detect ketamine in urine or blood.

Ketamine can be extracted from biological specimens using SPE (e.g., mixed-mode cation and C_8), or with typical alkaline L-L extraction. With appropriate instrumentation, specimen extraction volumes of 1–4 mL are usually sufficient for detection.

2. Instrumental Methods

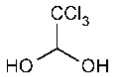
Cases of DFSA will require sufficient specificity of instrumental methods, which translates to some form of mass spectrometry with GC or LC input sources. Very low detection limits (0.03 ng/mL) can be achieved using instruments as ultra-performance liquid chromatography–mass spectrometry (UPLC–MS-MS) following extraction [94]. A 4-mL sample of urine was used followed by SPE (cation and C_8) resulting in a 250-L extract. Then 20 L were injected, leaving sufficient volume for other assays important in DFSA cases. The literature is replete with the other MS variations such as GC-MS [17], PCI [63], tandem MS [94,126], and turbo ion spray [124]. The mass spectrum of ketamine has principal peaks at m/z 180, 182, 209, 152, and 136 [17].

VI. CHLORAL HYDRATE

Reports of assault from victims of chloral hydrate (**Structure 6**) use date back to the early 19th century. An infamous example is Mickey Finn, the proprietor of the Chicago Lone Star Saloon, who was alleged to have drugged his customers with the addition of chloral hydrate to their ethanol-based beverages (i.e., "slipped them a Mickey") and subsequently robbed them. This concoction was also termed "knockout drops." The drug was developed for the specific purpose of inducing sleep. At therapeutic single doses, chloral hydrate has a rapid onset (30 min), produces minimal side effects, and is useful in inducing sleep. In general, it is a CNS depressant having sedative effects, but the exact mechanism of action is largely unknown [8].

A. Chemistry

Chloral hydrate (2,2,2-trichloroethane-1,1-diol) is chemical with sedative and hypnotic properties. It is formed from chloral (trichloroacetaldehyde) and one molecule of water. It was first synthesized in 1832, was one of the original depressants, and is still in use but has largely been replaced with newer sedative drugs. The chemical formula is $C_2H_3Cl_3O_2$ and molecular weight 165.4. Chloral hydrate is a colorless crystalline compound,



Structure 6. Chemical structure of chloral hydrate.

readily soluble in both water and alcohol, forming concentrated solutions. It is a synthetic commercial preparation supplied in capsule or liquid form. It has an unpleasant taste and thus the liquid form is flavored. The chemical structure is shown in Structure 6.

The boiling point is 97 ˚C and it decomposes, melting point: 57–60 ˚C and density: 1.9 g/cm≥. Quantitative analysis usually involves utilization of GC to find the conjugated and/or unbound metabolites trichloroethanol and trichloroacetic acid (which require derivitization) since the parent drug has a much shorter half-life.

B. Pharmacology

1. Administration

Chloral hydrate syrup, USP contains 500 mg/5 mL. The drug is also supplied as oral capsules of 250–500 mg or rectal suppositories of 324–648 mg. The recommended adult dose for a hypnotic effect is 500–1,000 mg (equivalent to 7–14 mg/kg). This large dose can be compared with the expected lethal dose of 10 g. The recommended dose for a child undergoing a medical or dental procedure is 50–100 mg/kg [6,33].

2. Pharmacokinetics

Chloral hydrate is rapidly and extensively metabolized in the liver and erythrocytes by alcohol dehydrogenase to its major active metabolite, trichloroethanol, and the glucuronated products. A small amount of chloral hydrate (11%) and a larger portion of trichloroethanol are oxidized to a minor, less active metabolite, trichloroacetic acid, in the liver and kidneys [87]. This metabolite is excreted in the urine and bile with free or conjugated trichloroethanol and possibly to a small amount of dichloroacetic acid (DCA) [22]. The average half-life of trichloroethanol in adults is 8 h, with a range of 4–12 h. The half-life in children is approximately 10 h. Trichloroethanol is 70–80% bound to plasma proteins and is widely distributed to all body tissues including CSF, breast milk, and placenta. The half-life of trichloroacetic acid is longer, up to 100 h. It is highly plasma protein bound (94%), primarily to albumin, and may be responsible for interactions with other highly protein bound drugs. Upon multiple dosing, trichloracetic acid can displace bilirubin or warfarin from binding sites, potentially resulting in hyperbilirubinemia or hypoprothrombinemia.

3. Pharmacodynamics

Eight male volunteers, aged 24–39, were administered single doses of 500 or 1,500 mg or a series of three doses of 500 mg given at 48-h intervals. The concentrations of chloral hydrate in plasma following low, high, or repeated oral administration of chloral hydrate in human volunteers were very low, but measurable amounts of chloral hydrate were detected in plasma following the 1,500-mg dose and the third dose of the repeated dose experiment over most of the first 24 h and the plasma half-life was found to be 9–10 h. There were multiphasic (three) elimination rates for trichloroethanol with half-lives ranging from 7–121 h [82].

There are a number of side effects, as expected, with this CNS depressant: drowsiness, slurred speech, slow reflexes, ataxia, lethargy, slurred speech, deep coma, respiratory depression, hypotension, and cardiac arrhythmias. Overdoses can be life-threatening from the severe respiratory depression, hypotension, and cardiac effects. It is irritating to the skin and mucous membranes and often causes gastric distress such as nausea and vomiting at normal doses.

Following a hypnotic dose, drowsiness occurs rapidly (10–15 min) and sleep usually occurs within 30–60 min. At low doses (20 mg/kg) symptoms may include relaxation, dizziness, slurred speech, confusion, disorientation, euphoria, irritability, and hypersensitivity rash. At higher doses (50 mg/kg) chloral hydrate can cause hypotension, hypothermia, hypoventilation, tachydysrhythmia, nausea, vomiting, diarrhea, headache, and amnesia [106].

The drug is used as a premedicant in children and infants, with sedation occurring within 15 min. Within 2 h the patient is fully awake. The recommended dose for a child undergoing a medical or dental procedure is 50–100 mg/kg [33]. Because of the rapid metabolism of chloral hydrate, trichloroethanol is responsible for the majority of the pharmacological activity [12].

4. Ethanol-Drug Interaction

Studies exist showing that ethanol ingestion alters the metabolism of chloral hydrate and increases subjective symptoms. Five male volunteers weighing 70–107 kg consumed ethanol (880 mg/kg), chloral hydrate (1 g, 9–14 mg/kg), or both. Blood pressure and cardiac rate did not vary significantly among treatments. In the presence of ethanol, the concentration of trichloroethanol in the blood rose more rapidly and reached a higher concentration, but the rate of depletion was not significantly changed. The increase in the concentration of trichloroethanol was not sufficient to produce a marked enhancement of the hypnotic effect. The volunteers reported symptoms (drowsiness, dizziness, blurred vision) and their severity during the 6-h observation period. At all time points, the rank order of effects were: ethanol plus chloral hydrate was greater than ethanol, which was greater than chloral hydrate.

If co-ingested with alcohol, chloral hydrate metabolism may be seriously impaired. Because ethanol and chloral hydrate are both metabolized by CYP2E1 and alcohol dehydrogenase, co-ingestion may not only exacerbate their clinical effects but also prolong their duration of action [90,106].

C. Methods of Analysis

1. Extraction Techniques

This drug and its metabolites are not typically found on any screening tests and must be targeted in order to be found. As previously mentioned, the parent drug is seldom found unless headspace GC with electron capture detection (or similar sensitive methodology) is used in sufficient time. A variety of techniques are published for MS determinations of the conjugated and unbound metabolites.

Several techniques have been used for isolation of chloral hydrate and its metabolites from biological samples, including headspace after equilibrium, LLE [27], SPE, and SPME. The headspace technique can also be used for the determination of "total" (unbound and conjugated) after samples are hydrolyzed chemically with sulfuric acid [106] or enzymatically [66]. Yan et al. developed a GC-MS method for analysis of human plasma and exposed samples to BF3-MeOH for derivatization of anions to their methyl esters [132]. Other sophisticated analytical methods (e.g., cryogenic sampling concentrate, thermal desorption) are used for sample preparation prior to chromatography [99].

2. Instrumental Methods

Headspace GC [98,106], GC-FID [12,46], GC-MS [83], and LC-MS-MS [4] have all been used for the determination of chloral hydrate and metabolites in biological specimens. In some of the tandem MS methods the LOD for chloral hydrate goes down below 1 ng/mL, using 1 mL specimen. The mass spectrum of chloral hydrate has principal peaks at m/z 83, 47, 111, and 147 [73].

CONCLUSIONS

A large number of diverse drugs may be used in the facilitation of a sexual assault. However, not all of these substances are the classic "sedatives-type" drugs; they may include a number of other drugs where sedation, muscle relaxation, cognitive impairment, and/or amnesia are the main side effects. Single or repeated oral doses of

barbiturates, opioids, antipsychotics, TCAs, ketamine, and chloral hydrate are capable of causing sedation, drowsiness, confusion, and impairment of various cognitive and psychomotor abilities.

Although the analysis of barbiturates, antipsychotics, opioids, TCAs, ketamine, and chloral hydrate is typically well documented in the literature, their detection and identification pose specific problems in the context of DFSA. Biological specimens may not have been collected until days after the alleged incident and the drugs and/or their metabolites are typically in low concentrations following a single dose. Also, analysis of the specimen often has to target one or more metabolites in addition to the parent drug.

When specimen volume is not an issue and/or when the identity of the alleged drug is unknown, the initial detection of barbiturates and opioids is usually accomplished with an immunoassay screening test, followed by a chromatographic confirmatory analysis. However, it is advised that laboratories be fully aware of the capabilities and limitations of their immunoassay systems, as several opioids may not cross-react sufficiently with morphine. In DFSA cases where TCAs, ketamine, chloral hydrate, antipsychotic, and/or opioid involvement is suspected, it is important to consider additionally testing for those drugs that require specialized analyses.

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Laboratory Management of Drug-Facilitated Sexual Assault Cases[a]

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Laboratory Management of Drug-Facilitated Sexual Assault Cases

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ABSTRACT: Over the past two decades, cases of drug-facilitated sexual assaults (DFSA) have increased in forensic laboratories in many parts of the world. Investigators of DFSA allegations know of the many challenges associated with these cases, but forensic toxicologists find that delays in the reporting of such crimes to law enforcement and subsequent lags in specimen collection are particularly important concerns. These delays are usually a result of the traumatic experience of sexual assaults, as well as the amnesic effect of the drugs typically used to commit DFSA. Unfortunately, such a delay in specimen collection may be the difference between detecting traces of a drug (or metabolite) and reporting a negative result. Therefore, it is imperative for toxicology laboratories to properly prepare for DFSA cases by developing forms, policies, and procedures to ensure that truly meaningful analyses are performed. This article provides guidance in the steps laboratories may take to best prepare themselves to analyze evidentiary specimens from DFSA investigations.

KEY WORDS: Drug-facilitated sexual assault, investigation, management.

INTRODUCTION

For forensic toxicologists, one of the more challenging aspects of drug-facilitated sexual assault (DFSA) cases is the lag in specimen collection for analysis often caused by delays in the reporting of such crimes to law enforcement entities. These delays are usually a result of the traumatic experience of sexual assaults, as well as the amnesiac effect of the drugs typically used to commit DFSA [1]. Unfortunately, a delay in specimen collection of as little as 1 hour may be the difference between detecting traces of a drug (or metabolite) and reporting a negative result [3]. Therefore, it is imperative for toxicology laboratories to properly prepare for DFSA cases by developing forms, policies, and procedures to address these cases in ways that ensure that truly meaningful analyses are performed.

The typical DFSA case involves a victim experiencing a period of blackout or unconsciousness during which he or she believes he/she was sexually assaulted [2]. Alcohol consumption is often involved; however, it is not always clear if alcohol alone caused the CNS depressant effects experienced by the victim [5,9,10]. Outside of these basic consistencies among many DFSA cases, each investigation has different facts and histories that require consideration by forensic toxicologists when determining if their laboratory is best suited to accept the case for analysis [8].

In 1999, a group of forensic toxicologists published recommendations for investigators, medical professionals, and toxicologists working on DFSA cases [6]. Those guidelines served as the first attempt to communicate the needs of forensic toxicologists to investigators and medical professionals in order to ensure that more meaningful toxicological analyses could be conducted. Most of the recommendations from this publication have been recognized and incorporated into federal, state, and local policies on evidence collection in alleged DFSA cases; however, it is now the responsibility of laboratories to ensure that they are adequately prepared to handle these cases. Therefore, to assist laboratories in their self-assessment as to whether to attempt to analyze biological specimens from DFSA investigation, the following steps are recommended. The recommendations presented here will help to prepare a toxicology laboratory for samples from DFSA investigations.

I. DEVELOP AN INFORMATION COLLECTION FORM AND CASE ACCEPTANCE POLICY

As a first step in preparing a toxicology laboratory to accept samples from DFSA investigations, it is recommended to develop an information collection form and case acceptance policy. These tools will allow a laboratory's staff to readily access the needs of a given case against the laboratory's capabilities so that the best use of available specimens is made. Unfortunately, in many DFSA cases, laboratories have consumed valuable specimens by performing meaningless testing. For example, in a DFSA investigation, it makes little sense to analyze for a drug such as gamma-hydroxybutyrate (GHB) in a blood sample when the specimen was collected 20 hours after the alleged assault [3]. It is also questionable to test for zolpidem using a method with a detection limit

of 300 ng/mL on a urine specimen collected 72 hours after an alleged DFSA [4]. This is not to suggest that such analyses are meaningless; however, their results generally would not answer the question as to which, if any, drugs were present at the time of the assault.

The information collection form can be used to easily differentiate cases that should be worked in a laboratory from those that should not be worked. Important information that may be collected includes:

- Symptoms experienced by the alleged victim;
- Estimated length of time that the victim had amnesia or was unconscious;
- Specimens that were collected;
- Estimated length of time that passed between the alleged drugging and specimen collection;
- Amount of alcohol that was voluntarily consumed (as specific as possible);
- Types and amounts of drugs (recreational, prescription, or over-the-counter) voluntarily consumed;
- If the victim recalls urinating before the specimens were provided and, if so, the approximate number of times;
- Drugs the alleged suspect may have available; and
- Contact information for the investigator most knowledgeable about the case.

Collection of this information is important in that it opens the door for good communication between the toxicology laboratory and the investigators. It also allows the investigators to serve as a first-line "screen" to focus the toxicological analyses in incoming DFSA cases.

Figure 1 provides an example of an information collection form that may be used or modified by a toxicology laboratory conducting examinations on specimens from DFSA investigations. Once the form is implemented in a laboratory, it is fairly straightforward to establish the case acceptance policy for that laboratory. Generally, the following items should be considered when defining the DFSA case acceptance policy:

- Specimens and volumes that are required by the laboratory;
- Length of time that passed before the specimens were collected; and
- Specific drugs that the investigation is focusing on.

The latter, of course, is dependent on the next step that helps the assessment of a laboratory's true capabilities with DFSA samples – validation of standard operating procedures.

II. VALIDATE STANDARD OPERATING PROCEDURES FOR DFSA DRUGS

A. Routine DFSA Drugs

Any drug that has direct or indirect central nervous system (CNS) depressant effects may be used to commit drug-facilitated crimes. In 2005, the Society of Forensic Toxicologists' (SOFT) DFSA Committee published a list of some of the drugs (and their metabolites) potentially used to facilitate crimes, with recommended detection limits for testing urine specimens [11]. The detection limits were based on published methods using typical laboratory instrumentation, as well as the professional judgment of the members of the DFSA Committee. The goal of this list was twofold: (a) to encourage laboratories to evaluate their current capabilities and make improvements where needed and (b) to improve consistency in results among different laboratories analyzing DFSA cases [11].

Obviously, some drugs are more likely to be used in DFSA cases than are others. The mass media have actively portrayed flunitrazepam, gamma-hydroxybutyrate, and ketamine as "date rape" drugs. Other drugs, such as zolpidem and alprazolam, are more likely to be used simply due to their availability as popular prescription medications. Nonetheless, it is imperative to determine the drugs that a laboratory will consider as "routine" and, therefore, will analyze for in most DFSA investigations. Once that list is determined, a thorough validation study should be undertaken to establish that the laboratory's method(s) can reliably detect and identify each of these drugs and their metabolites at the SOFT DFSA Committee-recommended detection limit. Following validation, the routine drugs and metabolites that the laboratory is able to detect at the SOFT-recommended levels will be established.

B. Non-Routine DFSA Drugs

Logically, not all of the drugs included in the SOFT DFSA Committee's list should be routinely tested for in every DFSA case. From time to time, a particular DFSA case may surface in which the history of the case warrants the inclusion of "non-routine" DFSA drugs in the toxicological analysis. As with the routine DFSA drugs, a method should also be thoroughly validated to establish its ability to find the non-routine DFSA drugs and metabolites at the recommended detection limits.

Drug-Facilitated Sexual Assault
Information Collection Worksheet

Agency: _____ City: _____ State: _____

Contact Person: _____ Phone: _____

Name of Victim: _____ Name of Suspect(s): _____

Case Number(s): _____ Date and Time(s) of Assault: _____

Date of Contact: _____ Examiner Collecting Information: _____

---

1. Were any specimens collected and what were they? _____

2. When were the specimens collected (date and times)? _____

3. What symptoms did the victim describe? _____

_____

4. Were there any witnesses? If so, how did they describe the victim? _____

_____

5. Did the victim report amnesia or loss of consciousness? If so, how long? _____

6. Did the victim consume any alcohol? If so, how much (types of alcohol, size of drinks, over how many hours, etc.)? _____

_____

7. Did the victim voluntarily take any drugs (recreational, prescription, or over-the-counter)? If so, which ones, how much, and when? What is known about the victim's drug-use history?_____

_____

8. Did the victim urinate prior to providing any specimens? If so, approximately how many times? _____

9. What is known about the suspect in regard to occupation, hobbies, drug history, and medical history? _____

_____

10. What recreational and prescription drugs does the suspect have ready access to? _____

_____

11. Additional Notes of Interest: _____

_____

_____

_____

Figure 1. Sample DFSA information collection form**.**

III. IMPROVING SENSITIVITY OF ANALYTICAL METHODS TO DETECT DFSA DRUGS

For those drugs in which a laboratory's current methods are unable to meet the SOFT DFSA Committee's recommended cutoff concentration, it must be decided if there will be an attempt to improve the method's ability to reach these levels, or if these additional drugs and metabolites will be excluded from the laboratory's list of detectable drugs.

To improve a method's sensitivity, the following approaches may be taken [6]:

- Increase specimen volume for the analysis;
- Hydrolyze urine specimens for detecting conjugated metabolites (e.g., benzodiazepines);
- Derivatize the drug and/or metabolite;
- Take advantage of selective detectors (e.g., electron capture detectors for benzodiazepines);
- Use selected ion monitoring (SIM) or tandem mass spectroscopy (MS/MS) analyses; and
- Upgrade to newer technology.

IV. PREPARE A CUSTOMIZED LIST OF ROUTINE AND NON-ROUTINE DFSA DRUGS

After it has been established which drugs a laboratory can properly analyze as "routine" and "non-routine" DFSA drugs, a customized list should be generated. This list readily communicates the strengths and limitations of the laboratory to the investigators relying on its results and, to be effective, the list should be made readily available to the laboratory's clients. **Table 1** is an example of such a list.

V. PUTTING POLICIES AND PROCEDURES INTO ACTION

Once the foundation has been established for acceptance of DFSA cases into a forensic toxicology laboratory, standard practices should be put into place for the day-to-day analysis of specimens from these cases. Generally speaking, most DFSA cases can follow the flowchart illustrated in **Figure 2**.

Using the laboratory's DFSA information collection form, communication with the DFSA investigator should be initiated to obtain the pertinent details allowing for a decision about the acceptance of the case for internal analysis. If a particular drug is suspected, then a target analysis for that drug and its related metabolites should be initiated [8]. Otherwise, the laboratory's "routine" DFSA drugs and metabolites should be the starting point of the analysis.

Table 1. Sample list of routine and non-routine DFSA drugs

Routine DFSA drug	Non-routine DFSA drug
Ethanol	Barbiturates
GHB	Amobarbital
Benzodiazepines:	Butalbital
Alprazolam	Phenobarbital
Diazepam	Pentobarbital
Chlordiazepoxide	Secobarbital
Clonazepam	Sedative Antihistamines:
Flunitrazepam	Brompheniramine
Lorazepam	Chlorpheniramine
Nordiazepam	Diphenhydramine
Temazepam	Doxylamine
Triazolam	Sedative Antidepressants:
Amphetamines	Amitriptyline
Amphetamine	Desipramine
Methamphetamine	Nortriptyline
Cocaine	Zolpidem
Narcotics	
Codeine	
Fentanyl	
Hydrocodone	
Meperidine	
Methadone	
Morphine	
Oxycodone	
THC	
Ketamine	

Since urine is the most useful specimen for most DFSA cases [6,7], analysis should focus on this sample when it is available. Positive findings must be confirmed following good laboratory practices and a decision should be made as to whether the confirmed findings are significant to the case. If they are, then any additional useful specimens should be analyzed for the presence of the drugs that were detected so that a complete understanding of their significance can be recognized.

If the routine screens fail to detect any significant drugs or metabolites, the toxicologist should reevaluate the case with the investigator to determine whether the case history warrants testing of the submitted specimens for the laboratory's "non-routine" DFSA drugs and metabolites. Once all testing is complete, it is important that the results are clearly reported in such a manner that the laboratory's results are not misrepresented. For example, in some instances laboratories may put a disclaimer in their reports that says negative findings should not be used as proof that the victim was not exposed to a drug. This is particularly important as the time interval between the alleged assault and collection of evidence increases [6].

CONCLUSION

While toxicologists will never be able to completely overcome the challenges associated with DFSA cases, proper preparation for acceptance of these cases into the laboratory is vital to these investigations. The recommendations presented here can help ensure that the best possible analyses are conducted in DFSA investigations.

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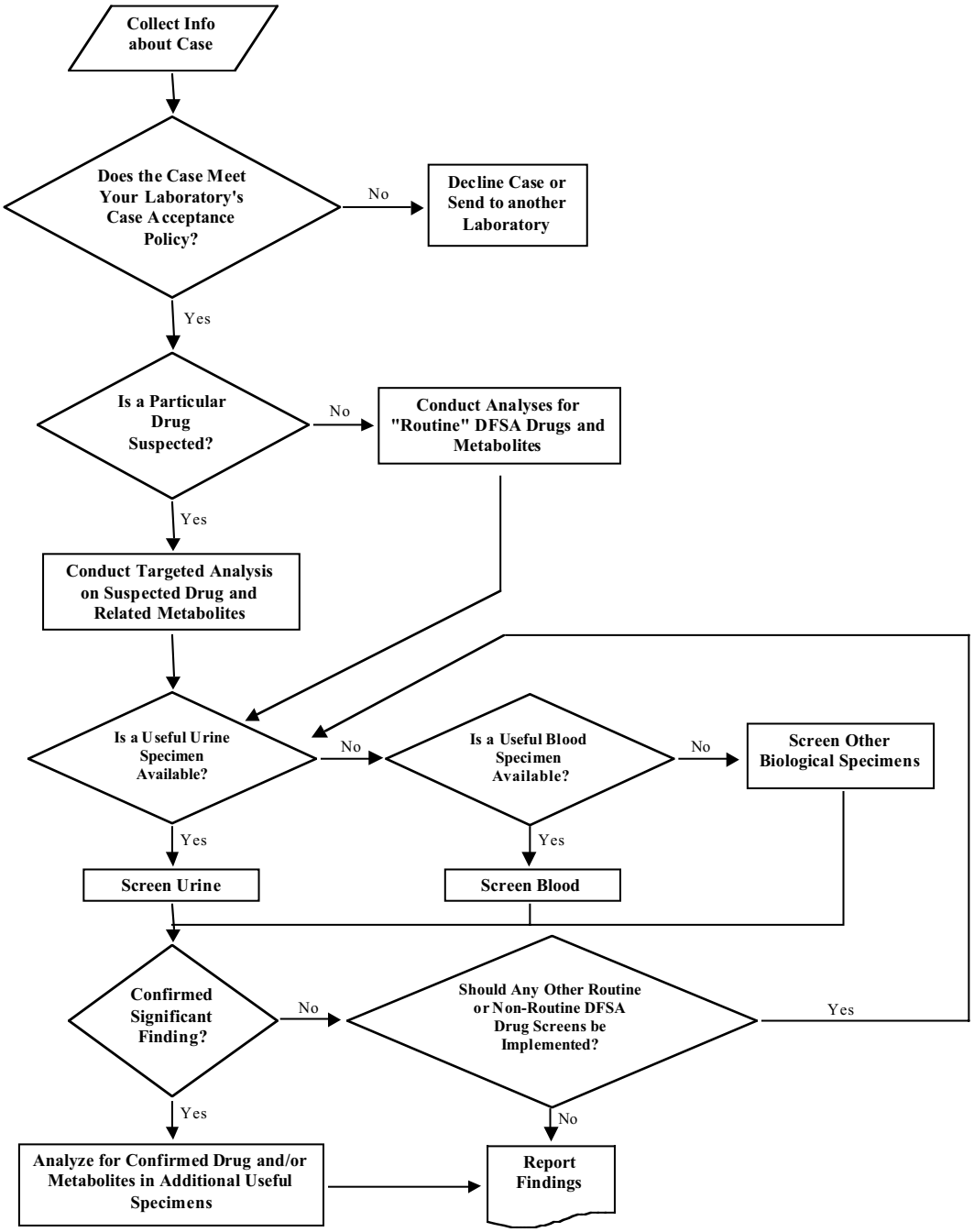

Figure 2. Sample approach to handling the toxicological analysis of a typical DFSA case.

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Marc A. LeBeau holds a B.A. degree in chemistry and criminal justice from Central Missouri State University (Warrensburg, MO; 1988) and an M.S. degree in forensic science from the University of New Haven (West Haven, CT; 1990). In 2005, he received his doctorate in toxicology from the University of Maryland–Baltimore. Dr. LeBeau is currently the chief of the FBI LaboratoryÕs Chemistry Unit.

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