

PAPER**TOXICOLOGY**

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Toxicological Findings in Cases of Sexual Assault in the Netherlands*

ABSTRACT: Reports on cases of alleged drug-facilitated sexual assault (DFSA) have increased since the mid-1990s. The aim of this study was to identify the extent and types of drugs found in cases of alleged sexual assault (DFSA) in the Netherlands. In total, 135 cases of alleged DFSA were identified. Most of the victims were women (94%), and the mean age of the victims was 25 years. Blood and urine samples were tested for the presence of alcohol, drugs (drugs of abuse and prescription drugs), or both. In 27% of the cases, no alcohol and/or drugs were found. With increasing time delay, more cases were found to be negative. Alcohol is the most commonly found drug followed by nonopioid analgesics, illicit drugs, and benzodiazepines. In some cases, the absence of alcohol and drugs may represent false-negative results owing to the time delay between alleged sexual assault and sampling.

KEYWORDS: forensic science, sexual assault, alcohol, drugs, drug-facilitated sexual assault, toxicology

Reports on cases of alleged drug-facilitated sexual assault (DFSA) have increased internationally since the mid-1990s (1–3). DFSA can be defined as offenses in which victims are subjected to nonconsensual sexual acts, while they are incapacitated or unconscious because of the effects of alcohol and/or drugs and are therefore prevented from resisting or are unable to consent (4). At least two possible types of DFSA have been identified, referred to as proactive and opportunistic. Proactive DFSA is defined as “the covert or forcible administration to a victim of an incapacitating or disinhibiting substance by an assailant for the purpose of sexual assault” (5, p. 497; 6, p. 291) and opportunistic DFSA as “sexual activity by an assailant with a victim who is profoundly intoxicated by his or her own actions to the point of near or actual unconsciousness” (5, pp. 497–498; 6, p. 291).

The Society of Forensic Toxicologists prepared a list of drugs that could be or have been used in DFSA (1). This list comprises about 50 compounds, including illicit, prescription, and over-the-counter drugs. The prevalence and types of drugs encountered during investigations of alleged sexual assault are likely to differ between countries depending on social norms and the use of drugs in society. Alcohol alone, or together with other drugs, has been a common finding in many previous studies in America, Australia, and Europe (Northern Ireland, Sweden, and the U.K.) (2,5,7–12). Reviews and comments on DFSA studies were published in the studies by LeBeau and Montgomery (3) and Hall and Moore (6).

In studies from America, a high incidence of alcohol in DFSA cases has been recorded (2,7,8). In one study published in 1999, 1179 urine samples were collected between May 1996 and June 1998 (2). Approximately 20 different substances were identified in 60% of these samples, the most common drug being alcohol,

followed by cannabinoids, cocaine metabolite, benzodiazepines, amphetamines, gamma-hydroxybutyric acid (GHB), and opiates. In 2001, an update of this study was presented. At that time, the database had grown to 3303 urine samples with 2026 samples (61%) testing positive for one or more substances (7). The samples were collected over a period from June 1996 until February 2000. Alcohol, either alone or in combination with other drugs, was by far the most common drug found, being present in 1358 urine samples (41%). Cannabinoids were the second most prevalent drug identified, present in 613 urine samples (19%). In a third American study, 61% of the samples contained alcohol and/or drugs (8). In this study, 2003 samples were tested in the period May 1996–March 1999. The predominant substances found were alcohol, present in 821 of the samples (41%), and cannabinoids in 364 samples (18%).

In an Australian study, 76 cases of suspected DFSA were identified from 434 cases of adult sexual assault from April 2002 to April 2003. Both blood and urine were collected if there was <24 h between assault and sampling and urine only if the delay was >24 h. Alcohol consumption in the hours prior to the assault was reported in 77% of the suspected DFSA cases and confirmed by toxicological analysis in 37% of these cases. Forty-nine percent reported using prescription medication, and 26% reported the use of recreational drugs (9). In 20% of the suspected cases, drugs were detected which were not reported by the victim including cannabis, antidepressants, amphetamines, benzodiazepines, and opiates.

In Northern Ireland, 294 cases of sexual assault were submitted for toxicological analysis over the years 1999 up to 2005. Both blood and urine samples were analyzed. The percentages of cases containing alcohol, drugs, or both increased from 66% in 1999 to 78% in 2005 (5). The largest group of drugs identified was the analgesic group (acetaminophen, codeine, and dihydrocodeine) followed by benzodiazepines and cannabinoids.

In a study from Sweden, the results of 1806 female victims over a 5-year period (2003–2007) showed that in 770 cases (43%),

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alcohol was the only drug identified in blood and urine (10). In 215 cases (12%), alcohol was present together with at least one other drug. Drugs other than alcohol were identified in 262 cases (15%). Amphetamine and cannabinoids were the most common illicit drugs found.

In a study from the U.K., the results of 1014 cases of claimed DFSA over a 3-year period (2000–2002) showed that alcohol (either alone or with an illicit and/or medicinal drug) was detected in 46% of the cases (11,12). Both blood and urine samples were submitted in this study. Illicit drugs were detected in 34% of the cases, with cannabinoids being the most commonly detected (26%) followed by cocaine (11%).

In this study, the toxicological results were examined from cases of DFSA submitted to the Netherlands Forensic Institute (NFI) between January 2004 and December 2006. The aim of this study was to identify the extent and types of drugs found in cases of alleged sexual assault. Included were those cases with an indication of sexual assault in the archives and the presence of a blood sample, a urine sample, or both for analysis.

Methods

Selection of Cases and Samples

Cases for review were selected from the archives of the NFI for the years 2004 through 2006. Included were those cases with an indication of sexual assault and the presence of a blood sample, a urine sample, or both for analysis. Most of the urine and blood samples were collected and stored in containers with sodium fluoride as preservative. The blood containers also contained sodium heparin as anticoagulant. Samples were sent to the NFI by mail or special delivery and stored at -20°C until analysis. Depending on the case description, information, and request from the police, the type of biological material, the amount of sample available, and the time delay between the alleged sexual assault and sampling, analysis of alcohol, GHB, and/or screening for drugs of abuse and prescription drugs were performed.

This resulted in the following numbers of analysis. In 108 of the 135 cases, samples were tested for alcohol. In 134 cases, samples were screened for drugs (drugs of abuse and prescription drugs) using the general screening method and/or enzyme-linked immunosorbent assay (ELISA). In two of these 134 cases, only ELISA was performed, and thus, only drugs of abuse could be identified in these two cases. In 109 cases, samples were tested for GHB. In 108 cases, samples were tested for both alcohol and drugs.

Analytical Procedures: Drug Screening

In the laboratory, two procedures for screening purposes were available. The first method, screening for selected classes of drugs of abuse and benzodiazepines, was performed only in blood samples. Blood samples were screened using ELISA (Cozart reagents) for amphetamines, benzodiazepines, cannabinoids, cocaine metabolite, methadone, methamphetamines, and opiates. Threshold levels for the classes of drugs (calibrators stated between brackets) expressed in ng/mL were 25 (*D*-amphetamine), 10 (oxazepam), 10 (9-carboxy-11-nor- Δ^9 -tetrahydrocannabinol), 50 (benzoyllecgonine), 10 (methadone), 25 (methamphetamine), and 10 (morphine), respectively.

The second method, general screening for drugs of abuse and prescription drugs, was performed in blood as well as in urine samples. Before analysis, urine samples were hydrolyzed with

β -glucuronidase/arylsulfatase (from *Helix pomatia*, 30 U/mL β -glucuronidase and 60 U/mL arylsulfatase [Merck, Schiphol-Rijk, The Netherlands], 12.5 $\mu\text{L}/5\text{ mL}$ urine) overnight at 37°C . After solid-phase extraction (SPE), acid and alkaline extractions were obtained and samples were analyzed by gas chromatography with mass spectrometric (GC-MS) detection after derivatization with bis(trimethylsilyl)trifluoroacetamide (BSTFA) and/or by high-performance liquid chromatography with diode array detection (HPLC-DAD) (13). To identify the unknown compounds, our own reference library containing *c.* 300 different compounds and commercially or freely on the Internet available libraries was used. The drugs listed by the Society of Forensic Toxicologists can be identified using these libraries (1). Limits of detection for this method differ for the different compounds and were *c.* 50 ng/mL.

Analytical Procedures: Drug Confirmation, Alcohol, and GHB Analysis

The presence of the various drugs and all ELISA screening positives were confirmed using the methods described below. Amphetamine derivatives (amphetamine, methamphetamine, 3,4-methylenedioxy-*N*-ethylamphetamine [MDEA], 3,4-methylenedioxyamphetamine [MDA], and 3,4-methylenedioxymethamphetamine [MDMA]) were identified and quantified after SPE and derivatization with heptafluorobutyric anhydride (HFBA) using GC-MS. The limit of quantification (LOQ) for all compounds was 10 ng/mL (14) (<http://www.tiaft.org/tiaft97/proceedings/abstract/posters/138.html>). The benzodiazepines diazepam, oxazepam, temazepam, and desmethyldiazepam were identified and quantified after matrix-assisted liquid-liquid extraction using HPLC-DAD. The LOQ for all benzodiazepines was 10 ng/mL (15). The cannabinoids Δ^9 -tetrahydrocannabinol (THC), 11-hydroxy- Δ^9 -tetrahydrocannabinol (11-OH-THC), and 9-carboxy-11-nor- Δ^9 -tetrahydrocannabinol (THC-COOH) were determined by gas chromatography with tandem mass spectrometric detection (GC-MS/MS) after protein precipitation, SPE, and derivatization. The LOQ for THC and THC-COOH was 2 ng/mL, and 1 ng/mL for 11-OH-THC (16). Cocaine, benzoyllecgonine, methylecgonine, and lidocaine were identified and quantified using GC-MS after SPE and derivatization with hexafluoroisopropanol/pentafluoropropionic anhydride (HFIP/PFPA). The LOQ for cocaine and metabolites was 25 ng/mL (17). Opiates (morphine, codeine, normorphine, and 6-acetylmorphine) were identified and quantified using GC-MS after SPE and derivatization with BSTFA. The LOQ for opiates was 10 ng/mL. Beginning in 2006, a method for the simultaneous analysis of amphetamine, MDEA, MDA, MDMA, cocaine, benzoyllecgonine, methadone, morphine, and codeine was used instead of the above-described single-drug group analyses. These drugs of abuse were identified and quantified using GC-MS after SPE and derivatization with hexafluoroisopropanol/trifluoroacetic anhydride (HFIP/TFAA). The LOQ for all tested drugs was 50 ng/mL in this assay. In some cases, specific drugs were identified and quantified: zolpidem was analyzed using HPLC-DAD after SPE (LOQ was 10 ng/mL) and ketamine was analyzed by using high-performance liquid chromatography with tandem mass spectrometric detection (LC-MS/MS) after protein precipitation with acetone (LOQ was 1 ng/mL).

In 108 cases, concentrations of alcohol were determined by headspace gas chromatography with flame ionization detection on two different columns. The LOQ for alcohol was 2 mg/dL. In 109 cases, mostly initiated by specific information from the police, samples were tested for the presence of GHB. GHB was identified and quantified in blood and urine using GC-MS after

liquid-liquid extraction and derivatization with BSTFA. Valproic acid can be determined simultaneously. The LOQ for GHB and valproic acid was 3 mg/L (18). Because of the endogenous nature of GHB, it can be present in low concentrations in biological samples. Therefore, only GHB concentrations in blood higher than 5 mg/L and concentrations of GHB in urine higher than 10 mg/L were considered indicative for administration or consumption of GHB (19–21).

Retrograde Extrapolation of Alcohol Concentrations

In cases where alcohol was detected in blood or urine, calculations were performed to estimate the alcohol concentration in blood at the time of the alleged sexual assault. The amount of alcohol that would have been eliminated during the time period between the alleged sexual assault and sampling was added to the measured alcohol concentration for each individual case. If the alleged sexual assault took place over a period of time, the midpoint of the time interval was taken for the calculations. If only the date of the assault was known, no calculation was performed. The retrograde extrapolation of alcohol concentrations requires making assumptions such as the existence of the postpeak declining phase at both time points, no consumption of alcohol after the alleged sexual assault and alcohol disappearance from the blood occurring at zero-order kinetics. An average alcohol elimination rate of 0.015 g/dL/h was used because most of the victims were young women with probably little prior experience of alcohol consumption (10,22,23). For urine samples, a ratio of 1.33 for urine to blood ratio was used to convert the concentration to the equivalent blood alcohol concentration (BAC) (12). Retrograde extrapolation was not performed if alcohol concentrations were <0.02 g/dL in blood and <0.03 g/dL in urine because at these concentrations, the alcohol metabolizing enzymes are not saturated, and therefore, no zero-order kinetics should be applied (9,23).

Results

Number of Cases and Seasonal Variation

Between January 2004 and December 2006, 135 cases of alleged DFSA were identified; 35 cases were included in 2004, and in the years 2005 and 2006, 50 cases per year were included. In 28 of the submitted cases, only blood samples were available; in 50 cases, only urine samples; and in 57 cases, both blood and urine samples were present for analysis. Table 1 provides specific information on the cases identified. From 2004 to 2006, a decrease was seen in the percentage of cases in which only blood samples were present for analysis, whereas an increase was seen in the percentage of urine samples. Figure 1 shows the distribution of the number of cases of alleged sexual assault. There was no clear seasonal variation in the number of reported cases of alleged sexual assault. Statistically, this was confirmed by performing a two-way analysis of variance, and no statistical significance was found between years and months.

TABLE 1—Annual and total numbers (and percentages) of blood samples, urine samples, or both sent to the NFI.

Year	2004	2005	2006	2004–2006
Number of cases included	35	50	50	135
Blood (%)	10 (29)	11 (22)	7 (14)	28 (21)
Urine (%)	10 (29)	18 (36)	22 (44)	50 (37)
Blood and urine (%)	15 (43)	21 (42)	21 (42)	57 (42)

Gender and Age of the Victims

Most of the victims were women (94%), and the mean age of the victims was 25 years (range 4–69 years; median age 24 years). In Table 2, detailed information of gender and age of the victims of alleged sexual assault is presented for each year and the 3-year period. No clear differences in gender and age were seen over the study period.

Negative Toxicology Results and Time Delay

In 135 cases, samples were tested for the presence of alcohol, drugs (drugs of abuse and prescription drugs), or both. In 27% (36 of 135) of the cases, no alcohol and/or drugs were found. The relationship between these negative toxicology results and time delay between alleged sexual assault and sampling was examined. If a range of times was given, the longest time interval was used for classification. The results are presented in Fig. 2. Including all 135 cases (blood or urine or both present for analysis), 11% (six of 54) of the cases were negative if the time delay was <12 h. With a time delay between 12 and 24 h, 25% (seven of 28) of the cases were negative, and with a time delay of >24 h, 47% (14 of 30) showed negative results. In 39% (nine of 23) of the cases, the time delay was unknown. The results show that, with increasing time delay, more cases were found to have negative toxicology results.

Looking into more detail into the relationship between negative toxicology results and time delay, the 135 cases were split up into two groups. In the first group, only those cases in which blood samples were available were included (28 cases) and the second group consisted of cases in which urine samples were available

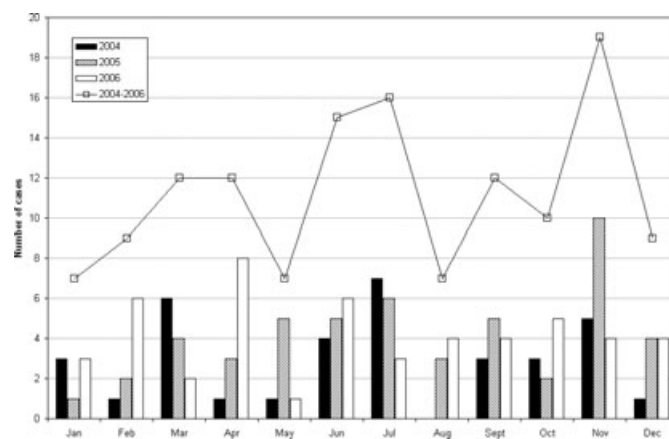


FIG. 1—Distribution of the number of cases of alleged sexual assault by month of the year.

TABLE 2—Gender and age of the victims of alleged sexual assault over the years.

Year	2004	2005	2006	2004–2006
Number of cases, gender known	32	48	48	128
Female (%)	31 (97)	43 (90)	46 (96)	120 (94)
Male (%)	1 (3)	5 (10)	2 (4)	8 (6)
Number of cases, age known	33	40	41	114
Range age (years)	4–41	15–60	15–69	4–69
Mean age (years)	20.7	27.1	27.6	25.4
Median age (years)	19.0	23.5	25.0	23.5

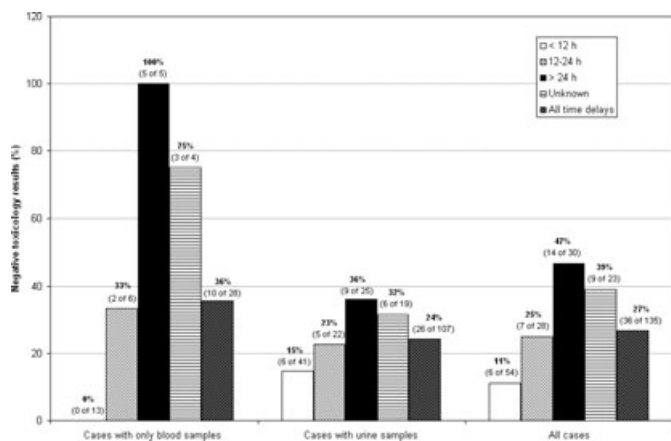


FIG. 2—Relationship between negative toxicology results and time delay between alleged sexual assault and sampling (% = percentage of negative toxicology results, n of m with n = number of cases with negative toxicology cases and m = number of cases per category). If a range of times was given, the longest time interval was used for classification.

with or without a blood sample (107 cases). As shown in Fig. 2, 100% of the cases had negative toxicology results when blood alone was collected more than 24 h after the alleged sexual assault whereas in cases in which urine samples were available, 36% of the cases showed negative toxicology results with the same sample collection time delay.

Alcohol Presence and Concentrations

In 108 cases, blood and/or urine samples were tested for alcohol. Not all 135 cases were tested for the presence of alcohol mainly because the time delay between alleged sexual assault and sampling was more than 24 h or the police did not request alcohol analysis. In Table 3, the results of alcohol presence and concentrations in blood and urine are summarized. In 47% (51 of 108) of these cases, alcohol was detected in blood or urine or both. In 62 of 108 cases, blood samples were analyzed for the presence of alcohol: 33 cases tested negative and 29 tested positive with a mean and median BAC of 0.121 and 0.116 g/dL, respectively. In cases in which urine samples were tested for the presence of alcohol, 43 cases of 79 tested negative and 36 tested positive with a mean and median urine alcohol concentration (UAC) of 0.146 and 0.139 g/dL, respectively. In 33 cases, blood and urine samples were tested for the presence of alcohol. In 16 cases, no alcohol was detected in both matrices, and in three cases, alcohol was detected in urine but not in blood. In the remaining 14 cases, alcohol was detected in both blood and urine.

In cases in which alcohol was detected in blood or urine, retrograde extrapolation of alcohol concentrations was performed to give an estimate of the BAC at the time of the alleged sexual assault. This resulted in very high mean and median BAC of 0.20 and 0.20 g/dL (22 cases) from measurements in blood and 0.22 and 0.22 g/dL (21 cases) from measurements in urine, respectively. For all cases in which retrograde extrapolation of alcohol concentrations could be performed, the time delay between the assault and sampling was <12 h.

Drugs Found in Blood and Urine

In 134 of 135 cases, blood and/or urine samples were screened for drugs. In one case, only GHB analysis was performed and no general drug screening or alcohol analysis because of a special request from the police and the limited amount of sample available. In two of 134 cases tested for drugs, samples were only analyzed using ELISA drug screening because the amount of sample was too small to perform screening for drugs of abuse and prescription drugs. Because the general screening method is required to identify many of the prescription drugs, the percentages of positive cases were calculated using 134 cases for drugs of abuse and 132 cases for prescription drugs.

In 54% (73 of 134) of the cases screened for drugs (drugs of abuse, prescription drugs, or both), at least one drug was detected. The most common group of drugs identified was the nonopiate analgesic group with acetaminophen and ibuprofen being the most frequently found in respectively 27 and 15 cases. Cocaine, MDMA, and THC or metabolites were the most commonly found illicit drugs. In 13 of 14 MDMA-positive cases, MDA was found, most probably because of metabolism from MDMA. MDA was not detected without the presence of MDMA. GHB is often associated with DFSA but was detected in only two cases of the 109 tested.

Benzodiazepines were detected in 14 cases. In six cases, combinations of diazepam, desmethyldiazepam, temazepam, and oxazepam were found consistent with the intake of at least one benzodiazepine (diazepam). In four cases, oxazepam was the only benzodiazepine identified. Other benzodiazepines detected were the following: bromazepam and metabolite (one case), temazepam (one case), and midazolam and metabolite (one case). In only one case, flunitrazepam and its metabolite were detected together with the benzodiazepines oxazepam and desalkylflurazepam. The results are summarized in Tables 4 and 5. Table 4 shows the number of cases in which drugs were identified, most common drug first. Table 5 shows the number of cases in which drugs were identified, divided into four groups, alcohol, illicit drugs, sedative, and nonsedative therapeutic drugs.

TABLE 3—Summary of the results of alcohol analysis in blood and urine.

	All Cases	Cases with Blood Samples	Cases with Urine Samples	Cases with Both Blood and Urine Samples
Total number investigated	108	62	79	33
Cases with negative alcohol analysis: number (%)	57 (53)	33 (53)	43 (54)	19 (58)
Cases with positive alcohol analysis: number (%)	51 (47)	29 (47)	36 (46)	14 (42)
Measured alcohol concentration (g/dL):				
mean ± SD; median; number of cases		0.121 ± 0.065;	0.146 ± 0.094;	
		0.116; 29 cases	0.139; 36 cases	
Retrograde extrapolation to estimate blood alcohol concentrations at time of the assault (g/dL):		0.20 ± 0.07;	0.22 ± 0.08;	
mean ± SD; median; number of cases*		0.20; 22 cases	0.22; 21 cases	

For assumptions, see Methods.

TABLE 4—The number of cases in which drugs were identified, most common drug first.

Drugs	Classification	Number of Cases Analyzed	Number of Positive Cases	% Positive of Cases Analyzed
Alcohol	Alcohol	108	51	47
Acetaminophen	Analgesics, nonopiate	132	27	20
Cocaine (metabolite)	Illicit drugs	134	19	14
Ibuprofen	Analgesics, nonopiate	132	15	11
Benzodiazepines	Hypnotics, anxiolytics, anticonvulsants	134	14	10
MDMA (and MDA)	Illicit drugs	134 (134)	14 (13)	10 (10)
Cannabinoids	Illicit drugs	134	13	10
Amphetamine	Illicit drugs	134	5	4
Paroxetine	Antidepressants	132	3	2
GHB	Illicit drugs	109	2	2
Lidocaine	Local anesthetics	132	2	2
Amitriptyline	Antidepressants	132	1	1
Codeine	Analgesics, opiate	134	1	1
Ketamine	Illicit drugs	132	1	1
Methadone	Analgesics, opiate	134	1	1
Naproxen	Analgesics, nonopiate	132	1	1
Olanzapine	Antipsychotics	132	1	1
Quinine/quinidine	Antimalarials/antiarrhythmics	132	1	1
Salbutamol	Bronchodilators	132	1	1
Venlafaxine	Antidepressants	132	1	1
Zolpidem	Hypnotics	132	1	1

TABLE 5—The number of cases in which drugs were identified, classified into four groups, alcohol, illicit drugs, sedative, and nonsedative therapeutic drugs.

Classification	Drugs	Number of Cases Analyzed	Number of Positive Cases	% Positive of Cases Analyzed
Alcohol	Alcohol	108	51	47
Illicit drugs	Cocaine (metabolite)	134	19	14
	MDMA (and MDA)	134 (134)	14 (13)	10 (10)
Sedative therapeutic drugs	Cannabinoids	134	13	10
	Amphetamine	134	5	4
	GHB	109	2	2
	Ketamine	132	1	1
	Benzodiazepines	134	14	10
	Amitriptyline	132	1	1
	Codeine	134	1	1
	Methadone	134	1	1
	Zolpidem	132	1	1
	Nonsedative therapeutic drugs	Acetaminophen	132	27
Ibuprofen		132	15	11
Paroxetine		132	3	2
Lidocaine		132	2	2
Naproxen		132	1	1
Olanzapine		132	1	1
Quinine/quinidine		132	1	1
Salbutamol		132	1	1
Venlafaxine		132	1	1

Combination of Alcohol and Drugs

In 108 cases, blood and/or urine samples were tested for both the presence of alcohol and drugs (drugs of abuse or prescription

TABLE 6—The number of cases in which alcohol and drugs are present of the 108 cases tested for both alcohol and drugs.

	Number of Cases	Drugs (Number of Cases)
Alcohol only	25	—
Alcohol + 1 drug	14	Acetaminophen (4) Cocaine (2) Paroxetine (2) Ibuprofen (1) Ketamine (1) Lidocaine (1) MDMA (and MDA) (1) Naproxen (1) Quinine/quinidine (1)
Alcohol + 2 drugs	7	Acetaminophen + Benzodiazepines (1) Acetaminophen + Cocaine (1) Acetaminophen + Ibuprofen (1) Acetaminophen + MDMA (and MDA) (1) Cannabinoids + Cocaine (1) Cannabinoids + Ibuprofen (1) MDMA (and MDA) + Amphetamine (1)
Alcohol + 3 drugs	3	Acetaminophen + Ibuprofen + Cocaine (1) Acetaminophen + Ibuprofen + Cannabinoids (1) Cannabinoids + MDMA (and MDA) + Benzodiazepines (1)
Alcohol + 4 drugs	2	Acetaminophen + Cocaine + Codeine + Benzodiazepines (1) Acetaminophen + Cocaine + Ibuprofen + Lidocaine (1)
No alcohol + 1 drug	16	Acetaminophen (3) Ibuprofen (3) Benzodiazepines (2) Cannabinoids (2) Cocaine (2) MDMA (and MDA) (2) GHB (1) Olanzapine (1)
No alcohol + 2 drugs	13	Cocaine + Cannabinoids (2) Ibuprofen + Cocaine (2) Acetaminophen + MDMA (and MDA) (2) Cocaine + Benzodiazepines (1) Cannabinoids + Benzodiazepines (1) Ibuprofen + Benzodiazepines (1) Ibuprofen + MDMA (1) MDMA (and MDA) + Amphetamine (1) Acetaminophen + Benzodiazepines (1) Acetaminophen + GHB (1)
No alcohol + 3 drugs	1	MDMA (and MDA) + Amphetamine + Cannabinoids (1)
No alcohol + 4 drugs	2	Acetaminophen + Cocaine + Benzodiazepines + Ibuprofen (1) Acetaminophen + Cocaine + Benzodiazepines + Cannabinoids (1)
No alcohol or drugs	25	—

drugs). In Table 6, details of the drug combinations are presented. In 23% (25 of 108) of the cases, alcohol was the only drug identified. In 24% (26 of 108) of the cases, alcohol and at least one drug were tested positive: in 14 cases, alcohol was found together with one drug; in seven cases with two drugs; in three cases with three drugs; and in two cases with four drugs. In 30% (32 of 108) of the cases, no alcohol was found but at least one drug was tested positive: in 16 cases, one drug was found; in 13 cases two drugs; in one case three drugs; and in two cases four drugs. And in 23% (25 of 108) of the cases, no alcohol or drugs were found.

Discussion

In this study, 135 cases of alleged DFSA were identified over a 3-year period from January 2004 until December 2006. The number of cases per year is comparable with the number of cases presented in the study from Northern Ireland (5) but much smaller than the numbers reported in most other studies (2,7,8,10,11). There was no clear seasonal variation in the number of reported cases of alleged sexual assault. This is in contrast to other literature reports showing peaks during summer months or in December during the festive season (10,24). This may be due to differences between countries or the relatively small sample size in our study.

Most of the victims in this study were women (94%), which is comparable with the study from Australia in which 95% of the victims were women (9). The mean and median age of the victims was 25 and 24 years, respectively, which was also similar to other studies (7,9).

Of the 135 cases tested for alcohol, drugs, or both, 36 (27%) were negative. This percentage is lower compared with the percentage of negative toxicology results in other countries. In America (2,7,8), 39–40% tested negative, 31% in Sweden (10), and 35% in the U.K. (11). Only in Northern Ireland was a lower percentage of negative toxicology results reported with 34% in 1999 and 22% in 2005. Most studies included no data on the time delay between the assault and the time of sampling; therefore, no comparison can be made. As expected, our results show that with increasing time delay, more cases were found to be negative. If only blood samples were available for analysis, 100% tested positive for alcohol or drugs when collected within 12 h of the alleged assault, and 100% tested negative if collected more than 24 h after the alleged sexual assault. In cases in which urine samples were available, 36% of the cases had negative toxicology results with collection times >24 h. These data support the collection of a urine sample if the time delay after the alleged assault is >24 h. In the study from Northern Ireland (5), 44–74% of the cases had negative toxicology results if the time delay was >12 h. The results of our study indicate that some cases may represent false-negative results because of the time delay. Laboratories may want to evaluate the analysis of blood samples taken more than 24 h after the alleged sexual assault.

As in many previous studies (23), our study shows that alcohol alone or together with other drugs was the most common finding. Of the 108 cases tested for the presence of alcohol, 51 cases (47%) tested positive for alcohol, either alone or in combination with other drugs, in blood or in urine or in both. In America (2,7,8), this percentage varied between 38% and 41%. In the studies from Europe, the following percentages of cases that tested positive for alcohol were obtained: in Northern Ireland (5), 55%; in Sweden (10), 55%; and in the U.K. (11,12), 46%. In the Australian study (7), alcohol consumption prior to the assault was reported by 77% of the subjects and alcohol was identified in 37% of the cases when subsequently examined.

In our study, high mean alcohol concentrations of 0.121 g/dL and 0.146 mg/dL were measured in blood (29 cases) and urine (36 cases), respectively. When retrograde extrapolation was performed, this resulted in very high estimated mean BAC at the time of the alleged sexual assault of 0.20 and 0.22 g/dL, respectively. Such high concentrations of alcohol could have caused disorientation, potential memory loss, or even loss of consciousness. These results confirm that cases of alleged DFSA often occur in the setting of heavy alcohol consumption. This might contribute to the question whether the victim was in the position to give informed consent. Internationally, comparable alcohol concentrations were found. In

Australia, the average BAC was 0.11 g/dL at the time of examination and the estimated concentration at the time of the alleged assault was 0.22–0.33 g/dL (9). In the study from Northern Ireland, estimated average BAC per year were reported from 1999 to 2005 varying from 0.17 to 0.22 g/dL (5). In Sweden, the average measured BAC was 0.124 g/dL and the retrograde estimated BAC was 0.199 g/dL using an average alcohol elimination rate of 0.015 g/dL/h and assuming a fixed time delay of 5 h (10). Using the low and high elimination rates of alcohol (0.010 and 0.025 g/dL/h), the lowest and highest estimated BAC were 0.174 and 0.249 g/dL, respectively (9). In the U.K., 60% of the cases in which alcohol was tested positive had estimated BAC >0.15 g/dL at the time of the alleged assault (12).

A wide range of drugs was found in cases of alleged DFSA in the Netherlands. The most common group of drugs identified was the nonopioid analgesic group with acetaminophen and ibuprofen being the most frequently found. These pain-relieving substances are over-the-counter preparations and are frequently used in the Netherlands. In the study from Northern Ireland (5), the authors assumed that these drugs were consumed voluntarily, possibly after the assault. In our study, the information from the police was incomplete and could not verify this.

The most frequently encountered illicit drugs were, respectively, cocaine, MDMA, THC, or metabolites, followed by amphetamine. GHB is often associated with DFSA but was detected in only two cases of the 109 tested, and the drug ketamine that is also abused recreationally was found in one case. In the group of sedatives, benzodiazepines were the largest group, detected in 14 cases. The other sedatives found were amitriptyline, codeine, methadone, and zolpidem. These results are in line with other studies, as summarized in the introduction of this study. It is important to realize that the use of illicit drugs as well as sedatives might have influenced the victim's behavior or consciousness although the use of stimulant illicit drugs is not a typical DFSA scenario. Stimulant illicit drugs do not possess the pharmacological effects typically associated with DFSA drugs, but drugs like MDMA may be administered in a DFSA case in an attempt to increase the libido of the victim or make the victim more amenable to sexual activity (11). However, we could not distinguish voluntary ingestion or therapeutic use from involuntary intake; that is, we could not discriminate between proactive and opportunistic DFSA.

As in other studies, we also found that combinations of drugs often occur. In nearly half of the cases that tested positive for drugs, more than one drug was present. Combinations with acetaminophen were the most frequently encountered. In most cases, acetaminophen was probably taken as an analgesic. In a number of cases, combinations were found of drugs with sedative properties, for example, alcohol, GHB, benzodiazepines, and cannabinoids, which might lead to increased intoxication. In a few other cases, it is not possible to predict the effect because combinations were found for drugs with sedative properties and drugs that could cause lowering of inhibitions.

In conclusion, the results show that alcohol is the most commonly found drug in alleged sexual assault cases in the Netherlands followed by nonopioid analgesics, illicit drugs, and benzodiazepines. Although it was not possible to distinguish between voluntary and involuntary ingestion, the presence of drugs may contribute to the victims' vulnerability. In some cases, the absence of alcohol and drugs may represent false-negative results owing to the time delay between alleged sexual assault and sampling. As the time delay between alleged sexual assault and sampling increases and exceeds 24 h, laboratories are more likely to identify drugs in urine samples.

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