Where the drug meets the road : actual driving, psychomotor performance and roadside drug screening methods in cannabis and ecstasy users

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Where the drug meets the road:

Actual driving, psychomotor performance and roadside drug screening methods in cannabis and ecstasy users

"If I know what love is, it is because of you."

Hermann Hesse

Voor de liefde in mijn leven:

Roos, Guido, pap en mam

COLOPHON

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Actual driving, psychomotor performance and roadside drug screening methods in cannabis and ecstasy users

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CHAPTER 1: GENERAL INTRODUCTION

Illicit drugs and traffic safety

Cannabis and ecstasy are frequently used recreational drugs. Cannabis is even the most widely used illicit substance worldwide. Globally, it was estimated that lifetime prevalence was between 129-191 million persons (2.9-4.3%) aged 15-64 years (UNODC, 2010). Use of ecstasy is less common, although the number of persons initiating ecstasy use is increasing (e.g. SAMHSA, 2010). The party-drug ecstasy is especially popular among young people. In most European countries lifetime prevalence of ecstasy of persons aged 15-24 years is between 2.1-6.8% (EMCDDA, 2010) and 12.4% among 18-25 year old persons in the US (SAMHSA, 2010).

Considering the frequent use of cannabis and ecstasy, the prevalence of these substances in traffic might also be substantial. The prevalence of cannabis in the general driving population is indeed the highest after alcohol, especially among young drivers (e.g. EMCDDA, 2008b; Lacey, et al., 2009). In a worldwide meta-analysis a median of 30% of young drivers reported they had ever driven under the influence of cannabis (EMCDDA, 2008b). Prevalence of ecstasy in traffic is rarely assessed, but one study reported that 0.1%-4.6% of the European general driving population asserted ever driving under the influence of ecstasy (EMCDDA, 2008b). Moreover, increased risk of an accident or being responsible for an accident was found for cannabis, benzodiazepines, amphetamines (including ecstasy), heroin and cocaine (EMCDDA, 2008b).

The problem of drugged driving was also recognized by the World Health Organization (WHO) in their world report on road traffic injury prevention (WHO, 2004). The WHO however also identified a number of scientific issues that have troubled determination of causal relations between drug concentrations in bodily fluids and driving safety, including absence of specific concentration-effect relations for many drugs that produce impairment, large inter-individual differences in response to a drug and synergistic or antagonistic effects after combined drug use. The WHO concludes that urgent research is needed to elucidate these issues.

Thus, driving under the influence of cannabis or ecstasy is not uncommon and might jeopardize traffic safety. Especially since research has demonstrated that cannabis impairs driving performance in occasional cannabis users (Ramaekers, Berghaus, van Laar, & Drummer, 2004; Ramaekers, Robbe, & O'Hanlon, 2000). Studies assessing the effect of ecstasy on driving performance are sparse, but demonstrated mild stimulatory effects during day-time testing (Kuypers, Samyn, & Ramaekers, 2006). However, ecstasy is generally taken during the night at so called 'rave parties'. The effects of the consequent sleep loss in combination with ecstasy on actual driving and driving related performance are largely unknown (Brookhuis, de Waard, & Samyn, 2004; Kuypers, Wingen, & Ramaekers, 2008; Kuypers, Wingen, Samyn, Limbert, & Ramaekers, 2007). The studies in this dissertation aim to investigate the relation between drug concentrations (i.e. Δ^9 -tetrahydrocannabinol (THC) and 3,4-methylenedioxymethamphetamine (MDMA) the active ingredients of cannabis and ecstasy respectively) and traffic safety. The effects of orally administered and smoked THC in occasional and/or heavy cannabis users and MDMA in combination with sleep deprivation on actual driving and psychomotor performance was assessed using experimental methods.

Cannabis and ecstasy

Cannabis sativa L. (Marijuana) is a herbaceous plant. The plant contains at least 85 different cannabinoids (El-Alfy, et al., 2010). The major active cannabinoid, THC, has been used as a recreational and medicinal drug since ancient times (Zuardi, 2006). THC is primarily responsible for the psychoactive effects by activating a cannabinoid (CB₁) receptor in the central nervous system (Di Marzo, 2009). This generally leads to relaxation, a sense of wellbeing, and slowing of thought processes. For medical use two formulations exist nowadays, i.e. dronabinol and nabilone, which are both orally administered synthetic forms of THC. Oral administration of dronabinol is the only US Federal Drug Administration (FDA) approved formulation of medicinal cannabis (DEA, 2011). Medicinal cannabis is prescribed for cancer patients undergoing chemotherapy to prevent emesis and as an appetite stimulant for patients suffering from AIDS or other wasting diseases. The pharmacokinetic effects differ depending on the route of administration. Smoked THC has an onset of action within 5-10 minutes, when it reaches peak plasma levels (McGilveray, 2005; Ramaekers, Moeller, et al., 2006). Subjective effects also peak within 5-10 minutes and significantly decrease after 1 hour (Ramaekers, Kauert, Theunissen, Toennes, & Moeller, 2009). The bioavailability of smoked THC is on average 30% (McGilveray, 2005). Orally administered THC has a slower onset of action of approximately 0.5-1 hour, but the effects last up to 4-6 hours. Peak effects occur around 2-4 hours and the bioavailability is only 4-12%.

The main psychoactive ingredient of the synthetic party-drug ecstasy is MDMA. Its action in the central nervous system is rather complex. MDMA produces its effects mainly by massive serotonin (5-HT) release. Additionally it blocks re-uptake of 5-HT by inhibiting the 5-HT transporter (SERT). This produces euphoria, enhanced mood, sociability and empathy acutely (de la Torre, et al., 2004). After the acute release, 5-HT is temporarily depleted for 24 hours to 7 days after drug intake. This is subjectively experienced as lowered mood (Curran & Travill, 1997). Pharmacokinetic analysis has shown that peak plasma levels of MDMA are attained at 2 hours post-drug. The elimination half-life is about 8-9 hours (Mas, et al., 1999).

Acute effects of THC and MDMA on driving (related) performance

Experimental studies have demonstrated that acute administration of cannabis causes driving and psychomotor impairment in occasional cannabis users (Grotenhermen, et al., 2007; Hunault, et al., 2009; Ramaekers, et al., 2004; deza Ramaekers, Kauert, et al., 2009; Ramaekers, Kauert, et al., 2006; Ramaekers, Moeller, et al., 2006; Ramaekers, et al., 2000; Sewell, Poling, & Sofuoglu, 2009). Heavy users seem largely tolerant to the impairing effects of cannabis on driving and psychomotor performance (Hart, et al., 2002; Ramaekers, Kauert, et al., 2009; Ramaekers, et al., 2010; Sewell, et al., 2009). Clearly, cannabis use history plays an important role in the effects of acutely administered smoked cannabis on these performance measures.

Since cannabis is gaining more and more interest as a medicine, orally administered cannabis, i.e. dronabinol, was developed for that purpose. The few studies that assessed the effects of dronabinol on psychomotor performance found that it had no effect in heavy users (Gray, Hart, Christie, & Upadhyaya, 2008; Hart, et al., 2002), but significant impairing effects in occasional users (Menetrey, et al., 2005) compared to placebo. This dissertation will assess whether actual driving

performance in occasional and heavy cannabis users is also impaired under the influence of dronabinol.

Acutely administered MDMA is known to impair performance on several neuropsychological tasks (Kalechstein, De La Garza, Mahoney, Fantegrossi, & Newton, 2007; Morgan, 2000; Zakzanis, Campbell, & Jovanovski, 2007). Research has also shown that MDMA has stimulating properties, e.g. it improved tracking performance acutely (Lamers, et al., 2003). The effects of MDMA on driving performance are mixed as well: road tracking performance generally improved, but car following performance was impaired after an acute dose of MDMA (Kuypers, et al., 2006; Ramaekers, Kuypers, & Samyn, 2006). However, MDMA is often used during the night and in combination with other substances such as alcohol and cannabis. Previous studies showed that the stimulating effects of MDMA are not strong enough to overcome alcohol or cannabis induced impairments (Dumont, et al., 2010; Kuypers, et al., 2006). Whether a single dose of MDMA is strong enough to compensate for psychomotor and driving impairment due to sleep loss was investigated in this dissertation.

Long-term effects of THC on psychomotor performance

As mentioned above, heavy cannabis users are generally tolerant to the acutely impairing effects of cannabis on psychomotor performance. One mechanism for tolerance is down-regulation of receptor density in the brain (Julien, Advokat, & Comaty, 2008). Down-regulation of CB₁ receptors was recently demonstrated in chronic, daily cannabis users (Hirvonen, et al., 2011). Whether these changes in the brain can cause long-term, irreversible cognitive deficits after abstinence is still debated.

Previous studies showed that compared to control subjects, abstinent heavy cannabis users performed worse on tasks measuring memory and attention during one week of abstinence (Pope, Gruber, & Yurgelun-Todd, 2001; Solowij & Battisti, 2008; Solowij, et al., 2002). However, after 4 weeks of abstinence heavy cannabis users' neuropsychological performance was the same as performance of a control group (Pope, Gruber, Hudson, Huestis, & Yurgelun-Todd, 2001). Therefore, the authors concluded that impairment of heavy users is most likely due to residual effects rather than long-term damage.

Furthermore, a recent positron emission tomography (PET) study found that CB₁ receptor density was down-regulated in cortical and limbic areas in heavy cannabis users, which returned to normal after 4 weeks of abstinence compared to controls (Hirvonen, et al., 2011). This indicates that, at least for these regions, the effects of heavy cannabis use are reversible with prolonged abstinence. However, down-regulation in motor areas was not demonstrated and thus psychomotor impairment might be more persistent. This is consistent with a study that found diminished brain activation in motor cortical circuits up to 4 weeks of abstinence (Pillay, et al., 2008). In this dissertation a study that assessed the presence and extent of psychomotor impairment in chronic, daily cannabis users during 3 weeks of abstinence was conducted to test the hypothesis that psychomotor impairment would last throughout abstinence.

Effect of THC on Standardized Field Sobriety Tests

Psychomotor impairment may be related to driving impairment. Roadside screening methods assess whether people are driving under the influence of drugs. In North America the Standardized Field Sobriety Tests (SFST) is therefore used to obtain validated indicators of driver impairment and to establish probable cause for arrest. The SFST consists of three tests, i.e. horizontal gaze nystagmus, one-leg-stand, walk-and-turn, which are administered and evaluated in a standardized manner (Stuster, 2006). The SFST was originally developed to detect drivers' impairment due to alcohol. Whether the SFST can detect drug induced impairment is largely unknown. A previous study showed a moderate relation between smoked cannabis and performance on the SFST in occasional users (Papafotiou, Carter, & Stough, 2005a). However, simulated driving impairment could not be accurately predicted based on SFST performance (Papafotiou, Carter, & Stough, 2005b). The present dissertation includes two studies that assessed the sensitivity of the SFST for THC induced impairment, either by smoked cannabis in heavy cannabis users or by orally administered dronabinol in heavy and occasional cannabis users.

Measuring THC and MDMA in oral fluid

Besides the SFST as a roadside screening method, oral fluid testing is gaining interest to determine whether people are driving under the influence of drugs. A large-scale European Union study, called Roadside testing assessment (Rosita-1), aimed to identify the best method for roadside drug testing. Oral fluid was preferred by most countries (Verstraete & Raes, 2006). During Rosita-2 several oral fluid devices were tested in Europe and the US for suitability as a roadside screening method, but none was found to be adequate (Verstraete & Raes, 2006). Since then oral fluid tests were improved and Australia was the first to implement them for random roadside drug testing (Drummer, et al., 2007), even though the cutoffs, especially for THC, were still deficient compared to proposed guidelines (Pil, Raes, Van den Neste, & Verstraete, 2007; SAMHSA, 2004; Walsh, Verstraete, Huestis, & Morland, 2008). Nowadays, the cutoffs of the oral fluid tests for THC are approaching these guidelines. However, in order to be able to interpret oral fluid test findings collected along the road, controlled drug administration studies are needed. These studies can for example indicate reliability of the oral fluid tests, windows of detection and analyte stability. Studies presented in this dissertation always included oral fluid collection to determine drug concentrations and/or sensitivity of oral fluid point of collection devices.

Methods

Most studies in this dissertation employed an experimental randomized, double-blind, placebo-controlled, within-subjects design. That is, subjects received placebo and several acute doses of either MDMA or THC in random order. Neither the subjects nor the experiment leaders were aware which treatment was received at the various times of testing. The advantage of an experimental design is that a causal relationship can be demonstrated. When subjects perform differently under the influence of a substance compared to placebo, the effect is attributable to the actions of the drug.

Performance testing occurred at peak plasma levels and consisted of psychomotor performance and actual driving. The study presented in Chapter 5 was conducted according to a mixed design, i.e. within and between subjects, in which effects of sustained cannabis abstinence was measured without treatment administration in comparison to control subjects. Psychomotor performance was assessed within subjects after 1, 2 and 3 weeks of abstinence.

The psychomotor performance tests used were the critical tracking task (Chapters 5 and 6), a divided attention task (Chapters 5 and 7), a stop-signal task, the rapid information processing task and the psychomotor vigilance task (Chapter 7). These tasks measure different aspects of psychomotor performance such as tracking (critical tracking and divided attention task), response inhibition (stop-signal task) and reaction time (psychomotor vigilance and rapid information processing task).

Actual driving performance was assessed during the road tracking and car following tests (Chapters 3 and 6). The road tracking test (O'Hanlon, 1984) consists of driving for approximately one hour in a specially instrumented car. Subjects have to maintain a constant speed of 95 km/h and drive as straight as possible on the right lane of a primary highway. The dependent measure is the Standard Deviation of Lateral Position (SDLP), a measure of road tracking control (i.e. weaving). Performance on this task is compared to findings from an alcohol calibration study (Louwerens, Gloerich, de Vries, Brookhuis, & O'Hanlon, 1987). This allows drug induced impairment to be expressed relative to impairment under the influence of 0.5 mg/mL BAC. When SDLP difference scores exceed the alcohol criterion of 2.4 cm, the impairment is defined as clinically relevant.

In the car-following test (Brookhuis, de Waard, & Mulder, 1994; Ramaekers, Muntjewerff, & O'Hanlon, 1995) subjects drive behind a leading vehicle for 25 minutes while maintaining a constant headway during a series of speed decelerations/acceleration that are initiated by the experimenter in the leading vehicle. Sinusoidal speed changes reaching an amplitude of -10% and returning to the starting level within 50 sec. are repeated 6-10 times. Time to Speed Adaptation (TSA) is the primary measure and is calculated from the phase delay between speed signals from the leading and following vehicle.

Roadside drug screening methods are used to determine driving under the influence of drugs. With the SFST (Chapters 3 and 4) drug induced impairment can be established. Oral fluid point of collection testing devices can be used to establish drug concentrations (Chapter 4). Both methods are evaluated in two experimental studies conducted according to a placebo-controlled, within-subjects design.

Outline

Chapter 2 is a review of available oral fluid tests for drugs of abuse. The advantages and limitations, the progress that has occurred and the remaining issues of the oral fluid tests are outlined. The review focuses on collection, screening, confirmation and interpretation of tests for cannabinoids, opioids, amphetamines, cocaine and benzodiazepines.

Chapter 3 is a study on the acute effects of dronabinol on actual driving performance and the SFST. Subjects were heavy (N=12) and occasional (N=12) cannabis users. They received placebo, 10 and 20 mg orally administered dronabinol. Blood and oral fluid concentrations were measured. It was hypothesized that occasional users would show impairment on both

performance tests after both doses dronabinol compared to placebo. Heavy users were included as a model for chronic use of dronabinol and expected to be tolerant to the effects of both doses dronabinol on both performance tests.

Chapter 4 describes a study on the effects of smoked cannabis with and without alcohol on SFST performance and on sensitivity of two oral fluid point of collection testing devices, i.e. Dräger Drug Test® 5000 and Securetec Drugwipe® 5. Subjects were heavy cannabis users (N=20), who received alcohol doses to reach 0, 0.5 and 0.7 mg/mL BAC in combination with 400 µg/kg bodyweight THC. It was hypothesized that subjects would be tolerant to the impairing effects of THC on the SFST, but that they would show impairment on the SFST under the influence of the combination of alcohol and THC.

Chapter 5 is a study on sustained cannabis abstinence. The effects of 1, 2 and 3 weeks of abstinence in chronic, daily cannabis users (N=19) on psychomotor performance is described. Psychomotor performance of the abstinent cannabis users is compared to a control group of non-intoxicated occasional drug users. Blood and oral fluid concentrations were measured. It was hypothesized that psychomotor impairment would last throughout abstinence in chronic, daily cannabis users.

Chapter 6 and Chapter 7 describe two studies on the effects of 25, 50 and 100 mg MDMA in occasional ecstasy users (N=16) before and after a night without sleep. In both studies blood and oral fluid concentrations were measured. In Chapter 6 actual driving performance was assessed while Chapter 7 investigated psychomotor performance. Driving performance was measured in the evening and the next morning, psychomotor performance in the evening, in the middle of the night and the next morning. It was hypothesized that MDMA would improve performance acutely in the evening, but impair performance after sleep deprivation compared to placebo.

In Chapter 8 general conclusions are drawn and the findings of the studies are discussed in a broader context. Additionally, suggestions for future research are made.

CHAPTER 2: ORAL FLUID TESTING FOR DRUGS OF ABUSE

Wendy M. Bosker and Marilyn A. Huestis

BACKGROUND: Oral fluid (OF) is an exciting alternative matrix for monitoring drugs of abuse in workplace, clinical toxicology, criminal justice, and driving under the influence of drugs (DUID) programs. During the last 5 years, scientific and technological advances in OF collection, point-of-collection testing devices, and screening and confirmation methods were achieved. Guidelines were proposed for workplace OF testing by the Substance Abuse and Mental Health Services Administration, DUID testing by the European Union's Driving under the Influence of Drugs, Alcohol and Medicines (DRUID) program, and standardization of DUID research. Although OF testing is now commonplace in many monitoring programs, the greatest current limitation is the scarcity of controlled drug administration studies available to guide interpretation.

CONTENT: This review outlines OF testing advantages and limitations, and the progress in OF that has occurred during the last 5 years in collection, screening, confirmation, and interpretation of cannabinoids, opioids, amphetamines, cocaine, and benzodiazepines. We examine controlled drug administration studies, immunoassay and chromatographic methods, collection devices, point-of-collection testing device performance, and recent applications of OF testing.

SUMMARY: Substance Abuse and Mental Health Services Administration approval of OF testing was delayed because questions about drug OF disposition were not yet resolved, and collection device performance and testing assays required improvement. Here, we document the many advances achieved in the use of OF. Additional research is needed to identify new biomarkers, determine drug detection windows, characterize OF adulteration techniques, and evaluate analyte stability. Nevertheless, there is no doubt that OF offers multiple advantages as an alternative matrix for drug monitoring and has an important role in DUID, treatment, workplace, and criminal justice programs.

Oral fluid (OF)¹ is a suitable alternative matrix to test drugs of abuse in clinical, workplace, driving under the influence of drugs (DUID), drug treatment, and criminal justice settings. The main advantages of OF are the simplicity and noninvasiveness of sample collection, which can be easily observed (Choo & Huestis, 2004), obviating the need for special restroom facilities and same-sex collectors and making adulteration more difficult (Gallardo & Queiroz, 2008). Infection risk is lower than for blood (Langel, et al., 2008), and OF may better reflect recent drug use. The parent drug is frequently prominent in OF and may reflect free plasma concentrations, providing a better correlation with pharmacodynamic effects, such as impaired performance (Verstraete, 2005). It is difficult to differentiate heroin from morphine or codeine ingestion with urine drug testing, whereas 6-acetylmorphine (6AM) and heroin are frequently present in OF, clearly delineating heroin abuse.

Weak bases are detected in higher concentrations and for longer times in OF than in plasma because of ion trapping. Depending on a drug's pKa and lipophilicity, ion trapping occurs because of pH differences in blood (7.4) and OF (4–6). Free uncharged drug is in equilibrium across membranes separating blood and OF. At the lower OF pH, weak bases ionize, increasing total OF drug concentrations.

For clinical toxicology applications, including drug treatment, physician office, and emergency room testing, onsite OF testing offers rapid availability of results for diagnostic purposes and ability to confront treatment patients with immediate indicators of drug relapse. In 2004, Substance Abuse and Mental Health Services Administration (SAMHSA) proposed recommended guidelines (Table 1) for mandated federal workplace OF testing (SAMHSA, 2004). Lack of resolution of important scientific questions delayed final approval, but SAMHSA guidelines, including low cutoff concentrations, are frequently applied in non-regulated settings. The guidelines encompass mandated cutoffs and procedures for sample collection, custody, and control to ensure sample identity and integrity; sample validity tests; testing facility requirements; analytical method result review and reporting; alternative medical explanations; and laboratory certification (Bush, 2008). In the US, OF testing is expanding at a rapid pace in non-regulated workplace testing, treatment, and driving under the influence of drugs (DUID) programs.

One major incentive for improving OF testing is the increasing problem of DUID. Drugs and alcohol were contributing factors in up to 22% of motor vehicle crashes in the US (NIDA, 2008). Unfortunately, an even higher prevalence is likely, because additional analyses are usually not conducted for drugs if alcohol is above the legal limit. OF testing offers a new tool to improve traffic safety with rapid, easy roadside drug testing of drivers. ROadSide Testing Assessment (ROSITA) was the first European Union effort to determine the best means of identifying drugged drivers at the roadside. OF was selected over urine or sweat as the best matrix, although OF

¹Nonstandard abbreviations: OF, oral fluid; DUID, driving under the influence of drugs; 6AM, 6-acetylmorphine; SAMHSA, Substance Abuse and Mental Health Services Administration; ROSITA, ROadSide Testing Assessment; POCT, point-of-collection testing; DRUID, DRiving Under the Influence of Drugs, alcohol, and medicines; THC, Δ^9 -tetrahydrocannabinol; DDS, Drug Detection System; BE, benzoylecgonine; LOQ, limit of quantification; LC-MS/MS, liquid chromatography-tandem mass spectrometry; APCI, atmospheric pressure chemical ionization; ESI, electrospray ionization; THCCOOH, 11-nor-9-carboxy-THC; 11-OH-THC, 11-hydroxy-THC; GC-MS/MS, gas chromatography-tandem mass spectrometry; CBD, cannabidiol; CBN, cannabinol; MDMA, 3,4-methylenedioxymethamphetamine; MDA, 3,4-methylenedioxyamphetamine; EME, ecgonine methylester.

Table 1: SAMHSA, DRUID & Talloires recommended oral fluid cutoffs.^a

Drug/ analyte	SAMHSA screen (µg/L)	SAMHSA confirmation (µg/L)	DRUID confirmation (µg/L)	Talloires confirmation (µg/L)
Cannabinoids	4	2 ^b	1 ^b	2
Opiates	40			
Morphine		40	20	20
Codeine		40	20	20
6-acetylmorphine	4	4	5	5
Methadone		-	20	20
Phencyclidine	10	10	-	-
Amphetamines	50			
Amphetamine		50	25	20
Methamphetamine		50 ^c	25	20
MDMA (ecstasy)	50	50	25	20
MDA		50	25	20
MDEA		50	25	20
Cocaine or benzoylecgonine	20	8	10	10
Benzodiazepines	-			
Flunitrazepam		-	1	-
Diazepam		-	5	-
Alprazolam		-	1	-
Oxazepam		-	5	-
Nordiazepam		-	1	-
Lorazepam		-	1	-
Clonazepam		-	1	-

^a See references: SAMHSA (SAMHSA, 2004), DRUID (Pil, et al., 2007), Talloires (Walsh, et al., 2008).

^b THC.

^c Specimen must also contain amphetamine ≥ method limit of detection.

collection and testing was then considered inadequate (Verstraete & Puddu). ROSITA-2 in Europe and the US specifically evaluated available OF point-of-collection testing (POCT) devices, concluding that none were yet adequately reliable (Verstraete & Raes). The latest European initiative, Driving Under the Influence of Drugs, Alcohol, and Medicines (DRUID), promotes research and scientific support to reduce European Union road deaths by 50%. Guidelines were proposed for DUID testing in the DRUID program, as shown in Table 1. In Victoria, Australia, OF DUID testing began in 2003, with public acceptance and reductions in impaired driving, despite less than optimal performance of collection devices and assays (Drummer, et al., 2007). The Talloires recommendations for OF cutoff concentrations (Walsh, et al., 2008) also are included in Table 1. In 2006, international experts met to draft guidelines for conducting research on drugged driving. Currently, it is difficult to compare results across studies owing to the lack of standardization in experimental methods. Recommendations focused on 3 broad areas, i.e., behavior, epidemiology, and toxicology. Included among the 80 recommendations for toxicological contributions were suggested OF drug cutoff concentrations.

OF testing does have disadvantages. Drugs may reduce salivation, limiting sample volume and necessitating sensitive analytical methods to quantify multiple analytes in 1 assay. Drug concentrations are frequently lower in OF than in urine (Verstraete, 2005). Other issues include contamination from ingested food and beverages, and unknown adulteration techniques (Crouch, 2005). Drugs that are smoked, inhaled, insufflated, or taken orally also may contaminate the oral mucosa and OF, increasing detection, but reducing correlation with blood concentrations for 30–60

min. We observed that OF concentrations of Δ^9 -tetrahydrocannabinol (THC) greatly exceeded plasma concentrations for approximately 30 min after cannabis smoking (Huestis & Cone, 2004). The magnitude and duration of contamination have not been clearly defined for many drugs, and require additional controlled drug administration studies. Stimulation of salivary flow leads to increased OF pH due to increased bicarbonate excretion and reduced drug concentrations due to dilution (Crouch, 2005).

In this review, we focus on advances, primarily those occurring in the last 5 years, in OF measurement and interpretation of OF concentrations of cannabinoids, opioids, amphetamines, cocaine, and benzodiazepines. We also summarize progress in OF testing and unresolved issues. Our aim is to inform laboratories adding OF collection and testing, improve interpretation of OF test results, and promote evidence-based drug policies and relevant traffic safety legislation.

OF Collection

There are 2 main approaches to OF testing. One approach involves OF collection by expectoration or with a specialized device, and transfer of the sample for conventional laboratory-based immunoassay screening and chromatographic confirmation. The other approach is a system for collection and initial screening of OF at the site of collection (POCT), followed by a laboratory-based chromatographic confirmation at a later time. Collecting OF without a specialized device can be achieved by the passive drool technique or by expectoration. The passive drool technique best reflects drug concentrations in excreted saliva, because expectoration increases the rate of salivary excretion to a minor extent. Although samples collected without a specialized device are useful from a scientific perspective, the collection process is distasteful for donors and collectors. Thus OF collection devices are now used for large-scale testing applications. As interest grew in testing this alternative matrix, a wide variety of OF collection devices were developed, with variable success. Major problems are variability of OF collected and deficiency of sample amounts.

Many devices collect <1 mL OF, limiting available sample for multiple drug confirmations. However, as chromatographic methods and instruments improve in analytical sensitivity, smaller sample volumes are being required. In addition, the trend is to develop assays that quantify multiple drug classes simultaneously, reducing the needed sample size. Another factor affecting adequate sample volume is the expected OF drug concentrations. Depending on a drug's potency and physiochemical characteristics, concentrations range from nanograms per liter to micrograms per liter. Therefore, the determination of adequate sample volume must take into consideration the drug being tested.

Some OF collection devices have built-in volume-adequacy indicators, facilitating appropriate collection, whereas other manufacturers report only approximate amounts collected. The Acro Biotech device has a mark on the vial indicating the amount collected (Langel, et al., 2008), and the Immunalysis Quantisal™ and StatSure Saliva Sampler™ contain indicators for collection of 1 mL. With the use of these indicators, the collector can keep the device in the donor's mouth for the time required to collect an adequate volume, despite individual variability in OF excretion. A similar indicator on the Cozart® Drug Detection System (DDS) documents 340 (60) μ L OF (Kintz, et al., 2009). Another approach is gravimetric determination by weighing the device before and after OF collection (Dickson, et al., 2007; Kauert, Iwersen-Bergmann, & Toennes, 2006). With the Greiner device a novel tactic is employed that determines OF amount based on dilution

of a dye in the extraction solution (Langel, et al., 2008). The Greiner device differs from other collection devices in that the sample donor rinses the mouth with the extraction solution, which is then expectorated with the OF into a collection beaker. The absorbance of the resulting solution is read in a spectrophotometer to calculate collected OF amount.

Even more problematic than the amount collected is variability in OF volume obtained within and between devices. The Orasure Intercept® Drugs Of Abuse oral sample collection device was reported to collect from 0.38 –1.53 g (Kauert, et al., 2006). A recent evaluation of 3 collection devices provided within and between collection device variability ranging from 1.045–1.667 g (Dickson, et al., 2007). This variability reflects imprecision in elution buffer volume included in the device, and more importantly, inconsistency in donor OF amount. Obviously, drug concentration is dependent upon the degree of dilution. Some manufacturers now provide assurances of the degree of variability in OF collection; within-device variability of <10% for the Quantisal collection device and <5% for the Cozart and StatSure devices were reported (Langel, et al., 2008).

OF drug concentration also is dependent on OF excretion stimulation, which may occur even to a small extent by placement of a collector in the mouth. Thus, it is impossible to prevent an increase in OF excretion during collection, except by employing passive drool. Older methods of increasing OF excretion included chewing on paraffin, and newer approaches embed citric acid or other chemicals onto collection devices. Early studies, including those in our laboratory, used devices that stimulated OF excretion; however, we learned that stimulation ultimately lowered rather than increased drug concentrations and complicated interpretation of results.

One of the most important limitations of OF collection devices is adsorption of drugs to the device, which frequently leads to false-negative test results (Gallardo & Queiroz, 2008). This effect was unknown early in OF test development, leading to low sensitivity. Manufacturers addressed the problem, with mixed results, by eluting drugs off the device with buffers. Buffers also reduce OF viscosity, improving measurement accuracy, but also dilute analyte concentrations. Many buffers and surfactants also interfere with direct injection techniques for LC-MS methods by increasing matrix effect. Generally, the more lipophilic a drug, the greater the adsorptivity to the collection device, but recoveries must be empirically determined. Device materials and buffers are proprietary, and it is not possible to predict when recovery might be an issue.

As is true for all toxicological analyses, the analysis is only as good as the sample. Specific challenges for OF collection devices include collection of adequate volume with good imprecision; although readily achievable, as demonstrated with some currently available devices, the speed of collection also is important. For DUID testing, one of the major challenges for manufacturers, and the source of complaints by police, is the time required for collection and testing at the roadside. Although elution buffers appear necessary for effective removal of drugs from the collection device, the resulting drug dilution and presence of salts that must be removed before LC-MS analysis are disadvantages. Although variability is inherent in OF collection and contributes to the difficulty in interpreting OF drug concentrations, is it appropriate to hold OF testing to a higher standard than is achieved with the currently accepted urine testing technology?

Laboratory-Based Drug Screening

The importance of screening OF for parent drugs and metabolites has been clearly documented by controlled drug administration data. With most RIAs for commonly abused drugs removed from

Table 2: Evaluation of immunological methods for analysis of drugs in oral fluid, including sample type, confirmation method, screening and confirmation cutoffs, diagnostic sensitivity (SN), diagnostic specificity (SP), and efficiency (EFF).

Method	Type	Confirm	Cannabinoids		Opiates		AMP ^a		COC		Benzodiazepines		Reference
			screen	confirm	screen	confirm	screen	confirm	screen	confirm	screen	confirm	
			THC 1	-	Methadone 5 Opiates 10	-	MAMP 40; AMP 100	-	BE 5	-	nDZP 1	-	
OraSure Intercept ELISA	-	GCMSMS LCMSMS	-	-	-	-	MAMP 40; AMP 100	-	BE 5	-	nDZP 1	-	Clarke and Wilson , 2005
Roche KIMS	-	-	-	Opiates 40	-	-	MAMP & AMP 50	-	-	-	-	-	Ananias, et al., 2007
Cozart Microplate ELISA	E	GCMS	-	Opiates 2.5, 10, 20, 30, 40	COD, nCOD, MOR, nMOR 2.5, 20, 30, 40	-	-	-	73.4-91.5	88.6-99.2	89.3-95.6	-	Barnes, et al., 2003
Cozart RapiScan	E	GCMS	-	Opiates 30	COD, nCOD, MOR, nMOR 2.5, 15, 30, 40	-	-	-	56.1-99.0	96.2-99.9	98.5.7-98.2	-	Kacinko, et al., 2004
Cozart Microplate ELISA	T	GCMS	-	Opiates 30	Opiates 30	-	-	-	99.1	94.4	96.8	-	Cooper, et al., 2005b
Cozart Microplate ELISA	T	GCMS	-	Methadone 20 & 30	Methadone 30	-	-	-	91.3-92.2	97.9-100	94.9-95.5	-	Cooper, et al., 2005a
IDS BUP One-step ELISA	T	GCMS	-	BUP 0.5	-	-	-	-	100	100	100	-	De Giovanni, et al., 2005
Cozart Microplate ELISA	E	LCMSMS	-	-	-	MDMA & MDA 51	MDMA & MDA 10	-	98.6	98.6	98.6	-	Laloup, Tilman, et al., 2005
Cozart Microplate ELISA	F	GCMS	-	-	-	AMP 45	AMP 30	-	87.2	97.8	93.5	-	Cooper, et al., 2006
Bio-Quant Direct ELISA F	-	-	-	-	-	MAMP & AMP ≤ 6 (1 in PBS)	-	-	-	-	-	-	Apollonio, et al., 2007
Cozart Microplate ELISA	E	GCMS	-	-	-	BE 10, 20, 30	COC, BE, EME 2.5, 8, 10, 15	-	89.8-99.0	58.8-89.6	80.0-89.7	-	Kim, et al., 2003
Cozart RapiScan	E	GCMS	-	-	-	BE 20, 30	COC, BE, EME 2.5, 8, 15, 30	-	66.5-95.5	82.7-98.1	74.1-92.0	-	Kolbrich, et al., 2003
Cozart Microplate ELISA	T	GCMS	-	-	-	BE 20	COC, BE, CE 30	-	99.0	100	99.5	-	Cooper, et al., 2004
EMIT II Plus	D	LCMSMS	-	-	-	-	-	-	-	-	-	-	Smink, et al., 2006

^aAMP, amphetamine; COC, cocaine; MAMP, methamphetamine; nDZP, nordiazepam; E, experimental; COD, codeine; nCOD, norcodeine; MOR, morphine; nMOR, normorphine; T, treatment; BUP, buprenorphine; F, fortified; CE, cocaethylene; D, DUUD; LMP, lorazepam; APZ, alprazolam; MZL, midazolam; DZP, diazepam; TZP, temazepam; OZP, oxazepam.

the market, new ELISA assays became available for screening drugs in blood; many were adapted for OF testing. Adaptation of blood rather than urine assays is preferable in many cases because of the presence of greater proportions of parent drug in blood and OF. Manufacturers that simply tried to adapt urine assays that primarily target metabolites had difficulty in meeting the required detection requirements, especially for cannabinoids. However, pH differences in blood and OF yield distinct biomarker disposition. For example, benzoylecgonine (BE) concentrations are higher than cocaine in blood, whereas the reverse is true in OF.

Another important consideration in the use of OF is expected drug concentrations. In general, drug concentrations in OF are much lower than in urine, although oral mucosal contamination for 30–45 min after smoking a drug can produce microgram per liter concentrations. Oral or sublingual administration also may contaminate the oral mucosa, although we observed little increase in OF concentrations after sample donors had ingested encapsulated or coated pills. Thus, challenges include achieving low limits of detection to extend the window of drug detection and expanding the linear range or validating dilution procedures to account for high concentrations found after smoked or oral doses.

In addition, it is essential that calibrators and controls be prepared in the same matrix as authentic samples. Matrix effects are important considerations, especially with LC-MS, and require documentation that analyte recovery and assay imprecision are equivalent for calibrator, QC, and authentic samples. Many manufacturers prepare calibrators and QC in synthetic OF; laboratories should validate performance against calibrators prepared in human OF.

Most OF assays are non-homogeneous, presenting challenges for large-scale automation. To meet the high workplace testing demands in the US, rapid throughput and automation have enabled the achievement of low costs per test for urine analysis. Fully automated procedures, including pipetting, incubation, washing, and absorbance measurement, are available for 96-well ELISA plates. Evaluations of drug immunoassays are included in Table 2.

POCT

The promise of worldwide OF testing spurred commercial research and development of POCT devices, and commercial devices were rushed to market before much of the basic science of drug excretion into OF was known. A POCT device includes OF collection and a built-in system for screening multiple drug classes. A large number of POCT devices have been evaluated (Table 3), although many are no longer available or were substantially modified (Crouch, 2005; Verstraete & Raes). The major problems with early generation OF POCT included inadequate limits of detection, specificity, and efficiency for identifying cannabinoids, amphetamines, and benzodiazepines; poor performance in bad weather; difficult-to-read results; complicated testing procedures; insufficient sample volume and prevalent device failures (Verstraete & Raes). Many deficiencies were revealed, leading to additional research and modifications, which led to improvement of available products. The ROSITA-2 project evaluated the performance of multiple POCT devices and set acceptance criteria for diagnostic sensitivity, diagnostic specificity, positive and negative predictive values of $\geq 90\%$, and efficiency of $\geq 95\%$ (Verstraete & Raes).

For all evaluations reported since 2004, Table 3 contains POCT devices, drug classes evaluated, confirmation methods employed, diagnostic sensitivities, diagnostic specificities, efficiencies, recoveries, and device failures, when reported. In addition, the types of samples tested

Table 3: Evaluation of collection and POCT devices for drugs in OF including the types of samples tested, drug classes evaluated, diagnostic sensitivity (SN), diagnostic specificity (SP), efficiency (EFF), recovery of drug from the device, % device failures and references.

POCT Devices	Type	THC	OP ^a	AMP	COC	BENZO	Confirmation	SN (%)	SP (%)	EFF (%)	Recovery (%)	Failures (%)	Reference
American Bio Medica OralStat	F	x	x	x	x	x	HPLC GCMS	0-100	100	71.4-100	-	-	Walsh, et al., 2007
Biomar Toxiquick	D	x	x	x	x		GCMS** HPLC-DAD	42.1-100	63.9-88.7	64.3-86.1	-	-	Biermann, et al., 2004
Branan Oratect	F	x	x	x	x		GCMS or LCMSMS	0-100	33.3-100	71.4-100	-	-	Crouch, et al., 2005
Cozart DDSV	T	x					GCMS	44.4	100	60	-	0	Kintz, et al., 2009
Cozart RapiScan	T		x		x		GCMS	Opiates 96.7 COC 98.2	100	Opiates 98.0 COC 99.0	-	-	Cooper, et al., 2005
Cozart RapiScan	E		x				ELISA & GCMS	ELISA 98.6 GCMS 91.3	ELISA / GCMS: 98.1/98.9	ELISA / GCMS: 98.2/97.5	-	-	Kacinko, et al., 2004
Cozart RapiScan	F	x	x	x	x	x	GCMS	5.0-100	40.0-100	45.7-100	-	-	Walsh, et al., 2007
Cozart RapiScan	-			x			GCMS	98.3	96.9	97.3	-	-	Wilson, et al., 2007
Dräger Drug Test	D	x	x	x	x		LCMS	22.2-90.1	89.8-100	77.7-97.3	-	10.2	Concheiro, et al., 2007
Dräger Drug Test	D	x					LCMSMS	49.5	100	55.0	-	-	Laloup, et al., 2006
Envitec SmartClip	F		x	x	x		HPLC or GCMS	65-100	33.3-100	65.7-97.1	-	-	Walsh, et al., 2007
LifePoint Impact	F	x	x	x	x		HPLC or GCMS	70.0-100	33.3-100	71.4-100	-	-	Walsh, et al., 2007
OraSure Uplink	F	x	x	x	x		MS	10.0-100	33.3-100	74.3-100	-	-	Crouch, et al., 2005
Securetec DrugWipe	D	x	x	x	x		ELISA/ MS ^b	7.7-42.9	97.4-99.6	86.1-98.1	-	0.7	Crouch, et al., 2008
Securetec DrugWipe	D	x	x	x	x	x	GCMS	50.0-100	84.2-99.3	79.2-98.6	-	5.3	Engblom, et al., 2007
Securetec DrugWipe	F	x	x	x	x		GCMS	50.0-100	100	71.4-100	-	-	Walsh, et al., 2007
Sun OraLine IV s.a.t.	T	x					GCMS	69.2	91.7	80.0	-	10.3	Grimele, et al., 2006
Sun OraLine IV s.a.t.	F	x	x	x	x		HPLC or GCMS	100	0-100	54.3-100	-	-	Walsh, et al., 2007
Ulti-Med SalivaScreen	F	x	x	x	x		GCMS	65.0-100	33.3-100	71.4-94.3	-	-	Walsh, et al., 2007
Varian OraLab	D	x	x	x	x		LCMS	50.0-100	97.9-100	71.4-100	-	24.5	Concheiro, et al., 2007
Ansys OralLab	F	x	x	x	x		GCMS	40.0-100	33.3-100	71.4-100	-	-	Walsh, et al., 2007

Table 3: Evaluation of collection and POCT devices for drugs in OF including the types of samples tested, drug classes evaluated, diagnostic sensitivity (SN), diagnostic specificity (SP), efficiency (EFF), recovery of drug from the device, % device failures and references. (Continued).

Collection devices	Type	THC	OP ^a	AMP	COC	BENZO	Confirmation	SN (%)	SP (%)	EFF (%)	Recovery (%)	Failures (%)	Reference
Acro Biotech Salicule	F	x	x	x	x	x	GCMS	-	-	-	>90 except THC 45.9	-	Langel, et al., 2008
Cozart	F	x	x	x	x	x	GCMS	-	-	-	66.0-91.6	-	Langel, et al., 2008
Cozart DDS	T	x	x	x	x	x	GCMS or LCMS	-	-	-	91.1-100	-	Speedy, et al., 2007
Dräger DCD 5000	F	x	x	x	x	x	LCMSMS	-	-	-	>90	-	Kempf, et al., 2009
Greiner Bio-One Saliva Collection System	F	x	x	x	x	x	GCMS	-	-	-	73.6-98.5; >80, except THC	-	Langel, et al., 2008
Immunalysis Quantisal TM	F	x	x	x	x	x	GCMS	-	-	-	>80, except THC 55.8	-	Langel, et al., 2008
Immunalysis Quantisal TM	F	x	x	x	x	x	GCMS	-	-	-	86.7-96.6	-	Moore, et al., 2007a
Immunalysis Quantisal TM	Fs	x	x	x	x	x	LCMSMS	-	-	-	81.3-109.4	-	Quintela, et al., 2006
Malvern Medical OraCol	F	x	x	x	x	x	GCMS	-	-	-	<12.5-81.5	-	Langel, et al., 2008
Orasure Intercept [®]	F	x	x	x	x	x	GCMS	-	-	-	53.0-83.4	-	Kauert, et al., 2006
Orasure Intercept [®]	F	x	x	x	x	x	GCMS	-	-	-	>80, except THC 37.6	-	Langel, et al., 2008
Sarstedt Salivette [®]	T	x	x	x	x	x	LCMSMS	-	-	-	OP 73.9-95.3 except PV 30.8-35.2, NC 56.4-63.4 COC 74.3-114.9 except pOHBE 47.4-49.6	-	Dams, et al., 2007
Sarstedt Salivette [®]	F	x	x	x	x	x	GCMS	-	-	-	<12.5-51.8	-	Langel, et al., 2008
StatSure Saliva.Sampler TM	F	x	x	x	x	x	GCMS	-	-	-	81.3-91.1	-	Langel, et al., 2008
Varian OraTube	F	x	x	x	x	x	GCMS	-	-	-	39.8-86.7	-	Langel, et al., 2008

^a OP, opioids; AMP, amphetamines; COC, cocaine; BENZO, benzodiazepines; F, fortified; T, treatment; D, DUID; DAD, diode array detection; E, experimental; s, synthetic OF; PV, papaverine; NC, noscapine; pOHBE, p-hydroxybenzoyllecgonine.

^b Confirmation in blood

are described, including fortified blank OF or authentic samples from drug treatment, DUID, or after experimental controlled drug administration. Critical factors must be taken into account when referencing these data: (a) Data are relevant for devices available at the time of testing. (b) Many early devices simply failed to operate correctly. (c) Only a few devices included adequate volume indicators. Collected OF volume varied substantially within and between devices, limiting ability to quantify OF concentrations. (d) Devices may perform differently with fortified authentic or synthetic OF than with authentic OF samples collected after drug ingestion, because the spectrum of analytes present may differ. Knowledge of antibody cross-reactivity data is key for understanding device performance. (e) Drug recovery from the device is a major issue, especially for lipophilic drugs like cannabinoids. (f) Many evaluations judged device performance only against stated manufacturer cutoff concentrations that may be unreasonably high compared to proposed SAMHSA, DRUID, or DUID research recommendations. Other evaluations assessed performance against low laboratory limits of quantification (LOQs), providing a better idea of false-negative rates. (g) Some devices performed poorly at night or in poor weather conditions, or had endpoints that were difficult to discriminate. (h) Some investigators compared OF POCT device performance to serum drug concentrations collected up to several hours after OF. (i) In some investigations, for later confirmation additional OF samples were collected by expectoration or with the Intercept® device.

Most POCT devices obtained good results for opioids with cutoffs at or below SAMHSA cutoffs, although false-negative results may have been missed if all negative screening tests were not subjected to confirmation (Table 3). Reported efficiencies do not always agree, owing to differences in populations, analytes, and concentrations tested and cutoffs used. Opioids have a wide range of potency, leading to differences in sensitivities for various analytes.

Many POCT devices (Table 3) performed well for amphetamines, with diagnostic sensitivities, diagnostic specificities, and efficiencies in the range of 70%–100%. Notable exceptions occurred, however: diagnostic sensitivity was too low for the Varian OraLab® and Dräger Drug Test® (Concheiro, de Castro, Quintela, Cruz, & Lopez-Rivadulla, 2007) and the Securetec Drug-Wipe® (Crouch, Walsh, Cangianelli, & Quintela, 2008). The Biomar Toxiquick® (Biermann, Schwarze, Zedler, & Betz, 2004) and 4 devices evaluated by Walsh et al. (2007) performed poorly, with low diagnostic sensitivities, diagnostic specificities, and efficiencies. Development of a POCT for amphetamines is challenging owing to the large number of over-the-counter and prescription drugs sharing similar sympathomimetic amine structures. The challenge is to detect drugs of abuse without detecting multiple therapeutic drugs.

POCT devices (Table 3) performed variably for cocaine. The Cozart RapiScan had diagnostic sensitivities ranging from 5% (Walsh, et al., 2007) to 98.2% (Cooper, Wilson, Reid, Main, & Hand, 2005) and efficiencies of 45.7% (Walsh, et al., 2007) to 99% (Cooper, Wilson, Reid, Main, et al., 2005). Diagnostic specificity was generally good, with a range from 88.7% for cocaine/BE with the Biomar Toxiquick® (cutoff 50 µg/L) (Biermann, et al., 2004) to 100% for 6 different devices (Walsh, et al., 2007). Diagnostic specificities were poor for the Sun OraLine® IV substance of abuse test and Ansys OralLab® (Walsh, et al., 2007).

Other important OF testing issues have been poorly investigated. For example, adulteration of OF samples and potential interferences with POCT devices have not been systematically investigated. It is clear that considerable effort is expended to produce false-negative urine test

Table 4: Chromatographic methods for measurement of drugs in OF.

Method	Extraction	Cannabinoids	Opiates	LOQ ($\mu\text{g/L}$)			Reference
				Amphetamines	Cocaine	Benzodiazepines	
GCMS	SPE	THC 2	6AM, FEN 2; BUP, NBUP 4; Pholcodine 5; Methadone, MOR, COD, EtMOR 20	AMP, MAMP, MDMA, MDA, MDEA 25	COC, BE 8	DZP, nDZP, LZP, MZL, OZP, PZP, TZP 2; APZ, CZP, NZP 5	Gunnar, et al., 2005
LCMSMS (ESI)	SPE		MOR, 6AM, COD 2	AMP, MAMP, MDMA, MDA 2	COC, BE 2		Wood, et al., 2005
LCMSMS (ESI)	SPE		Methadone 2.2; EDDP 0.8; 6AM 1.0; NBUP 1.6; MOR 1.7; COD, BUP 1.8; nMOR, MOR, nCOD, COD, 6AM, Heroin, AC 1	AMP 1.2; MAMP 1.3; MDMA 2.5; MDA 3.3; MDEA 4	EME 0.9; COC 3.1; CE 3.4; BE 11.4	DZP 0.9; nDZP 1.2; CLP, TZP 1.6; OZP 2.5	Wylie, Torrance, Anderson, et al., 2005
LCMSMS (APCI)	PP				COC, EME, BE, CE 1		Dams, et al., 2007
LCMSMS (ESI)	LLE	THC < 0.16	6AM 0.82; BUP 1.2; Methadone 4.0; COD 6.5; MOR 7.1	MAMP 3.0; MDA <3.6; MDMA <3.9; MDEA <4.1 A MP< 6.8	COC <0.78; BE 7.2	APZ 0.093; OZP <1.4; FTP 0.15; NZP 0.23; CZP 0.26; LZP 0.32; DZP 0.41; FZP 0.75; BZP 7.9	Øiestad, et al., 2007
GCMS	SPE	CBD 0.9; THC 1.9; THCCOOH 4.8; CBN 5.6; 11-OH-THC 12.7	6AM 2.9; MOR 6.5; COD 6.6	MDMA 8.9; MDA 14.4; MDEA 15.0 A 20.6; MAMP 20.9	COC 4.1; BE 8; CE 7.2; EME 12.7	FLP 10.9; DZP 16.3	Pujadas, et al., 2007
LCMSMS (ESI)	SPE	THC 1	6AM 1; MOR, COD 5	AMP, MAMP, MDMA, MDA, MDEA 5	COC, BE 5	APZ, CZP, DZP, nDZP, FZP, LZP, OZP 1	Concheiro, et al., 2008
LC-DAD	LLE/MAE		MOR, 6AM, Methadone, EDDP 50		COC, BE, CE 50		Fernandez, et al., 2008
LCMSMS (ESI)	LLE/SPE		COD, MOR, HCOD, HMOR, OCOD, OMOR 10	AMP, MAMP, MDMA, MDA 10	COC, BE 2		Kala, et al., 2008
LCMSMS (ESI)	PP	THCCOOH 3.5; THC 3.7	MOR 1.2; 6AM 1.9	MAMP 0.4; MDE 0.5; AMP, MDMA, MDA 1.5	COC, BE 0.5		Sergi, et al., 2009
LCMS (ESI)	LLE	THC 2					Concheiro, et al., 2004
GCMSMS	SPE	THC 0.75					Niedbala, et al., 2004
LCMS	SPE	THC 2					Teixeira, et al., 2004
LCMSMS (ESI)	LLE	THC 0.1 (500 μL), 0.5 (100 μL)					Laloup, Ramirez Fernandez, et al., 2005
LCMS (ESI)	SPE	THC 2					Teixeira, et al., 2005
GCMSMS (NCI)	SPE	THCCOOH 0.01					Day, et al., 2006
GCMS	SPE	THC 2.4					Kauert, et al., 2006

Table 4: Chromatographic methods for measurement of drugs in OF. (Continued).

Method	Extraction	LOQ ($\mu\text{g/L}$)				Reference
		Cannabinoids	Opiates	Amphetamines	Cocaine	Benzodiazepines
GC/GCMS (ECI)	SPE	THCCOOH 0.02				Moore, Coulter, et al., 2006
GCMS	SPE	11-OH-THC 0.3; THC 0.5; THCCOOH 6.2				Kauert, et al., 2007
GCMS	SPE	Conjugated THCCOOH 0.5				Moore, Rana, et al., 2007
GCMS	SPE	THC, CBN 0.5; CBD, 2-carboxyTHC 1				Moore, et al., 2007b
LC/MSMS (ESI) ^b	LLE	THC 0.1; THCCOOH 0.5				Quintela, et al., 2007
LCMS (ESI)	SPE	THC 5				Teixeira, et al., 2007
GCMS (PCI)	LLE		6AM 2; COD 2.3; MOR 6.7			Campora, et al., 2006
LC/MSMS (ESI)	none		6AM, COD, AC 2; MOR 6; Heroin 10			Phillips and Allen, 2006
GCMS	SPE		Propoxyphene 5			Rana, et al., 2006
GCMS	SPE		MEP, TRA, OCOD 10			Moore, et al., 2007a
CEP	LLE		EDDP 7.6; Methadone 8.1			Martins, et al., 2008
GCMS	LLE			AMP, MAMP, MDMA, MDA, MDEA 20		Kankaanpää, et al., 2004
LC-FD	LLE			MDMA, MDA, MDEA 10		Concheiro, et al., 2005
GCMS	SPE			MDMA, MDA 5; HMMA, HMA 25		Scheidweiler and Huestis, 2005
GCMS	SPE			AMP, MAMP, MDMA, MDA, MDEA 5; HMA, HMMA 25		Scheidweiler and Huestis, 2006
GCMS	SPE			MAMP 2.5		Huestis and Cone, 2007
GCMS	SPE			AMP, MAMP, MDMA, MDA, MDEA 25		Moore, Coulter and Crompton, 2007
GCMS (NCI)	LLE			MDA 5; AMP, MAMP, MDMA, MDEA 25		Peters, et al., 2007

Table 4: Chromatographic methods for measurement of drugs in OF. (Continued).

Method	Extraction	LOQ ($\mu\text{g/L}$)				Reference
		Cannabinoids	Opiates	Amphetamines	Cocaine	
LCMSMS (ESI)	SPE				COC, BE, CE 10	Clauwaert, et al., 2004
GCMS	SPE				BE 0.75	Robarge, et al., 2005
GCMSMS (PCI)	SPE					Cognard, et al., 2006
LCMS (ESI)	LLE				COC, CE 2; EME 5	Quintela, et al., 2004
LCMSMS (ESI)	LLE					MZL 0.2
LCMS (ESI)	LLE					APZ, BZP, DZP, nDZP, LZIP, LMP, MZL, OZP, TZP, TRZ, al., 2005
LCMSMS (APCI)*	LLE					TZL 0.1; CBZ 0.2
LCMSMS (ESI)	LLE					DZP, FTP, MZL, TRZ, TZL Quintela, et al., 2005
LCMSMS (ESI)	LLE					0.2; APZ, BZP, LZIP 0.5
LCMSMS (ESI)	LLE					NZP 0.1; APZ, LZIP 1.2;
LCMSMS (ESI)	LLE					LMP 1.3; MZL 2.3; DZP 6.9; Smink, et al., 2006
LCMSMS (ESI)	LLE					nDZP 7.6; TZP 8.1; OZP 13.1
LCMSMS (ESI)	LLE					DZP, nDZP, OZP, 0.05; TZP, Laloup, et al., 2007
LCMSMS (ESI)	LLE					TRZ 0.2
LCMSMS (ESI)	LLE					MZL 0.05
LCMSMS (ESI)	SPE					APZ, nDZP, FTP, FLP, Moore, Coulter, 2007
LCMSMS (APCI)	SPE					MZL, TZP, TZL 0.5; BZP, Crompton and Zumwalt, 2007
LCMSMS (ESI)	PP					CZP, DZP, NZP 1; LZIP, OZP 5
LCMSMS (APCI)	SPE					APZ, CZP, DZP, nDZP, FTP, Ngwa, et al., 2007
LCMSMS (ESI)	PP					LZIP, OZP, TZP 0.1
LCMSMS (ESI)	PP					OZP glucuronide 0.25; Smink, et al., 2008
LCMSMS (ESI)	PP					OZP 0.5
LCMSMS (ESI)	PP					LZIP 130; DZP 290; BZP 370; Uddin, et al., 2008
LCMSMS (ESI)	PP					CZP, FTP 390; APZ 450

^a SPE, solid phase extraction; FEN, fentanyl; BUP, buprenorphine; nBUP, norbuprenorphine; MOR, morphine; COD, codeine; EtMOR, ethylmorphine; AMP, amphetamine; MAMP, methamphetamine; MDEA, 3,4-methylenedioxy-ethylamphetamine; COC, cocaine; DZP, diazepam; nDZP, nordiazepam; LZIP, lorazepam; MZL, midazolam; OZP, oxazepam; PZP, phenazepam; TZP, temazepam; APZ, alprazolam; CZP, clonazepam; EDDP, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine; CE, cocaethylene; CLP, citalopram; PP, protein precipitation; nMOR, normorphine; nCOD, norcodeine; AC, acetylcodeine; LLE, liquid liquid extraction; FLP, flurazepam; FZP, fenazepam; BZP, bromazepam; DAD, diode array detection; MAE, microwave-assisted extraction; HCOD, hydrocodone; HMOR, hydromorphone; OCOD, oxycodone; OMOR, oxymorphone; MDE, methylenedioxy-N-ethylamphetamine; NCI, negative chemical ionization; ECCL, electron capture chemical ionization; PCI, positive chemical ionization; MEP, meperidine; TRA, tramadol; CEP, capillary electrophoresis; HMMA, 3-hydroxy-4-methoxymethamphetamine; HMA, 3-hydroxy-4-methoxyamphetamine; FD, fluorescence detection; LMP, lormetazepam; TRZ, triazepam; TZL, triazolam; CBZ, clobazam; FTP, flunitrazepam; NZP, nitrazepam.

^b Synthetic OF

results, with numerous products available. Certainly similar efforts will be directed at falsifying OF tests. The risk of test falsification requires initial characterization and continuous surveillance of potential interfering substances and adulterants. Specified foods, drinks, mouthwash, and cigarettes did not interfere with the Cozart RapiScan and the Cozart Microplate ELISA assays for opiates (Cooper, Wilson, et al., 2005b), cocaine (Cooper, et al., 2004), and amphetamines (Cooper, Wilson, Reid, Hand, & Spiehler, 2006), nor with the Branam Oratect[®] collection device (Wong, Tran, & Tung, 2005). Alcohol and hemoglobin did not affect the Cozart assays and the RapiScan collection device (Cooper, et al., 2004; Cooper, Wilson, et al., 2005b; Cooper, et al., 2006). Toothpaste, lipstick, gum, and 2 commercially available adulterants also had no effect on Branam Oratect results (Wong, et al., 2005). These few reports provide to date the only available data on adulterants and interferences. Additional research is critically needed to characterize potential problems with OF collection devices and immunological and chromatographic assays.

Chromatographic Confirmation and Quantification of Drugs in OF

From 2004 to 2008, reports of 71 OF drug assays were published, documenting increasing interest in OF as an alternative matrix for identifying and quantifying drug exposure. Chromatographic methods, analytes, extraction procedures, and LOQs for quantifying OF drug biomarkers are described in Table 4. Most new methods utilize liquid chromatography-tandem mass spectrometry (LC-MS/MS), which permits simultaneous analysis of multiple, nonvolatile, labile, polar, and/or high molecular weight compounds in a limited OF volume (Dams, Murphy, et al., 2003; Maurer, 2005; Samyn, Laloup, & De Boeck, 2007; Wood, et al., 2006). Comprehensive methods for multiple drugs also limit optimization for individual drugs, sometimes yielding insufficient detection capabilities (Gunnar, Ariniemi, & Lillsunde, 2005; Kempf, Wuske, Schubert, & Weinmann, 2009). The major disadvantage of LC-MS is matrix enhancement or suppression, which is best managed by inclusion of deuterated internal standards for all analytes; however, this technique may not fully compensate for matrix effects (Dams, Huestis, Lambert, & Murphy, 2003). OF is less complex than blood, with fewer proteins, characteristics suggesting the possibility of successful measurement with limited sample preparation, such as dilution, protein precipitation, or centrifugation and direct injection. Unfortunately, more extensive sample preparation is frequently required to limit matrix suppression. Our laboratory found atmospheric pressure chemical ionization (APCI) to be less susceptible to matrix effects than electrospray ionization (ESI) (Dams, Huestis, et al., 2003). Owing to more efficient ionization, however, ESI is preferred for some analytes, i.e., glucuronides. Many laboratories use ESI routinely and do not consider the advantages of APCI in reducing matrix effects. In addition to LC-MS, many GC-MS assays for drugs in OF also are available, but generally require biomarker derivatization, which increases cost and analysis time.

OF Testing of Specific Drug Classes

With various classes of drugs that are measured in OF, including cannabinoids, amphetamines, opiates, cocaine, and benzodiazepines, specific issues arise with regard to collection, screening, POCT, and result confirmation and interpretation. Controlled drug administration provides a scientific database to guide interpretation of OF tests. The disposition of illicit drugs in OF is

affected by metabolic, physiological, and chemical processes (Cone & Huestis, 2007), including biomarkers of interest, minimal detectable doses, expected concentrations, detection windows, and collection device requirements. The major research findings are detailed by drug class. Specific studies are referenced in Table 5.

Table 5: Controlled drug administration studies with oral fluid collection.

THC (cannabinoids)	Opioids	Amphetamines	Cocaine	BENZO ^a	Doses	Study participants, N	Reference
x					1 cannabis joint, potency unknown	3	Jehanli, et al., 2001
x					20-25mg smoked THC	15	Niedbala, et al., 2001
					20-25mg THC (brownies)	3	
x					0, 1.75 & 3.55% smoked THC	6	Huestis and Cone, 2004
x					Passive cannabis smoke exposure	4	Niedbala, et al., 2004
x					Passive cannabis smoke exposure	4	Niedbala, et al., 2005
x					0, 250 & 500µg/kg smoked THC	10 10	Ramaekers, Moeller, et al., 2006; Kauert, et al., 2007
x					1 cannabis joint, potency unknown	1	Moore, Coulter, et al., 2006
	x				30mg oral COD	3	Sharp, et al., 1983
	x				60 & 120mg intramuscular COD	1	Cone, 1990
	x				Oral COD	8	Chen, et al., 1991
	x				30mg oral liquid COD phosphate	17	O'Neal, et al., 1999
	x				30mg oral liquid COD phosphate	37	O'Neal, et al., 2000
	x				16mg oral COD	5	Jehanli, et al., 2001
	x				Single & multiple 60 & 120mg/ 70kg COD	19	Kim, et al., 2002; Kacinko, et al., 2004
	x				Poppy seed bagels & poppy seeds	7	Rohrig and Moore, 2003
		x			10 or 20mg MAMP	8	Schepers, et al., 2003
		x			0, 75 & 100mg oral MDMA	18	Laloup, Tilman, et al., 2005; Wood, et al., 2005
		x			Single & multiple 10 & 20mg MAMP	8	Huestis and Cone, 2007
		x			75mg MDMA	12	Peters, et al., 2007
			x		25mg smoked, 32mg intravenous & 42mg insufflated cocaine	6	Jufer, et al., 2006
					Multiple oral cocaine up to 2000mg/day	6	
			x		1 mg oral flunitrazepam	4	Samyn, et al., 2002
			x		50mg oral TZP or 10mg oral DZP or repeated 25mg oral TZP	7	Laloup, et al., 2007
			x		15 & 30mg OZP	8	Smink, et al., 2008

^a BENZO, benzodiazepines; MAMP, methamphetamine; MDMA, 3,4-methylenedioxymethamphetamine; DZP, diazepam; TZP, tetrazepam.

CANNABINOIDS

THC is the primary analyte for cannabinoids in OF, whereas 11-nor-9-carboxy-THC (THCCOOH) predominates in urine. Most available antibodies from urine immunoassays are directed toward THCCOOH and have poor cross-reactivity with THC, limiting diagnostic sensitivity.

Recovery of the sample from the collection device is a major issue, especially for lipophilic drugs like cannabinoids, although 94.5% THC recovery from the Cozart DDS device (Speedy, Baldwin, Jowett, Gallina, & Jehanli, 2007) and up to 91.4% recovery from the Quantisal device at a

concentration of 4 µg/L (Quintela, Crouch, & Andrenyak, 2006) were recently reported. It is suggested that manufacturers evaluate each lot of OF collection devices and publish the data in the package insert, which would alleviate each laboratory from performing the same function. To our knowledge, only THC recovery from devices has been examined, although THCCOOH recovery may be equally important.

Initially, THC was thought to be present in OF only from oral mucosal contamination from cannabis smoke. No radiolabel was detected in human OF following radiolabeled intravenous THC administration (Hawks & Chiang, 1986). Using GC-MS with 0.5 µg/L LOQs, we detected no THC metabolites, 11-hydroxy-THC (11-OH-THC) or THCCOOH, in OF after 6 participants smoked a 1.75% or 3.55% THC cigarette (Huestis, Dickerson, & Cone, 1992). However, later we observed that after approximately 30–60 min, gross oral mucosal contamination following cannabis smoking was cleared and OF and plasma concentrations were temporally correlated (Huestis & Cone, 2004). THC concentrations as high as 5800 µg/L were observed 12 min after cannabis smoking, falling to 81 µg/L by 20 min, documenting rapid drug clearance. Mean (SD) THC OF/ plasma ratio in simultaneously collected samples was 1.18 (0.62) (range 0.5–2.2) 0.3 to 4 h after cannabis smoking. Similarity in OF and plasma THC concentrations following contamination dissipation likely indicates a physiological link (transmucosal THC absorption into blood) between these matrices.

Other investigators recently reported a strong linear correlation ($r=0.84$, $p<0.001$) in OF and serum THC concentrations up to 6 h after sample donors had smoked 250 or 500 µg/kg THC (Ramaekers, Moeller, et al., 2006). Mean (SD) THC OF concentrations were 900 (589) and 1041 (652) µg/L, respectively, in samples collected 15 min after donors had smoked, decreasing to 18 (12) µg/L during the next 6 h (Kauert, Ramaekers, Schneider, Moeller, & Toennes, 2007). OF/serum ratios were markedly higher than previously reported at 46 (27) (250 µg/kg) and 36 (20) (500 µg/kg), with a longer period of THC OF detection in OF compared to serum samples. It is not yet clear why the OF/plasma (Huestis & Cone, 2004) and OF/serum (Kauert, et al., 2007) ratios are so discrepant between these studies; additional research is underway. We noted that although plasma and OF THC concentrations were temporally correlated, there was too much variability to predict plasma concentrations from single OF results.

Another major issue is the possibility of false-positive OF test results in sample donors exposed to environmental cannabis smoke. With a 0.5 µg/L gas chromatography–tandem mass spectrometry (GC-MS/MS) cutoff, passive cannabis smoke exposure produced positive THC OF test results in 4 nonsmokers after they spent 15–30 min in a sealed room with 5 cannabis smokers (Niedbala, et al., 2004). Contamination cleared rapidly, with negative results for all later samples. In another passive exposure study in which sample donors were exposed to cannabis smoke while in an unventilated van, OF samples were collected with the Intercept device inside the van 1 h after smoking cessation (study 1) and outside the van after up to 72 h (study 2), with 4 study participants smoking 39.5 mg THC mixed with tobacco (study 1) or 83.2 mg THC (study 2) cigarettes (Niedbala, et al., 2005). Peak OF THC concentrations in passively exposed sample donors were up to 7.5 µg/L (study 1) and 1.2 µg/L (study 2); THC was not quantifiable by 30 – 45 min after exposure. Collection devices environmentally exposed in the van contained 3–14 µg/L in study 1. When OF samples were collected outside the van after the 1 h exposure, all OF samples

were negative, suggesting that earlier studies may have used environmentally contaminated collection devices. OF results were positive for active smokers for 0 – 8 h.

More recently, THCCOOH (Moore, Coulter, Rana, Vincent, & Soares, 2006; Moore, Ross, et al., 2006) and its glucuronide metabolite (Moore, Rana, Coulter, et al., 2007) were quantified in OF in nanogram per liter concentrations. This finding was important, because THCCOOH is not in cannabis smoke. Demonstrating THCCOOH in OF could clearly differentiate passive exposure from active smoking. Until recently, no analytical procedures existed for simultaneously extracting and quantifying THC and THCCOOH from the same OF sample. We recently developed and validated an assay for simultaneous identification and quantification of THC, cannabidiol (CBD), cannabinol (CBN), 11-OH-THC, and THCCOOH in OF collected with the Quantisal device (Milman, Lowe, & Huestis, 2008). Simultaneous analysis was problematic owing to varying physiochemical characteristics and concentration ranges. THC, 11-OH-THC, CBD, and CBN were eluted with hexane/acetone/ethyl acetate (60:30:20 vol/vol/vol), derivatized with N, O-bis-(trimethylsilyl) tri-fluoroacetamide and quantified by 2-dimensional GC electron-impact MS with cryotrapping. Acidic THCCOOH was separately eluted with hexane/ethyl acetate/acetic acid (75:25:2.5 vol/vol/vol), derivatized with trifluoroacetic anhydride and hexafluoroisopropanol, and quantified by the more sensitive 2-dimensional GC-MS– electron capture negative chemical ionization method. Linearity was 0.5–50 µg/L for THC, 11-OH-THC, and CBD and 1–50 µg/L for CBN. The linear dynamic range for THCCOOH was 7.5–500 ng/L. This new analytical method is being applied to OF samples collected in our cannabinoid controlled drug administration studies, and we hope it will provide new insights into cannabinoid disposition in OF.

Acceptance of OF testing for cannabinoids has been difficult for multiple reasons, including short detection times. The mean time for the last positive result for THC in OF, collected with the Intercept OF collection device and analyzed by GC-MS/MS (LOQ 0.5 µg/L), was 34 (11) h (range 1–72 h) after the sample donor had smoked a 20–25 mg THC cigarette (Niedbala, et al., 2001). However, study participants left the laboratory after 8 h and were instructed not to smoke cannabis, but they were not monitored. Mean OF THC concentrations 1 h after smoking were 27.8 (6.2) and 22.6 (6.2) µg/L from the right and left sides of the mouth, with good concordance throughout 72 h after smoking (Niedbala, et al., 2001). OF samples were positive up to 72 h after eating 20–25 mg THC-laced brownies; concentrations were <6.9 µg/L THC (Niedbala, et al., 2001). Currently, few data are available for detection windows after acute cannabis smoking, and no data are available for detection after ingestion of THC-containing pharmacotherapies or after chronic, heavy cannabis smoking. The lack of such data limits our ability to interpret OF test results.

Cannabinoid cutoffs were proposed before THCCOOH was detected in OF. Certainly quantification of THCCOOH requires a highly sensitive assay, but such an assay may prove to be the answer for cannabinoid OF testing. The window of detection and prevalence of THCCOOH in OF is still unknown. Perhaps an immunoassay screen for THC and confirmation for THCCOOH would provide definitive identification of cannabis use, although this must be proven. Because THC and cannabinoids are the most prevalent drugs in clinical, workplace, and DUID testing (Walsh, 2008), it is difficult to move to an alternative matrix if cannabinoids are not adequately and specifically identified. These limitations have slowed acceptance of OF for multiple drug testing applications.

Opioids have many therapeutic applications, but these drugs also are abused. Heroin has a short half-life and is rapidly metabolized to 6AM and then morphine. Although 6AM can be detected in urine for a short time after exposure, one of the main advantages of OF over urine testing for opiates is that 6AM and sometimes heroin are frequently detected in OF. SAMHSA increased the mandated urine testing cutoff from 300 to 2000 $\mu\text{g/L}$ because Medical Review Officers acted on so few opiate-positive tests; most were attributed to codeine use or poppy seed ingestion rather than heroin abuse.

Recoveries of opioids from collection devices were approximately 80% for most devices, except the Sarstedt Salivette® (35.2% for morphine and 39.0% for codeine) (Langel, et al., 2008). Existing immunoassay antibodies adequately target morphine, codeine, and 6AM in OF. A problem for opioid OF and urine testing is the wide range of analytes that are abused, including heroin, codeine, oxycodone, buprenorphine, hydromorphone, and hydrocodone. Additional specific assays are available that target oxycodone (Moore, Rana, & Coulter, 2007a) and buprenorphine (De Giovanni, Fucci, Scarlata, & Donzelli, 2005). Multiple screening assays must be conducted to adequately cover this large class of abused drugs.

There are few controlled opiate administration studies to guide interpretation of OF tests. Our laboratory administered placebo, low (60 mg/70 kg) and high (120 mg/70 kg) oral codeine sulfate to 19 participants and then collected OF for up to 72 h by citric acid-stimulated expectoration and by stimulated and unstimulated Salivette. Samples were analyzed by GC-MS with LOQs of 2.5 $\mu\text{g/L}$ for codeine, norcodeine, morphine, and normorphine (Kacinko, et al., 2004; Kim, et al., 2002). At 0.08–1.0 h after administration, codeine was detected with all collection methods after both doses. Mean peak OF codeine concentrations did not differ significantly between collection methods and were 638.4 (64.4) $\mu\text{g/L}$ at 1.7 (0.23) h (low) and 1599.3 (241.0) $\mu\text{g/L}$ at 1.6 (0.14) h (high). During the 1–12-h period after administration, the OF/plasma ratio was approximately 4. Codeine is a basic drug and is ion trapped in OF, increasing its detection. OF and plasma codeine concentrations were significantly correlated ($r=0.22$, $p<0.0001$), but variability precluded predicting plasma concentrations from OF concentrations. Mean codeine half-lives were 2.5 (0.21) h and 1.8 (0.19) h, similar to plasma half-lives. Mean codeine OF detection time was 21 h (using the LOQ), but only 7 h at proposed SAMHSA cutoffs. Norcodeine/codeine ratios increased over time from 0.5–8 h, ranging from 0.3%–31%. Norcodeine OF/ plasma ratios were lower than those of codeine owing to increased polarity and a lower pK_a , conditions that decrease ion trapping. Morphine and normorphine have not been detected in OF or plasma after codeine administration, whereas late in the time course of urinary excretion of codeine, low morphine concentrations may be present without measurable codeine, making interpretation difficult. This does not occur in OF after codeine ingestion, another advantage of OF compared to urine testing for opiates.

These data document similar opiate detection windows for OF and plasma, making OF a good matrix for DUID, because the OF detection window better reflects drug intoxication than urinary detection windows. OF also is a good alternative matrix for drug treatment, owing to the shorter opiate half-lives in OF than urine. For the same reason, consecutive OF samples are less likely to be positive after a single opiate exposure than consecutive urine samples. A difficult problem in drug treatment programs is differentiating new opiate use from residual opiate

excretion in urine. Our controlled drug administration data support that such differentiation would be much less problematic with OF than with urine testing.

Another problem with opiate urine testing is that positive results may occur after sample donors have ingested poppy seed foodstuffs. A recent study showed that ingestion of poppy seed bagels and poppy seeds (9.8 –20.8 g) also can produce positive OF tests, albeit only for up to 1 h after ingestion, when a morphine OF cutoff of 40 µg/L is applied (Rohrig & Moore, 2003).

Thus, OF opiate testing offers multiple advantages to urine testing, including observed, easy collection, even at the roadside; enhanced identification of heroin abuse; better differentiation of new opiate exposure from residual drug excretion; and reduced possibility of positive tests following ingestion of opiate-containing food. Urine testing for opiates offers the advantage of higher drug concentrations and greater availability of data on controlled administration.

AMPHETAMINES

Amphetamine is the primary abused sympathomimetic amine in Europe, whereas methamphetamine is more prominent in the Americas, Asia, and Australia. Cross-reactivity with methamphetamine, amphetamine, and 3,4-methylenedioxymethamphetamine (MDMA) (ecstasy) is considered an advantage because only one screen adequately covers the three most commonly abused drugs, while cross-reactivity with phenylpropanolamine, ephedrine and other common cold medication ingredients is a disadvantage, due to the need for expensive and time-consuming confirmatory procedures to rule out these drugs. Analytes of interest are the same in urine and OF, thus, cross-reactivity is a major testing issue for both. Also, drug potencies and doses are similar for the analytes of interest, making sensitivity less of a problem.

One of the problems encountered in OF testing after stimulant abuse is dry mouth. During routine OF testing and controlled dosing research, mouth dryness in sample donors may lead to low sample volumes and/or long collection times. Recovery from collection devices was rarely problematic for amphetamines, with percentages generally above 75%. However, the Sarstedt Salivette had low recoveries of 51.8%/26.5% for amphetamine/MDMA, as did the Malvern Medical OraCol (69.1%/52.0%) for the same analytes (Langel, et al., 2008).

Amphetamines are weak bases and are subject to ion trapping in OF, providing the advantage of higher concentrations in OF than plasma. We first detected methamphetamine in expectorated OF with citric acid stimulation within 0.08–2 h after sample donors had ingested 10 or 20 mg sustained-release oral S-(+)-methamphetamine hydrochloride (GC-MS LOQs 2.5 µg/L) (Schepers, et al., 2003). We found that samples collected with the use of citric acid stimulation had amphetamine concentrations 1.5-fold lower than samples collected without stimulation. The decrease in OF pH that occurs with citric acid should increase basic amphetamine concentrations, but stimulation had a greater effect on salivary volume. For samples collected by expectoration, mean peak OF concentrations after single 10- or 20-mg doses were 106.1 (24.7) µg/L (range 25–312 µg/L) during the period of 4–8 h after dosing and 192.2 (120.8) µg/L (range 75–322) 2–12 h after dosing. The linear correlation between OF and plasma concentrations, although significant, was only 0.222. The median OF/plasma methamphetamine ratio was 2.0 and was highly variable within and between participants. Methamphetamine was detected in the OF of all study participants for 24 h; amphetamine was detected in only 62.5% (low) and 100% (high) of samples after administration at 8.6 (6.5) µg/L (range 4 –21 µg/L) and 14.3 (6.1) µg/L (range 3–20 µg/L).

Interestingly, OF was not contaminated by oral administration of this preparation, as noted by a lag time before first drug detection. It was thought that swallowing the methamphetamine pill would lead to contamination of the oral mucosa, but such contamination did not occur. If methamphetamine was smoked, extensive contamination of the oral cavity would be expected. The OF detection rate at 50 µg/L (proposed SAMHSA cutoffs) was only 4% after 10 mg and 28.6% after 20 mg methamphetamine, which are therapeutic doses and quite low compared to abuse doses (Huestis & Cone, 2007). Mean detection times until last positive OF after the last of 4 daily 10- or 20-mg methamphetamine doses (using the LOQ) were 24.6 (9.9) h and 74.1 (17.0) h, respectively, and with a 50-µg/L methamphetamine and 2.5-µg/L amphetamine cutoff were 4.0 (1.8) h and 20.6 (3.2) h. Detection times for OF were much shorter than those for urine.

In contrast to the controlled dosing data available for methamphetamine, MDMA doses spanning the recreational range have been administered. Median MDMA and 3,4-methylenedioxyamphetamine (MDA) OF concentrations collected with the OraSure Intercept® device 1.5 h after sample donors had received 75 or 100 mg oral MDMA were 447.6 µg/L (range 44.1–3993.1 µg/L) and 7.7 µg/L (range 1– 42.4 µg/L), respectively (Laloup, Tilman, et al., 2005; Wood, et al., 2005). By 5.5 h, median MDMA concentrations were 315.7 µg/L (range 42.4 –3078.9 µg/L) and MDA concentrations were 16.5 µg/L (range 1.5–102.6 µg/L). Unfortunately, no later samples were collected, negating the determination of MDMA OF detection windows.

Determination of MDMA detection windows also was not possible in a study in which racemic MDMA (75 mg) was administered to 9 participants and OF was collected by expectoration from 1–5 h after administration (Peters, Samyn, Kraemer, Riedel, & Maurer, 2007). Interestingly, however, MDMA and MDA enantiomers were quantified by GC-MS. Mean peak OF concentrations 1–4 h after ingestion were approximately 600 µg/L, with R-(-)-MDMA significantly ($p<0.05$) exceeding S-(+)-MDMA (approximately 400 µg/L) concentrations in all samples. Enantiomer ratios (R/S) increased over time and ranged from 1.04–1.92. R-(-)-MDA concentrations were <5 µg/L in 78% of samples and never >21.4 µg/L, whereas S-(+)-MDA concentrations were quantified up to 74.7 µg/L in 72% of samples.

As weak bases, amphetamines are ion trapped in OF, leading to increased concentrations and windows of drug detection. OF testing appears to be a good alternative matrix to urine, although data informing interpretation of methamphetamine, amphetamine, and MDMA OF concentrations, including windows of drug detection, are limited.

COCAINE

Cocaine is a highly abused drug in the US and South America, and prevalence of abuse has increased in Europe in recent years (EMCDDA). Because cocaine is a weak base and stimulant, ion trapping in OF is expected, as is the incidence of dry mouth and restricted sample volume. In simultaneously collected plasma and OF samples, we found higher concentrations of cocaine in plasma than OF, and the reverse for the primary, more polar, BE metabolite (Kolbrich, et al., 2006; Kolbrich, et al., 2005). Recovery of cocaine from collection devices was reported as good (85.6%–100%), except for the Malvern Medical OraCol device (Langel, et al., 2008).

Although cocaine is an important analyte in emergency toxicology, drug treatment, criminal justice, and workplace drug testing programs, there are only 2 reports of cocaine OF concentrations after controlled drug administration. In the first, cocaine and metabolite disposition

in OF after single 25-mg intravenous, 32-mg intranasal, and 42-mg smoked doses of cocaine was determined in OF collected by stimulated expectoration before and up to 12 h after dosing (Jufer, Walsh, Cone, & Sampson-Cone, 2006). Mean last detection times for cocaine were short, 4.7 (0.6), 6.3 (1.2), and 4.1 (1.0) h with an 8 µg/L proposed SAMHSA cutoff. However, these administered doses are low compared to recreational doses and doses self-administered by tolerant users. BE mean last detection times were 6.7 (1.9), 8.7 (1.5), and 5.0 (2.1) h at the same cutoff. Cocaine and BE OF disposition also were evaluated following up to 5 daily escalating doses of oral cocaine, beginning with 100 mg and increasing by 25 mg to a maximum single dose of 400 mg and a maximum total dose of 2000 mg/day for up to 16 days (Jufer, et al., 2006). OF detection times with stimulated expectoration after repeated dosing were 4-fold and 7-fold longer for cocaine and BE compared to single doses. The authors suggested that detection times based on single doses underestimate the utility of OF for identifying cocaine use because high, repeated doses are frequently self-administered.

In the other investigation of controlled cocaine administration, participants each received placebo or 75 and 150 mg/70 kg cocaine sulfate on different days, administered subcutaneously for safety purposes (Kolbrich, et al., 2005). OF collection occurred for 48 h after each dose and samples were obtained by expectoration with citric acid candy stimulation. OF was analyzed by GC-MS with 2.5 µg/L LOQs for cocaine, BE, and ecgonine methylester (EME). OF was positive for cocaine within 5 min in 65% of low and 100% of high-dose samples. Mean onset times of EME and BE were similar, approximately 0.1 h in plasma and 0.3 h in OF. OF cocaine maximum concentrations were 1322.3 (848) and 3130.7 (2228.2) µg/L after the 75 and 150 mg/70 kg doses. Cocaine and EME maximum concentrations were significantly higher in OF than plasma, whereas BE maximum concentrations were higher in plasma. Mean BE OF maximum concentrations were 154.7 (100.3) and 308.0 (163.8) µg/L. Cocaine maximum concentrations was slightly lower in plasma than in OF. Although cocaine concentrations exceeded those of BE in OF, detection times were generally longer for BE in this matrix. Cocaine, EME, and BE were ≥ LOQ in 93% of participant samples at 8 h. After study participants received the high dose, cocaine OF/plasma ratios tended to be 1–2 up to 0.25 h and >3 at 0.5–8 h; about half remained >3 up to 48 h.

Cocaine is a weak base and thereby is subject to OF ion trapping, which leads to increased concentrations and windows of drug detection. Reduced salivary volume follows the use of this stimulant. Abuse of smoked crack cocaine, insufflation of cocaine hydrochloride, and oral cocaine abuse will contaminate the oral cavity, with resulting high initial OF concentrations compared to concentration that occur after intravenous cocaine abuse. There are no data describing how long cocaine concentrations remain increased after mucosal contamination. OF appears to be a good alternative matrix to urine, with reasonable detection windows of 1–2 days, even after recreational single 100-mg doses.

BENZODIAZEPINES

Benzodiazepines encompass a large variety of drugs with differing pharmacodynamic effects and pharmacokinetic profiles. SAMHSA does not include benzodiazepines in workplace drug testing, but DRUID recommends cutoffs (Table 1). Benzodiazepines have a high prevalence in DUID cases (Verstraete, 2005) and appear to be especially impairing during the first few weeks of therapy (Busto, et al., 2000). Monitoring benzodiazepine exposure with OF is especially challenging owing

to the wide range of available benzodiazepines with highly variable potencies, low concentrations, legal prescription status, high lipophilicity, analyte instability in non-preserved OF, and variable cross-reactivities with antibodies. Many jurisdictions, especially in Europe, strongly advocate inclusion of benzodiazepines in testing strategies because of their contribution to DUID. However, few data are available for OF benzodiazepines.

Research on collection devices for benzodiazepines is limited and results are more variable (Table 3). Langel et al. (2008) reported 97.1% and 96.9% recoveries for diazepam and alprazolam from OF collected by expectoration, and 95.7% and 95.8% with the Acro Biotech Salicula™ device. The Varian OraTube, Sarstedt Salivette, and Malvern Medical OraCol were not suitable for benzodiazepine collection, with recoveries <49% (Langel, et al., 2008). Recoveries for the Cozart device were 66.0% for alprazolam but 91.6% for diazepam, results that highlight the variability within this class (Langel, et al., 2008). Speedy et al. (2007) found a recovery of 92.1% for temazepam with Cozart DDS and Quintela et al. (2006) 101.3% for oxazepam with Immunalysis Quantisal.

Flunitrazepam was detected only up to 6 h in expectorated OF after oral dosing of 1 mg. Concentrations were <0.6 µg/L if OF was preserved with 2% sodium fluoride. Concentrations of flunitrazepam's metabolite 7-aminoflunitrazepam were <3.1 µg/L (Samyn, DeBoeck, Cirimele, Verstraete, & Kintz, 2002). OF was positive for tetrazepam from 0.25–10 h after a 50-mg dose, and no diazepam or nordiazepam metabolites were measurable in OF, but these metabolites were positive in urine (Laloup, et al., 2007). Diazepam was found as a pill contaminant, but also may have derived from metabolism of tetrazepam. Three study participants receiving 10 mg diazepam in the same study had positive OF samples for >10 h. Other investigators reported that after study participants received 15 or 30 mg oxazepam, oxazepam and oxazepam glucuronide were detected in expectorated OF for >8.5 h, in much lower concentrations than in simultaneously collected blood (Smink, et al., 2008). OF/blood ratios ranged from 0.04–0.07 for oxazepam and from 0.002–0.006 for oxazepam glucuronide.

These data indicate that OF testing for benzodiazepines will be challenging indeed. Low OF concentrations, a multitude of drugs within the class, instability of some parent drugs, and potential recovery issues from collection devices must be addressed. Although a single study has addressed glucuronide-benzodiazepine conjugates, the available data suggest that glucuronide concentrations may be an order of magnitude lower than free drug in OF, and hydrolysis of OF samples may not be necessary. Alternatively, LC-MS/MS may permit direct measurement of glucuronide conjugates; however, matrix suppression and coelution with endogenous matrix components are adverse events that frequently occur with early elution of conjugates on the most common reversed-phase columns.

Applications

Applications of OF testing in DUID, treatment, and workplace settings reported in the last 5 years are listed in Table 6. The number of such reports is increasing, providing excellent data on the usefulness of this new technology, the prevalence of drugs in various populations, and the performance of various onsite tests. It is hoped that recommendations proposed at the Talloires meeting of experts for standardizing research DUID methods will be followed for future studies, allowing better comparison of data across studies than can be currently accomplished.

Table 6: Application studies with oral fluid testing.

Type	No. of samples	Screening	Confirmation	Prevalence, %					Reference
				THC	Opiates	Amphetamines	Cocaine	Benzodiazepines	
D: I/S ^a	302	Toxiquick	GCMS*	56.8	8.0	48.3	5.7		Biermann, et al., 2004
D: Hos	300	Cozart® Microplate ELISA	GCMS & LCMSMS	≥ 1.7	≥ 1.4	≥ 1.4	≥ 0.4	≥ 6.7	Bernhoft, et al., 2005
D	61	DrugWipe & Intercept	GCMS	29.5					Kintz, Bernhard, et al., 2005
D: I/S	131	Dräger DrugTest	GCMS	54.2	17.6	32.8	14.5		Toennes, Kauert, et al., 2005
D: I/S	177	Dräger DrugTest	GCMS	54.2	19.8	44.1		16.4	Toennes, Steinmeyer, et al., 2005
D	55	-	LCMSMS		3.6	27.3	20.0		Wood, et al., 2005
D: Ra	1396	Cozart® Microplate ELISA	GCMS	3.7	≥ 3.2	≥ 4.6	1.6	2.0	Wylie, Torrance, Seymour, et al., 2005
D: Rd	468	Dräger DrugTest	LCMS	≥ 38.7	≥ 3.5	≥ 7.0	≥ 35.5		Concheiro, et al., 2007
D: Rd	468	OraLab	LCMS	≥ 9.5	≥ 3.5	≥ 1.2	≥ 18.5		Concheiro, et al., 2007
D: R	13,176	DrugWipe & RapiScan	GCMS	≥ 0.7		≥ 2.0			Drummer, et al., 2007
D: I/S	114	DrugWipe	GCMS			87.7			Engblom, et al., 2007
D: I/S	40	DrugWipe	ELISA & MS ^b	37.5	12.5	5.0	20.0		Crouch, et al., 2008
D: I/S	267	DrugWipe	ELISA & MS ^b	14.6	2.6	4.1	14.6		Crouch, et al., 2008
D: R	10,816	-	LCMSMS	0.6	1.1	0.3	0.1	1.4	Gjerde, et al., 2008
D: I/S	148	DrugWipe	GCMS	15.5	2.7	90.5	1.4	50.6	Pehrsson, et al., 2008
T	217	Cozart Microplate ELISA	GCMS				53.5		Cooper, et al., 2004
T	358	Cozart RapiScan	GCMS		25.4		31.6		Cooper, et al., 2005
T	198	Cozart Microplate ELISA	GCMS		57.1				Cooper, et al., 2005a
T	216	Cozart Microplate ELISA	GCMS		50.5				Cooper, et al., 2005b
T	27	Sun OraLine IV s.a.t.	GCMS	52.0					Cirimele, et al., 2006
T	135	Cozart Microplate ELISA	GCMS			57.8			Cooper, et al., 2006
T	513	Orasure Intercept	LCMSMS		57.9				Phillips and Allen, 2006
T&W	635,000	Orasure Intercept	GCMS(MS)	≥ 0.1	≥ 1.1	≥ 0.1	≥ 0.2	≥ 0.1	Cone, et al., 2007
T	403	Sarstedt Salivette	LCMSMS		15.4		32.3		Dams, et al., 2007
T	134	Cozart Microplate ELISA	GCMS & LCMS	73.1		23.9			Speedy, et al., 2007
T	20	Cozart DDSV	GCMS	90.0					Kintz, et al., 2009

^a D, DUID; Hos, hospitalized drivers; I/S, impaired or suspected drivers; R, random; Ra, random at high-risk accident area; Rd, random near discotheques; T, treatment; W, workplace testing;

^b Screening with OF, but confirmation with blood

DUID studies have tested impaired or suspected drivers (Biermann, et al., 2004; Crouch, et al., 2008; Engblom, Gunnar, Rantanen, & Lillsunde, 2007; Pehrsson, et al., 2008; Toennes, Kauert, Steinmeyer, & Moeller, 2005; Toennes, Steinmeyer, Maurer, Moeller, & Kauert, 2005), random drivers at road-blocks (Drummer, et al., 2007; Gjerde, et al., 2008), and drivers near discotheques (Concheiro, et al., 2007) and high- risk accident areas (Wylie, Torrance, Seymour, Buttress, & Oliver, 2005), and have evaluated injured hospitalized drivers (Bernhoft, et al., 2005). Percentages of positive tests were highly variable (range 0.1%–90.5%) owing to differences in study populations, collection and analytical methods, LOQs, times between stop and OF collection, and DUID laws (Wylie, Torrance, Seymour, et al., 2005).

In 46.7% of reports, cannabinoids were the most prevalent drug. THC concentrations ranged from 0.7 (Kintz, Bernhard, et al., 2005) to 6484 µg/L (Drummer, et al., 2007). The higher

concentrations appear to be attributable to contamination of the oral mucosa from cannabis smoke immediately after use; median concentration was 81 µg/L (Drummer, et al., 2007). Concheiro et al. (2007) evaluated the Varian OraLab® and Dräger Drug Test®, but also collected OF for confirmation by expectoration, eliminating the variable of cannabinoid recovery from the collection device.

Opioids are one of the least commonly identified drug classes in DUID. Detected concentrations have ranged from 4 µg/L for codeine to 7600 µg/L for 6AM, the latter possibly due to oral contamination, because high-purity heroin may be smoked, inhaled, or snorted (Wylie, Torrance, Seymour, et al., 2005). Opioids are one of the most investigated drug classes in treatment settings (54.5% of studies).

Amphetamine prevalence was as high as 90.5% (Pehrsson, et al., 2008) and 87.7% (Engblom, et al., 2007) in 2 Finnish studies of DUID suspected drivers. In a study by Engblom et al. (2007) OF concentrations up to 131 000 µg/L for amphetamine were observed, most likely due to oral contamination, because the median concentration was 7440 µg/L.

Cocaine was one of the least commonly reported drugs in DUID, having the lowest prevalence rate in 50% of worldwide studies. In 2 populations, however, Spain 18.5% (Concheiro, et al., 2007) and Utah 14.6% (Crouch, et al., 2008), it was the most commonly found drug.

Benzodiazepines are an important drug class to monitor because these drugs are frequently reported in DUID cases (Ojaniemi, Lintonen, Impinen, Lillsunde, & Ostamo, 2009), yet they are some of the most commonly prescribed medications (Cook, Marshall, Masci, & Coyne, 2007). Another difficulty is that an individual may be highly impaired when first taking a benzodiazepine, but rapidly develop tolerance to the drug's impairing effects (Busto, et al., 2000). Thus, determining an appropriate cutoff concentration that reflects impairment is difficult for this and most other drug classes. Reported concentrations ranged from 4 µg/L for temazepam, nordiazepam, or oxazepam to 221 µg/L for nordiazepam in DUID (Wylie, Torrance, Seymour, et al., 2005). In treatment and workplace settings, concentrations ranged from 0.5 µg/L for diazepam, nordiazepam, oxazepam, and temazepam to 14 301 µg/L for diazepam, with a median of 2.8 µg/L (Cone, Clarke, & Tsanaclis, 2007). In this interesting study, which compared the sensitivity of drug detection in 635 000 treatment and workplace urine and OF samples, investigators concluded that OF was as efficient as urine for identifying drug use.

In conclusion, the technology of OF collection devices, laboratory-based assays, on-site testing devices, and chromatographic confirmation methods has greatly advanced in the last 5 years. POCT devices are improving, with better recoveries, especially for THC, and more accurate performance. However, successful development of a POCT device that performs acceptably for all drug classes is a challenge. Currently available chromatographic methods quantify multiple analytes at increasingly lower concentrations, and important new analytes (e.g., THCCOOH) have been identified. Perhaps the greatest current limitation for OF testing is the small number of controlled drug administration studies available to inform interpretation of OF tests. Additional research is needed to identify new biomarkers, determine drug detection windows, characterize OF adulteration techniques, and evaluate analyte stability in OF. There is no doubt that OF offers multiple advantages as an alternative matrix for monitoring licit and illicit drug use, and that OF testing has an important role in DUID, treatment, workplace, and criminal justice programs.

**CHAPTER 3: MEDICINAL THC (DRONABINOL) IMPAIRS ON-THE-ROAD
DRIVING PERFORMANCE OF OCCASIONAL AND HEAVY CANNABIS USERS
BUT IS NOT DETECTED IN STANDARDIZED FIELD SOBRIETY TESTS**

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Bosker, W. M., Kuypers, K. P. C., Theunissen, E. L., Surinx, A., Blankespoor, R. J., Skopp, G., Jeffery, W. K., Walls, H. C. van Leeuwen, C. J. & Ramaekers, J. G. (submitted). Medicinal THC (dronabinol) impairs on-the-road driving performance of occasional and heavy cannabis users but is not detected in Standardized Field Sobriety Tests.

CONTEXT Dronabinol (Marinol®) is an orally administered cannabinoid used for the treatment of chronic pain, anorexia in AIDS and other wasting diseases, and as an antiemetic medication in cancer patients undergoing chemotherapy. The active ingredient dronabinol is synthetic Δ^9 -tetrahydrocannabinol (THC), which is known to cause driving impairment when smoked.

OBJECTIVE Assess the acute and chronic effects of dronabinol on actual driving performance and the Standardized Field Sobriety Tests (SFST). It was hypothesized that occasional users would be impaired on these tests and that heavy users would show less or no outward signs of impairment due to tolerance.

DESIGN, SETTING AND PARTICIPANTS Double-blind, placebo-controlled, randomized, 3-way cross-over study. Twelve occasional and twelve heavy cannabis users (14 males/ 10 females) performed a road tracking and car-following task in normal traffic and SFST following use of dronabinol and placebo.

INTERVENTIONS Single doses of placebo, 10 and 20 mg dronabinol.

MAIN OUTCOME MEASURES Standard Deviation of Lateral Position (SDLP; i.e. weaving) is the primary measure of road tracking control. Time to speed adaptation (TSA) is the primary reaction time measure in the car-following test. Percentage of impaired individuals on the SFST and subjective high on a visual analogue scale were secondary measures.

RESULTS Superiority tests showed that SDLP ($p=0.008$) and TSA ($p=0.011$) increased after dronabinol in occasional users. Non-inferiority tests demonstrated that dronabinol induced increments in SDLP, were bigger than impairment associated with the Blood Alcohol Content of 0.5 mg/mL (0.05 grams %) criterion in occasional and heavy users, although the magnitude of driving impairment was generally less in heavy users. The SFST did not discriminate between treatments. Levels of subjective high were comparable in occasional and heavy users.

CONCLUSION Dronabinol impairs driving performance in occasional and heavy users in a dose-dependent way, but to a lesser degree in heavy users due to tolerance. SFST are not sensitive to detect clinically relevant driving impairment observed after oral dronabinol.

Introduction

Cannabis is one of the most widely used drugs of abuse worldwide. The 2008 national survey on drug use and health reported that about 6% of the general US population aged 12 and older admitted to cannabis use in the previous month (SAMHSA, 2009). The widespread use of cannabis has also increased the prevalence of cannabis among drivers in the general driving population. Overall, 6.8% of the general driving population tested positive for Δ^9 -tetrahydrocannabinol (THC), the active ingredient of cannabis, in blood. In young drivers aged 16-20 years prevalence rates even increased to 15.2% (Lacey, et al., 2009). Moreover, 13% of drivers involved in fatal accidents were positive for THC (NHTSA, 2009). High prevalence rates of THC among drivers may pose a serious problem since both experimental and epidemiological studies have demonstrated that THC increases driving impairment and crash risk in a dose related manner (Grotenhermen, et al., 2007; Ramaekers, et al., 2004; Ramaekers, et al., 2000).

Prevalence of drivers under the influence of THC is even likely to increase in countries that have passed laws to regulate cannabis distribution and consumption for medicinal use. Pharmaceutical companies have developed synthetic cannabinoids for a number of medical indications that are now readily available on the market as prescription drugs. Dronabinol (Marinol®) is a synthetic cannabinoid that is used to treat anorexia in AIDS and other wasting diseases, emesis in cancer patients undergoing chemotherapy and chronic pain. The active ingredient dronabinol is pure, synthetic THC. Dronabinol is taken orally in therapeutic doses that range between 2.5 and 20 mg/day. The pharmacokinetic profile of dronabinol following oral administration differs markedly from that of smoked cannabis. After smoked cannabis, the peak THC concentration is reached within 5 minutes. Blood THC concentrations then rapidly decline to about 10% of peak blood THC concentrations (T_{max}) within 1-2 hrs. After oral doses of dronabinol, T_{max} is achieved within 2-4 hrs after administration. Maximum concentrations are generally less than those observed after smoking but these levels remain on a plateau for up to 6 hrs after administration (McGilveray, 2005; Ramaekers, Kauert, et al., 2009; Ramaekers, Moeller, et al., 2006). Hence, oral administration of THC has a slower onset of and blunted effect profile but persists longer over time as compared to smoked cannabis.

Oral equipotent doses of THC are expected to produce similar effects on human performance as smoked THC. Yet only few studies to date assessed the effects of oral doses of THC on human performance. Several studies have demonstrated that oral doses of THC ranging from 5-15 mg produce subjective feelings of high (Curran, Brignell, Fletcher, Middleton, & Henry, 2002; Gray, et al., 2008; Kirk & de Wit, 1999). Low doses (i.e. 5 and 10 mg) did not produce impairments in a number of neuropsychological performance tests (Gray, et al., 2008), but at higher doses (15-60 mg) there have been reports of memory impairment and tracking impairment in a driving simulator (Curran, et al., 2002; Menetrey, et al., 2005).

The present study was primarily designed to assess the effects of orally administered dronabinol within the range of normal therapeutic doses, i.e. 10 and 20 mg, on driving performance in a standardized on-the-road driving test that was performed in normal traffic. This standardized driving test has been successfully employed in the past to demonstrate dose related impairments after smoked cannabis (Ramaekers, et al., 2000; Robbe, 1998). Oral doses of dronabinol were administered to occasional and heavy cannabis users in order to model acute THC effects in naïve THC users and in chronic THC users who generally develop some behavioral

tolerance to the impairing effects of THC (Hart, van Gorp, Haney, Foltin, & Fischman, 2001; Ramaekers, et al., 2010). A second objective was to determine whether THC induced impairments that were expected during on-the-road driving could be assessed using Standardized Field Sobriety Tests (SFST). SFST are a battery of three tests administered and evaluated in a standardized manner to obtain validated indicators of impairment and establish probable cause for arrest. SFST are routinely conducted by the US police and several countries around the world to identify drivers under the influence.

Subjects and Method

SUBJECTS

Twelve occasional and twelve heavy cannabis users (14 males/10 females evenly distributed over both user groups) with mean (SE) lifetime use of 274.1 (89.6) and 2444.2 (708.8) times respectively, and mean (SE) age of 23.6 (0.6) years, participated. Almost all heavy users were daily smokers (range 7.7-23.1 joints per week), except subjects 21 and 23 who smoked respectively on average 4.7 and 6.3 joints per week. Inclusion criteria were experience with cannabis, i.e., 5-36 times yearly for occasional users and >160 times yearly for heavy users; free from psychotropic medication; good physical health; absence of any major medical condition; BMI between 18 and 28; possession of valid driving license; and written informed consent. Exclusion criteria were history of drug abuse or addiction to non-cannabinoids; pregnancy or lactation; cardiovascular abnormalities; excessive drinking; hypertension; history of or current psychiatric disorder; and allergy to sesame oil.

This study was conducted according to the code of ethics on human experimentation established by the declaration of Helsinki (1964) and amended in Seoul (2008). Approval for the study was obtained from the Medical Ethics committee of the Academic Hospital of Maastricht and Maastricht University. A permit for obtaining, storing, and administering THC was obtained from the Dutch drug enforcement administration.

STUDY DESIGN

The study was conducted according to a double-blind, placebo-controlled, randomized, three-way, cross-over design. Treatments consisted of single doses of placebo, 10 and 20 mg dronabinol and were administered orally in identically appearing capsules. Treatment orders were balanced over subjects and treatment periods. The wash-out period between treatments was at least four days to prevent interference from preceding treatments.

PROCEDURE

Subjects were asked to refrain from any drugs one week before the medical examination until study completion, except heavy users who continued their cannabis consumption. Subjects were not allowed to drink alcohol or caffeine during a 24-h period prior to testing. Subjects were tested for alcohol in breath and drugs in urine upon arrival at the laboratory. THC treatments were only given when occasional users tested negative and heavy users positive on THC and negative on other drugs. Assessment of subjective high and blood samples were taken at baseline (30 minutes before) and 1.5, 4.25 and 6 hours after drug administration. The driving tests were performed between 2-4 hours and the SFST 4.5-5 hours post-drug. At the end of a testing day subjects were driven home.

ACTUAL DRIVING TESTS

The road tracking test (O'Hanlon, 1984) consists of driving for approximately one hour in a specially instrumented car. Subjects have to maintain a constant speed of 95 km/h (60 miles/h) and drive as straight as possible on the right lane of a primary highway. The dependent measure is the Standard Deviation of Lateral Position (SDLP), a measure of road tracking control (i.e. weaving). Speed and Standard Deviation of speed (SD Speed) are recorded as secondary control measures. This standardized test has been applied in more than 60 studies to determine drug effects on driving (Louwerens, et al., 1987; O'Hanlon, Haak, Blaauw, & Riemersma, 1982; Ramaekers, 2003). The road tracking test has been calibrated for the effects of alcohol in such a way that drug effects can be expressed in blood alcohol concentration (BAC) equivalents. A BAC of 0.5 mg/mL (0.05 grams %) has been shown to increase SDLP with 2.4cm (Louwerens, et al., 1987). Drug induced changes in SDLP that exceed this alcohol criterion values are qualified as a clinical relevant drug effect.

In the car-following test (Brookhuis, et al., 1994; Ramaekers, et al., 2002) subjects drive behind a leading vehicle on a primary highway for 25 minutes while maintaining a constant distance between vehicles during a series of speed decelerations/acceleration that are initiated by the experimenter in the leading vehicle. The speed of the leading car is controlled by a cruise-control system and is set to maintain a constant speed of ± 100 km/h (62 miles/h). Sinusoidal speed changes reaching an amplitude of -10% and returning to the starting speed within 50 sec. are repeated 6-10 times. Time to Speed Adaptation (TSA) is the primary measure and is calculated from the phase delay between speed signals from the leading and following vehicle. Secondary measures are gain and coherence. Gain is the amplification factor between both speed signals collected from the leading and following vehicle. Coherence is a control measure for correspondence between both speed signals.

STANDARDIZED FIELD SOBRIETY TESTS

An investigator (WMB) received extensive training by two forensic toxicologists with extensive training in SFST (WKJ and HCW) to administer and evaluate the SFST in a standardized manner as defined in the training manuals used by all US police agencies and laboratory practicals. The three tests of the driving under the influence SFST, i.e. Horizontal Gaze Nystagmus (HGN), Walk-and-Turn (WAT) and One-Leg Stand (OLS), are described and validated by Stuster and Burns (1998). A subject is scored as impaired whenever he shows 4 out of 6 signs of impairment on HGN, 2 out of 8 on WAT and 2 out of 4 on OLS (Stuster & Burns, 1998). When a subject is impaired on 2 or more of these tests, he is classified as impaired overall by the SFST program. Percentages of impaired subjects on each test, i.e. HGN, WAT, OLS and overall SFST, were the dependent variable.

SUBJECTIVE MEASURES

A Visual Analogue Scale (VAS) was used to measure subjective high. On a 100 mm line anchored with "not at all" and "most ever", subjects indicated the effect of the drug by marking the line vertically.

PHARMACOKINETIC ASSESSMENT

Blood samples (8 mL) were collected at baseline (heavy users only), 1.5, 4.25 and 6 hours post-drug. The blood sample was centrifuged and the resulting serum was frozen at -20°C until analysis. THC, 11-OH-THC and THCCOOH concentrations were determined afterwards with LOD/LOQ of 0.24/0.73, 0.11/0.26 and 0.98/2.99 µg/L respectively.

STATISTICAL ANALYSIS

All statistical analyses were performed using SPSS 18.0 for Mac. The general statistical analyses consisted of two steps: 1) Assessment of an overall dronabinol effect by means of superiority testing. Driving data entered a GLM repeated measures ANOVA procedure with Dronabinol (3 levels) as main within subject factor and Cannabis use history (2 levels) as between group factor. For subjective high an additional within subject factor, Time after drug administration (4 levels) was added to the model in order to account for test repetitions. Following this overall analysis, the Dronabinol (3 levels) effects were also tested separately in occasional and heavy users. If the sphericity assumption was violated, the Greenhouse-Geisser correction was used. 2) Non-inferiority testing of dronabinol effects was based on difference scores from placebo relative to a predefined alcohol criterion (i.e. driving impairment equivalent to a BAC of 0.5 mg/mL (Louwerens, et al., 1987). Basically, this analysis assessed whether the alcohol criterion value, i.e. 2.4 cm, falls within the 95% CI of the drug effect. If yes, then the drug effect was considered inferior, i.e. comparable to or greater than a BAC of 0.5 mg/mL, and thus clinically relevant for traffic safety. If the upper limit of the 95% CI was below the alcohol criterion value then a drug effect was considered non-inferior, i.e. not clinically relevant. The sample size provided a power of at least 80% to detect a clinically relevant drug effect, given a mean population standard deviation of 4.0 cm, a test-retest correlation ≥ 0.70 and a non-inferiority margin of 2.4 cm at a two-sided α -level of 0.05.

The SFST were evaluated using χ^2 tests to determine whether a relationship existed between performance and THC dosing protocol. In case of a significant relationship Spearman's coefficient (ρ) was calculated to determine strength and direction of the relationship (Papafotiou, et al., 2005a).

Results

ACTUAL DRIVING PERFORMANCE

Overall, Dronabinol increased SDLP ($F_{2,40}=7.812$, $p=0.001$) during road tracking and TSA ($F_{2,24}=3.083$, $p=0.064$) during car-following, although the latter effect only approached significance. The interaction between Dronabinol and Cannabis use history did not reach significance. However, separate ANOVAs in occasional and heavy users demonstrated that the impairing effects of dronabinol on road tracking and car-following were prominent in occasional users (resp. $F_{2,18}=6.493$, $p=0.008$; $F_{2,10}=7.269$, $p=0.011$) but less so in heavy users. Other driving measures were not affected by Dronabinol. Means (SE) for driving measures in the two driving tests in all treatment conditions are given in Table 1.

Table 1: Mean (SE) performance on road tracking test and car following test and percentage of subjects impaired on SFST in every treatment condition. Significance indicated by p-value.

Test	Cannabis use history	Drug			ANOVA (overall)	
		Placebo	10 mg dronabinol	20 mg dronabinol	Dronabinol x Cannabis use history	Dronabinol
Road tracking						
SDLP (cm)	Occasional	17.9 (0.8)	20.4 (1.2)	22.1 (1.4)	NS ^a	0.008
	Heavy	19.7 (1.3)	21.0 (1.2)	21.4 (1.2)		NS
Mean speed (km/h)	Occasional	96.1 (0.4)	95.9 (0.3)	96.1 (0.6)	NS	NS
	Heavy	95.8 (0.2)	95.8 (0.4)	95.6 (0.3)		NS
SD Speed (km/h)	Occasional	2.1 (0.1)	2.2 (0.2)	2.1 (0.1)	NS	NS
	Heavy	2.2 (0.2)	2.3 (0.2)	2.3 (0.2)		NS
Car following						
TSA (s)	Occasional	2.5 (0.4)	4.1 (0.3)	2.9 (0.3)	NS	0.011
	Heavy	3.1 (0.1)	3.6 (0.4)	3.8 (0.8)		NS
Coherence	Occasional	0.9 (0.01)	0.9 (0.02)	0.9 (0.01)	NS	NS
	Heavy	0.9 (0.01)	0.9 (0.02)	0.9 (0.01)		NS
Gain	Occasional	1.2 (0.07)	1.2 (0.05)	1.2 (0.07)	NS	NS
	Heavy	1.2 (0.06)	1.2 (0.04)	1.4 (0.10)		NS
SFST					χ^2	
Overall SFST (% impaired)	Occasional	8.3	8.3	8.3	NS	
	Heavy	0.0	16.7	16.7	NS	
HGN (% impaired)	Occasional	0.0	8.3	0.0	NS	
	Heavy	0.0	0.0	0.0	NS	
WAT (% impaired)	Occasional	8.3	33.3	33.3	NS	
	Heavy	16.7	41.7	50.0	NS	
OLS (% impaired)	Occasional	16.7	16.7	25.0	NS	
	Heavy	8.3	25.0	16.7	NS	

^a NS, not significant; SDLP, standard deviation of lateral position; TSA, time to speed adaptation; SFST, standardized field sobriety tests; HGN, horizontal gaze nystagmus; WAT, walk and turn; OLS, one leg stand.

Mean (95% CI) changes in SDLP after dronabinol in occasional and heavy users are shown in Figure 1. Non-inferiority tests demonstrated that the upper limit of the 95% CI associated with change SDLP relative to placebo exceeded the alcohol criterion limit (BAC 0.5 mg/mL) after both dronabinol doses in occasional users. In heavy users the mean change from placebo was relatively small, but the 95% CI associated with change SDLP after both doses of dronabinol included the alcohol criterion value. Small mean changes and wide CIs indicate large inter-individual variation in response to dronabinol in heavy users. This was also confirmed by plots of individual change scores that are shown in Figure 2.

STANDARDIZED FIELD SOBRIETY TESTS

The analysis of the SFST did not reveal any significant effects of Dronabinol or Cannabis use history. Percentage of individuals who were impaired is shown in Table 1.

Figure 1: Mean (95% CI) change in SDLP after single doses of dronabinol in occasional and heavy users. * non-inferiority not shown, upper bound of the 95% CI is above the non-inferiority margin of 2.4cm.

Figure 2: Individual data of change SDLP in occasional and heavy users after single doses of dronabinol. Change score >0 indicates impairment, change score <0 indicates improvement. The dotted horizontal line represents impairment equivalent to BAC of 0.5 mg/mL.

SUBJECTIVE MEASURES

Dronabinol significantly increased subjective high in occasional and heavy users to similar degrees (respectively $F_{2,20}=9.160$, $p=0.001$; $F_{2,20}=6.664$; $p=0.006$). Subjective high significantly decreased as a function of time after dronabinol administration ($F_{3,60}=15.780$, $p<0.001$). Mean (SE) subjective high are given in Figure 3.

Figure 3: Mean (SE) subjective rating of high as a function of time after drug administration in all treatment conditions.

PHARMACOKINETIC ASSESSMENT

Mean (SE) THC, 11-OH-THC and THCCOOH concentrations are displayed in Table 2. Baseline THC concentrations for heavy users did not differ significantly between treatment conditions ($F_{1,086,8.686}=1.971$, $p=0.196$). Overall, a significant difference in drug concentrations between the drug

Table 2: Mean (SE) serum concentrations (µg/L) for THC, 11-OH-THC and THCCOOH in occasional and heavy users for every treatment and measurement time.

	Time post-drug (h)	Occasional users			Heavy users		
		THC	11-OH-THC	THCCOOH	THC	11-OH-THC	THCCOOH
Placebo	-0.5	-	-	-	15.3 (8.5)	5.6 (2.4)	58.2 (17.5)
	1.5	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	6.4 (2.7)	2.8 (0.9)	37.4 (9.9)
	4.25	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	4.9 (1.7)	2.0 (0.6)	37.2 (11.6)
	6	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	4.4 (1.6)	1.6 (0.5)	31.2 (9.4)
10 mg dronabinol	-0.5	-	-	-	7.6 (3.6)	2.9 (1.2)	41.8 (11.2)
	1.5	3.2 (0.8)	4.2 (0.6)	22.6 (5.6)	9.2 (2.1)	6.2 (0.9)	54.4 (8.8)
	4.25	2.1 (0.7)	1.8 (0.3)	18.5 (2.8)	4.6 (1.1)	3.1 (0.4)	43.4 (7.4)
	6	1.2 (0.3)	1.7 (0.3)	16.8 (1.3)	4.2 (1.0)	2.3 (0.4)	37.2 (7.6)
20 mg dronabinol	-0.5	-	-	-	10.3 (4.9)	3.6 (1.5)	46.2 (12.7)
	1.5	6.2 (1.3)	6.9 (1.4)	37.2 (11.4)	10.2 (2.2)	7.1 (1.1)	54.0 (10.1)
	4.25	1.8 (0.3)	2.6 (0.5)	24.0 (3.7)	6.3 (1.2)	4.5 (0.5)	52.0 (8.9)
	6	2.8 (0.6)	3.1 (0.5)	30.2 (4.3)	5.7 (1.1)	3.9 (0.5)	56.2 (9.1)

conditions was shown ($F_{1,430,22.881}=10.567$, $p=0.001$) as well as a significant effect in drug concentrations as a function of time after dosing ($F_{1,152,18.433}=7.015$, $p=0.013$).

Discussion

This study was designed to assess the effects of typical dosing of dronabinol on driving performance in occasional and heavy cannabis users. The doses administered were within the normal range prescribed for patients suffering from anorexia in AIDS and other wasting diseases, emesis due to chemotherapy in cancer patients and for chronic pain.

Superiority tests revealed an overall effect of dronabinol on SDLP, independent of Cannabis use history. ANOVAs in occasional and heavy users separately indicated that dronabinol effects were most prominent in occasional users. Dronabinol significantly impaired road tracking performance in occasional cannabis users. Mean SDLP increased with 2.5 and 4.2 cm respectively after doses of 10 and 20 mg. Non-inferiority tests demonstrated that 95% CIs associated with the mean change in SDLP included equivalent BAC effects of 0.8 and 1.0 mg/mL. This suggests that road tracking impairments after dronabinol were meaningful and of significant relevance to safe operation of a motor vehicle. Dronabinol also increased TSA during car-following of occasional users indicating that drivers under the influence of dronabinol needed more time to react and adjust to speed changes of a leading vehicle. The present data are in accordance with previous driving studies that assessed the influence of smoked cannabis in occasional users and confirm THC significantly increases SDLP in a dose related manner (Ramaekers, et al., 2004; Ramaekers, Kauert, et al., 2009; Ramaekers, et al., 2000).

In heavy users, mean SDLP did not differ between treatments according to superiority tests. However, non-inferiority tests demonstrated that the 95% CI associated with change SDLP after both doses of dronabinol included the criterion value equivalent to BAC of 0.5 mg/mL. A relatively wide range of these 95% CIs was caused by large inter-individual variations in change SDLP observed after both doses. Inspection of individual data indicated that approximately 25% of the heavy users demonstrated impairment in road tracking performance that was equivalent to or worse than that observed in drivers with a BAC of 0.5 mg/mL (0.05 grams %). Together, these data show that THC induced impairments of road tracking performance in heavy cannabis are less as compared to those observed in occasional users. This reduction in sensitivity to the impairing effects of THC in heavy users has been reported before and has been interpreted as a demonstration of behavioral tolerance that develops after repeated cannabis use (Gray, et al., 2008; Hart, et al., 2001; Hart, et al., 2002; Ramaekers, Kauert, et al., 2009; Ramaekers, et al., 2010). This study however also demonstrates that behavioral tolerance was not complete in every heavy cannabis user as indicated by large inter-individual differences in driving impairments observed in these drivers.

Performances on the SFST consisting of the WAT, OLS and HGN did not differ between treatments. The absence of any observable impairments in SFST appear to indicate that these tests are not sensitive to the impairing effects of THC, even when objective impairment levels are comparable to BAC > 0.8 mg/mL (0.08 grams %). These findings confirm results from a previous placebo controlled study demonstrating that the number of impairments observed in subjects during THC intoxication was very comparable to the number of impairments observed in the same

subjects during placebo (Papafotiou, et al., 2005b). The low propensity of SFST to discriminate between THC use and placebo may be related to the fact that these tests have been validated against alcohol impaired drivers at different BACs (Stuster & Burns, 1998). The present results further underscore this notion and demonstrate that current SFST are not sufficiently sensitive to detect THC induced driving impairment following oral administration of dronabinol. This is why in North America the Drug Recognition and Evaluation Program has included more indicators of drug use besides the 3 SFST to detect driving impairment.

It is highly significant that the driving impairments after dronabinol use were evident even though THC plasma concentrations were relative low. Overall, mean THC concentrations varied between 10 and 2 µg/L during onset and completion of the road driving tests. This finding confirms the results from a previous report showing that impairment of neurocognitive functions, i.e. tracking, impulse control and decision making, can be observed when THC in plasma ranges between 2-5 µg/L (Ramaekers, Moeller, et al., 2006). For patients using dronabinol this practically means that risk of driving impairment is present at any therapeutic dose, independent of THC concentration.

To sum up, it is concluded that a single doses of dronabinol severely impairs driving performance of drivers with a history of occasional cannabis use. Behavioral tolerance to the impairing effects of dronabinol was observable in heavy cannabis users, although not in every individual. About 25% of heavy users still displayed driving impairments comparable to or worse than a BAC of 0.5 mg/mL (0.05 grams %). The general advice to patients using dronabinol is not to drive, irrespective of dose, serum concentration or THC use history. This study clearly point to the need for the development of new tests that can be used in the field to detect impairment caused by drugs like THC.

CHAPTER 4: A PLACEBO CONTROLLED STUDY TO ASSESS STANDARDIZED FIELD SOBRIETY TESTS PERFORMANCE DURING ALCOHOL AND CANNABIS INTOXICATION IN HEAVY CANNABIS USERS AND ACCURACY OF POINT OF COLLECTION TESTING DEVICES FOR DETECTING THC IN ORAL FLUID

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Bosker, W. M., Theunissen, E. L., Conen, S., Kuypers, K. P. C., Jeffery, W. K., Walls, H. C., Kauert, G. F., Toennes, S. W., Moeller, M. R. & Ramaekers, J. G. (submitted). A placebo controlled study to assess Standardized Field Sobriety Tests performance during alcohol and cannabis intoxication in heavy cannabis users and accuracy of point of collection testing devices for detecting THC in oral fluid.

RATIONALE Standardized Field Sobriety Tests (SFST) and oral fluid point-of-collection testing devices are used to screen for driving impairment and for roadside drug detection respectively. SFST have been validated for alcohol, but its sensitivity for other drugs is relatively unknown. Sensitivity and specificity of most oral fluid devices for detection of Δ^9 -tetrahydrocannabinol (THC) have been low.

OBJECTIVE This study assessed the effects of smoked cannabis alone and in combination with alcohol on SFST. Additionally, presence of THC in oral fluid collected with two point-of-collection testing devices (Dräger Drug Test® 5000 and Securetec Drugwipe® 5) was determined.

METHODS Twenty heavy cannabis users participated in a double-blind, placebo-controlled study assessing percentage of impaired individuals on the horizontal-gaze-nystagmus, one-leg-stand, walk-and-turn and overall SFST and sensitivity of the two oral fluid devices. They received alcohol doses to reach peak values of 0, 0.5 and 0.7 mg/mL BAC in combination with 400 µg/kg bodyweight THC.

RESULTS Cannabis was significantly related to performance on the one-leg-stand. The combination of alcohol and cannabis was significantly related to impairment on horizontal-gaze-nystagmus. The Dräger Drug Test® 5000 demonstrated a high sensitivity for THC, whereas sensitivity of the Securetec Drugwipe® 5 was low.

CONCLUSIONS SFST are mildly sensitive to impairment from cannabis in heavy users. Lack of sensitivity is probably due to tolerance and time of testing. SFST were sensitive to low doses of alcohol. The Dräger Drug Test® 5000 appears a promising tool for detecting THC in oral fluid as far as correct THC detection is concerned.

Introduction

Cannabis is one of the most widely used drugs. About 15.2 million persons or 6% of the US general population aged 12 and older admitted to past month use (SAMHSA, 2009). In Europe this was estimated to be 12 million (3.6%) (EMCDDA, 2009). Because of its widespread use, the prevalence of cannabis in the general driving population is also one of the highest after alcohol: driving under the influence of cannabis (DUIC) was reported by 2.4% of the general driving population (EMCDDA, 2008b). Among young drivers the prevalence of DUIC was as high as 30%. This is a serious cause for concern, since experimental and epidemiological studies showed that cannabis impairs driving performance in a concentration related manner (e.g. Drummer, et al., 2004; Grotenhermen, et al., 2007; Laumon, Gadegbeku, Martin, & Biecheler, 2005; Ramaekers, et al., 2004; Ramaekers, Kauert, et al., 2009; Ramaekers, Moeller, et al., 2006).

Due to high prevalence of cannabis in drivers and its impairing effect on driving performance, roadside screening devices are needed to determine DUIC. Oral fluid is gaining interest as a matrix for roadside drug testing. In contrast to urine, the advantages include ease of use, noninvasiveness observable sample collection, difficulty to adulterate and demonstration of recent drug use. However, at present many immunoassays for detecting Δ^9 -tetrahydrocannabinol (THC) in oral fluid do not have high reliability (Bosker & Huestis, 2009; Verstraete, 2005). This is mainly due to THC adsorption to the collection device, which makes recovery from the device difficult. In addition, the currently used cutoffs of point of collection testing devices, although improving, are too high to reliably detect THC in oral fluid. Furthermore, the time window for detection is unknown and this makes interpretation of the results of oral fluid collection devices difficult. Therefore, controlled drug administration studies are needed (Bosker & Huestis, 2009).

Standardized Field Sobriety Tests (SFST) are currently in use in the US for documenting impairment in drivers suspected of driving under the influence. The SFST has been validated for detection of alcohol impairment (Stuster, 2006; Stuster & Burns, 1998) but its sensitivity for other drugs is relatively unknown. One study reported moderately positive relationship between smoked cannabis dose and performance impairment on the SFST right after smoking (Papafotiou, et al., 2005a). A second study found that performance on the SFST under the influence of cannabis was a moderate predictor of impaired driving performance in a simulator. Although SFST were quite accurate in predicting impaired performance, the prediction of non-impaired driving was less good (Papafotiou, et al., 2005b). In cannabis users who were treated with the synthetic cannabinoid dronabinol, SFST were unable to detect THC induced impairment (Bosker, et al., submitted). THC concentrations after dronabinol were much lower as those generally observed after smoking cannabis, but still produced objective driving impairment comparable to BAC levels > 0.8 mg/mL.

The present study was designed to assess the effects of alcohol and THC on SFST performance and to determine the reliability of two point of collection testing devices, i.e. Dräger Drug Test® 5000 and Securetec Drugwipe® 5, for detecting THC in oral fluid. It was part of larger protocol to assess tolerance and cross-tolerance of heavy cannabis users to the impairing effects of THC and alcohol on neurocognitive performance. These data have been published elsewhere (Ramaekers, et al., 2010).

Subjects and methods

SUBJECTS

Twenty heavy users with mean (SE) age of 24.3 (1.4) years participated in the study (15 males and 5 females). Subjects were recruited by advertisements at Maastricht University and/or at coffee-shops and were paid upon completion of the study. Before enrollment, all subjects were screened by means of a telephone interview to determine whether they qualified for the study. The inclusion criteria were experience with cannabis, i.e., >4 days/week in the previous year; free from psychotropic medication; good physical health as determined by a medical examination; absence of any major medical, endocrine, and neurological condition; body mass index between 18 and 28 kg/m²; possession of valid driving license without revocation; and written informed consent. Furthermore, they needed to test positive for cannabis, i.e. THC, 11-OH-THC and/or THCCOOH, in serum on screening and in urine on every testing day. The exclusion criteria were history of drug abuse or addiction (except cannabis) as assessed by means of a medical questionnaire; pregnancy or lactation; cardiovascular abnormalities on electrocardiogram; excessive drinking, i.e., more than 25 alcoholic consumptions a week; hypertension; history of or current psychiatric disorder; and non cigarette smokers. If subjects met the inclusion criteria, they received a medical history and a drug questionnaire. Finally, subjects underwent a medical examination and took part in a training session to get familiar with the tests.

The study was conducted according to the code of ethics on human experimentation established by the declaration of Helsinki (1964) and amended in Seoul (2008). Approval for the study was obtained from the Medical Ethics committee of the Academic Hospital of Maastricht and Maastricht University. A permit for obtaining, storing, and administering cannabis was obtained from the Dutch drug enforcement administration.

STUDY DESIGN

The study was conducted according to a double-blind, placebo-controlled three-way design with 3 alcohol conditions (see Ramaekers, et al., 2010). Subjects underwent three alcohol-dosing conditions that were designed to achieve steady state blood alcohol concentration (BACs) of about 0, 0.5, and 0.7 mg/mL during a 5-h time window. The order of alcohol-dosing conditions was counterbalanced across subjects. In addition, subjects smoked a THC cigarette (400 µg/kg) at 3 h post-onset of alcohol dosing, in each alcohol condition. Alcohol dosing started at 10:30 hours in the morning with placebo alcohol, 0.5 or 0.7 g/kg alcohol. Additional alcohol booster doses of about 0.1 g/kg or alcohol placebo were given on an as needed basis at approximately every half hour up until 4.5 h after onset of alcohol dosing in order to keep BAC at the desired level. On average, subjects received 5.4 additional booster doses containing alcohol. Alcohol was administered as “pure” ethanol (96%) mixed with orange juice to a volume of 300 mL for the initial dose. Total volumes of booster doses mixed with orange juice were approximately 80 mL. The marijuana cigarettes were prepared beforehand for each individual from stock provided by the Dutch Bureau for Medicinal Cannabis. Marijuana contained 11% THC, a standard potency for marijuana used recreationally and sold at Dutch pharmacies for medical use. The total amount of cannabis was individually weight calibrated and mixed with tobacco to achieve a standard cigarette size and weight. THC smoking started at 3 h post-onset of alcohol dosing and lasted for about 15 min. Subjects were instructed to smoke the cigarette according to a standardized procedure (Ramaekers,

Kauert, et al., 2006) in order to minimize the subject's possibility of dose titration and to increase optimal absorption of THC, i.e. inhale for 4 s, hold breath for 10 s, and exhale/break for 15 s. This sequence was repeated until the cigarettes were smoked as completely as possible. The wash-out period between treatments was at least four days.

PROCEDURE

Subjects were asked to refrain from any drugs except cannabis 1 week before the medical examination until study completion. Subjects were not allowed to drink alcohol and caffeine or smoke tobacco during a 24-h period prior to testing. Subjects were always tested for alcohol and drugs, i.e., tetrahydrocannabinol, opiates, amphetamine/ecstasy, benzodiazepines, cocaine, and methamphetamine/ecstasy, in breath and urine respectively upon arrival at the laboratory on test days. Treatments were only administered when subjects were positive for THC and negative for all other drugs. The procedure on a test day is described elsewhere (Ramaekers, et al., 2010). The SFST was performed approximately 2 hours after cannabis smoking, which is approximately 5.5 hours after the first alcohol dose. In addition, baseline SFST performance was assessed in all individuals on a separate day prior to study treatments.

STANDARDIZED FIELD SOBRIETY TESTS

The SFST is a battery of three tests administered and evaluated in a standardized manner to obtain validated indicators of impairment after alcohol consumption and establish probable cause for arrest. The tests of the SFST are Horizontal Gaze Nystagmus (HGN), Walk-and-Turn (WAT) and One-Leg Stand (OLS).

HGN is an involuntary jerking of the eye that occurs naturally as the eyes gaze to the side. Under normal circumstances, nystagmus occurs when the eyes are rotated at high peripheral angles. However, when a person is impaired by alcohol, nystagmus is exaggerated and may occur at lesser angles. An alcohol-impaired person will also often have difficulty smoothly tracking a moving object. In the HGN test, the eyes of a subject are observed as the subject follows a slowly moving object such as a pen or small flashlight, horizontally with his or her eyes. The examiner looks for three indicators of impairment in each eye: if the eye cannot follow a moving object smoothly, if jerking is distinct when the eye is at maximum deviation, and if the angle of onset of jerking is within 45 degrees of center. If, between the two eyes, four or more clues appear, the subject likely has a BAC of 0.8 mg/mL or greater and is indicated as impaired in the current study. Research found that this test allows proper classification of approximately 88 percent of subjects (Stuster & Burns, 1998).

In the WAT test, the subject is directed to take nine steps, heel-to-toe, along a straight line. After taking the steps, the subject must turn on one foot and return in the same manner in the opposite direction. The examiner looks for eight indicators of impairment: if the subject cannot keep balance while listening to the instructions, begins before the instructions are finished, stops while walking to regain balance, does not touch heel-to-toe, steps off the line, uses arms to balance, makes an improper turn, or takes an incorrect number of steps. Research indicates that 79 percent of individuals who exhibit two or more indicators in the performance of the test will have a BAC of 0.8 mg/mL or greater (Stuster & Burns, 1998). When subjects show two or more signs of impairment he/she is indicated as impaired in the current study.

In the OLS test, the subject is instructed to stand with one foot approximately six inches (15 cm) off the ground and count aloud by thousands (1001, 1002, etc.) until told to put the foot down. The examiner times the subject for 30 seconds and looks for four indicators of impairment: swaying while balancing, using arms to balance, hopping to maintain balance, and putting the foot down. Research indicates that 83 percent of individuals who exhibit two or more such indicators in the performance of the test will have a BAC of 0.8 mg/mL or greater (Stuster & Burns, 1998). The time it took to count to 1030 is also noted. Subjects showing two or more indicators of impairment are classified as impaired on this test. Whenever a subject was impaired on 2 or more of these tests, the overall SFST score was also rated as indicative of impairment. Percentages of impaired subjects on each test, i.e. HGN, OLS, WAT and overall SFST, were the dependent variables.

PHARMACOKINETIC ASSESSMENTS

Oral fluid and blood samples (6 mL) were collected at baseline, 15, 30, 45 and 60 minutes during the first hour after smoking and subsequently every 30 minutes between 1 and 4 hours after smoking. Oral fluid from half of the subjects was collected using the Dräger Drug Test® 5000 and the other half with the Securetec Drugwipe® 5 device. With the Securetec Drugwipe® 5 two results are produced: one for oral fluid taken from the cheek and the other from the tongue. The cutoff for cannabinoids as stated by the manufacturers of the Dräger Drug Test® 5000 is 5 ng/mL and of the Securetec Drugwipe® 5 is 30 ng/mL. The blood sample was centrifuged and the resulting serum was frozen at -20°C until analysis. THC, 11-OH-THC and THCCOOH concentrations were determined afterwards (Toennes, Ramaekers, Theunissen, Moeller, & Kauert, 2008). BAC was also assessed in serum at regular intervals (see Toennes, et al., 2011), but only the BAC at the time of the SFST will be reported here.

STATISTICAL ANALYSIS

All statistical analyses were performed using SPSS 18.0 for Mac. For each SFST and for the overall impairment score separate chi-square (χ^2) tests were performed to determine whether a relationship existed between SFST performance and treatment conditions. SFST performance in each alcohol condition (i.e. 0.5 mg/mL + THC and 0.7 mg/mL + THC), and in the THC only condition (alcohol placebo) was compared to baseline. In case of a significant treatment effect, Spearman's coefficient (ρ) was calculated to determine the strength and direction of the relationship between treatment condition and SFST performance. A positive relationship indicates that impairment increases with increasing dose and a negative relationship that impairment decreases with increasing dosage.

For the oral fluid tests the percentages false negatives for THC, i.e. oral fluid tests that indicated the subject was negative for THC divided through the total number of oral fluid tests at every time-point with all treatment conditions collapsed, were calculated. Sensitivity was calculated by dividing true positives, i.e. oral fluid tests that indicated a subject was positive for THC, through true positives + false negatives.

Results

DROPOUTS

Two subjects dropped out after the first treatment condition for reasons unrelated to the study. Incomplete data from drop-outs did not enter statistical analysis.

STANDARDIZED FIELD SOBRIETY TESTS

Percentages impaired subjects on the SFST and associated significances are displayed in Table 1. Cannabis alone was significantly related to OLS impairment ($\chi^2=4.364$, $p=0.037$) and a trend was shown for HGN ($\chi^2=3.399$, $p=0.065$). Both relationships were positive ($\rho=0.3$, $p=0.019$; $\rho=0.3$, $p=0.034$ respectively).

Table 1: Percentages of subjects who showed impairment under the influence of two doses of alcohol + THC and THC alone on overall SFST, HGN^a, OLS and WAT. Significance indicated by p-value (2-sided).

Test	Baseline	THC	0.5 mg/mL BAC + THC	0.7 mg/mL BAC + THC	χ^2 THC	χ^2 Alcohol + THC
Overall SFST	14	30	21	44	NS	0.085
HGN	0	15	11	28	0.065	0.029
OLS	19	50	53	44	0.037	0.072
WAT	57	35	37	67	NS	NS

^a HGN, horizontal gaze nystagmus; OLS, one leg stand; WAT, walk and turn; NS, not significant.

The results showed that the combinations of alcohol and cannabis were significantly related to HGN impairment ($\chi^2=7.110$, $df=2$, $p=0.029$). A trend was shown on overall SFST ($\chi^2=4.939$, $df=2$, $p=0.085$) and OLS impairment ($\chi^2=5.249$, $df=2$, $p=0.072$). All correlations were positive ($\rho=0.3$, $p=0.004$; $\rho=0.3$, $p=0.018$; $\rho=0.2$, $p=0.043$ respectively).

Table 2: Percentages false negatives (FN) and sensitivity (SN) of the Dräger Drug Test® 5000 and Securetec Drugwipe® 5 (from cheek and tongue) and mean THC, 11-OH-THC and THCCOOH concentrations (µg/L) in serum relative to time after smoking collapsed over treatment conditions.

Time after smoking (h)	Dräger Drug Test 5000		Securetec Drugwipe 5 (cheek)		Securetec Drugwipe 5 (tongue)		Drug concentrations		
	FN	SN	FN	SN	FN	SN	THC	11-OH-THC	THCCOOH
baseline	-	-	-	-	-	-	7.1 (1.4)	2.9 (0.7)	50.1 (8.4)
-03:15	28	72	48	52	50	50	-	-	-
-02:45	22	78	46	54	54	46	-	-	-
-00:05	48	52	58	42	54	46	9.1	69.9	5.1
00:15	3	97	8	92	8	92	101.1	98.3	17.5
00:30	3	97	29	71	13	88	47.4	101.4	15.2
00:45	6	94	46	54	38	63	30.1	95.0	12.5
01:00	3	97	48	54	35	65	22.1	90.4	10.7
01:30	0	100	42	58	33	67	18.7	85.3	9.3
02:00	3	97	42	58	38	63	13.1	81.1	7.5
02:30	3	97	54	46	50	50	10.3	77.4	6.2
03:00	10	90	52	48	38	63	8.2	67.4	5.1
03:30	18	82	50	50	43	57	8.5	66.7	5.1
04:00	7	93	52	48	46	54	8.0	65.6	4.6

PHARMACOKINETIC MEASURES

The percentages false negatives and sensitivity of the Dräger Drug Test® 5000 and Securetec Drugwipe® 5 and the associated concentrations of THC, 11-OH-THC and THCCOOH in serum are shown in Table 2. The Dräger Drug Test® 5000 generally performed well after acute administrations of THC. The percentages of false negatives and sensitivities ranged from 0-10% and 90-100% respectively between 15 minutes after smoking up to 3 hours after smoking. However, the test was less accurate at the baseline measures, i.e. before drinking/smoking. At baseline the percentages false negatives ranged from 22-48% and sensitivities from 52-78% while mean THC serum concentration was 7.1 µg/L (range 0-20 µg/L). The performance of the Securetec Drugwipe® 5 was less accurate. Performance was best 15 minutes after smoking, i.e. 8% false negatives, sensitivity 92%. As time after smoking progressed, the percentages false negatives increased and sensitivities were low. At baseline accuracy was low as well.

Mean BAC concentrations during SFST after THC, THC + alcohol 0.5 mg/mL and THC + alcohol 0.7 mg/mL were 0, 0.37 and 0.51 mg/mL respectively. Mean THC concentrations during these conditions were 13.4, 14.4 and 11.6 µg/L respectively.

Discussion

This study was designed to assess the effects of THC and the combination of alcohol and THC on the SFST. Two oral fluid tests, i.e. Dräger Drug Test® 5000 and Securetec Drugwipe® 5, were assessed for their accuracy in determining the presence of THC.

The SFST was mildly sensitive to the effects of cannabis alone. A dose of 400 µg/kg bodyweight THC significantly increased the percentage of subjects displaying impairments in OLS compared to baseline performance from 19 to 30%. THC also increased percentage of individuals showing impairment on HGN from 0 to 15%, relative to baseline, but this change only approached statistical significance. WAT and the overall score on SFST did not discriminate between THC and baseline. These findings appear in line with previous studies that reported a relation between impairment on the SFST and presence of THC in blood. A study that assessed which signs of the Drug Evaluation and Classification (DEC) evaluations predicted various drug categories (including cannabis) best showed that OLS contributed significantly to the prediction, but HGN and WAT did not (Porath-Waller, Beirness, & Beasley, 2009). Papafotiou et al. (2005a) assessed SFST performance in 40 healthy subjects that received low and high doses of THC in a placebo controlled study. On average, blood THC concentrations obtained after the highest dose were comparable to serum THC concentrations achieved in the present study after smoking cannabis. Yet, THC significantly affected performance on OLS, HGN and WAT and appeared to be more prominent as compared to the current study. For example, in that study THC produced impairments on overall SFST in up to 50% of the participants (Papafotiou, et al., 2005a) but in only 30% of the subjects of the present study. These differences may be explained in terms of differences in cannabis use history. In the study by Papafotiou et al. (2005a), the reported frequency of cannabis use of the subjects varied from once a week to once every 2–6 months. The present study however only included heavy cannabis users, who smoked cannabis on at least 4 occasions per week. Previous studies demonstrated that heavy cannabis users develop tolerance to the impairing effects of THC on neurocognitive measures (Hart, et al., 2001; Ramaekers, et al., 2010). It is likely

that many of the subjects that participated in the present study, in part or in total, developed tolerance to impairing effects of THC. In such a scenario, the failure of the SFST to demonstrate robust effects of THC is not necessarily an indicator of poor sensitivity, but may reflect the chronic cannabis use of the participants. Alternatively, it should be mentioned that SFST were performed 2 hrs after smoking in the present study. Performance impairment has been shown to occur for 3-4 hrs after smoking THC (e.g. Ramaekers, Kauert, et al., 2009; Ramaekers, Kauert, et al., 2006), but is maximal during the first hour after smoking. SFST thus may be more sensitive for the effects of THC if conducted right after smoking.

In general, these data indicate that SFST are mildly sensitive to the effects of THC depending on dose and cannabis use history. It is noteworthy however, that SFST were unable to discriminate performance impairments produced by synthetic cannabinoids (dronabinol) (Bosker, et al., submitted). In this placebo controlled study, occasional and heavy users, received oral doses of dronabinol that produced significant on-the-road driving impairments that were comparable to those observed after BACs > 0.8 mg/mL. THC concentrations after oral dronabinol however were much lower (< 10 mg/mL) than those achieved after smoking cannabis. Also, THC concentrations during SFST testing were twice as low in the dronabinol study compared to the present study. This fits with the general conclusion that SFST are mildly sensitive to the impairing effects of THC, but that impairments may go undetected in some individuals, particularly at lower THC concentrations.

THC with alcohol generally increased the number of individuals displaying impairment on HGN, OLS and total SFST score. Relative to baseline, percentages of impaired individuals increased after both alcohol combinations with THC in a dose dependent manner. HGN was the only measure that did not reveal any impairment in subjects during baseline. It should be remembered here that all subjects included were heavy cannabis users who were always positive for THC, also during baseline. On average, baseline THC levels were 7.1 ng/mL. HGN may thus be an interesting parameter to separate residual THC use from recent THC intoxication when OLS and overall SFST show impairment. In general, impairments observed after the combination of THC and alcohol are most likely attributable to alcohol since most of the current subjects may have developed tolerance for the impairing effects of THC on performance. As such, the present data confirm the sensitivity of the SFST for alcohol induced impairment.

The point of collection testing devices provided mixed results. The Dräger Drug Test® 5000 generally performed quite well and only produced false negative in 6% of the measurements during 2.5 hrs after smoking. Securetec Drugwipe® 5 however performed poorly in detecting THC. The rate of false negatives was already 8% 15 minutes after smoking and rapidly increased to about 40-50% within the hour. The difference in sensitivity between both devices may well be related to differences in cut-off levels which are 5 ng/mL in the Dräger Drug Test® 5000 and 30 ng/mL in the Securetec Drugwipe® 5 according to the manufacturers. It should be noted that in this study we did not determine the rate of false positives for both devices because our subject were never drug free. Even during baseline low levels of THC were present in all subjects. A recent roadside study reported about 2% false positives for the Dräger Drug Test® 5000 and about 3% false negatives. Sensitivity, specificity and accuracy were 93, 71 and 90% respectively (Wille, Samyn, Ramirez-Fernandez Mdel, & De Boeck, 2010). Together these data indicate that point of collection testing devices for detecting THC in oral fluid are making large improvements relative

to some of the previous devices that were on the market (Bosker & Huestis, 2009; Ramaekers, Moeller, et al., 2006). Still it has been argued in the ROSITA-2 project that the sensitivity and specificity of oral fluid devices should be higher than 90% (Verstraete & Raes, 2006). According to these criteria, the Dräger Drug Test® 5000 would still fall short on specificity (Wille, et al., 2009). However, compared to the alternative matrix urine, oral fluid drug concentrations have a better correlation with blood concentrations and recent use of cannabis and are therefore still the preferable matrix for evaluating THC presence in drivers (Bosker & Huestis, 2009; Toennes, Kauert, et al., 2005).

Taken together the results indicate that the SFST are mildly sensitive to THC use in heavy users, probably because many of the subjects have developed behavioral tolerance to THC induced impairments. SFST were sensitive to low levels of alcohol in combination with THC as indicated by increments in the number of subjects rated as impaired on HGN, OLS and total SFST score. The Dräger Drug Test® 5000 achieved a high sensitivity for THC after acute THC administration in the present study, but sensitivity in case of the Securetec Drugwipe® 5 was low.

CHAPTER 5: SUSTAINED ABSTINENCE IMPROVES ATTENTION AND MOTOR CONTROL IN CHRONIC DAILY CANNABIS SMOKERS

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Bosker, W. M., Karschner, E. L., Lee, D., Goodwin, R. S., Hirvonen, J., Innis, R. B., Theunissen, E. L., Kuypers, K. P. C., Huestis, M. A. & Ramaekers, J. G. (submitted). Sustained abstinence improves attention and motor control in chronic daily cannabis smokers.

CONTEXT Evaluation of performance impairment during abstinence from chronic daily cannabis smoking is confounded by residual Δ^9 -tetrahydrocannabinol concentrations, withdrawal symptoms, and persistent neuropsychological dysfunction due to cumulative lifetime cannabis use.

OBJECTIVE The present study assessed attentional and motor functions of chronic, daily cannabis smokers during 3 weeks of continuously monitored abstinence on a closed research unit. It was hypothesized that attentional and psychomotor performance of chronic, daily cannabis smokers would improve during abstinence.

DESIGN, SETTING AND PARTICIPANTS Psychomotor and attentional performance was assessed in nineteen male chronic, daily cannabis smokers at baseline and after 8, 14-16 and 21-23 days of continuously monitored abstinence. Performance was compared to a control group of non-intoxicated occasional drug users.

MAIN OUTCOME MEASURES Critical frequency (λ_c) of the critical tracking task and tracking error and control losses of the divided attention task were the primary outcome measures.

RESULTS Results showed that chronic cannabis smokers' baseline performance was impaired as compared to controls, although attention and motor performance improved during abstinence. Performance did not fully return to the level of occasional drug users over three weeks of abstinence.

CONCLUSIONS Sustained cannabis abstinence moderately improved critical tracking and divided attention performance in chronic, daily cannabis smokers, although impairment was still observable compared to controls.

Introduction

Cannabis is the most commonly used illicit substance in the world (UNODC, 2010). About 6.6% of the general US population 12 years and older and 3.7% of Europeans 15 years and older were recent cannabis smokers (EMCDDA, 2010; SAMHSA, 2010). Although most do not become regular users, it was estimated that in the US 36.7% and in Europe 25% of past month smokers used cannabis on 20 days or more in the previous month. Furthermore, approximately 4.3 million people or 1.7% of the US population aged 12 years or older was dependent on cannabis in 2009 (SAMHSA, 2010). In Europe 4 million people or >1% of Europeans smoked cannabis daily or almost daily (EMCDDA, 2010).

Chronic cannabis use was associated with neuropsychological (e.g. memory) deficits (e.g. Battisti, et al., 2010; Solowij & Battisti, 2008; Solowij, et al., 2002) and changes in brain morphology (Lorenzetti, Lubman, Whittle, Solowij, & Yucel, 2010), even when individuals were not intoxicated. Were these changes due to residual effects of recent cannabis use or long-term persistent cognitive deficits? Neuropsychological function was impaired for at least 7 but not 28 days of abstinence in long-term cannabis smokers (Pope, Gruber, Hudson, et al., 2001). It was concluded that these memory deficits were reversible and related to recent cannabis exposure, rather than irreversible and due to cumulative life time use. However, the same research group also reported diminished brain activation after 28 days of cannabis discontinuation in motor cortical circuits during a finger-tapping task (Pillay, et al., 2008). Thus, some cognitive deficits in long-term cannabis smokers are reversible, whereas motor impairments may persist during abstinence.

Animal research also demonstrated changes in the endocannabinoid system during abstinence. Chronic cannabis use produced desensitization and down-regulation of CB1 receptors in animals (e.g. Sim-Selley, 2003; Sim-Selley, et al., 2006). These changes were potentially reversible upon discontinuation of cannabis use. Receptor levels in mice recovered after approximately 1-2 weeks of abstinence (Sim-Selley, et al., 2006). In animal models, activation of CB1 receptors by either natural or synthetic agonists or the endogenous ligand anandamide induced alterations in motor behavior, such as catalepsy, immobility, ataxia or impairment of complex motor behavior (e.g. Giuffrida, Desarnaud, & Piomelli, 2000; Rodriguez de Fonseca, Del Arco, Martin-Calderon, Gorriti, & Navarro, 1998). In humans CB1 receptor density was downregulated in chronic, daily cannabis smokers, which returned to normal levels after 4 weeks of abstinence (Hirvonen, et al., 2011). Consequently, these changes in CB1 receptor density during abstinence also would be expected to affect motor control.

The motor function impairing effects of cannabis were extensively demonstrated in placebo-controlled experimental studies in occasional cannabis smokers. Such studies generally showed that acute cannabis administration impairs reaction time, tracking, divided attention and actual driving performance in a dose-dependent manner (Hunault, et al., 2009; Ramaekers, et al., 2004; Ramaekers, Kauert, et al., 2009; Ramaekers, Kauert, et al., 2006; Ramaekers, Moeller, et al., 2006; Ramaekers, et al., 2010). Δ^9 -tetrahydrocannabinol (THC) dosages ranged from 17.5-69.4 mg/joint in these studies of acute cannabis effects on motor performance. Currently, it is unclear whether the acute motor impairment persists for multiple days due to residual THC concentrations, or for a prolonged period due to cumulative lifetime use.

The present study was designed to assess the effects of sustained abstinence (3 weeks) on psychomotor performance of chronic daily cannabis smokers. This investigation was part of a

larger clinical project exploring CB1 receptor availability during cannabis dependence and extended abstinence (Hirvonen, et al., 2011). We hypothesized that CB1 receptors are downregulated in specific brain areas during chronic cannabis use and upregulated in specific brain areas during extended abstinence. Based on these assumptions, we predicted that psychomotor function of chronic, daily cannabis smokers would improve during abstinence.

Method

PARTICIPANTS

Nineteen chronic daily cannabis smokers, mean (SE) age 27.6 (6.4) years, participated in this sustained continuously monitored cannabis abstinence study. Participants self-reported smoking 10.9 (6.8) cannabis joints per day for the last 10.5 (5.1) years. Inclusion criteria were: male; 18-65 years of age; written informed consent; healthy based on history and physical examination; smoked cannabis at least 5 days/week for the 6 months prior to admission; and a positive urine cannabinoid test within 90 days of admission. Exclusion criteria were: history or presence of any clinically significant illness; past or present diagnosis of schizophrenia, bipolar illness or any other psychotic disorder; need for psychoactive medication within the preceding 28-day period (42 days for fluoxetine); current physical dependence on any substance other than cannabis, nicotine or caffeine; positive test for HIV; metallic foreign bodies or fear of enclosed spaces; head trauma resulting in a period of unconsciousness lasting longer than 10 minutes; history of fetal alcohol syndrome or other neurodevelopmental disorder; history of seizures; recent exposure to radiation, i.e. PET from other research; inability to lie flat on the camera bed for approximately 2.5 h; regular use of alcohol, i.e. ≥ 6 standard drinks per day four or more times per week in the month prior to study entry; currently interested in or participating in drug abuse treatment, or participation within 60 days preceding study enrollment.

The control group consisted of 30 occasional drug users (primarily cannabis and/or MDMA) who participated previously in double-blind placebo controlled studies conducted at Maastricht University, The Netherlands to assess the effects of single doses of cannabis or MDMA on psychomotor function (Bosker, Kuypers, Conen, & Ramaekers, 2010; Kuypers, et al., 2007; Ramaekers, Kauert, et al., 2009; Ramaekers, Moeller, et al., 2006). Performance during placebo treatment (i.e. subjects were negative for any drug) was taken as a general reference level of normal performance against which psychomotor performance of chronic daily cannabis smokers could be tested. Psychomotor test procedures were identical for controls and subjects in the abstinence group. Inclusion and exclusion criteria were generally the same for the control subjects as the abstinence group, except that control subjects were males and females and had experience with either MDMA, i.e., at least one time in the previous year, or cannabis, i.e., weekly use or less in the previous year. Controls' mean age was 23 years.

This study was conducted according to the code of ethics on human experimentation established by the declaration of Helsinki (1964) and amended in Seoul (2008). Approval for the study was obtained from the Institutional Review Board (IRB) of the National Institute of Mental Health (NIMH).

PROCEDURES AND STUDY DESIGN

Chronic daily cannabis smokers underwent screening procedures, including medical history, physical examination, clinical laboratory tests, standard 12-lead ECG, determination of psychological and drug use eligibility. When subjects were found eligible to participate, they were housed on the closed, secure Johns Hopkins Behavioral Pharmacology Research Unit (BPRU), Baltimore, USA for the duration of the study. Performance tests were administered at NIDA and subjects were under continuous medical supervision. Psychomotor tests for measuring attention and motor performance included critical tracking and divided attention tasks administered on days 1 or 2 (baseline), day 8, day 14, 15 or 16, and day 21, 22 or 23. Psychomotor tests were developed and installed at the US testing site by research team members from Maastricht University, The Netherlands. All subjects received extensive training on the psychomotor tests (at least 20 trials) before participation in the study until stable performance was achieved (less than 10% variance in 5 consecutive measurements).

CRITICAL TRACKING TASK

The critical tracking task measures the subject's ability to control a displayed error signal in a 1st-order compensatory tracking task. Error appears as horizontal deviation of the cursor from midpoint on a horizontal, linear scale. Compensatory joystick movements null the error by returning the cursor to the midpoint. The frequency of cursor deviations, and therefore its velocity, increases as a stochastic, linear function of time. The subject is required to make compensatory movements with a progressively higher frequency until the subject loses control. The frequency at which control loss occurs is λ_c (the critical frequency). The reciprocal of this frequency is theoretically the perceptual/motor delay lag for humans operating in a closed-loop system. The participant performs this test in five trials and the mean λ_c is recorded as the final score (Jex, McDonnell, & Phatak, 1966). The test has demonstrated sensitivity to the impairing effects of THC (Ramaekers, Kauert, et al., 2009; Ramaekers, Moeller, et al., 2006; Ramaekers, et al., 2010).

DIVIDED ATTENTION TASK

The divided attention task assesses the ability to divide attention between two tasks performed simultaneously. The primary task required the use of a joystick to continuously null the horizontal movement of a cursor from the center of a display as described above in the critical tracking task. The velocity of the cursor was kept constant at 50% of the participant's optimal performance ($\lambda_c / 2$). The dependent measure of this subtask was control losses, i.e. the number of times a participant could not keep the cursor within a predefined range. Tracking error, measured by the absolute distance (mm) between the cursor's position and the center was another dependent measure of this subtask. The secondary task involved monitoring 24 single-digit numbers (0-9) that were arranged in the four corners of the display. The numbers changed asynchronously every 5 seconds. The requirement was to react as rapidly as possible by lifting the foot from a pedal every time a target, i.e. the number 2, appeared. Average reaction time to target and percentages hit were recorded as the dependent measure (Moskowitz, 1973). The divided attention task also has demonstrated sensitivity to the impairing effects of THC (Ramaekers, Kauert, et al., 2009; Ramaekers, et al., 2010).

PHARMACOKINETIC ASSESSMENT

Oral fluid and blood were collected before the 4 test sessions, i.e. on days 1 or 2 (baseline), 8, 14-16 and 21-23. Blood samples were centrifuged and the resulting plasma was frozen at -20°C until analysis. Plasma specimens were analyzed for THC, 11-OH-THC and THCCOOH concentrations by a previously published analytical method (Lowe, Karschner, Schwilke, Barnes, & Huestis, 2007). Low dynamic ranges were linear with $r^2 > 0.990$ between 0.25-25 for THC and THCCOOH and 0.5-25 for 11-OH-THC. Analytes quantifying $> 25 \mu\text{g/L}$ were quantified on a higher calibration curve spanning 25-100 $\mu\text{g/L}$. Quality control samples were analyzed with each analytical run at 0.35, 2, 20, 30, 60 and 90 $\mu\text{g/L}$. Imprecision was $\leq 13.6\%$ (%relative standard deviation) and analytical bias was within $\pm 14\%$ of target for all analytes.

Oral fluid samples were collected with the Immunalysis Quantisal™ device, an absorptive cellulose pad mounted on a polypropylene stem with a volume adequacy indicator. The pad, placed into the participant's mouth until the designated volume [1.0 (0.1) mL] was collected, was then put into a plastic tube containing 3 mL elution and stabilizing buffer, yielding a 1:4 oral fluid dilution. The tube was capped and refrigerated for at least 24 h. Pads were squeezed dry with a serum separator before decanting into a Nunc® cryotube and stored at -20°C before analysis. Oral fluid samples were analyzed for THC, CBD, CBN, and THCCOOH according to a previously published 2-dimensional gas chromatography-mass spectrometry (2D-GC-MS) method employing two analytical systems with different ionization techniques (Milman, Barnes, Lowe, & Huestis, 2010). Linear ranges were 0.5–50 $\mu\text{g/L}$, with the exception of cannabitol (1–50 $\mu\text{g/L}$) and THCCOOH (7.5–500 ng/L). Analytical recovery and imprecision were evaluated across the linear range with three QC samples (1.5, 7.5 and 30 $\mu\text{g/L}$ for THC, CBD and CBN; 15, 75 and 300 ng/L for THCCOOH) (Milman, et al., 2010). Analytical recovery ranged from 99.1-113.8%. Inter-assay and intra-assay imprecision (%RSD) ranged from 2.2 to 6.6% and 0.6-5.2%, respectively.

STATISTICAL ANALYSIS

All statistical analyses were performed with SPSS 19.0 for Mac. A general linear model (GLM) repeated measures analysis of variance (ANOVA) was performed with days of abstinence (3 levels: baseline, day 8, day 14-16) as the main within subjects factor to assess overall differences in performance over the test days. If the sphericity assumption was violated, the Greenhouse-Geisser correction was used. Assessments of overall effects of days of abstinence were followed by simple contrasts comparing performance on day 8 and 14-16 versus baseline. Data collected on days 21-23 were not included in the overall analyses but contrasted separately to baseline, because multiple subjects withdrew from the study prior to 3 weeks abstinence. Inclusion in the overall analysis would have reduced the overall number of subjects that completed all test days to N=12. Differences between the control and experimental groups at baseline and during abstinence were tested using a one-way ANOVA with planned comparisons.

Results

MISSING VALUES

Nineteen subjects had complete data sets on the divided attention task from baseline until day 14-16. Two subjects had missing data on the critical tracking task (one on day 8 and one on day 14-16) and thus, seventeen subjects entered the repeated measures analysis. By day 21-23, seven

subjects voluntarily withdrew from the study for family reasons or because they no longer wanted to remain on the closed research unit. Consequently, twelve subjects were included in the analysis comparing performance on the critical tracking and the divided attention tasks between baseline and day 21-23.

Table 1 shows mean (SE) performance on the critical tracking and divided attention tasks of controls and chronic, daily cannabis smokers at baseline, and after 8, 14-16, and 21-23 days of abstinence.

Table 1: Mean (SE) critical tracking task and divided attention performance of chronic, daily cannabis smokers and controls. Performance of chronic cannabis smokers was assessed at baseline and after 8, 14-16, 21-23 days of abstinence.

Cannabis use history	Chronic cannabis smokers				Controls
Days of abstinence	Baseline	Day 8	Day 14-16	Day 21-23	
Critical tracking task					
λ_c (rad/s)	2.8 (0.2)	3.1 (0.2)	3.1 (0.1)	3.0 (0.3)	3.8 (0.2)
Divided attention task					
Control loss (N)	23.4 (8.2)	8.7 (3.1)	5.6 (1.6)	8.8 (2.1)	1.2 (0.4)
Tracking error (mm)	19 (1.0)	18 (1.2)	17 (1.2)	17 (1.8)	14 (0.8)
Hit (%)	87.2 (2.8)	89.5 (2.6)	87.0 (4.4)	85.4 (5.7)	94.6 (1.3)
RT (ms)	1892 (72)	1828 (70)	1854 (101)	1830 (74)	1880 (53)

CRITICAL TRACKING TASK

Mean λ_c demonstrated a trend effect of Days of abstinence ($F_{2,32}=2.540$, $p=0.095$). Simple contrasts indicated that subjects performed better after 8 days ($F_{1,16}=3.203$, $p=0.092$), after 14-16 days ($F_{1,16}=3.487$, $p=0.080$) and after 21-23 days of abstinence ($F_{1,11}=5.096$, $p=0.045$) compared to baseline. The chronic, daily smokers also differed significantly from the control group ($F_{4,92}=5.430$, $p=0.001$). Planned comparisons noted differences at baseline ($p<0.001$), after 8 ($p=0.004$), 14-16 ($p=0.006$) and 21-23 days of abstinence ($p=0.008$) compared to controls. Mean (SE) performance is shown in Figure 1.

Figure 1: Mean (SE) critical frequency of the critical tracking task as a function of time of abstinence. # indicates significant difference ($p<0.05$) from baseline; * indicates significant difference ($p<0.05$) from control group.

DIVIDED ATTENTION TASK

Overall, the number of control losses decreased during abstinence and approached statistical significance ($F_{1,186,20.160}=3.495$, $p=0.070$). Simple contrasts showed that the number of control losses significantly decreased after 14-16 days of abstinence ($F_{1,17}=4.611$, $p=0.046$), but trended at 21-23 days of abstinence ($F_{1,11}=3.850$, $p=0.076$), relative to baseline. Compared to the control group, chronic, daily cannabis smokers had significantly more control losses ($F_{4,93}=5.051$, $p=0.001$). Planned comparisons indicated this was true at baseline ($p=0.014$), 8 ($p=0.029$), 14-16 ($p=0.017$) and 21-23 days of abstinence ($p=0.004$). Mean (SE) number of control losses in both groups are displayed in Figure 2.

Figure 2: Mean (SE) number of control losses on the divided attention task as a function of time of abstinence in chronic daily cannabis smokers. # indicates significant difference ($p<0.05$) from baseline; * indicates significant difference ($p<0.05$) from control group.

Tracking error decreased during abstinence ($F_{2,34}=7.226$, $p=0.002$). Simple contrasts indicated that tracking error of daily cannabis smokers significantly decreased after 8 ($F_{1,17}=5.382$,

Figure 3: Mean (SE) tracking error on the divided attention task as a function of time of abstinence in chronic daily cannabis smokers. # indicates significant difference ($p<0.05$) from baseline; * indicates significant difference ($p<0.05$) from control group.

p=0.033) and 14-16 days of abstinence ($F_{1,17}=13.370$, $p=0.002$). The decrease in tracking error after 21-23 days of abstinence approached significance ($F_{1,11}=4.028$, $p=0.070$). Compared to controls, chronic daily cannabis smokers displayed significantly larger tracking error ($F_{4,93}=4.315$, $p=0.003$). Planned comparisons showed significant increments in tracking error of chronic cannabis smokers at baseline ($p<0.001$), after 8 ($p=0.008$), 14-16 ($p=0.024$) and 21-23 days of abstinence ($p=0.037$) compared to controls. Mean (SE) tracking error in both groups is displayed in Figure 3.

Reaction time and percentages hits of the experimental group did not differ during abstinence or in comparison to the control group.

PHARMACOKINETIC ASSESSMENT

Mean THC, 11-OH-THC and THCCOOH plasma concentrations and THC and THCCOOH oral fluid concentrations are shown in Table 2. At baseline, 1 subject (5.3%) was negative for THC in plasma, this increased to 15.8% of subjects after 8 and 14-16 days of abstinence. After 21-23 days of abstinence, 33.3% of subjects were plasma THC negative.

Table 2: Mean (SE) THC, 11-OH-THC and THCCOOH concentrations and percentage THC positive subjects in plasma and THC and THCCOOH concentrations in oral fluid of chronic daily cannabis smokers as a function of days of abstinence.

Days of abstinence	Plasma				Oral fluid	
	% THC negative	THC (µg/L)	11-OH-THC (µg/L)	THCCOOH (µg/L)	THC (µg/L)	THCCOOH (pg/mL)
Baseline (N=19)	5.3	5.3 (1.2)	2.1 (0.4)	54.2 (9.0)	29.5 (11.5)	48.2 (8.4)
Day 8 (N=19)	15.8	1.3 (0.2)	0.2 (0.1)	6.9 (1.0)	0.0 (0.0)	4.4 (2.8)
Day 14-16 (N=19)	15.8	0.8 (0.2)	0.1 (0.04)	4.0 (0.6)	0.0 (0.0)	5.1 (2.8)
Day 21-23 (N=12)	33.3	0.4 (0.2)	0.0 (0.0)	2.2 (0.7)	0.0 (0.0)	2.5 (2.5)

Discussion

This study was designed to assess the effects of cannabis abstinence on psychomotor performance and attention in chronic, daily cannabis smokers. Their performance on the critical tracking and divided attention tasks was compared to an occasional drug-using control group. Results showed that attention and motor performance of chronic, daily cannabis smokers improved throughout 3 weeks of abstinence. However, their performance was significantly poorer than a control group of occasional drug users.

The critical tracking task is a measure of perceptual motor control. The primary dependent variable is λ_c , which defines the perceptual/motor delay lag for humans operating in a closed-loop system. Real world tracking is prominent in many aspects of vehicle control such as bicycles, cars and airplanes. In essence, the task assesses human operator performance where the person may perceive a discrepancy or error between a desired state and an actual state (i.e. cursor deviates from the middle position or a car deviates from the middle lane position) and aims to reduce the error by compensatory movement during a continuous closed-loop system. Chronic, daily cannabis smokers performed relatively poorly on this task, indicating that they were slow in initiating a compensatory response to error signals when compared to controls. Mean λ_c also significantly increased after 3 weeks of abstinence, demonstrating critical tracking performance

recovery in chronic cannabis smokers. However, critical tracking performance did not fully recover to normal levels after 3 weeks of abstinence and was still significantly worse compared to critical tracking of the control group.

Similar results were obtained in the divided attention task. At baseline, tracking performance and tracking control were impaired in chronic, daily cannabis smokers compared to a control group of occasional drug users; i.e. tracking error and number of control losses were significantly higher in the chronic smokers. During abstinence, tracking performance and control significantly improved. These improvements were clearly visible after 1, 2 and 3 weeks of abstinence. They only failed to reach significance in the 3rd week, likely because of a loss of statistical power resulting from the large number of withdrawals during this period. However, tracking performance of chronic, daily cannabis smokers remained impaired throughout abstinence as compared to controls, albeit the differences became smaller in magnitude.

Together, these results indicate prolonged impairment of attention and motor function in chronic cannabis smokers that only partially recovered over 3 weeks of continuously monitored abstinence on a closed research unit. In the past, several explanations were given for impairments observed in chronic cannabis smokers. First, it was suggested that such impairments may arise from withdrawal from daily cannabis use (Pope, Gruber, & Yurgelun-Todd, 2001). These authors also noted that withdrawal symptoms were most prominent between 3-7 days after abstinence onset. Thus, withdrawal might have affected performance of chronic smokers, but primarily during the 1st week of abstinence. The effect of withdrawal on psychomotor performance after 3 weeks of abstinence in chronic, daily smokers is probably minimal, but cannot be excluded.

Second, it was suggested that impairments in chronic cannabis smokers may result from residual THC concentrations in blood (Pope, Gruber, Hudson, et al., 2001), which may remain present in sustainable amounts for several days after last use (Karschner, Schwilke, Lowe, Darwin, Herning, et al., 2009; Karschner, Schwilke, Lowe, Darwin, Pope, et al., 2009; Ramaekers, Kauert, et al., 2009). This finding also is illustrated by the fact that in the present study, 8 of 12 chronic smokers were still positive for THC after 3 weeks of abstinence. However, mean THC concentrations were low (i.e. $<1 \mu\text{g/L}$ in plasma). Such low concentrations are below THC thresholds (i.e. $2\text{--}5 \mu\text{g/L}$ in serum), above which THC impairment becomes apparent in psychomotor functions (Ramaekers, Kauert, et al., 2009; Ramaekers, Moeller, et al., 2006). Residual THC concentrations, therefore, may not account for the psychomotor impairment observed in chronic cannabis smokers in the present study.

Alternatively, prolonged impairment may have resulted from cumulative lifetime intake and reflect persistent changes in attentional and motor functions in chronic cannabis smokers. Previous reports indicated that recently abstinent smokers had reduced activation in motor cortical areas and that such deactivation was still observed after 28 days of cannabis discontinuation (Pillay, et al., 2008). Similar results were shown by Hirvonen et al. (2011) who also demonstrated that CB1 receptor density in motor areas did not downregulate due to chronic, daily cannabis smoking. Moreover, subjects in the present study started their cannabis use early in life (mean 14.7 years), suggested to be an additional risk factor for developing long-term neuropsychological dysfunction in chronic cannabis smokers (Pope, et al., 2003). Performance impairment may arise from lifetime cannabis smoking and persist for at least 3 weeks after discontinuation. However, based on these data, it is unknown whether such impairment is truly long-term and persistent, as

additional follow-up of subjects during longer periods of abstinence (i.e. 6-12 months) would be required to ensure that potentially confounding factors, such as residual THC concentrations, withdrawal symptoms, and psychological dependence, no longer interfere with neuropsychological performance. A strength of the current study was the residence of participants on a closed research unit to ensure no cannabis access.

Some limitations of the current study design should be noted. Subjects participating in the US (i.e. the daily, chronic cannabis smokers) were not fully matched with control subjects from a Dutch sample of occasional users. Both groups were reasonably comparable in terms of age (i.e. 23 vs. 28 years) but differed in terms of gender and race. Chronic cannabis smokers were all males, whereas the control group consisted of males and females. The Dutch sample consisted of Caucasian subjects, whereas the US sample contained a mixture of African-American and Caucasian subjects. It cannot be excluded that differences in subject demographics also contributed to between group differences observed at the psychomotor level.

In sum, attention and motor function of chronic cannabis smokers improved during 3 weeks of monitored abstinence, but did not recover to a normal performance level as assessed in a control group of occasional drug users.

**CHAPTER 6: MDMA (ECSTASY) EFFECTS ON ACTUAL DRIVING
PERFORMANCE BEFORE AND AFTER SLEEP DEPRIVATION, AS FUNCTION OF
DOSE AND CONCENTRATION IN BLOOD AND ORAL FLUID**

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RATIONALE Experimental research has shown that 3,4-methylenedioxymethamphetamine (MDMA) can improve some psychomotor driving skills when administered during the day. In real life however, MDMA is taken during the night, and driving may likely occur early in the morning after a night of “raving” and sleep loss.

OBJECTIVES The present study assessed the effects of MDMA on road tracking and car following performance in on-the-road driving tests in normal traffic.

METHODS Sixteen recreational MDMA users participated in a randomized double-blind placebo-controlled 4-way cross-over design. They received single, evening doses of 0, 25, 50 and 100 mg MDMA on separate occasions. Actual driving tests were conducted in the evening when MDMA serum concentrations were maximal and in the morning after a night of sleep loss.

RESULTS The primary measure of driving, i.e. Standard Deviation of Lateral Position (SDLP, a measure of weaving) was significantly increased during driving tests in the morning in all treatment conditions, irrespective of MDMA dose and concentration. The increments in SDLP were of high clinical relevance and comparable to those observed for alcohol at blood alcohol concentrations > 0.8 mg/mL. These impairments were primarily caused by sleep loss.

CONCLUSION In general, MDMA did not affect driving performance nor did it change the impairing effects of sleep loss. It is concluded that MDMA cannot compensate for the impairing effects of sleep loss and that drivers who are under the influence of MDMA and sleep deprived are unfit to drive.

Introduction

3,4-Methylenedioxymethamphetamine (MDMA) is the main psychoactive constituent of ecstasy. Ecstasy has stimulant and hallucinogenic effects, and has often been described as an entactogenic drug because of its characteristic that it makes people feel close to each other. Ecstasy is a popular drug: in 2007 9.5 million European adults had ever used ecstasy, which is 2.8% of the general population (EMCDDA, 2008b). In the USA a trend of increasing ecstasy use among adults in 2006-2007 is noticeable, which remained stable in 2008 (SAMHSA, 2009). In Europe as well as in the USA ecstasy is one of the most commonly used illicit drugs after cannabis.

The widespread use of ecstasy might have implications for traffic safety. Epidemiological studies show an increased risk of accidents while driving under the influence of drugs and/or alcohol (EMCDDA, 2008b). Data on illicit substances such as cannabis, amphetamines (including MDMA) and other stimulants in traffic seem to point out that driving home after a party under the influence of so called 'party drugs' is increasing (Morgan, 2000; Ojaniemi, et al., 2009; Walsh, de Gier, Christopherson, & Verstraete, 2004). A recent report of the National Highway and Traffic Safety Association reported that 10.5% of drivers during nighttime were under the influence of illicit drugs (Lacey, et al., 2009).

Driving is a complex task that requires several cognitive functions. There is ample evidence that long term use as well as acute use of MDMA detrimentally affects cognition. Multiple reviews have indicated that chronic MDMA use impairs cognitive performance on tasks measuring working and episodic memory, attention, frontal-executive functions, impulsiveness and psychomotor speed (e.g. Kalechstein, et al., 2007; Morgan, 2000; Parrott, 2006; Zakzanis, et al., 2007). Acute studies demonstrated attentional and memory deficits during MDMA intoxication. For example, Kuypers and Ramaekers (2005, 2007) reported impairments in verbal and spatial memory performance after a single dose of 75 mg MDMA. Dumont et al. (2008) also described acute impairment in memory as well as in attention after a single dose of 100 mg MDMA. In contrast, psychomotor performance improved after a single acute dose of MDMA (Lamers, et al., 2003), showing that MDMA also possesses stimulating properties.

A number of studies have assessed the effects of MDMA on driving performance in actual driving studies and driving simulator studies. Studies that assessed on-the-road driving performance showed that acute administration of regular recreational doses of 75 or 100 mg MDMA improved road tracking performance (Kuypers, et al., 2006; Ramaekers, Kuypers, et al., 2006). However, MDMA also impaired other aspects of driving such as car-following performance (Ramaekers, Kuypers, et al., 2006). Subjects overreacted to speed decelerations of a leading car as indicated by a significant 'overshoot' in their adaptive response. Another on-the-road driving study indicated that the stimulating effects of MDMA on driving performance were only mild, and not sufficient to counteract the impairing effect of alcohol when used in combination (Kuypers, et al., 2006). The latter finding was also reported by Brookhuis et al. (2004) who assessed simulated driving performance of rave party visitors before and after the party in a quasi controlled study. All of the participants used multiple drugs, including MDMA and alcohol. Drug users clearly had higher accidents rates and displayed more risk taking behaviors early in the morning, when compared to non-drug using controls. However it was difficult to determine whether these impairments in the drug using group resulted from MDMA use, polydrug use or sleep deprivation.

The present study was designed to assess effects of MDMA and sleep deprivation on actual driving performance, separately and in combination. In addition, the present study assessed the association between MDMA concentration in blood and oral fluid. In order to cover a wide range of concentrations three doses of MDMA (25, 50 and 100 mg) were included in the design. Driving performance was measured in the evening after administration of placebo or MDMA and in the morning after a night of sleep loss. It was expected that MDMA would produce stimulant effects in the evening, but impairment in the morning after a night of sleep loss.

Method

SUBJECTS

Eight males and eight females participated in this study (N=16). Their mean (SE) age was 22.0 (0.41) years and their mean (SE) lifetime MDMA use was 27.0 (8.4) times. Participants were recruited by advertisements at Maastricht University and were paid upon completion of the study. Before enrollment all subjects were screened by means of a telephone interview to determine whether they qualified for the study. The inclusion criteria were: experience with MDMA, i.e. at least 1 time in the last year; free from psychotropic medication; good physical health as determined by a medical examination; absence of any major medical, endocrine and neurological condition; normal weight, i.e. BMI between 18 and 28; valid driving license and written informed consent. The exclusion criteria were history of drug abuse or addiction; pregnancy or lactation; cardiovascular abnormalities on electrocardiogram; excessive drinking, i.e. more than 20 alcoholic consumptions a week; hypertension, i.e. systolic blood pressure over 170 mmHg or diastolic blood pressure over 100 mmHg; history of or current psychiatric disorder. If subjects met the inclusion criteria, they received a medical history and a drug questionnaire to get a more precise view on their health and drug use. Finally, participants underwent a medical examination and took part in a training session.

The study was conducted according to the code of ethics on human experimentation established by the declaration of Helsinki (1964) and amended in Seoul (2008). Approval for the study was obtained from the Medical Ethics committee of the Academic Hospital of Maastricht and Maastricht University. A permit for obtaining, storing and administering MDMA was obtained from the Dutch drug enforcement administration.

STUDY DESIGN

The study was conducted according to a double blind, placebo-controlled, randomized, 4-way cross-over design. Treatments consisted of single doses of placebo, 25, 50 and 100 mg MDMA. Treatment orders were balanced over subjects and treatment periods. Placebo and MDMA were administered orally in identically appearing formulations. MDMA was dissolved in 25 mL bitter orange peel syrup and placebo consisted of only the bitter orange peel syrup. The syrup was mixed with 200 mL juice before it was given to the participants. The wash-out period between treatments was at least one week.

PROCEDURE

Subjects were asked to refrain from any drugs one week before the medical examination until two weeks after study completion. Subjects were not allowed to drink alcohol and caffeine or smoke

tobacco during a 24 hour period prior to testing. Subjects were always tested for alcohol and drugs in breath and urine upon arrival (4:30 pm) at the laboratory on test days. In case of a positive result, subjects were send home and asked to come back on another day. This happened in case of one subject. After repeated violation this subject was excluded and replaced. At 5:00 pm participants received a light, standard dinner and at 5:15 pm MDMA or placebo was administered. Driving performance was assessed in the evening and in the morning after a night of sleep loss and psychomotor performance in the evening, in the middle of the night and in the morning. Subjective questionnaire were administered throughout the night. The timeline for performance testing, questionnaires and blood draws is displayed in Figure 1. An additional blood sample was drawn one week after each testing day to monitor renal and liver function. A test day ended at 9:00 am the next morning at which time participants were driven home.

Figure 1: Timeline for blood samples, questionnaires and performance tests relative to drug administration. KSS, Karolinska sleepiness scale; Ph, physiological measures; CTT, critical tracking task; TQ, treatment questionnaire.

ACTUAL DRIVING TESTS

The road tracking test (O'Hanlon, 1984) consists of driving in a specially instrumented car with a constant speed of 95 km/h and as straight as possible on the right lane of primary highway during a 1 hour test ride. A video-camera mounted on the rear end of the car registers its lateral position relative to the road delineation. The images are recorded onto a hard drive in the car with a frequency of 4 Hz and are transformed into a file containing the measures of the lateral position. An off line editing routine is applied for removal of all data segments that reveal signal loss, disturbance or occurrence of passing maneuvers. The edited dataset is then used to calculate means and variances for lateral position. The primary dependent measures of this test is the Standard Deviation of Lateral Position (SDLP; i.e. a measure of weaving). Speed and Standard Deviation of speed (SD Speed) are recorded as secondary control measures. The highway driving test has been calibrated in a manner allowing expression of any sedative drug effect in terms of the blood alcohol concentration (BAC) required to achieve the equivalent level of driving impairment (Louwerens, et al., 1987). The alcohol calibration curve demonstrates that drinkers' mean SDLP rises exponentially with BAC. Results from the alcohol calibration study can be used for describing drugs' effects on SDLP in terms of respective BAC equivalencies. The change in SDLP at a BAC of 0.5 mg/ml (i.e. 2.4 cm) has been used as a criterion level to quantify drug effects. Any drug induced changes in SDLP that exceed this criterion value are defined as clinically relevant impairing drug effect in the present study.

The car-following test (Brookhuis, et al., 1994; Ramaekers, et al., 1995) consists of two cars driving in tandem on a secondary road. The leading vehicle is operated by a study staff member, the following vehicle is operated by the subject who is accompanied by a driving instructor. The

test begins with the two vehicles traveling in tandem at speeds of 70 km/h on a secondary highway. Subjects attempt to drive 15-30 m behind the preceding vehicle and to maintain that headway as it executes a series of deceleration maneuvers. During the test, the speed of the leading car is automatically controlled by a modified cruise-control system. At the beginning it is set to maintain a constant speed of 70 km/h, and by activating a microprocessor, the investigator can start sinusoidal speed changes reaching an amplitude of -10% and returning to the starting level within 50 sec. The maneuver is repeated 6-10 times. Speed signals collected during speed maneuvers enter a power spectral analysis for yielding phase-delay between the vehicle's velocities at the maneuver cycle frequency (0.02 Hz). Phase delay converted to a measure of Time to Speed Adaptation (TSA, in s) is the primary measure. Gain and coherence are secondary control measures. Gain is the amplification factor between both speed signals collected from the leading and following vehicle and indicates the magnitude of overshoot in reaction. Coherence is a measure to control for correspondence between both speed signals. Test duration is 25 minutes.

CRITICAL TRACKING TASK

The critical tracking task measures the participant's ability to control a displayed error signal in a 1st-order compensatory tracking task. Error appears as horizontal deviation of the cursor from midpoint on a horizontal, linear scale. Compensatory joy-stick movements null the error by returning the cursor to the midpoint. The frequency of cursor deviations, and therefore its velocity, increases as a stochastic, linear function of time. The participant is required to make compensatory movements with a progressively higher frequency until the participant loses control. The frequency at which control loss occurs is λ_c (the critical frequency). The reciprocal of this frequency is theoretically the perceptual/motor delay lag for humans operating in a closed-loop system. The participant performs this test in five trials and the mean λ_c is recorded as the final score (Jex, et al., 1966).

KAROLINSKA SLEEPINESS SCALE

The Karolinska sleepiness scale is a subjective rating scale with scores that range from 1 "extremely alert" to 9 "very sleepy, great effort to keep alert, fighting sleep" (Åkerstedt & Gillberg, 1990). Participant are instructed to report their experienced sleepiness during the preceding 10 minutes. Reyner and Horne (1998) modified the original scale by adding verbal descriptions to intermediate steps, which do not have any descriptions in the original version.

PHARMACOKINETIC ASSESSMENT

Blood samples (10 mL) and oral fluid (1-2 mL) samples were collected throughout a testing day/night i.e. at 1.5 and 11 hours post-drug. Blood sample was centrifuged immediately and the resulting serum was frozen at -20°C until analyses for pharmacokinetic assessments. MDMA concentrations and its main metabolite 3,4-methyleendioxyamfetamine (MDA) were determined in the corresponding serum samples using solid phase extraction and gas chromatography with mass spectrometric (GC-MS) detection with a limit of quantification of 16.8 µg/L. Oral fluid was collected with the Orasure Intercept® device for a quantitative analysis of MDMA concentrations by GC-MS.

STATISTICAL ANALYSES

All statistical analyses were conducted by means of SPSS 16.0 for Mac. Statistical analyses consisted of 2 steps: 1) Assessment for overall treatment effects by means of superiority testing; 2) Equivalence or non-inferiority testing of drug effects based on difference scores from placebo (within group) relative to the pre-established alcohol criterion, and 3) determination of concentration effect relations. Steps 2 and 3 were only conducted in case of treatment effects, and only for the primary measures of driving performance.

During step one all data entered the general linear model (GLM) repeated measures ANOVA procedures with MDMA (4 levels) and hours of sleep loss (2 levels for driving tests, 3 levels for cognitive test and 8 levels for subjective test) as main within subject factors. If the sphericity assumption was violated or not applicable, the Greenhouse-Geisser correction was used. In case of an overall effect of MDMA, separate drug-placebo contrast analyses were conducted for each MDMA dose.

Step two assessed whether a pre-established alcohol criterion falls within the 95% confidence interval (CI) of the drug effect. If yes, then the drug effect was considered to be equivalent or bigger than a BAC of 0.5 mg/mL, and thus relevant for traffic safety. If the 95% CI was lower than the alcohol criterion value, then a drug effect was considered of no clinical relevance.

In step 3, concentration-effect relations were determined according to the following procedure. Data collected during different doses of a drug were converted to change scores from placebo for analyses of the association between drug concentration and performance. A linear regression analysis was conducted to establish linear relationships between changes (from placebo) in task performance during drug treatment and log-transformed drug concentrations in serum. The total number of data points included in these equations was defined by the number of subjects x maximal number test repetitions x the number of drug doses. Individual drug concentrations in serum prior to performance assessments in each of the drug dose conditions were divided over three mutually exclusive categories covering the full range of drug concentrations. The concentration ranges in serum were 0-50, 50-100 and >100 µg/L during evening sessions and 0-25, 25-50 and >50 µg/L during morning sessions. The concentration ranges in oral fluid were 0-250, 250-1000 and >1000 µg/L during evening sessions and 0-100, 100-500 and >500 µg/L during morning sessions. Corresponding change scores of task performance were then classified either as showing "impairment" or "no impairment" for all individual cases within each of these categories. Impairment was defined as a positive change score from placebo. Binomial tests were applied to measure whether the proportion of observations showing impairment or no impairment significantly differed from the hypothesized proportion. It was hypothesized that in case of no effect of a drug on task performance the proportion of observations showing impairment or no impairment would be equal, i.e. 50%.

Results

DROPOUTS AND MISSING DATA

Two participants dropped out. One was positive for Δ^9 -tetrahydrocannabinol (THC) in urine. The other indicated that the sleep loss interfered too much with his daily life. Both drop outs were

replaced. Data was missing in the car following test on some occasions. These missing values were replaced by the overall group mean for the respective treatments.

DRIVING TESTS PREMATURELY TERMINATED

Table 1: Drug conditions of and reasons for prematurely termination of the highway driving test, mean SDLP and distance traveled until termination.

Subject number	Drug condition (mg MDMA)	Time of testing	Reason/ terminated by	mean SDLP (cm)	Distance traveled (km)
3	25	morning	sleepiness/ instructor	26.59	92
3	100	morning	sleepiness/ subject	22.50	64
4	0	morning	sleepiness/ instructor	23.88	99
4	50	morning	sleepiness/ instructor	23.30	29
6	50	morning	sleepiness/ subject	18.69	31
6	100	morning	sleepiness/ instructor	22.25	88
7	0	morning	sleepiness/ subject	24.94	66
7	100	evening	anxiousness/ subject	20.02	61
7	100	morning	sleepiness/ instructor	33.72	11
9	0	morning	sleepiness/ instructor	29.53	25
9	50	morning	sleepiness/ instructor	28.95	32
9	100	morning	sleepiness/ instructor	23.49	43
10	0	morning	sleepiness/ subject	22.57	48
10	25	morning	sleepiness/ subject	21.23	31
10	50	morning	sleepiness/ instructor	17.25	32
10	100	morning	sleepiness/ instructor	31.24	13
11	25	morning	sleepiness/ instructor	21.83	59
11	50	evening	sleepiness/ instructor	28.62	63
11	50	morning	sleepiness/ instructor	28.85	54
15	0	morning	sleepiness/ instructor	29.43	14
15	25	morning	sleepiness/ instructor	26.60	31
15	50	evening	sleepiness + bad weather/ instructor	23.30	61
15	50	morning	sleepiness/ instructor	25.76	29
15	100	morning	sleepiness/ instructor	22.61	9
16	0	morning	sleepiness/ instructor	25.89	47
16	25	morning	sleepiness/ subject	19.74	65

Table 1 shows the highway driving tests that were prematurely terminated, the drug condition in which the test was terminated, the reasons for termination, the initiator of termination, the measured SDLP and distance travelled before termination.

The car following test was prematurely terminated in 2 cases (1.6%), once in the placebo condition and once in the 25 mg MDMA condition. Both tests were terminated in the morning and by the instructor, because of sleepiness of the subject. In case of a premature stop, available data till time of test termination were used for statistical analysis.

DRIVING TESTS

Mean (SE) performances on the primary (SDLP and TSA) and secondary measures of the highway driving and car-following test are shown in Table 2.

Table 2: Mean (SE) of the driving and psychomotor tests and subjective measure for the treatment conditions and measuring times. Significance indicated by p-value.

Measuring time, ergo, performance, mood and mood by p-value.								
Test	Test repetitions	Drug conditions				ANOVA		
		Placebo	25 mg MDMA	50 mg MDMA	100 mg MDMA	Sleep deprivation	MDMA	MDMA x sleep deprivation
Road tracking								
SDLP ^a (cm)	1	18.2 (0.7)	18.4 (0.8)	18.1 (1.0)	18.0 (0.6)	<0.001	NS	NS
	2	22.8 (0.8)	22.9 (0.6)	21.7 (0.9)	22.4 (1.1)			
SD Speed (km/h)	1	1.70 (0.13)	1.74 (0.14)	1.88 (0.17)	2.14 (0.14)	<0.001	NS	0.020
	2	2.68 (0.23)	2.62 (0.19)	2.66 (0.18)	2.46 (0.19)			
Car following								
TSA (s)	1	2.8 (0.2)	3.0 (0.2)	3.0 (0.3)	2.7 (0.2)	NS	NS	NS
	2	3.3 (0.4)	3.3 (0.4)	3.2 (0.3)	3.1 (0.5)			
Coherence	1	0.9 (0.01)	0.9 (0.01)	0.9 (0.01)	0.9 (0.01)	NS	NS	NS
	2	0.9 (0.01)	0.9 (0.01)	0.9 (0.01)	0.9 (0.01)			
Gain	1	1.1 (0.04)	1.2 (0.03)	1.0 (0.03)	1.1 (0.03)	NS	NS	NS
	2	1.1 (0.03)	1.1 (0.03)	1.1 (0.04)	1.2 (0.06)			
Critical tracking task								
λ_c (rad/s)	1	3.77 (0.13)	3.83 (0.16)	3.70 (0.15)	3.78 (0.19)	<0.001	NS	NS
	2	3.63 (0.16)	3.56 (0.18)	3.73 (0.16)	3.77 (0.16)			
	3	3.09 (0.19)	2.87 (0.22)	3.09 (0.19)	3.36 (0.17)			
Karolinska sleepiness scale								
score	1	2.94 (0.21)	2.94 (0.27)	2.88 (0.36)	2.94 (0.25)	<0.001	NS	NS
	2	3.13 (0.32)	2.69 (0.25)	2.19 (0.21)	1.81 (0.25)			
	3	3.62 (0.32)	3.69 (0.29)	3.62 (0.38)	3.62 (0.41)			
	4	4.44 (0.35)	4.62 (0.38)	4.25 (0.41)	5.13 (0.40)			
	5	5.31 (0.40)	5.81 (0.45)	5.44 (0.35)	6.12 (0.42)			
	6	5.88 (0.39)	6.50 (0.45)	6.19 (0.38)	5.81 (0.44)			
	7	7.38 (0.30)	7.69 (0.37)	7.50 (0.32)	7.56 (0.33)			
	8	7.38 (0.44)	7.88 (0.36)	7.62 (0.40)	7.62 (0.42)			

^a SDLP, standard deviation of lateral position; NS, not significant; TSA, time to speed adaptation.

MDMA did not affect any of the driving measures. Sleep loss significantly affected SDLP ($F_{1,15}=40.833$, $p<0.001$) and SD Speed ($F_{1,15}=29.905$, $p<0.001$). SDLP and SD Speed were markedly higher in the morning as compared to the evening. The interaction between MDMA and sleep loss only reached significance for SD Speed ($F_{3,45}=3.607$, $p=0.020$). SD speed was higher in the morning as compared to the evening, particularly after treatment with MDMA 100 mg ($p=0.017$). In general, variations in mean SD Speed were very small and ranged between 1-2.5 km/h.

Equivalence testing demonstrated that increments in SDLP in the morning in all treatment conditions were equivalent to a BAC of 0.8 mg/mL when compared to placebo performance in the evening. The upper limits of the 95% CI of changes induced by sleep loss clearly exceeded the pre-established inferiority margin of 2.4 cm. Mean change SDLP and 95% CI in every treatment condition are shown in Figure 2.

Regression analysis of MDMA levels in serum and SDLP change scores showed a general lack of correlation between the measures. Scatter plots showing the linear relationship between MDMA levels in serum and oral fluid and changes in SDLP are shown in Figure 3.

Binomial tests showed a significant increase in the proportion of observations showing impairment in the highway driving test when conducted in the morning for serum MDMA

Figure 2: Mean (95% CI) SDLP difference from placebo after single doses of MDMA during the road tracking test in the evening and in the morning after a night of sleep loss. (* = non-inferiority not shown, upper bound of the 95% CI is above the non-inferiority margin of 2.4 cm).

Figure 3: Correlations between change SDLP and log converted MDMA concentrations in serum (left panels, a-b) and saliva (right panels, c-d) during driving tests in the evening (upper panels, a and c) and in the morning (lower panels, b and d).

concentrations between 0-25 $\mu\text{g/L}$ and $>50 \mu\text{g/L}$ ($p<.05$). In oral fluid all concentration ranges were associated with impairment ($p<.05$). Impairments were only apparent when compared to placebo SDLP during evening sessions. Distributions of observations showing 'impairment' and 'no impairment' in the highway driving test as a function of MDMA in serum and oral fluid are shown in Figure 4.

Fig. 4 Percentage of observations showing MDMA induced impairment or MDMA induced improvement of SDLP in the road tracking task, as a function of MDMA concentrations in serum (left panels, a-b) and saliva (right panels, c-d) during driving tests in the evening (upper panels, a and c) and in the morning after a night of sleep loss (lower panels, b and d). * indicates significance ($p<0.05$).

CRITICAL TRACKING TASK

Mean λ_c (critical frequency) of the critical tracking task was significantly affected by sleep deprivation ($F_{2,30}=34.516$, $p<0.001$). Mean critical frequency generally decreased during the night, indicating diminished psychomotor control. Critical tracking was not affected by MDMA or MDMA $\times$ sleep loss. Mean (SE) performance on the critical tracking task is displayed in Table 2.

SUBJECTIVE MEASURES

The Karolinska sleepiness scale showed a significant effect of sleep loss ($F_{2,972,44.576}=108.717$, $p<0.001$). The Karolinska sleepiness scale also demonstrated a significant dose-related MDMA

effect at T_{max} ($F_{3,45}=6.006$, $p=0.002$). Contrasts indicated that the 100 mg as well as 50 mg MDMA conditions differed significantly from placebo ($p=0.009$ and $p=0.023$ respectively). Mean (SE) performance on the Karolinska sleepiness scale is displayed in Table 2.

PHARMACOKINETIC ASSESSMENT

Pharmacokinetic analysis in serum revealed mean (SE) MDMA concentrations of 25.8 (3.3), 63.9 (6.4) and 157.2 (9.5) $\mu\text{g/L}$ at 1.5 hours after administration of a 25, 50 and 100 mg dose respectively. At 11 hours post drug mean MDMA concentrations were 14.2 (2.7), 34.0 (3.9) and 84.3 (6.7) $\mu\text{g/L}$ respectively. MDA concentrations were 3.5 (0.1), 3.9 (0.4) and 5.8 (0.2) $\mu\text{g/L}$ 1.5 hours post drug and 2.9 (0.5), 5.8 (0.2) and 9.7 (0.6) $\mu\text{g/L}$ after 11 hours for 25, 50 and 100 mg MDMA respectively.

Mean (SE) MDMA concentrations in oral fluid were 208.2 (72.4), 833.0 (270.9) and 3417.8 (694.2) ng/g and MDA concentrations were 3.2 (1.5), 13.9 (4.2) and 56.4 (13.5) for 25, 50 and 100 mg MDMA respectively at 1.5 hour post drug. The concentrations at 11 hours after drug intake were respectively 57.0 (16.3), 292.6 (79.0) and 925.3 (224.7) for MDMA and 3.6 (1.4), 18.9 (4.4) and 56.8 (12.8) for MDA.

Discussion

The present study demonstrated that sleep deprivation produced severe impairment in actual driving performance as expressed by a significant rise in SDLP and a large number prematurely terminated driving tests during early morning sessions. In general, MDMA did not affect actual driving performance and did not interact with the effects of sleep deprivation.

On average, SDLP increased with 4.2 cm in the morning after sleep deprivation, relative to SDLP before sleep deprivation. This increment is about 1.5-2 times greater than found in two recent driving under the influence of alcohol studies with blood alcohol concentrations between 0.29-0.5 mg/mL (Kuypers, et al., 2006; Ramaekers, et al., 2000). From a previous alcohol study that was conducted in order to calibrate SDLP for the dose-related effects of alcohol (Louwerens, et al., 1987) it can be concluded that a mean increase in SDLP of 4.2 cm is equivalent to a blood alcohol concentration of approximately 0.8 mg/mL. Equivalence testing even demonstrated that the upper limit of the 95% CI associated with the mean change in SDLP after sleep deprivation widely exceeded the criterion level of 1.0 mg/mL BAC. Together this indicates that sleep deprivation caused severe driving impairment comparable to driving under the influence of high to very high BAC.

These findings were corroborated by results from the critical tracking task. Critical tracking performance significantly decreased over the night, as a function of hours of sleep loss. Similar findings have previously been reported by Dawson and Reid (1997). They measured tracking performance as a function of hours of sleep loss and BAC intoxication. According to their model, tracking performance of subjects after 17-24 hours of wakefulness is equivalent to that observed at BAC between 0.5-1.0 mg/mL. This again indicates that a night of sleep deprivation causes serious impairment of driving skills. In terms of BAC equivalents, these impairments exceeded those observed at legal BAC limits of 0.5 mg/mL and 0.8 mg/mL that are currently in place for driving under influence of alcohol in most of Europe and the US.

The car following test was the only driving measure that did not show an effect of sleep loss. This diverging effect between the two driving tests, i.e., road tracking and car following,

could be explained by a study of Harrison and Horne (2000). They conducted a study in which they showed that dull and monotonous tasks are more sensitive to sleep deprivation than more complex, rule based tasks, because the latter ones generate more interest and effort to compensate for the effects of sleep deprivation. The highway driving test is monotone and subjects reported that it was more boring than the car-following test, which is more complex. This is corroborated by the fact that 20% of road-tracking and 2% of car-following tests were prematurely terminated of which the majority in the morning, i.e., after sleep deprivation. Therefore, this divergence between the two driving tests are in line with the results of Harrison and Horne (2000), in that the more monotone road-tracking test is more sensitive to sleep deprivation than the car-following test.

In general, MDMA did not produce any significant effects on driving parameters independent of dose or concentration. Stimulating effects of MDMA on actual driving parameters or psychomotor measures that have been demonstrated before (e.g. Dumont, et al., 2008; Kuypers, et al., 2006; Kuypers, et al., 2007; Lamers, et al., 2003; Ramaekers, Kuypers, et al., 2006). could not be clearly replicated in the present study. This discrepancy could be due to the fact that the present study also included lower doses of MDMA, i.e. 25 and 50 mg that may be less likely to produce stimulatory effects than MDMA between 75 and 125 mg that have been tested in the studies mentioned above. However, even in the present study some measures tended to show stimulatory effects of MDMA. For example, SD speed was significantly affected by an interaction between sleep loss and MDMA. Overall, SD speed increased after a night of sleep loss, but this increase was somewhat less after 100 mg MDMA. Also, binomial tests of SDLP change scores in the evening demonstrated that the majority of observations indicated a reduction in SDLP, particularly at lower MDMA concentrations. However, this effect failed to reach statistical significance.

It is also apparent from the present study that the stimulant effects of MDMA, if any, could not compensate for the impairing effect of sleep loss on driving performance. None of the primary driving measures demonstrated any significant MDMA x sleep loss interaction. The effects of sleep deprivation on driving were highly prominent during MDMA treatments and did not change as a function of dose and concentration. These findings are in line with those in a previous study that assessed the stimulant effects of MDMA on SDLP during alcohol intoxication (Kuypers, et al., 2006). That study demonstrated that stimulatory effects of MDMA did not compensate for driving impairment caused by alcohol. Together, these data indicate that the effects of MDMA on psychomotor functions are neutral or mildly stimulating, but that these effects are not sufficient to overcome impairments caused by other factors such as sleep deprivation or alcohol intoxication.

The latter notion is of crucial importance when evaluating driving under the influence (DUI) offenders involving the use of MDMA. Subjects in the present study were significantly impaired during MDMA treatments when deprived of sleep for one night. However, the prime factor causing these impairments was sleep deprivation rather than the use of MDMA itself. When applied in courts, one could rightfully pose the argument that such drivers should not be prosecuted for DUI since MDMA did not contribute to the impairments of the driver. There are a number of counter arguments that should be taken into consideration when evaluating MDMA cases in traffic. First, it has been demonstrated in previous studies that single doses of MDMA increase subjective feelings of arousal and mood (Bosker, et al., 2010; Kuypers, et al., 2008) and the current study showed a decrease in sleepiness from baseline at Tmax in the MDMA conditions. Such feelings may affect the subjective judgment of MDMA users on whether or not it is safe to

drive home after spending a night at a rave party. During MDMA intoxication, they may not be able to subjectively experience the debilitating effects of sleep loss to the same degree as drug free drivers, because they feel energetic. As a consequence, they may decide to drive because they feel alert, thereby neglecting the impairing effects of other impairing factors such as sleep deprivation or even alcohol use. Secondly, several studies have also demonstrated that cognitive functions such as working memory, spatial memory and timing of moving objects (Kuypers & Ramaekers, 2005; Lamers, et al., 2003; Ramaekers, Kuypers, Wingen, Heinecke, & Formisano, 2009) are impaired during MDMA intoxication. Such cognitive impairments may also affect a person's ability to reflect on his/her fitness to drive as well driving ability in general.

It is concluded from this study that drivers who are under the influence of MDMA and are sleep deprived are unfit to drive. The impairing effects of sleep deprivation during MDMA intoxication occurred independent of MDMA dose and concentration.

CHAPTER 7: DOSE-RELATED EFFECTS OF MDMA ON PSYCHOMOTOR FUNCTION AND MOOD BEFORE, DURING, AND AFTER A NIGHT OF SLEEP LOSS

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INTRODUCTION 3,4-methylenedioxymethamphetamine (MDMA) is known to improve psychomotor function and mood when measured during daytime. However, MDMA users tend to take this drug at dance parties while staying awake for prolonged periods of time.

SUBJECTS AND METHODS This study was designed to assess dose-related residual effects of MDMA on psychomotor function and mood after a night without sleep. Sixteen recreational MDMA users received single doses of 25, 50, and 100 mg MDMA in a randomized, double-blind, placebo-controlled cross-over study.

RESULTS Results showed that sleep loss significantly impaired psychomotor function. MDMA generally did not affect performance but did improve rapid information processing at the highest dose in the morning after administration. In the evening, MDMA also increased subjective ratings of positive mood at every dose and subjective arousal at the highest dose. These subjective effects were no longer present after a night of sleep loss.

DISCUSSION It is concluded that sleep deprivation impairs psychomotor function and that stimulant effects of MDMA are not sufficient to compensate for this impairment.

Introduction

3,4-methylenedioxymethamphetamine (MDMA) is the main psychoactive substance of the party drug ecstasy. Ecstasy is a popular drug: about 0.4% of the Dutch general population (40,000 people) has been described as current user in 2005 (National Drug Monitor, 2008) and 9.5 million European adults indicated to have ever used ecstasy, which is 2.8% of the general population (EMCDDA, 2008a). Ecstasy has been described as an entactogen, because of its subjective effect of feeling close to and connected with others. Other drug effects are enhanced visual and auditory perception: colors appear brighter and sounds seem more intense. This is why people generally take ecstasy at dance parties where rhythmic music is being played in combination with colorful lights. Also, the stimulant effects enable people to dance for longer periods of time.

An extensive body of literature showed that ecstasy may produce detrimental effects on cognitive and psychomotor functions. For example, Morgan et al. (2006) found that ecstasy users were impaired on several measures of impulsivity compared to groups of polydrug users and drug-naïve controls. Two meta-analytic reviews demonstrated that ecstasy users performed significantly worse on cognitive tasks measuring learning/memory, attention, executive function, and psychomotor performance compared to non-using control subjects, although effect sizes were medium to small (Kalechstein, et al., 2007; Zakzanis, et al., 2007). However, a large number of studies have also failed to exclusively link MDMA use to long-term cognitive impairments. Roiser, Rogers and Sahakian (2007) reported that performance of current ecstasy users, former-ecstasy users, polydrug users, and drug-naïve controls on tests measuring memory, executive function, and impulsivity did not differ between groups. Clark, Roiser, Robins and Sahakian (2009) reported no performance difference between ecstasy users and controls on a task that measured impulsivity. Hoshi et al. (2007) and Hanson, Luciana and Sullwold (2008) concluded that the subtle impairments of memory and impulse control in MDMA users were due to polydrug use rather than ecstasy use alone. Likewise, Lamers, Bechara, Rizzo and Ramaekers (2006) and Jager et al. (2008) concluded that impairments in cognitive and psychomotor function of MDMA users may be attributed to other drugs than MDMA, such as cannabis and amphetamine.

These conflicting findings of MDMA impairing performance in some, but not all, studies have been attributed to methodological problems inherent in cross-sectional designs in abstinent drug users. For example, the fact that most ecstasy users also take other drugs is an important confounder. Thus, the results of the aforementioned studies could be due to the use of other drugs than MDMA. Another related problem is causality: the nature of cross-sectional designs does not allow inferences on whether the effects are caused by ecstasy or another factor, e.g., premorbid factors that might make people more vulnerable to use illicit drugs (Morgan, 2000).

One way to overcome these problems is to study acute effects of MDMA on cognitive performance in placebo-controlled experimental designs. In such designs, changes in performance can be exclusively linked to the experimental drug. Experimental studies have previously shown that single doses of MDMA impair memory function (Dumont, et al., 2008; Kuypers & Ramaekers, 2005, 2007; Ramaekers, Kuypers, et al., 2009) and attention (Dumont, et al., 2008) and improve performance on tasks measuring impulse control (Ramaekers & Kuypers, 2006) and psychomotor function (Lamers, et al., 2003) when given during the day. The magnitude of impairment on memory and attention increased when MDMA was given during the night to subjects who stayed awake throughout the night. Moreover, stimulating effects of nocturnal doses of MDMA on

psychomotor function, vigilance, and sleepiness were very mild or no longer present after a night of sleep loss (Kuypers, et al., 2008; Kuypers, et al., 2007). In the latter studies, repeated doses of 75 and 50 mg MDMA were administered to subjects, which resulted in psychomotor impairment additive to impairment produced by sleep loss. However, MDMA also produced some mild stimulatory effects; it reduced subjective feelings of sleepiness and mildly increased vigilance performance in the morning.

At present, it is not clear at which MDMA dose or serum concentration residual impairments start to emerge. Residual effects are limited to the time window in which the drug is still present in blood, but at low concentrations. The objectives of the present study were to establish the residual effects of MDMA on cognitive performance early in the morning as a function of MDMA dose. Subjects were treated on four separate occasions with evening doses of 0, 25, 50, and 100 mg. Because identical MDMA doses can lead to different MDMA blood concentrations, it is important to study MDMA effects or its interaction with sleep deprivation as a function of MDMA concentrations rather than dose. We decided to include a broad range of (low) nocturnal doses in order to be able to measure residual MDMA effects at very low concentrations in the morning. This wide range of residual blood concentrations will help determine the MDMA concentration threshold at which residual performance effects will still be present. Performance was tested in the evening, in the middle of the night, and in the morning after a night of sleep loss.

Subjects and methods

SUBJECTS

Eight males and eight females participated in this study. Their mean (SE) age was 22.0 (0.41) years, and their mean (SE) lifetime MDMA use was 27.0 (8.4) times. Subjects were recruited by advertisements at Maastricht University and were paid upon completion of the study. Before enrollment, all subjects were screened by means of a telephone interview to determine whether they qualified for the study. The inclusion criteria were experience with MDMA, i.e., at least one time in the last year; free from psychotropic medication; good physical health as determined by a medical examination; absence of any major medical, endocrine, and neurological condition; body mass index between 18 and 28; and written informed consent. The exclusion criteria were history of drug abuse or addiction as assessed by means of a medical questionnaire by the physician at the medical checkup; pregnancy or lactation; cardiovascular abnormalities on electrocardiogram; excessive drinking, i.e., more than 20 alcoholic consumptions a week; hypertension, i.e., systolic blood pressure over 170 mmHg or diastolic blood pressure over 100 mmHg; and history of or current psychiatric disorder. If subjects met the inclusion criteria, they received a medical history and a drug questionnaire to get a more precise view on their health and drug use. Finally, subjects underwent a medical examination and took part in a training session to get familiar with the tests.

This study was conducted according to the code of ethics on human experimentation established by the declaration of Helsinki (1964) and amended in Seoul (2008). Approval for the study was obtained from the Medical Ethics committee of the Academic Hospital of Maastricht and Maastricht University. A permit for obtaining, storing, and administering MDMA was obtained from the Dutch drug enforcement administration.

STUDY DESIGN

The study was conducted according to a double-blind, placebo-controlled, randomized, four-way, cross-over design. Treatments consisted of single doses of placebo, 25, 50, and 100 mg MDMA. Treatment orders were balanced over subjects and treatment periods. Placebo and MDMA were administered orally in identically appearing formulations. MDMA was dissolved in 25 mL bitter orange peel syrup, and placebo consisted of only the bitter orange peel syrup. The syrup was mixed with 200 mL juice before it was given to the subjects. The wash-out period between treatments was at least 1 week.

PROCEDURE

Subjects were asked to refrain from any drugs 1 week before the medical examination until 2 weeks after study completion. Subjects were not allowed to drink alcohol and caffeine or smoke tobacco during a 24-h period prior to testing. Subjects were always tested for alcohol and drugs, i.e., tetrahydrocannabinol, opiates, amphetamine/ecstasy, benzodiazepines, cocaine, and methamphetamine/ecstasy, respectively, in breath and urine upon arrival (4:30 p.m.) at the laboratory on test days. At 5:00 p.m. subjects received a light, standard dinner, and at 5:15 p.m., MDMA or placebo was administered. Performance was assessed in the evening, the middle of the night, and in the morning after a night of sleep loss. The timeline for performance testing, questionnaires, and blood draws are displayed in Figure 1. Subjects watched television or movies, played games, read a book or magazine, or used the internet in between testing episodes during a test night. An additional blood sample was drawn 1 week after each testing day to monitor renal and liver function. A test day ended at 9:00 a.m. the next morning, at which time, subjects were driven home.

Figure 1: Timeline for blood samples, questionnaires, and laboratory tests relative to drug administration. GSS, Groninger sleep scale; POMS, profile of mood states; TQ, treatment questionnaire.

PSYCHOMOTOR ASSESSMENT

The stop signal task required subjects to make quick key responses to visually presented go signals and to inhibit their response when a visual stop signal was suddenly presented. The go signals were four 1.5 cm letters (A, B, C, and D) presented one at a time in the center of a computer screen. Subjects were required to respond to each letter as quickly as possible by pressing one of two response buttons. One button was pressed to indicate that A or C appeared and the other to indicate B or D. Letters were displayed for 500 ms, and the computer screen was blank for 1.5 s inter-stimulus interval. This provided a period of 2 s in which the subject could respond to a letter. A single test consisted of 176 trials in which each of the four-letter stimuli was presented equally often. A stop signal occurred in 48 trials during a test. The stop signal consisted of a visual cue, i.e.,

*, that appeared in one of the four corners of the screen. Stop signals were presented 12 times at each of the four delays after the onset of a letter: 50, 150, 250, and 350 ms. Trials always began with a 500-ms preparation interval in which a fixation point appeared in the center of the screen. The task lasted about 10 min. Dependent variables were the proportion of commission errors on stop trials and the reaction times (RT) on go as well as stop trials, i.e., stop RT. Stop RT represents the estimated mean time required to inhibit a response and is a measure of impulsivity (Fillmore, Rush, & Hays, 2002; Ramaekers & Kuypers, 2006).

The rapid information-processing task is a self-paced interactive working memory task that assessed subjects' information-processing capacity. A pseudo-random sequence of 250 digits (1–8) was presented in the center of the screen. The digits were presented one at a time for 67 ms with an initial inter-stimulus-interval (ISI) of 600 ms. Subjects had to press a button when they saw the third digit of a three-digit sequence (triad) that was comprised of even (e.g., 6, 2, and 4) and odd (e.g., 5, 1, and 7) digits. The entire 250-digit sequence contained 11 even-digit triads and ten odd-digit triads. Each correct response speeded up the presentation rate by decreasing the ISI with 33 ms, and each false response slowed it down with 33 ms. Task duration was 5 min; the 250-digit sequence was presented in a repeated loop. The initial presentation rate of the test was 90 digits per minute, and the dependent variable was the average number of digits per minute presented in the test (Fillmore, Kelly, & Martin, 2005).

The divided attention task assessed the ability to divide attention between two tasks performed simultaneously. The primary task required the use of a joystick to continuously null the horizontal movement of a cursor from the center of a display. The cursor traveled in both directions with irregular velocity, but on average, 50% of what the subject could just control. The dependent measures of this subtask were control losses and tracking error, which was measured by the absolute distance (millimeters) between the cursor's position and the center. The secondary task involved monitoring 24 single-digit numbers (0–9) that were arranged in the four corners of the display. The numbers changed asynchronously every 5 s. The requirement was to react as rapidly as possible by lifting the foot from a pedal every time a target, i.e., the number 2, appeared. Average reaction time to targets was recorded as the dependent measure (Moskowitz, 1973).

The psychomotor vigilance task assessed the reaction time in response to a visual stimulus. The visual stimulus was a counter in the center of a computer screen that ran in 1 min from 0 to 60 with a fixed inter-stimulus interval of 1 ms. The counter started at random intervals between 2 and 10 s, and the subject had to react to the onset of the counter as quickly as possible by pressing a response button. Duration of the task was 10 min. This task has often been used to assess the impact of sleep loss on performance (Loh, Lamond, Dorrian, Roach, & Dawson, 2004).

SUBJECTIVE MEASURES

The Groninger sleep scale assessed sleep quality and quantity (hours of sleep). It consisted of 15 dichotomous questions about sleep complaints and an open question concerning the duration of sleep (Mulder-Hajonides van der Meulen, Wijnberg, Hollanders, DeDiana, & Hoofdakker, 1980). The quality score ranged from 0 (best quality of sleep) to 15 (worst quality of sleep).

The profile of mood states is a self-assessment mood questionnaire with 72 five-point Likert scale items, representing eight mood states, i.e., anxiety, depression, anger, vigor, fatigue, confusion, friendliness, and elation. Three composite scales were derived, i.e., arousal ((anxiety +

vigor) – (fatigue + confusion)), positive mood (elation – depression), and a total score ((anxiety + depression + anger + fatigue) – vigor), which is a measure of malaise. The subject had to indicate to what extent these items were representing his/her mood (de Wit, Enggasser, & Richards, 2002).

PHARMACOKINETIC ASSESSMENT

Blood samples (8 mL) were collected two times throughout a testing day/night, i.e., at 1.5 and 11 h post-drug. MDMA and 3,4-methylenedioxyamphetamine (MDA) concentrations were determined afterwards in serum. The blood sample was centrifuged immediately, and the resulting serum was frozen at –20°C until analyses for pharmacokinetic assessments.

Table 1: Mean (SE) performance on the cognitive and psychomotor tests for the treatment conditions and measuring times. Significance indicated by p-value.

times. Significance indicated by p-value.								
Test	Measure	Drug conditions				ANOVA		
		Placebo	25 mg MDMA	50 mg MDMA	100 mg MDMA	Sleep deprivation	MDMA	MDMA x sleep deprivation
Stop signal task								
Commission errors (N)	1	4.8 (0.7)	4.5 (0.6)	5.1 (0.7)	5.3 (0.8)			
	2	5.5 (0.8)	3.0 (0.6)	4.5 (0.7)	4.8 (0.7)	-	-	-
	3	5.6 (0.7)	5.6 (0.6)	4.9 (0.6)	5.1 (0.7)			
RT go (ms)	1	543 (36)	540 (23)	528 (25)	523 (23)			
	2	551 (35)	595 (30)	552 (27)	542 (28)	0.034	-	-
	3	555 (34)	585 (29)	562 (26)	578 (30)			
stop RT (ms)	1	276 (20)	271 (11)	292 (23)	315 (33)			
	2	327 (22)	290 (11)	279 (18)	299 (22)	0.049	-	-
	3	309 (25)	297 (16)	320 (33)	334 (23)			
Rapid information processing								
processing speed (N/min)	1	529.0 (18.9)	525.2 (20.3)	506.0 (22.2)	522.1 (20.6)			
	2	519.4 (20.3)	511.8 (20.3)	496.1 (14.2)	517.3 (18.2)	0.012	-	0.022
	3	468.0 (18.3)	462.9 (22.1)	479.9 (17.1)	526.4 (20.6)			
Divided attention task								
control loss (N)	1	1.9 (0.7)	1.8 (0.5)	2.9 (1.3)	1.6 (0.5)			
	2	12.6 (8.9)	14.4 (6.2)	5.6 (1.8)	6.1 (2.9)	0.001	-	-
	3	48.4 (12.3)	33.4 (7.2)	47.9 (14.3)	24.7 (5.7)			
tracking error (mm)	1	15.2 (1.1)	15.7 (1.2)	15.2 (1.0)	14.1 (1.2)			
	2	15.9 (1.3)	17.3 (1.1)	16.1 (1.2)	15.5 (1.3)	0.001	-	-
	3	19.3 (0.9)	19.7 (0.9)	18.0 (0.8)	18.5 (1.0)			
RT (ms)	1	1822 (63)	1853 (79)	1885 (74)	1920 (70)			
	2	2046 (89)	2013 (79)	2017 (89)	1973 (74)	<0.001	-	-
	3	2180 (65)	2189 (66)	2246 (69)	2285 (73)			
Psychomotor vigilance task								
RT (ms)	1	289 (8)	286 (8)	295 (15)	301 (13)			
	2	384 (47)	493 (108)	456 (76)	349 (26)	<0.001	-	-
	3	464 (29)	463 (45)	544 (49)	515 (38)			

STATISTICAL ANALYSES

All statistical analyses were conducted by means of SPSS 16.0 for Mac. All data were entered in the general linear model (GLM) repeated measures analysis of variance (ANOVA) procedures with MDMA (four levels) and hours of sleep loss (three levels) as main within-subject factors. If the sphericity assumption was violated, the Greenhouse–Geisser correction was used. In case of an overall effect of MDMA or an interaction effect between MDMA and sleep loss, separate drug-placebo contrast analyses were conducted for each MDMA dose. In case of the profile of mood states, difference scores between post-drug and the pre-drug score entered the statistical analysis.

Results

PSYCHOMOTOR ASSESSMENT

Mean (SE) performance scores obtained from psychomotor tests are displayed in Table 1 along with p values associated with GLM ANOVA.

MDMA did not affect performance in any of the psychomotor tasks. Sleep loss impaired performance on a range of tasks. It increased RT ($F_{2,30}=6.415$, $p=0.005$) and stop RT ($F_{2,30}=3.685$, $p=0.037$) in the stop signal task, processing speed in the rapid information-processing task ($F_{2,30}=7.896$, $p=0.002$), and RT in the psychomotor vigilance task ($F_{2,30}=16.598$, $p<0.001$). Sleep loss also increased control losses ($F_{1,345,20,171}=14.272$, $p=0.001$), tracking error ($F_{2,30}=16.449$, $p<0.001$), and RT ($F_{2,30}=35.538$, $p<0.001$) in the divided attention task.

The interaction between MDMA and sleep loss was significant for processing speed ($F_{6,90}=2.616$, $p=0.022$) in the rapid information-processing task. Simple drug-placebo contrasts showed that this interaction effect was attributable to MDMA 100 mg ($p=0.001$). Performance during the placebo condition worsened during the night, while in the 100-mg MDMA condition, subjects' performance remained stable throughout the night and slightly improved in the morning (see Figure 2).

Figure 2: Mean (SE) performance on the rapid information-processing task in every treatment condition as a function of time after drug administration.

SUBJECTIVE MEASURES

There were no significant differences in sleep quality and quantity between any of the MDMA conditions as measured by the Groninger sleep scale. Subjects' mean (SE) quality of sleep and hours of sleep were 2.22 (0.36) and 8.5 (0.24), respectively.

ANOVA of the profile of mood states showed a significant interaction between MDMA and sleep loss on the arousal ($F_{3,45}=4.294$, $p=0.010$) and positive mood scale ($F_{3,45}=9.526$, $p<0.001$). Simple contrasts indicated that MDMA 100 mg increased arousal ($p=0.012$) in the evening but not in the morning, as compared to placebo. Drug-placebo contrasts also showed that all MDMA doses ($p<0.046$) increased positive mood in the evening after drug intake but not in the morning. Mean (SE) arousal and positive mood ratings during treatment are given in Figures 3 and 4, respectively.

Figure 3: Mean (SE) score on profile of mood states arousal scale in every treatment condition as a function of time after drug administration.

Figure 4: Mean (SE) score on profile of mood states positive mood scale in every treatment condition as a function of time after drug administration.

Pharmacokinetic analysis in serum revealed mean (SE) MDMA concentrations of 25.8 (3.3), 63.9 (6.4), and 157.2 (9.5) $\mu\text{g/L}$ at 1.5 h after administration of a 25-, 50-, and 100-mg dose, respectively. At 11.5 h post-drug, these concentrations were 14.2 (2.7), 34.0 (3.9), and 84.3 (6.7) $\mu\text{g/L}$, respectively. MDA concentrations were too low to detect.

Discussion

The present study showed significant impairing effects of sleep loss on various psychomotor measures. Performance deteriorated without exception on all tasks as the hours of sleep loss increased. RT in the stop signal, divided attention, and psychomotor vigilance task as well as processing speed in the rapid information-processing task slowed down over time. Tracking error increased in the divided attention task, which indicates that subjects were unable to allocate sufficient cognitive resources to perform the primary tracking task at a normal, placebo level, despite a general slowing of RT in the secondary task. The number of control losses increased dramatically in the morning after sleep deprivation. Together, this shows that sleep deprivation produced gross impairment of cognitive and psychomotor functions. Similar findings have previously been reported by Kuypers et al. (2008; 2007) and Dawson and Reid (1997). The latter even demonstrated that performance decrements for each hour of wakefulness between 10 and 26 h were equivalent to a performance decrement observed with a 0.004% rise in blood alcohol concentration. According to their model, performance of subjects in the present study after 17–24 h of sustained wakefulness would have decreased to a level equivalent to the performance impairment observed at a blood alcohol concentration of roughly 0.05–0.10%.

This study showed no main effects of MDMA on performance, neither in the evening after drug administration nor throughout the night. This contrasts somewhat with findings of previous studies who reported neutral or even stimulating effects on psychomotor function after single doses of MDMA, particularly around T_{max} (Kuypers, et al., 2007; Lamers, et al., 2003; Ramaekers & Kuypers, 2006; Ramaekers, Kuypers, et al., 2006). It should be noted however that the MDMA doses/concentrations in the present study were relatively low compared to previous studies. This was particularly true for MDMA concentrations early in the morning. In the present study, the mean MDMA concentration in the 100-mg MDMA condition was 84.3 $\mu\text{g/L}$ at 11.5 h post-dosing. This concentration was about 2.5 times lower than the concentration reported in a previous study assessing the effect of repeated doses of MDMA on psychomotor function after a night of sleep loss (Kuypers, et al., 2008; Kuypers, et al., 2007).

The only significant interaction between MDMA and sleep loss was caused by the highest dose of MDMA in the present study. Relative to placebo, performance in the rapid information-processing task slightly improved 14–15 h after MDMA 100 mg, after a full night of sleep loss. This interaction confirms some of the mild stimulatory characteristics that have been reported before when measuring MDMA effects at T_{max} (Kuypers, et al., 2006; Ramaekers, Kuypers, et al., 2006). In general, acute drug studies have shown that MDMA effects subside over time and normalize after about 6 h after administration when taken during the day (Dumont, et al., 2009; Hernandez-Lopez, et al., 2002). However, MDMA effects on cognition may last longer or become more noticeable when taken during the night or after a night without sleep, because they may add to or

even interact with the detrimental effects of sleep loss on cognition. Kuypers et al. (2007) demonstrated that nocturnal doses of MDMA significantly increased vigilance and decreased sleepiness in the morning after a night of sleep loss. These results confirm the residual, mild, stimulatory effects of MDMA on psychomotor function that were measured in the present study after a night of sleep loss. These data seem to indicate that mild stimulatory effects of MDMA may compensate somewhat for the impairing effects of sleep loss on performance. However, this effect of MDMA was very mild and only apparent in a single performance task. Overall, MDMA did not compensate for the detrimental effects of sleep loss on performance. This conclusion is in line with work of others who also showed that stimulant effects of MDMA are generally mild and not sufficient to counteract the impairing effects of other sources such as alcohol (Dumont, et al., 2008; Kuypers, et al., 2006).

The profile of mood states rating scales confirmed that subjects actively experienced an MDMA effect even after the lowest dose of 25 mg. Ratings of positive mood were significantly elevated after all MDMA doses between 1 and 2 h after dosing. Likewise, feelings of arousal also increased at T_{max}, albeit only after the highest dose. These positive feelings however did not last through the night. When rated in the morning mood and arousal were considerably lower as compared to the evening before, due to sleep loss, and not different from placebo. It again shows that residual concentrations of MDMA or stimulatory effects of MDMA on mood and arousal cannot compensate for decrements in subjective mood and arousal as a result of fatigue.

In summary, it can be concluded that sleep deprivation has a major impairing effect on cognitive and psychomotor performance. Low doses of MDMA generally failed to affect performance but produced some stimulatory effect on rapid information processing at the highest dose. MDMA increased ratings of positive mood and alertness. However, the stimulatory effects of MDMA were only mild and never sufficient to overcome decrements in performance, mood, or arousal due to sleep loss.

CHAPTER 8: GENERAL DISCUSSION

The primary aim of this dissertation was to investigate the relation between two frequently used substances, i.e. THC and MDMA, and traffic safety by assessing their effect on actual driving and psychomotor performance. Additionally, studies on the relation between THC and roadside drug screening methods, i.e. SFST performance and oral fluid point of collection testing devices, were presented.

Acute THC effects on actual driving performance

The study in Chapter 3 assessed actual driving performance under the influence of dronabinol in occasional and heavy cannabis users. It showed that acute administration of dronabinol caused driving performance impairment in cannabis users compared to placebo. The heavy cannabis users demonstrated less impairing effects than the occasional cannabis users, probably due to tolerance.

Tolerance is defined as the production of a decreased effect of a given dose of a drug after repeated administration. Thus, increasing dosages are needed to reach the same effect a lower dose could produce previously. Tolerance can be developed for some effects and not for others. In our study, most subjects developed tolerance to the impairing effects on driving performance, but not to the subjective effects of feeling high. This has been demonstrated in other studies as well (e.g. Ramaekers, Kauert, et al., 2009; Ramaekers, et al., 2010).

How subjects develop tolerance may depend on three mechanisms (Julien, et al., 2008). In *metabolic tolerance*, more enzyme is available to metabolize the drug. When *pharmacodynamic tolerance* occurs, the receptors in the brain adapt to different levels of the drug in the brain. When drug levels are high, the receptors are down-regulated, i.e. the number of available receptors is reduced or they become less sensitive. A third mechanism is behavioral conditioning, or *context-specific tolerance*, where context cues that are normally present when taking a drug, can cause a diminished drug effect. This context can be the actual surroundings or particular (learned) behavioral circumstances. Tolerance based on learned behavior is also called behavioral tolerance (cf. Vogel-Sprott, 1997). Thus, when taking a drug in a different context or under different circumstances, tolerance does not occur.

It is sometimes suggested that heavy cannabis users recognize their impairment and use compensatory behavior (i.e. behavioral tolerance) to correct for driving impairment, e.g. driving more slowly (Robbe, 1998; Sewell, et al., 2009), even though it has never been demonstrated that this leads to safer driving (Grotenhermen, et al., 2007; Ramaekers, et al., 2004; Ramaekers, et al., 2000). Chapter 3 underscores this by showing that even some heavy cannabis users were impaired. Moreover, such compensatory behavior was not demonstrated. Subjects were instructed to drive at a constant speed of 95 km/h. The results showed no significant effect of mean or standard deviation of speed and mean speed was virtually the same in all treatment conditions, i.e. between 95.6-95.8.

It cannot be excluded however that heavy cannabis users recruited more cognitive resources to accomplish the driving task. Driving is a complex task that involves several cognitive functions, such as memory, (divided) attention, psychomotor and executive functions. For example, these functions are used when continuously monitoring the lateral position of the car, remembering the maximum speed or responding to a sudden event such as another car that fails to yield. Previous fMRI studies have demonstrated that heavy cannabis users showed increased activation in the frontal cortex compared to matched controls during tasks related to executive

functions and attention (Abdullaev, Posner, Nunnally, & Dishion, 2010; Kanayama, Rogowska, Pope, Gruber, & Yurgelun-Todd, 2004; Schweinsburg, et al., 2010). Also, heavy cannabis users demonstrated activation in an alternative network during a working memory task (Kanayama, et al., 2004). Thus, the absence of impairment on the road-tracking task in some heavy cannabis users might be related to compensatory mechanisms in the brain.

Does abstinence recover THC induced psychomotor impairment?

In Chapter 5 psychomotor performance was assessed in chronic, daily users during abstinence. Performance of the chronic, daily users was compared to performance of non-intoxicated occasional drug users. It was found that although chronic, daily users' performance improved during abstinence, they did not reach the same level of performance as the control group after 3 weeks of abstinence. Consequently, after this period of abstinence, subjects either experience residual cannabis effects, withdrawal effects or long-term effects that are not reversible. Residual cannabis and withdrawal effects had probably a minimal effect on performance. Although low levels of THC were still present in plasma of most chronic, daily users after 3 weeks of abstinence, it was previously demonstrated that the psychomotor tests were not sensitive to impairment at those concentrations (Ramaekers, Moeller, et al., 2006). Withdrawal is most prominent during 3-7 days of abstinence (Pope, Gruber, & Yurgelun-Todd, 2001), but could not entirely be excluded as subjective withdrawal effects might last longer (Allsop, Norberg, Copeland, Fu, & Budney, in press). Thus, it seems that chronic, daily use of cannabis may cause long-term impairment which persisted throughout 3 weeks of abstinence.

This corroborates the finding from another study where attention did not recover after 3 weeks of abstinence, although other measures of cognitive performance did (Hanson, et al., 2010). Whether this impairment is irreversible could not be decided based on this study. However, another study showed that after 4 weeks of abstinence the difference in neuropsychological performance (i.e. memory, intelligence, attention and executive function) between chronic, daily users and a control group disappeared (Pope, Gruber, Hudson, et al., 2001). Moreover, a recent PET study showed that the cannabinoid receptor (CB₁) density in areas involved in the aforementioned functions, i.e. cortical and limbic areas, returned to normal levels after 4 weeks of abstinence in chronic, daily users (Hirvonen, et al., 2011). These findings indicate that after 4 weeks of abstinence, THC induced cognitive impairment and neural changes did not persist. Down-regulation of CB₁ receptors due to chronic, daily cannabis use in motor areas was not demonstrated, thus long-term persistent psychomotor effects cannot be excluded.

Detection of THC induced impairment with the SFST

In Chapters 3 and 4, the relation between SFST performance and THC serum concentration was assessed. In Chapter 3 SFST performance was assessed in occasional and heavy cannabis users receiving orally administered dronabinol. Chapter 4 assessed SFST performance in heavy cannabis users under the influence of smoked THC with and without alcohol. This study showed that SFST was mildly sensitive to THC effects without alcohol. Performance on the SFST correlated positively with THC concentrations. This indicates that when THC increased from baseline (i.e. mean 7.1 µg/

L) to the THC only condition (i.e. mean 13.4 µg/L), impairment on the SFST increased as well. This is in line with a study in occasional users that also showed a moderate relationship between THC dose and SFST performance (Papafotiou, et al., 2005a).

When THC was combined with alcohol, heavy cannabis users demonstrated moderate impairment on the SFST. This finding indicates that despite tolerance for THC effects, heavy cannabis users do not show cross-tolerance for alcohol. The absence of cross-tolerance for alcohol effects in heavy cannabis users was also demonstrated on psychomotor performance tests (Ramaekers, et al., 2010). It can therefore be speculated that heavy cannabis users would show driving impairment as well when THC and alcohol are combined. This is not an unlikely combination in traffic and was shown to impair driving performance of occasional cannabis users (Lamers & Ramaekers, 2001; Ramaekers, et al., 2004; Ramaekers, et al., 2000; Robbe, 1998).

However, SFST performance and dronabinol dose (Chapter 3) did not show a relation in either occasional or heavy cannabis users. Even though actual driving performance was clearly impaired in the occasional cannabis users, their performance on the SFST was not. This is most likely due to the different pharmacokinetics of dronabinol compared to smoked cannabis. THC serum concentrations after dronabinol are generally lower than after smoked THC (e.g. Goodwin, et al., 2006; Huestis, Henningfield, & Cone, 1992; Toennes, et al., 2008). Furthermore, the SFST was performed 4.5-5 hours after dronabinol administration. While the blood concentrations and effects of dronabinol peak later and last longer than smoked cannabis, it is possible that THC concentrations were already declining, causing the non-significant findings. Together, it seems that for roadside testing the SFST is not a sensitive measure to determine THC induced impairment. This was also demonstrated in another study in which all signs of the Drug Evaluation and Classification (DEC) program were correlated with 3 drug categories, i.e. cannabis, CNS stimulants and narcotic analgesics (Porath-Waller, et al., 2009). This study found that only the one-leg-stand of the SFST predicted the drug categories correctly, besides measures of the eyes (e.g. eyelids, convergence), pulse rate, blood pressure and injection sites. Thus, these additional measures are clearly needed besides or instead of the SFST to identify THC impaired drivers and improve traffic safety.

Oral fluid testing of THC

Oral fluid point of collection testing devices can be used as a roadside screening method to determine drug concentrations in drivers. The devices are developing rapidly. While Chapter 2 demonstrated that none of the devices available between 2004 and 2009 performed acceptable for roadside testing, nowadays problems with THC adsorption are being resolved and reliability of the devices is improving. Therefore, oral fluid testing has the potential of contributing to traffic safety in the near future. The Dräger Drug Test® 5000 has demonstrated a sensitivity of 94% for THC in the smoked cannabis study (Chapter 4). Sensitivity is a measure of how accurate an oral fluid test is in determining a positive specimen (i.e. true positives / true positives + false negatives). A previous study with the same device demonstrated a similar sensitivity (i.e. 93%), a specificity of 71% and efficiency of 90% (Wille, Samyn, Ramirez-Fernandez, & De Boeck, 2010). Specificity indicates the accuracy of an oral fluid test in determining when a specimen is negative (i.e. true negatives / true negatives + false positives) and efficiency is the proportion of positive and negative specimens (i.e. true positives + true negatives / total). Although the THC cutoff of the Dräger Drug

Test® 5000 is just above proposed guidelines (Pil, et al., 2007; SAMHSA, 2004; Walsh, et al., 2008) and the specificity needs improvement, it is quite accurate in determining THC positive specimens. On the other hand, the Securetec Drugwipe® 5 which was also assessed in Chapter 4 demonstrated lack of sensitivity to THC. It is therefore not recommended to use this device for roadside drug screening. In sum, some oral fluid devices, e.g. Dräger Drug Test® 5000, seem ready to be evaluated along the road. However, confirmatory blood analyses are needed in order to prevent false positives. It should also be realized that when the oral fluid test indicates a specimen is negative, it does not necessarily mean the driver is not under the influence of drugs due to a relatively high rate of false negatives. This can also be ascertained by a confirmatory blood analysis.

Acute MDMA effects on driving (related) performance

Chapters 6 and 7 describe two MDMA studies: one on driving performance and one on psychomotor performance. Subjects were tested before, during and after a night without sleep. Results of both studies demonstrated that sleep loss caused impairment on driving as well as psychomotor performance. In all conditions, i.e. 25, 50 and 100 mg MDMA and placebo, both measures worsened dramatically after sleep deprivation compared to the measures before sleep deprivation. This shows that driving after a night without sleep is comparable to driving under the influence of alcohol above the legal limit of 0.5 mg/mL BAC (cf. Louwerens, et al., 1987). This is in line with previous research that also showed the detrimental effects of sleep loss on performance (Dawson & Reid, 1997; Kuypers, et al., 2008; Kuypers, et al., 2007), especially on dull, monotonous tasks (Harrison & Horne, 2000) such as the road tracking task.

MDMA did not show an effect on driving and psychomotor performance, except on a task measuring rapid information processing. An interaction between sleep loss and MDMA demonstrated that the speed of information processing increased under the influence of the highest MDMA dose in the morning after sleep deprivation compared to placebo. This indicates that MDMA has stimulating properties. Previous studies also showed that MDMA improved psychomotor speed acutely (Dumont, et al., 2009; Kuypers & Ramaekers, 2007; Kuypers, et al., 2006; Kuypers, et al., 2008; Lamers, et al., 2003; Ramaekers, Kuypers, et al., 2006). However other measures demonstrated the acutely impairing effects of MDMA, most notably on memory and attentional processes (Dumont, et al., 2008; Kuypers & Ramaekers, 2005, 2007; Kuypers, et al., 2008; Kuypers, et al., 2007; Lamers, et al., 2003; Ramaekers, Kuypers, et al., 2009). There are several possible explanations why we did not find any effect of MDMA on the other measures of psychomotor performance. First, the MDMA doses administered were lower than in most other studies. The reason for choosing these lower doses was to have a broad range of blood concentrations in order to be able to determine cutoff concentrations for impairment. However, the highest MDMA dose was comparable to doses administered in other studies. Second, even after the highest dose, blood concentrations were markedly lower than in other studies. Apparently, individual differences in pharmacodynamics between the subjects of the various studies existed. It was suggested before that MDMA has a non-linear pharmacokinetic profile and that genetic differences are related to inter-individual differences in pharmacodynamics (de la Torre, et al., 2000; de la Torre, et al., 2004; Musshoff, Stamer, & Madea, 2010). Third, the fact that we tested in the evening and after sleep deprivation might be related to the different findings between studies.

As mentioned above, other studies that tested subjects during daytime found stimulating effects on psychomotor measures. Only two studies assessed the effects of MDMA and sleep deprivation on cognition and mood and found impaired tracking, divided attention and memory performance and improved response speed and positive feelings after MDMA administration, which returned to placebo levels after sleep deprivation (Kuypers, et al., 2008; Kuypers, et al., 2007). However, in these studies a repeated dose was administered. Since we did not find the stimulating effects, it seems that a single acute dose of MDMA is unable to compensate for the impairing effects of sleep deprivation.

Consequently, our results suggest that driving is impaired the morning after a night of partying with the party-drug ecstasy. The impairment is primarily due to sleep loss, but in this real-life situation the effects of the drug and the sleep loss cannot be dissociated. Furthermore, subjectively the drug has a stimulating effect, which causes subjects to feel less sleepy. Therefore they may think that they are able to drive a car, while objectively they cannot. Besides the stimulating effects, previous studies also showed acutely impairing effects of MDMA on spatial and working memory and movement perception, which are highly relevant cognitive functions for safe driving (Kuypers & Ramaekers, 2005; Lamers, et al., 2003). Moreover, people generally use ecstasy in combination with other drugs, e.g. alcohol, which might cause additive impairment (Dumont, et al., 2009; Dumont, et al., 2007; Kuypers, et al., 2006). Taking all these factors into account, traffic safety is compromised when drivers are under the influence of ecstasy, especially at night.

Future research

Experimental studies, like the ones described in this dissertation, can demonstrate direct causal relations between drug concentrations in bodily fluids and impairment. Future research should focus on common drug combinations. For example, the combination of ecstasy and cannabis is common in real life situations. Only few studies assessed the combined effect of MDMA and THC (Dumont, et al., 2010; Mohamed, Hamida, Cassel, de Vasconcelos, & Jones, in press). The implications of this combination for traffic safety are largely unknown (cf. Rizzo, et al., 2005). Synergistic effects can increase crash-risk significantly compared to either drug alone. Moreover, as demonstrated in this dissertation, drug use history should be taken into account as well. For example, the combination of cannabis and alcohol in heavy cannabis users on driving performance has not been investigated yet. These studies can inform legislative authorities on which drug combinations threaten traffic safety (most). Measures to identify people who drive under the influence of combinations of drugs can then be developed, such as oral fluid tests and the SFST.

Furthermore, the question whether long-term psychomotor impairment after abstinence in heavy cannabis users persisted was not indisputably answered. Longitudinal studies could provide us with an answer. When subjects are followed for a longer period of time (e.g. months), withdrawal and residual cannabis effects can definitely be excluded as reasons for impairment. Functional brain imaging and PET studies can also contribute to the knowledge of long-term impairment in heavy cannabis users, because with these techniques changes in the brain can be made visible. If long-term psychomotor impairment is established in heavy cannabis users, they might threaten traffic safety and measures to prevent them from participating in traffic should be taken.

The SFST does not seem sensitive to THC effects. However, it might be useful to determine impairment caused by other drugs such as amphetamines, cocaine or benzodiazepines. Studies on the relation between these drugs and SFST performance are lacking, even though they are frequently used and crash-risk is elevated (EMCDDA, 2008b). Furthermore, the relation between THC concentrations and performance on other tests such as hippus, rebound dilation and condition of the eyes as proposed by Porath-Waller et al. (2009) should be investigated in experimental within-subjects designs in order to assess the sensitivity of these tests. With this knowledge impaired drivers can possibly be better identified, which will improve traffic safety.

Finally, some hurdles still need to be taken as far as oral fluid tests are concerned. Besides improvements in THC specificity, issues such as evaluation of analyte stability, determination of windows of detection and characterization of adulteration techniques are largely unknown. Even though performance of the Dräger Drug Test® 5000 was quite accurate in laboratory tests, performance in various weather conditions also needs to be assessed, because it was previously demonstrated that cold and rainy weather can cause problems in reading the results of some devices (Verstraete & Raes, 2006). Once these issues are solved traffic safety can be improved by correctly identifying drugged drivers and taking them off the roads.

Conclusion

Overall, the studies in this dissertation indicate that traffic safety is compromised whenever people drive under the influence of cannabis or ecstasy in combination with sleep deprivation. Especially occasional drug users are at risk of experiencing acute driving impairment. However, heavy drug users contribute to diminished traffic safety as well, though to a lesser degree than occasional drug users. It was demonstrated that on average a heavy users group was tolerant for the effects of THC on driving performance. However, individual data indicated that this was not true for all heavy cannabis users. Some of these subjects showed clinically relevant driving impairment as they performed above the alcohol criterion of driving under the influence of 0.5 mg/mL BAC, i.e. an increase of ≥ 2.4 cm in standard deviation of lateral position compared to placebo (Louwerens, et al., 1987). Furthermore, even chronic, daily cannabis users who abstained from cannabis for 3 weeks, might jeopardize traffic safety as they performed worse than a control group on psychomotor performance tasks. Also, heavy cannabis users were not completely tolerant to the impairing effects of THC in combination with alcohol on the SFST. However, the SFST was generally mildly sensitive to the impairing effects of THC alone and therefore not suitable for roadside identification of THC impaired drivers. Oral fluid tests on the other hand can have a significant contribution to traffic safety by improving the correct detection of THC negative specimens. In sum, cannabis and sleep deprived ecstasy users threaten traffic safety due to impaired driving performance. Cannabis use history played a moderate role in aptness to drive, because heavy cannabis users' impairment was less severe but not absent.

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