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84 *Current Neuropharmacology*, **2018**, *16*, 84-96

REVIEW ARTICLE

Correlation between Blood and Oral Fluid Psychoactive Drug Concentrations and Cognitive Impairment in Driving under the Influence of Drugs

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Abstract: ***Background*:** The effects of drugs on driving performance should be checked with drug concentration in the brain and at the same time with the evaluation of both the behavioural and neurophysiological effects. The best accessible indicator of this information is the concentration of the drug and/or metabolites in blood and, to a certain extent, oral fluid. We sought to review international studies on correlation between blood and oral fluid drug concentrations, neurological correlates and cognitive impairment in driving under the influence of drugs.

***Methods*:** Relevant scientific articles were identified from PubMed, Cochrane Central, Scopus, Web of Science, Science Direct, EMBASE up to April 2017.

***Results*:** Up to 2010, no epidemiological studies were available on this matter and International scientists suggested that even minimal amounts of parent drugs in blood and oral fluid could affect driving impairment. More recently, epidemiological data, systematic reviews and meta-analysis on drugged drivers allowed the suggestion of impairment concentration limits for the most common illicit drugs. These values were obtained comparing driving disability induced by psychotropic drugs with that of established blood alcohol limits. Differently from ethyl alcohol where both detection methods and concentration limits have been well established even with inhomogeneity of ranges within different countries, in case of drugs of abuse no official cut-offs have yet been established, nor any standardized analytical protocols.

***Conclusion*:** Multiple aspects of driving performance can be differently affected by illicit drugs, and even if for few of them some dose/concentration dependent impairment has been reported, a wider knowledge on concentration/impairment relationship is still missing.

ARTICLE HISTORY

Received: June 17, 2017
Revised: August 09, 2017
Accepted: August 24, 2017

DOI:
10.2174/1570159X15666170828162057

Keywords: Cognitive impairment, driving under the influence of drugs (DUID), blood, oral fluid, cut-off.

1. INTRODUCTION

A recent report by The World Health Organization on road safety showed that in 2013, 3.16% and 15.03% of total road traffic deaths have been related to psychoactive drug use or alcohol consumption, respectively [1]. The association between alcohol drinking, impaired driving, and road accidents risk has been extensively investigated, leading to official international threshold limits for alcohol concentration in blood when driving, standardized analytical methods to determine breath alcohol concentration at roadsides and blood alcohol levels at emergency departments. In the last twenty years, awareness of road safety and psychoactive drug use has been increased, due to the evidence that these substances have the ability to affect the correct functioning of the central nervous system and may impair driving skills (*e.g.*, attention, time estimation, reaction time). Starting from 1999, several international research projects have been performed to investigate various aspects of psychoactive drugs and driving. Roadside surveys, self-reported questionnaires or interviews as well as epidemiological studies have involved both the general driving population and specific subsets of drivers to detect the different prevalence of drugs. It should be considered that direct comparison between different studies is difficult to achieve, because of the different approaches involved and considered parameters such as the time of sampling (*e.g.*, daytime or night-time), biological matrix chosen for analysis (*e.g.*, blood, oral fluid, urine), sample devices for collection, and also selected drugs and their cut-offs [2]. Recent studies have reported that the prevalence of drug use among drivers ranges from 3.9 to

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1570-159X/18 $58.00+.00

20.0% [3, 4], and among fatally injured people in a road traffic crash from 8.8% to 33.5% [3, 5]. Drugs relevant to impaired driving include prescription drugs (*e.g.*, benzodiazepines, opioid analgesics, antidepressants), illicit psychotropic drugs (*e.g.*, cocaine, heroin, amphetamines, cannabis), and new psychoactive substances (*e.g.*, synthetic cannabinoids or cathinones). Cannabis, cocaine, amphetamines, and opiates are the most detected drugs in general driving population [6]. In addition, a recent systematic review and meta-analysis of 66 international studies concluded that the use of amphetamines, benzodiazepines, cannabis, cocaine and opiates was associated to a higher risk of a fatal road crash [7]. Similarly, the "World Health Organization report on road safety" showed that amphetamine is responsible for 51% drug-related road traffic deaths, followed by cannabis (22%), cocaine (14%) and opioids (13%) [1]. Measures to reduce drug induced road injuries have been introduced worldwide, and involve awareness-raising about drug-driving injury, law provisions and their enforcement together with drug testing at roadside [8]. Blood is the matrix of choice for drug confirmation and quantification analysis in driving under the influence of drugs cases, due to a good correlation with pharmacologic effects of the drug at the central nervous system. Cut-offs for blood concentrations have been proposed by different countries and bodies [9, 10] and recently a comparison between different proposals evidenced great international inhomogeneity [11].

Since the 1970s, oral fluid (OF) has been studied as an alternative sample matrix to disclose current consumption of psychotropic drugs [12-16] and recently its use has been proposed as on-site test at roadside to identify drivers under the influence of drugs [17-19]. The main advantages of oral fluid are the easy and non-invasive sample collection, more difficult adulteration of the sample, and a lower infection risk compared to blood collection. Passage of drug molecule from blood to OF is created primarily by passive diffusion that depends on several physicochemical factors, such as: pH of blood and OF, pKa and lipid-solubility of the drug, its molecular weight, fraction bound to plasma proteins and salivary flow rate. As only unbound or free drugs are excreted into the oral fluid, there is evidence for some drugs that OF concentrations correlate with free drug plasma concentrations, so OF may better reflect recent drug use, providing a better correlation with pharmacodynamics effects, such as impaired performances. Limitations associated with OF testing include the difficulty in collecting proper volume, oral cavity contamination after oral or smoked administration and dry mouth following cannabis or stimulant drugs use [20, 21]. The performance of OF testing when driving under the influence of drugs is yet to be investigated in systematic studies [10, 19, 22-24]. Moreover major gaps exist, ranging from the relationship between drug concentration in oral fluid and blood, cognitive impairment and crash risk, standardized analytical methods and appropriate drug cut-off associated to alteration in driving skills.

Nevertheless, due to several advantages of OF testing (*e.g.*, on site testing at the roadsite, non- invasive collection not requiring skilled personnel, possibility to collect a second sample for laboratory confirmation), quite recently this matrix has been introduced in place of blood.

In this scenario, we sought to review international studies on correlation between blood and oral fluid drug concentrations, neurological correlates and cognitive impairment in driving under the influence of drugs.

2. METHODS

Relevant scientific articles were identified from PubMed, Cochrane Central, Scopus, Web of Science, Science Direct, EMBASE up to April 2017 using the following keywords: "cannabis", "marijuana", "heroin", "cocaine", "amphetamines", "amphetamine-type stimulants", "new psychoactive substances", "driving under the influence", "cognitive impairment", "neurocognitive correlates", "acute cognitive effects", "chronic cognitive effects" and "toxicological analysis". The main keywords were individually searched in association with each of the others. The papers not suitable for the purpose of the review were excluded; hand search was performed through the reference-lists of the included articles.

3. RESULTS

The experimental studies investigating the psychoactive effects of drugs on neurological performances, and how these effects correlate with blood and/or oral fluid concentrations and with driving impairment, have been reported for each psychotropic drug or class of drugs. Table **1** shows blood and oral fluid psychoactive drug concentrations related to cognitive impairment parameters in driving under the influence of drugs.

4. CANNABIS

Cannabis is a natural drug, consisting of the dried flowering, fruiting tops and leaves of the Cannabis sativa plant. Hashish is the dried resinous secretion of the plant, and cannabis oil is a solvent extract of cannabis. The main psychoactive substance is tetrahydrocannabinol (THC), which is highly lipophilic and can be distributed widely in the body [25]. Acute and chronic use of cannabis has been shown to impair psychomotor functions, memory and attention, often in a dose dependent manner [26, 27]. Since the '70 of the last century, experimental investigations have assessed the effects of cannabis on neurocognitive functions necessary in normal driving tasks [28]. Both acute and chronic use of cannabis has been linked to affect driving skills such as motor control, psychomotor speed, executive function, motor impulsivity, manual dexterity, visual processing, short-term memory, working memory, perception and balance [29-31]. Cognitive impairment following THC smoking can be detected several hours after its use; *e.g.*, in healthy volunteers, memory impairment persists for about 10 hours after the administration of 15 mg of THC [32]. Recent studies showed that chronic users of cannabis often present a long-term cognitive impairment that may persist even after a period of abstinence [26, 33, 34]. In a recent meta-analysis, cannabis use was found to be associated with a higher relative risk (RR) of being involved in fatal (RR 1.25) or injury (RR 1.08) road accidents [7].

Even if THC blood concentrations do not directly correlate with those in the brain responsible for cognitive

Table 1. Blood and oral fluid psychoactive drug concentrations related to cognitive impairment parameters in driving under the influence of drugs.

Psychoactive Substance	Impairment Parameters Reported	Blood Concentration	Impairment Correlation	Oral fluid Concentration and OF/B	Impairment Correlation	Refs.
Cannabis (THC)	Critical tracking task (perceptual motor control) Stop signal task (motor impulsivity) Tower of London task (cognition and judgment)	0 - >30 ng/ml	Critical tracking task: p=0.026, R=-0.13 Stop signal task: p<0.001, R=0.32 Tower of London task: p<0.001, R=-0.38	10-30 folds higher than serum THC (OF/B p<0.001, R= 0.84)	Critical tracking task: p=ns, R=-0.18 Tower of London task: p=0.006, R=-0.35	[37]
Cannabis (THC)	31 signs of impairment (attitude, body appearance, facial expression, speech, coordination, and eye signs)	Not tested	Not tested	≤ 3.00 - >100 ng/mL	OR 1.70 (THC ≤ 3.00 ng/mL), 3.78 (THC 3.01-25.00 ng/mL), 7.22 (THC 25.01-100 ng/mL), 16.55 (THC <100ng/mL)	[47]
Amphetamine/Methamphetamine	Clinical test for impairment (CTI, 25 tests related to common signs of drug impairment)	Median amphethamines concentration 0.53 mg/l (range 0.04-3.74 mg/l)	OR adjusted for age, gender and blood amphetamines concentration: 0.98 (0.96-1.00)	Not tested	Not tested	[125]
Cocaine	Walking performance, speech, mood, motor coordination, pupil's state	Not tested	Not tested	Mean: 4.12 mg/l M ± SD: 0.38 ± 28.28 mg/l Range: 0.01-345.64 mg/l	No significant correlation observed	[106]
Cannabis (THC)	15 signs by police observation 15 signs by medical examination	THC serum concentrations in impaired/not impaired users is reported in the next column	31.9% of users were classified as impaired by both police and medical officers (THC in serum 4.6 ± 4.8 µg/L, M ± SD) 27.7% were classified as impaired only by police officers (THC in serum 5.9 ± 4.9 µg/L) 31.9% appeared not to be impaired at all (THC in serum 5.0 ± 5.3 µg/L) However a correlation of the THC concentrations with the impairment was not possible	Oral fluid versus serum Accuracy 90.8% Positive predictive value 93.0% Negative predictive value 88.3%	Not reported	[50]
Opioids		Not reported	77.8% of users were classified as impaired by both police and medical officers. Opiods serum concentrations not reported	Oral fluid versus serum Accuracy 95.4% Positive predictive value 91.3% Negative predictive value 96.3%	Not reported	
Amphetamines		Not reported	50.0% of users were classified as impaired by both police and medical officers. Amphetamines serum concentrations not reported	Oral fluid versus serum Accuracy 93.1% Positive predictive value 81.4% Negative predictive value 98.9%	Not reported	

impairment [35], several studies have investigated the link between THC blood levels and impaired driving. Recent epidemiological studies have suggested that THC blood concentrations of 2-5 ng/ml are generally associated with driving impairment and increased accident risk [27, 36-40]. Proposed cut-offs for THC blood concentration range from 1 to 5 ng/ml [11] but several countries adopt a zero-tolerance policy that does not allow the presence of any amount of THC in blood while driving. Nevertheless, a significant correlation between THC blood concentrations and drivers impairment is still lacking mainly due to the complex pharmacokinetics and metabolism of THC and the inter-individual variability in blood concentrations of consumers of similar doses [37]. Moreover, it should be considered that the results of studies on the driving effects of cannabis use have been related to the investigation strategies applied and to the impairment parameters considered. Indeed, Battistella and coworkers recently suggested that driving skills correlate with the subjective feeling of confusion rather than with the blood level of THC [41], while Karschner *et al*. supported that THC's presence in blood may not correlate with its recent use and driving impairment since THC whole blood/plasma levels >1 ng/mL (range 1.2–5.5 ng/mL) could be measured in 50% chronic daily cannabis smokers even after 7 days of abstinence [42].

An experimental study on performance impairment as a function of THC in serum and OF by Ramarkers and collaborators showed a strong and linear correlation between THC in serum and OF; however they did not find a significant linear association between performance impairment and serum THC values. Nevertheless, when comparing the proportion of observations showing impairment or no impairment as a function of THC concentration, impairment was found to progressively increase as a function of serum THC in every performance task. Subsequently, at THC concentrations between 5 and 10 ng/ml, approximately 75–90% of the observations showed a significant impairment in every performance test. From all the gathered information, it was concluded that serum THC concentrations between 2 and 5 ng/ml represent the lower and upper range of THC limit for impairment [37].

With respect to OF, the possibility to predict the prevalence of blood THC concentrations above the chosen limits in a population by analysing oral fluid was investigated. Equivalent cut-off thresholds could be estimated in a regression model with concentration percentiles in OF as dependent variables and the corresponding concentration percentiles in blood as independent variables. The authors used paired OF and blood samples to determine accurate regression formulae for THC cut-off concentrations in oral fluid corresponding to 2.0, 4.0, 6.0, 8.0 and 10.0 ng/ml in blood. The accuracy was better than 100 ± 20% in comparison to actual prevalence in blood. However, the authors highlighted that the regression formula could be influenced by some factors, as the number of samples investigated, the oral fluid sampling method, and the time between cannabis smoking and sampling [43,44].

Finally, several studies tried to establish fixed ratios between the THC concentrations in oral fluid and blood and between oral fluid THC and driving impairment. Although some correlations have been described, large inter-individual variations in THC OF/ blood ratio have been reported, along with as a weak relationship between performance impairment and THC oral fluid concentration. Eleven -hydroxy-delta (9)-tetrahydrocannabinol (11-OH-THC) and 11-nor-9-carboxy-delta-9-tetrahydrocannabinol, (THC-COOH) must be measured in biological specimens in addition to THC. If both metabolites are present, it indicates that cannabis was consumed more recently and motor impairment may still be present [37, 45-53].

5. OPIOIDS

Opioids are primarily used as licit drugs in the treatment of moderate to severe pain [54, 55]. At the same time, both natural, synthetic and new synthetic opioid (*e.g*., fentanyl and derivatives) are a class of psychotropic substances that are widely mistreated [56, 57]. Cognitive functions and psychomotor abilities have been moderately affected by opioids' use, although the effects vary between different molecules and are generally most pronounced during the first few days after starting opioid therapy, before tolerance develops [58, 59]. An early studies of this century by Zacny *et al*. tested psychomotor and cognitive performance following the administration of commonly prescribed opioids to healthy, opioid naive subjects finding no evidence of statistically significant impairment with respect to placebo-administered subjects [60-62].

In a 2011 published meta-analysis, a single dose of morphine of up to 5 mg has been shown to cause only very few effects on driving performances tasks whereas higher doses corresponded to alteration of various tasks, but no clear direct dose-effect relationship was observed. Differently, single dose of methadone up to 10 mg impaired performance of drug-naïve, healthy subjects, but these effects were less pronounced in opioid users [63].

Concerning codeine, another driving simulator study showed that 120 to 270 mg daily use of this natural opioid does not impair driving abilities in patients with chronic pain [64]. Conversely, chronic heroin users showed impairment of planning function [65], reaction time [66], time perception [67], spatial working memory [68], executive functioning [68-70], and right-left discrimination [71] with some of these impaired skills persisting for more than 1 year after cessation of the drug's use [72].

Apart from these investigations carried out on driving simulators, international literature reports some epidemiological studies on the risk of it being involved in a traffic accident while driving under the influence of opioids. Increased accident relative risks were observed when driving under the influence of any opioid alone (morphine, heroin, codeine or methadone) [38, 73-75]. A recent control case study based on data from nine European countries estimated a moderate increased relative risk (RR 2-10) of serious in-

jury when driving under the influence of medicinal or illicit opioids [76].

OF gave an excellent alternative biological matrix for opioids detection in case of driving under the influence of these drugs. The biomarker of heroin consumption, 6-monoacetylmorphine (6-MAM), can be easily found in oral fluid [77], providing a useful tool for screening of opioids consumption at the roadside [10, 78, 79]. It is known that within a few minutes after intake, heroin is metabolized to 6-MAM and then further to morphine [77]. The short half-life makes 6-MAM only detectable for a short time, and it is rarely found in blood or urine. Its presence in oral fluid can be used to differentiate heroin use from the consumption of morphine alone or codeine in drivers suspected of opioids' use [10].

In another work, by Langel and collaborators, the median OF/B ratios for codeine (4.8), methadone (1.8), morphine (6.4) and tramadol (1.1) were found to be close to theoretical values based on the physicochemical properties of the drugs, and a statistically significant correlation between OF and blood concentrations was observed. However, some cases were only positive in oral fluid; most of them have been explained by the low concentration of the analyte in the OF sample, and due to longer detection times in the OF with the cut-offs used, the corresponding lower blood concentrations might not have been detected [51].

Toennes and collaborators found more than 90% accuracy in correlating opioids' detection in oral fluid and serum. Moreover, impairment symptoms considered in the study were detected in all the opioid user group both by police officers at the time of the offence and by medical officer at the time of sampling [80].

In North America (US and Canada), there is an epidemic of the abuse of prescribed opioids, *e.g*., fentanyl, oxycodone or hydrocodone. With respect to these substances, some studies have been performed.

For example, it has been found that fentanyl in concentrations used in surgical procedures (0.2 μg/kg) produced pronounced cognitive impairment (auditory reaction time, signal detection, sustained attention, recognition) when compared with placebo [81]. Similarly, attention, reaction, visual orientation, motor coordination and vigilance were not affected by the long-term use of transdermal fentanyl in patients with continuous non-cancer pain [82, 83].

Several investigations in different countries agreed that psychomotor functioning, crucial for safe driving, was not impaired in patients with chronic pain receiving effective pain relief with controlled release oxycodone therapy [84-87].

Likewise, a modest psychomotor impairment has also been shown with increasing (0, 0.33, 0.65, 1.3 mg/70 kg) intravenous hydromorphone in non-drug-abusing volunteers [88]. This study has been subsequently confirmed by Byas-Smith *et al*., [89], who evidenced that many patients with chronic pain, even if treated with potent analgesics such as morphine and hydromorphone, show comparable driving ability as normals.

Finally, a very recent systematic review of experimental studies to define blood opioid concentrations related to impairment in opioid-naïve subjects concluded that plasma morphine concentration of 14.3 ng/ml could represent at a threshold concentration, under which there is little related road traffic risk. A single dose of 5 mg intravenous morphine and analgetic equivalence doses of fentanyl, hydromorphone, oxycodone and oxymorphone did not present traffic-relevant effects [90].

6. COCAINE

Cocaine, the psychoactive drug extracted from the leaves of Erythroxylum Coca shrub, is the most popular drug of abuse in Europe after cannabis. It belongs to the group of "stimulant drugs", increasing mood and feelings of well-being, energy and alertness [91]. Only few experimental studies investigated cognitive effects of acute cocaine use in naive users especially due to ethical issues since cocaine shows a considerable risk of addiction [92]. Hence, few systematic studies investigated the eventual driving ability under the influence of this drug.

Acute cocaine administration in users seems to improve response inhibition and a speed component in psychomotor tasks but only when intranasally administered [93-97]. Long term cocaine use has been associated with impairment in sustained attention, visuospatial perception, cognitive flexibility, response inhibition, memory and psychomotor performances [91, 98-101]. In agreement with the previous controlled observations, epidemiological studies demonstrated that cocaine may increase the risk of being involved in driving accidents [76, 102, 103]. In a meta-analysis carried out by Elvik *et al*. in 2013, the best estimate of the relative risk of accident involvement with cocaine was 2.96 (95% CI 1.18–7.38) for fatal accidents, 1.66 (95% CI 0.91–3.02) for injury accidents and 1.44 (95% CI 0.93–2.23) for crashes resulting in property damage [7].

In 370 fatally injured drivers in Washington State, the mean and median blood cocaine concentrations were 0.72 mg/l and 0.31 mg/l, respectively (range <0.01- 1.08 mg/l) [104]. Similarly in 1425 German drivers under suspicion of driving under the influence of psychoactive drugs, mean and median blood value for cocaine were 0.836 mg/l and 0.379 mg/l (range 0. 005-2 mg/l), respectively [105]. In 1791, Spanish drivers who were tested as positive for cocaine with mean and median cocaine concentration of 4.12 mg/l and 0.38 mg/l (range 0.01-345.64 mg/l), respectively, clinical impairment symptoms such as motor coordination, walking, speech, mood and state of pupils were not significantly correlated with cocaine concentrations [106]. Proposed cut-off values for cocaine in blood range from 10 up to 80 ng/mL depending on different international studies as reported by Busardò and collaborators [11].

The correlation between OF and blood cocaine concentrations is still not well established. Cocaine is a weak base

and is subject to OF ion trapping; however the use of cocaine leads to reduced salivary volume (dry mouth), and abuse of smoked crack cocaine, insufflation of cocaine hydrochloride, and oral cocaine abuse can lead to a contamination of the oral cavity resulting in high initial OF levels compared to concentrations that occur after intravenous cocaine administration [12]. In controlled administration studies, cocaine was identified in OF after smoking, intravenous, intranasal and oral administration [107-109]. After subcutaneous administration, cocaine was identified in OF 0.08-0.32 h after dosing, with a half-life of 1.1-3.8 h; the correlation between cocaine OF and blood concentrations was significant, although no correlation with clinical symptoms was described [110]. A cocaine OF/Blood ratio of 22 (range 4-119) was reported in a sample of people driving under suspicion of drugs [52] and a similar OF/blood ratio value (17) was also obtained by Langel and collaborators [51]. These data indicate that OF is a good alternative biological matrix to test cocaine use at the roadside or in drivers involved in car crashes, providing an immediate evidence of driving under the influence of this psychostimulant drug.

Cocaine can partially diminish performance impairments caused by alcohol consumption. The use of a combination of alcohol and cocaine decreases psychomotor impairment and improves performance on cognitive tests when compared with the use of alcohol alone [111,112]. Cocaine use also reduces the subjective feeling of drunkenness caused by alcohol [111,112]. Chronic use of alcohol or cocaine selectively affects performance on different neurobehavioural tests in a dose-dependent way [113]. However, their combined use may not cause additional negative effects on the brain, as subjects addicted to only cocaine demonstrate similar or greater neurocognitive impairments than those who abuse both alcohol and cocaine [114-116].

7. AMPHETAMINES

Amphetamine and amphetamine-type substances such as methamphetamine and 3,4-methylendioxymethamphetamine (MDMA, ecstasy) are most commonly abused due their stimulant effect on the central nervous system, that results in sociability enhancement, increased mood, auditory and/or visual perceptions, and energy boosting as reported by recreational users during controlled administrations or recreational settings (discos, dance parties, sex parties and raves) [117-120]. Acute or chronic use of amphetamines has been implicated in increased dangerous driving, with an estimated relative risk of 8.67 (95% CI 3.23 - 23.32) for crashes resulting in property damage, 6.19 (95% CI 3.46 - 11.06) for injury accidents, and 5.17 (95% CI 2.56 - 10.42) for fatal accidents [7]. In contrast with the above reported data, experimental studies showed that amphetamines like MDMA, methamphetamine and dexamphetamine, can improve certain aspects of cognitive/driving performances, while impairing other aspects at the same time. Skills such as tracking, impulse control and reaction time can be generally improved, whereas cognitive functions such as working memory and movement perception can be impaired [121-123]. Nevertheless, there is a limitation in these experimental investigations: doses used are not representative of the doses consumed by users in real-life scenarios.

High dose effect of amphetamines on driving performances cannot be assessed because of medical and ethical constraints, and concentrations of amphetamines in the blood of subjects driving under the influence of these drugs have been reported about or above 10-fold higher than those used in experimental studies [124]. Blood cut-off values for amphetamines have been recently proposed, ranging from 20 to 600 ng/mL for amphetamine, from 20 to 200 ng/mL for methamphetamine, and from 20 to 300 ng/mL for MDMA [11].

Gustavsen and collaborators investigated the concentration-effect relationship between blood amphetamine levels (range 0.04 - >1.00 mg/l) and impairment in 878 cases with amphetamine as the only drug present in the blood samples of impaired drivers. 73% of cases were judged as impaired and a significant positive concentration-effect relationship was found, with a ceiling effect above 0.27-0.53 mg/l [125].

Amphetamines are lipid-soluble, weakly basic drugs with high pKa and low plasma-protein-binding, and can be detected in oral fluid, generally with oral fluid concentrations several times higher than in blood. Recent studies showed the possibility of estimating the prevalence of blood amphetamine concentrations above the chosen limits in a population by analysing oral fluid. In addition, equivalent cut-off thresholds could be estimated using a regression model with concentration percentiles in the OF as the response variable and the corresponding concentration percentiles in blood as the predictor variable. They used paired OF and blood samples to determine accurate regression formulae to calculate amphetamine cut-off concentrations in oral fluid corresponding to 200, 400, 600, 800 and 1000 ng/ml in blood; the accuracy was better than 100 ± 20% compared to the actual prevalence in blood. However, the authors highlighted that the regression formula could be influenced by some factors, as the number of samples investigated, the oral fluid sampling method and the time between consumption and sampling [43, 44].

A significant OF/B correlation has been found in several studies [10, 51, 52, 126]; it was also reported that when similar detection cut-off values were used for both oral fluid and blood, some positive amphetamines samples were missed when analysing only the blood specimen, due to the larger detection time window in oral fluid and/or oral contamination [127, 128]. To the best of our knowledge, there are no studies investigating the relationship between amphetamines in OF and impairment signs associated to driving.

8. NEW PSYCHOACTIVE SUBSTANCES

New psychoactive substances (NPS) are generally reported as substances that are “not specifically controlled under the existing legislation, with the capacity to stimulate or depress the central nervous system resulting in hallucinations, dependence or significant changes to motor function, thinking or behaviour” [129, 130].

NPS are also known and sold under the names of "legal highs", "bath salts", "herbal highs", and "research chemicals"; most of them are not regulated through the International Drug Control Convention so that their legal status is differently settled in each country.

First classes of NPS were represented by cathinones, phenethylamines, tryptamines, piperazines, and synthetic cannabinoids molecules, recently followed by aminoindanes, arylalkylamines and arylcyclohexylamines molecules [131]. These substances are mainly sold over the Internet and despite the numerous reports, mainly by the users themselves, about the effects, pharmacology of NPS is not well known. In addition, knowledge regarding the potential toxic and lethal concentrations of these drugs after ingestion is absent for the majority of the different substances [132-134]. As previously mentioned, all the drugs that affect the CNS – whether prescribed or illegal drugs – may cause driving impairment by affecting crucial driving skills such as the reaction time, judgment and processing simultaneous tasks [135].

Driving under the influence of drugs has a major detrimental impact on traffic all over the world and the problems of driving under the influence of NPS constitute at the moment an open research area. Some investigators support the idea that since on site tests are still not able to detect NPS, it is not possible to exclude the presence of NPS in a driver even if the test at roadside shows a negative result to alcohol and classic psychotropic drugs [136].

Synthetic cathinones are the most frequently detected NPS in Europe together with synthetic cannabinoids. Notwithstanding this fact, the only cathinone investigated for its impact on driving has been alpha-pyrrolidinovalerophenone (α-PVP). This synthetic stimulant has been identified as the cause of a traffic accident involving the driver and four passengers. Variable concentrations of α-PVP were detected in blood samples of all individuals, ranging from 230-360 ng/ml for n. 3 surviving subjects and 290-650 ng/ml for the two deceased individuals [137].

Adamowicz and collaborators reported α-PVP blood levels of 9 drivers using exclusively this drug; a mean concentration of 20 ng/ml was not associated to any impairment sign, whereas a mean concentration of 39 ng/ml was associated to staggering, unsteady gait, confusion, and slurred speech. In 14 drivers, α-PVP has been detected in blood together with other drugs, but with mean concentrations similar to α-PVP-only cases (28 ng/ml for drivers with observed symptoms, and 38 ng/ml for drivers without symptoms) [138].

Synthetic cannabinoids are also frequently consumed NPS due to their marijuana-like effect; they are available as herbal products sprayed with these molecules that have been shown to be functionally similar to THC [139].

To date, synthetic cannabinoids are not included in any specific road legislation; their prevalence in DUID cases is arising and international studies have reported the association between positive chemical–toxicological analysis and various signs of impairment.

In 2014, Musshoff and collaborators reported several DUID cases involving synthetic cannabinoids. Drivers were found impaired by the police or medical personnel; toxicological analysis in blood allowed the identification and quantification of several synthetic cannabinoids, as AM 2201 (concentration range 0.31 - 4.6 ng/ml), JWH-018 (concentration range 0.17 - 1.9 ng/ml), JWH 210 (concentration range 0.66 - 6.2 ng/ml), JWH122(0.26 - 28ng/ml), JWH-019 (concentration 1.7 ng/ml), and JWH-307 (concentration 1.1 ng/ml) [140].

Other synthetic cannabinoids of newer chemical classes have been measured in the blood of six impaired drivers one year later by Karinen *et al*. UR-144 levels were 0.22 mg/l and 0.47 mg/l in two drivers; 5F-APINACA level was 5.3 mg/l in one case, whereas concentrations of 0.9 mg/l, 6.5 mg/l, and 2.2 mg/l were detected in three drivers together with APINACA concentrations of 2.2 mg/l, 0.24 mg/l, and 24.5 mg/l respectively.

In addition, AM-2201, a synthetic cannabinoid of first generation, was detected in blood samples of three drivers with concentrations ranging between 0.11 and 0.13 mg/l, close to previously reported ranges [132].

A potential reduction in driving abilities in subjects consuming synthetic cannabinoids has been proposed, since impairment signs as a delayed reaction of pupils to light, blurred speech, dizziness, instable appearance and retarded sequence of movements have been detected in these users [141].

Another important class of NPS is phenethylamines. The consumption of these compounds can lead to psychic alterations similar to those observed after the use of amphetamine or amphetamine-type substances such as MDMA, including different neurological and psychological symptoms which can affect a safe driving behaviour.

In a road accident involving a driver and one passenger, the phenethylamine 2C-B was detected at blood concentrations of 1.6 and 14 ng/ml, together with amphetamine levels of 23 and 38 mg/ml, respectively [131].

Methoxetamine, a dissociative drug that has been sold as a new designer drug, is a non-competitive NMDA receptor antagonist, reuptake inhibitor of different neurotransmitters [142]. This is also one of the NPS detected in the blood (10 ± 0.3 ng/ml, M ± SD) of a car driver with a polydrug intoxication who presented a compromised ability in controlling the motor vehicle [143]. A recent work indicates that the range of blood concentrations of methoxetamine in living individuals is similar in the U.S.A. and Europe (0.2– 0.5 ng/ml), despite the consumed dosage was unknown. A high methoxetamine level (8.6 ng/g) was found in a femoral blood sample in a post-mortem case [144].

Fassette and collaborators described a case of a female subject driving a vehicle under the effect of acute intoxication

after ingesting methoxetamine earlier in the day. Loss of legs control, difficulty in talking, confusion and excessive sweating were present. Screening of the blood sample showed positivity for amphetamines and confirmation analysis by GC/MS revealed methamphetamine (90 ng/ml), amphetamine (20 ng/ml), dextromethorphan (20 ng/mL) and methoxetamine at the concentration of 151 ng/mL [145].

A major observation can be made on impaired driving and NPS: to date, the lack of analytical data, pure standards, and studies on impaired performances following consumption, make NPS detection a major toxicological challenge. Although few data exist for NPS measurement in blood, no international literature exists regarding the determination of these substances in oral fluid at the roadside. Although this biological matrix has been demonstrated for many basic drugs to more closely mimic the time course of detection in the whole blood, no epidemiological data could verify if this could be true for NPS.

CONCLUSION

Driving is a complex task where the driver continuously elaborates and responds to information received from the external surround; so it is clear that driving performance can be influenced by any substance able to influence brain functions and/or mental processes. Multiple aspects of driving performance can be differently affected by illicit drugs, and even if for few of them, some dose/concentration dependent impairment has been reported, a wider knowledge on concentration/impairment relationship is still missing.

Blood is the matrix of choice when investigating DUID cases [52, 146] due to its detection time-window, and for most drugs, it has been shown a good correspondence with the oral fluid matrix.

It is well known that the passage of a drug from blood to oral fluid, reported as the concentration ratio between oral fluid and blood (OF/B ratio), is determined principally by some physicochemical properties of the molecule (pKa, protein binding, lipophilicity, molecular weight and spatial configuration) [21].

Although OF/B ratios of many drugs have been theoretically calculated, experimental studies have showed significant inter- and intra-subject variability in OF/B ratios, even when collecting OF in a controlled manner, making the estimation of drug concentrations inaccurate in blood from its concentrations in oral fluid.

Different classes of drugs have been found in the blood of drivers, with cannabis, cocaine, opiates, and amphetamines representing the most prevalent in the EU countries [147].

Efficient and reliable on-site tests to detect drivers under the influence of drugs are continuously developed. Roadside testing using urine sample is usually performed but it is time-consuming and has the risk of infections and potential disease transmission. OF testing has also been proposed as an alternative to blood and has shown its feasibility in roadside studies [138, 139, 148-152].

However, different studies underlined that a reliable correlation of pharmacologic effects can only be based on blood/serum concentrations as oral fluid concentrations are elevated shortly after drug-use because of contamination of the oral cavity [21, 48, 147] and the complexity in defining fixed OF/B ratios, that makes oral fluid drug concentration an inappropriate tool for impairment evaluation [52, 148]. Finally, the difficulties of testing some drugs independently of the biological matrix on-site have made some programs to include blood testing for very specific drugs. This approach will be of relevance in the future for controlling NPS misuse.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

The authors thank Michele Sciotti, Simonetta di Carlo and Antonella Bacosi for technical assistance in the preparation of the manuscript.

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