Presence of psychoactive substances in oral fluid from randomly selected drivers in Denmark.

Simonsen, Kirsten Wiese; Steentoft, Anni; Hels, Tove; Bernhoft, Inger Marie; Rasmussen, Brian Schou; Linnet, Kristian

Presence of psychoactive substances in oral fluid from randomly selected drivers in Denmark

K. Wiese Simonsen [a,*], A. Steentoft [a], T. Hels [b], I.M. Bernhoft [b], B.S. Rasmussen [a], K. Linnet [a]

[a] Section of Forensic Chemistry, Department of Forensic Medicine, Faculty of Health Sciences, University of Copenhagen, Frederik V's vej 11, 3. DK-2100 Copenhagen, Denmark
[b] Technical University of Denmark, Department of Transport, Bygningstorvet 116V, DK-2800 Lyngby, Denmark

A R T I C L E   I N F O

A B S T R A C T

This roadside study is the Danish part of the EU-project DRUID (Driving under the Influence of Drugs, Alcohol, and Medicines) and included three representative regions in Denmark. Oral fluid samples (n = 3002) were collected randomly from drivers using a sampling scheme stratified by time, season, and road type. The oral fluid samples were screened for 29 illegal and legal psychoactive substances and metabolites as well as ethanol. Fourteen (0.5%) drivers were positive for ethanol (alone or in combination with drugs) at concentrations above 0.53 g/l, which is the Danish legal limit. The percentage of drivers positive for medicinal drugs above the Danish legal concentration limit was 0.4%; while, 0.3% of the drivers tested positive for one or more illicit drug at concentrations exceeding the Danish legal limit. Tetrahydrocannabinol, cocaine, and amphetamine were the most frequent illicit drugs detected above the limit of quantitation (LOQ); while, codeine, tramadol, zopiclone, and benzodiazepines were the most frequent legal drugs. Middle aged men (median age 47.5 years) dominated the drunk driving group, while the drivers positive for illegal drugs consisted mainly of young men (median age 26 years). Middle aged women (median age 44.5 years) often tested positive for benzodiazepines at concentrations exceeding the legal limits. Interestingly, 0.6% of drivers tested positive for tramadol, at concentrations above the DRUID cut off; although, tramadol is not included in the Danish list of narcotic drugs. It can be concluded that driving under the influence of drugs is as serious a road safety problem as drunk driving.

© 2012 Published by Elsevier Ireland Ltd.

1. Introduction

In recent years there has been increasing focus on the detection of psychoactive substances among drivers [1–9]. Many studies have shown that the use of psychoactive substances is a major risk factor for traffic accidents [10–13]. Some studies have shown that driving under the influence of drugs has become more prevalent than drunk driving [1,2]. In Denmark, about 300 people are killed in traffic accidents every year. It is estimated that 25% could have been saved if drunk driving did not occur [14]. Since the introduction of legal concentration limits for psychoactive substances in Denmark in 2007, there has been a fivefold increase in samples sent for toxicological analysis by the police, and positive results for one or more drugs have been found in about 75% of the samples [3]. Yet, little is known about the extent that psychoactive drugs are used by drivers in general traffic. Driving under the influence of alcohol and drugs is a global problem. In this context, the European Commission within the Sixth Framework Programme (2002–2006) initiated the project DRUID (Driving under the Influence of Drugs, Alcohol, and Medicines) [15]. One aim of the DRUID project was to evaluate the number of drivers that tested positive for alcohol and psychoactive drugs. Therefore, the prevalence of psychoactive substances among the general driving population was evaluated in a roadside survey comprising 13 European countries. Oral fluid was used as the sample matrix because the collection method is easy and noninvasive. A uniform design was followed by all participating countries regarding the sample device, choice of psychoactive substances, and sampling. The same 24 legal and illegal drugs and some metabolites as well as alcohol were analyzed in all participating countries as part of the protocol. In addition, about four to six substances could be chosen that were specific for each country [16]. This article reports the results of the Danish part of the study.

2. Materials and methods

2.1. Sampling design

Drivers of passenger cars and vans were randomly selected using a stratified multistage sampling design. Three police regions were chosen that were assumed to be representative of Denmark with regard to substance use and traffic distribution. Within these regions, a number of survey locations were selected in cooperation with the police to make sure the entire region was covered. Subjects were stopped at random at these survey locations in a number of sessions and were asked to

Table 1
LOQ, DRUID cut offs and estimated legal concentration limit in oral fluid (calculated from legal concentrations in blood).

Substance	LOQ in oral fluid (μg/l)	Recommended equivalent cut off in oral fluid (μg/l)	Recommended equivalent cut-off in whole blood (μg/l)	Conversion factor: F = oral fluid equiv. cut-off/blood equiv. cut-off	Calc. legal conc. limit in oral fluid (μg/l)
Ethanol	0.053 g/l	0.1 g/l	0.12 g/l	0.820[a]	0.43 g/l
Morphine	0.53	95	10	9.5	143
Codeine	0.53	94	10	9.4	
6-MAM	0.53	16	10	1.6	
Cocaine	0.53	170	10	17	510
Benzoylecgonine	0.53	95	50	1.9	
Amphetamine	0.53	360	20	18	540
Methamphetamine	0.53	410	20	20.5	615
MDMA	0.53	270	20	13.5	405
MDA	0.53	220	20	11	330
MDEA	0.53	270	20	13.5	
THC	0.53	27	1.0	27	40.5
Methadone	0.53	22	10	2.2	165
Buprenorphine	0.53	1.0			
Tramadol	0.53	480	50	9.6	
Zolpidem	0.53	10	37	0.27	32.4
Zopiclone	0.53	25	10	2.5	37.5
Alprazolam	0.53	3.5	10	0.35	2.6
Bromazepam	0.53	5.0			
Chlordiazepoxide	0.53	10			
Clonazepam	0.53	1.7	10	0.17	1.3
7-Aminoclonazepam	0.53	3.1	10		
Diazepam	0.53	5.0	140	0.036	5.4
Nordiazepam	0.53	1.1	20	0.055	8.3
Flunitrazepam	0.53	1.0	5.3	0.19	1.4
7-Aminoflunitrazepam	0.53	1.0	8.5		
Lorazepam	0.53	1.1	10	0.11	3.3
Nitrazepam	0.53	1.0			
7-Aminonitrazepam	0.53	1.0			
Oxazepam	0.53	13	50	0.26	39

[a] Verstrate et al. (unpublished observation).

participate in the study. The study population samples in each survey region were stratified into eight time periods over the week. The time periods did not overlap and covered all days of the week and all times of day, as well as each season during the year. The distribution of the study population by time periods was proportionate to the distribution of seriously injured drivers during three previous years [17]. Information (among others: age, gender, nationality) on each subject and the type of vehicle was collected, as well as an oral fluid sample. If the driver refused to participate the reason was noted. Age and gender distribution did not vary between the respondents and those who refused.

In Denmark, the survey was carried out during the period from March 2008 through May 2009, covering one whole year in each police jurisdiction. The police officers were in charge of stopping passenger cars and vans and breath testing the driver for alcohol. Then, research personnel employed by the Technical University of Denmark, Department of Transport were in charge of filling in driver information and taking the saliva samples. Driver participation in the road side survey was voluntary and anonymous. Drivers with an alcohol concentration above 0.53 g/l were charged with driving under the influence of alcohol and taken into police custody for a blood sample. These drivers was also asked by the research personnel to provide an oral fluid sample and driver information for the DRUID project before being taken to the medical doctor for blood sampling.

The evaluation of the data is based on raw percentages of detection contrary to calculating weighted prevalence. A χ^2 test was used to test the age distribution of all sampled drivers and of positive drivers above the limit of quantitation (LOQ). A P-value <0.05 was considered significant.

2.2. Toxicological analysis of legal and illegal drugs

Oral fluid was collected by the Statsure saliva sampler (Statsure Diagnostic Systems, Framingham, MA, USA). In this device, a variable amount of oral fluid (300–1500 mg) was diluted with 1 ml buffer. For analyses, 200 mg of oral fluid corrected for buffer was used. The minimum accepted amount of oral fluid was 600 mg for analysis in duplicate. 2812 samples fulfilled this requirement. The sample volume can influence the prevalence of drugs and it has been found that alcohol and drugs were more frequently detected in small oral fluid volume than in large volumes [18]. To avoid important results to be lost a minimum of 320 mg oral fluid was accepted and a single analysis was made in 190 samples. The amount of oral fluid was below 320 mg in 29 samples and these were not included in the project. All oral fluid samples were stored in a cooled box and transferred to a freezer (−20 °C) after finalizing each session until analysis took place. The analytical

method was based on solid phase extraction followed by ultra performance liquid chromatography tandem mass spectrometry (UPLC–MS/MS) as described in detail by Badawi et al. [19]. Twenty-nine medicinal drugs and illicit substances, including some metabolites, were measured (Table 1). This study was planned as a part of the DRUID project and 24 of the drugs were agreed upon for the project; while, 4 medical drugs (buprenorphine, bromazepam, chlordiazepoxide, and nitrazepam/7-amino-nitrazepam) were chosen specifically for Denmark because the drugs were used frequently (the benzodiazepines) or to investigate the use (buprenorphine) among drivers. The toxicological laboratories in all participating countries had to pass a proficiency test, which was sent to all laboratories twice a year during the sample analysis process. Within the DRUID project, equivalent cut-off concentrations for blood and oral fluid were developed for the substances in question [20].

To evaluate driving under the influence, legal concentration limits in oral fluid were calculated from legal blood concentration limits after multiplication with conversion factors [16,20,21]. The conversion factors should be used with caution because in some cases they were determined from few studies (MDA, MDMA, 6-MAM, and zopiclone) and no conversion factor was determined for MDEA, therefore, the MDMA factor was used. The conversion factors can only be used for epidemiological studies and not for individual cases because of large individual variations.

2.3. Method of BAC quantification

The calculated blood alcohol content (BAC) was measured in breath by means of hand-held alcometers used by the police teams. The following four types from Lion Laboratories were used: SM-3, S-D2, S-300, and S-500. The alcometers used by the Danish police are calibrated to show a reading of 0.6 g/l ethanol when calibrated by means of breath containing 0.35×10^{-3} g/l. Furthermore, it was decided in the common protocol to use the factor 1/2100 to convert between breath and blood. Thus, the calculated blood ethanol (BAC) in g/l was equal to the reading on the alcometer (mg/l) × 2.10/(0.6/0.35), which equals the reading on the alcometer (mg/l) × 1.225.

However, due to missing information from the police, a breath test was not carried out in 194 cases. An Agilent GC-FID 6890 equipped with a head-spacesampler G1888 was used for determination of ethanol in these 194 oral fluid samples. The concentration in oral fluid was multiplied by 1.22 to calculate the equivalent alcohol concentration, BAC, in blood (Verstraete et al., unpublished observations).
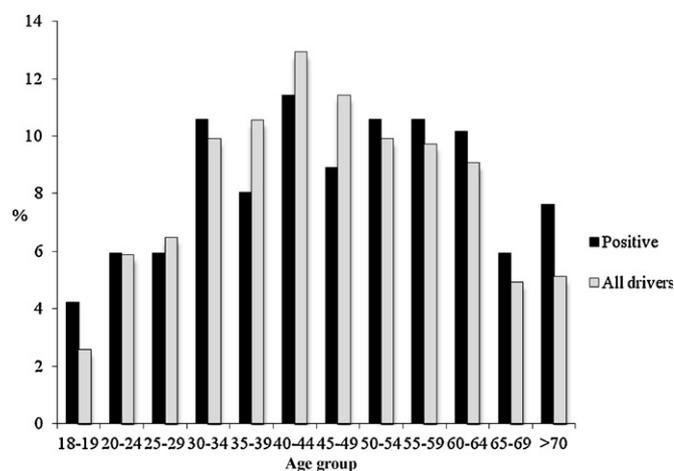
Fig. 1. Age distribution for drivers positive for one or more illicit and or medicinal drugs above the LOQ and in all drivers. Note: Drivers positive for ethanol or ethanol in combination with other substances are not included.

3. Results

The survey included 3002 voluntary drivers from whom oral fluid could be analyzed. Men constituted 66% and women 34%. The average age was 45 years (median: 45 and range 18–91 years) (Fig. 1).

3.1. Frequencies of drug concentrations exceeding the LOQ

Table 1 gives an overview of the LOQs, DRUID cut offs, and legal concentration limits in Denmark for oral fluid, which were calculated from the official legal blood concentration limits – if any – after multiplication with the respective conversion factors (including the addition of 50% for compensation for analytical variation, according to the current practice in Denmark for drivers accused of impaired driving) [16,20,21]. Not including ethanol, 236 (7.9%) drivers were positive for one or more illicit drugs and/or medicines (Table 2). Concentrations above the LOQ sometimes occurred for more than one compound in each sample; therefore, in total, 314 recorded concentrations were above the LOQ. Poly-drug use was observed in 35 (1.2%) drivers. The median age for all positive drivers was 47 years, and the range was 18–86 years. The age distributions of all sampled drivers and drivers positive above the LOQ were not significantly different ($\chi^2 = 1.88$, df = 11, $P > 0.99$), Fig. 1. Women constituted 34% of the drivers that were positive above the LOQ for one or more illicit drugs or medicines.

Codeine (2.1%), tramadol (1.6%), and zopiclone (0.9%) were the most frequently detected medical drugs; while, tetrahydrocannabinol (THC) (1.3%), cocaine (1%, including benzoylecgonine), and amphetamine (0.5%) were the most frequent illicit drugs (Table 2). Only one sample was positive for 6-monoacetylmorphine, which confirms recent heroin use. Heroin metabolizes very quickly to 6-monoacetylmorphine and further to morphine; therefore, some of the positive morphine samples could reflect heroin abuse. Few samples were positive for other stimulant drugs (1 for MDMA and 2 for methamphetamine). In total, 85 (2.8%) of the drivers tested positive for one or more illicit drug (morphine included). The median age was 33 years and 19% of the drivers positive for illicit drugs were women. Opioids like morphine, methadone, and tramadol were more frequently detected among older drivers and women in the study population; while, THC and the CNS stimulants, cocaine and amphetamines, were more common in young male drivers (Table 3). The most frequent combinations (0.53%) were combinations between THC,

Table 2
Number of positive concentrations above LOQ in oral fluid samples (in brackets above DRUID cut offs) and concentration range, average and median values.

Substance	N above LOQ (N above DRUID cut off)	Conc. range (μg/l)	Average conc. (μg/l)	Median conc. (μg/l)
Ethanol	95 (81)	0.07–1.28 g/l (calc in OF[a])	0.33 g/l (calc in OF[a])	0.28 g/l (calc in OF[a])
Morphine	17 (0)	0.7–52	3.9	2.0
Codeine	64 (8)	0.6–236	118	8.5
6-MAM	1 (0)	4.6		
Cocaine	29 (2)	0.5–390	28	2.5
Benzoylecgonine	15 (0)	0.6–85	18	8.1
Amphetamine	16 (2)	2–1014	39	25
Methamphetamine	2 (0)	5.5–6.8	6.2	6.2
MDMA	1 (0)	1.5	1.5	1.5
MDA	0 (0)	–	–	–
MDEA	0 (0)	–	–	–
THC	40 (10)	0.67–2280	90	5.2
Methadone	3 (1)	1.9–670	225	3.5
Buprenorphine	0 (0)	–	–	–
Tramadol	49 (18)	0.5–9300	1012	175
Zolpidem	6 (1)	0.8–16	5.4	3.3
Zopiclone	28 (7)	0.8–1693	78	13
Alprazolam	4 (3)	0.8–23	9.8	7.8
Bromazepam	5 (5)	5.6–307	76	9.0
Chlordiazepoxide	1 (0)	4.1	4.1	4.1
Clonazepam	2 (1)	1.5–3.9	2.7	2.7
7-Aminoclonazepam	2 (2)	3.2–5.9	4.6	4.6
Diazepam	9 (2)	0.8–13	3.9	1.1
Nordiazepam	11 (9)	0.3–26	5.2	1.9
Flunitrazepam	1 (1)	4.1	4.1	4.1
7-Aminoflunitrazepam	0 (0)	–	–	–
Lorazepam	0 (0)	–	–	–
Nitrazepam	1 (1)	2.1	2.1	2.1
7-Aminonitrazepam	4 (3)	0.6–2.8	1.8	1.5
Oxazepam	3 (0)	1.4–25	9.9	3.3

Note. An oral fluid sample can be positive for more than one substance.
[a] Calc in OF: ethanol (alcohol) concentration in oral fluid was calculated from the blood alcohol concentration (BAC) using the divisor 1.22 (Verstrate et al., unpublished observation).

Table 3
Age and gender for drivers positive above LOQ for various substances.

Substance	N above LOQ	Age range	Age average	Age median	Females (% N)
Ethanol	95	19–78	48	48	11
Morphine	17	31–80	56	46	53
Codeine	64	20–80	50	52	36
6-MAM	1	32			0
Cocaine	29	18–62	30	25	6.3
Benzoylecgonine	15	18–35	26	25	0
Amphetamine	16	18–63	41	25	12
Methamphetamine	2	23–25	24	24	0
MDMA	1	18			0
MDA	0	–	–	–	–
MDEA	0	–	–	–	–
THC	40	18–69	35	31	13
Methadone	3	31–55	41	36	100
Buprenorphine	0	–	–	–	–
Tramadol	49	25–75	47	48	50
Zolpidem	6	39–75	53	63	33
Zopiclone	28	37–86	60	59	43
Alprazolam	4	40–57	48	48	0
Bromazepam	5	58–80	68	66	20
Chlordiazepoxide	1	47			100
Clonazepam	2	31–45	38	38	50
7-Aminoclonazepam	2	31–45	38	38	50
Diazepam	9	23–76	50	44	11
Nordiazepam	11	23–76	50	47	27
Flunitrazepam	1	53			100
7-Aminoflunitrazepam	0	–	–	–	–
Lorazepam	0	–	–	–	–
Nitrazepam	1	24			0
7-Aminonitrazepam	4	24–80	49	47	25
Oxazepam	3	37–40	38	37	33

Note: An oral fluid sample can be positive for more than one substance.

cocaine and amphetamine. These combinations were only observed in young male drivers, median age 24.

Sixty (2%) drivers tested positive for one or more benzodiazepines and/or z-hypnotics. The median age of this group was 56 years and 35% were women. Diazepam was the most frequently detected benzodiazepine (0.3%) (Table 2). The z-hypnotics like zolpidem (0.2%) and zopiclone (0.9%) were frequently detected. Women constituted 33–43% of the drivers that were positive for zolpidem and zopiclone, with median ages of 59 and 63 years, respectively (Table 3).

3.2. Frequencies of drug concentrations exceeding the DRUID cut off limits

The percentage of drivers that tested positive for one or more illicit drugs and/or medicines exceeding the DRUID cut off was 2.3% (Table 2). The DRUID cut off limits (Table 1) are higher than the LOQs; accordingly, fewer samples exceeded the limits, but the same tendencies were observed. Two thirds of the positive drivers

were men (66%). The median age (range) was 46.5 years (20–86 years). Tetrahydrocannabinol, amphetamine, and cocaine were the only illicit drugs (Table 2) detected; while, codeine, tramadol, diazepam/nordiazepam, and zopiclone were the most frequent medicinal drugs. Morphine was not detected above the DRUID cut-off. Nordiazepam is not marketed as a medicinal drug in Denmark and is normally only detected as a metabolite of either diazepam or chlordiazepoxide. In this study, it was considered a metabolite of diazepam, if chlordiazepoxide was not detected.

The percentage of drivers with one or more illicit drugs exceeding the DRUID cut off was 0.4%. All drivers positive for one or more illicit drugs were men (100%), and the median age was 26. Seventeen drivers (0.6%) were positive for one or more benzodiazepines, at concentrations exceeding the DRUID cut off, and 29% of these samples were from women. The median age of this group was 44 years. Women represented 56% of the tramadol cases exceeding the DRUID cut off (median age 49.5 years). Eighty-one drivers (2.7%) had ethanol concentrations alone or in combination with other psychoactive drugs that exceeding the DRUID cut off. By

Table 4
Number of positive concentrations above the calculated legal concentration limit in oral fluid samples.

Substance	Number	Concentration (range μg/l)	Average concentration (μg/l)	Median concentration (μg/l)
Ethanol	14	0.47–1.28 g/l (calc. in OF[a])	0.77 g/l (calc. in OF[a])	0.73 g/l (calc. in OF[a])
Amphetamine	2	780–1014	897	897
THC	9	42.7–2280	373	158
Methadone	1	670		
Zopiclone	2	130–1693	912	912
Alprazolam	3	4.3–23	13	11
Clonazepam	2	1.5–3.9	3.0	3.0
Diazepam	2	9–13	11	11
Nordiazepam	2	8.4–25.8	17	17
Flunitrazepam	1	4.1		

Note: An oral fluid sample can be positive for more than on substance.
[a] Calc in OF: ethanol (alcohol) concentration in oral fluid was calculated from the blood alcohol concentration (BAC) using the divisor 1.22 (Verstrate et al., unpublished observation).

far, most of the alcohol positive drivers were males (89%), and the median age (range) was 48 years (19–78 years).

3.3. Frequencies of drug concentrations exceeding the legal concentration limits

Table 4 shows the number of oral fluid samples with results exceeding the legal concentration limits in Denmark (with 50% added as compensation for analytical variation). Formally, there are no legal limits for oral fluid, but the values for blood multiplied by conversion factors have been used. The number of drivers that tested positive for one or more compound above the computed legal limits was 22 (0.7%). This number is to be regarded as a minimum number because conversion factors were not available for all compounds (Tables 1 and 4) [16,20,21]. The median age (range) of this group was 39.5 years (20–57 years), and 18% of the positive drivers were women.

Tetrahydrocannabinol was the most frequently detected drug (0.3%). In two cases, amphetamine exceeded the legal limit. Overall, 0.3% of the drivers tested positive for one or more illicit drug, exceeding the legal limit. They were all men, and the median age was 26 years. For medicinal drugs, 0.4% of the drivers exceeded the legal concentration limit and benzodiazepines constituted the major drug class (0.3%) (Table 4). Women constituted 33% of this group and the median age was 44.5 years. It should be noted that subjects having a prescription for a legal drug are allowed to drive. It is only in cases of illegal use of prescription drugs that drug concentrations are forbidden above the legal limits [22].

Fourteen (0.5%) subjects tested positive for ethanol (alone or in combination with other psychoactive drugs) at concentrations above 0.53 g/l, which is the Danish legal limit. The median age of this group was 47.5 years, the range was 23–71 years, and women constituted 7%.

4. Discussion

The present study on the presence of alcohol and medicinal and illegal drugs in oral fluid of randomly selected drivers provided data on driving under the influence of drugs and alcohol in Denmark. To provide a reliable overview of the situation, a systematic sampling design and a valid approach for collecting and analyzing oral fluid were critical factors. In this study, sampling was conducted in early mornings, during daytime, and nights on both work days and weekends during each season to attain a balanced estimate of the occurrence of drug and alcohol use. The Statsure saliva sampling device was chosen for sampling the oral fluid based on a study on 9 sampling devices, which showed this device provided reliable recoveries for important drug classes [23]. Finally, a validated analytical procedure with good recovery and accuracy for all studied drugs was used [19].

Codeine and tramadol were the most frequently used medicinal drugs. This is not surprising since these drugs are some of the most commonly used pain killers in Denmark [24]. The next-most frequently detected medicinal drug was zopiclone, a widely used hypnotic. Other frequently prescribed drugs in Denmark, such as zolpidem, diazepam, and methadone, occurred less frequently. This could be explained by the short half-life of zolpidem [2] and the high degree of protein-binding for benzodiazepines that result in low concentrations in oral fluid [25,26]. However, methadone has a long half-life; therefore, the low frequency in this study might reflect the driving warnings associated with this drug [27].

The frequency of illicit drugs observed in this study is consistent with other surveys on randomly selected drivers [2,4]. THC, cocaine/benzoylecgonine, and amphetamine were most frequently detected. Morphine was also rather frequently detected, but it cannot be determined whether this was a result of illegal use

(heroin) or a legal drug preparation. The drugs mentioned above generally have a high concentration in oral fluid compared to blood. THC has a very high concentration shortly after smoking, but the concentration declines toward the level in blood [28,29]. An overrepresentation of young men driving under the influence of illicit drugs was found [2,4]; while, older drivers and women made up most of the group of drivers positive for medicinal drugs. In agreement with an earlier study in Queensland, Australia, poly-drug use was more common among young men [1]. Concerning alcohol, 95 (3.2%) subjects had detectable levels, but only 14 (0.5%) exceeded the Danish legal limit. Men also dominated the group with alcohol concentrations exceeding the legal limit, but they tended to be 10–20 years older than the male drivers that tested above the legal limits for illicit drugs.

This study detected illegal and medicinal drugs in oral fluid, but it is of interest to evaluate whether the detected concentrations correspond to levels exceeding the legal limits in blood, which were instituted in 2007 by the Danish traffic legislation and replaced the earlier impairment criterion based on a clinical examination by a medical doctor. The legal concentration limits introduced in 2007 were based on the lower limit of the therapeutic blood concentration interval for medicinal drugs and the lower limit of blood concentrations usually observed within some hours after intake of an ordinary dose for illicit drugs [22]. Thus, the limits were not based on the analytical limits of quantification in the laboratory. The relationship between oral fluid and blood concentrations is characterized by considerable uncertainty, because the conversion factors depend not only on physiologic conditions such as pH in oral fluid, but also on the sampling technique [21,30,31]. In the DRUID project, conversion factors from experimental studies on the Statsure devices were used [16,20,21]. Our study showed that 0.7% of the drivers had drug concentrations corresponding to a level exceeding the Danish legal limits. In an earlier study, comprising 1000 drivers in one police district in Denmark, about 1% drivers were estimated to be impaired by drugs and constituted a risk to road safety [5]. It should be noted that neither of these estimates included tramadol and codeine, because these drugs are not included in the Danish legal list of narcotic drugs. In addition, medicinal drugs included on the Danish list of narcotic drugs are legal when the driver has a prescription by a medical doctor [22]. In a recent survey of oral fluid samples from 12,000 drivers in Norway, it was suggested that driving under the influence of drugs (~1%) had become more prevalent than drunk driving (0.3%) [2]. The focus on preventing drunk driving over the years in Norway and Sweden, which have a low legal limit of 0.2 g/l, may be the reason for the very low percentage of drunk drivers observed.

In addition to the presented frequencies (raw percentages) of detection of drugs and alcohol, weighted prevalence was estimated by taking the volume of traffic into account. In this context, the substances have been grouped into eight mutually exclusive substance groups and a weighted prevalence calculated for each group according to traffic volume. This way, the weighted prevalence reflected the actual prevalence in traffic. The eight substance groups were: alcohol, amphetamines, cocaine (incl. benzoylecgonine), tetrahydrocannabinol, illicit opiates, benzodiazepines, Z-drugs, medicinal opioids, and two groups referring to combined use: alcohol and one or more drugs and more than one drug [17]. The weighted prevalence of these substance groups, based on the equivalent legal cut-offs in oral fluid (Table 1), was calculated by dividing the weighted number of positives for the substance group in question by the weighted total of samples. Confidence intervals were calculated using the Wilson formula [16]. This resulted in the following overall prevalence: alcohol (0.1–0.49 g/l): 2.05%, alcohol ($\geq$0.5 g/l): 0.48%, illicit drugs alone: 0.22%, medicine alone: 1.58%, combined use: 0.16%. These results

show that the weighted prevalence in general is lower than the unweighted (raw) percentages.

The number of drug-positive drivers found in this study must be regarded as a minimum number, as the survey on drug use was voluntary. This could cause bias, leading to underestimation of the number of drug-positive drivers.

5. Conclusion

Legal or illegal psychoactive drugs (alcohol excluded) were detected relatively frequently (7.9%) in this roadside survey. A smaller proportion of these cases corresponded to violations of the Danish traffic legislation, i.e., illegal drugs (THC and amphetamine) were detected in concentrations exceeding the legal limits in 0.3% of subjects compared with 0.5% of subjects that tested positive for alcohol over the legal limit. In agreement with other studies, most of the drivers that tested positive for illicit drugs were young male drivers, while middle aged women were more represented among drivers under the influence of legal drugs. Men dominated the group of drivers exceeding the legal limit for alcohol, but they were about 20 years older than the male drivers that tested positive for illicit drugs.

The present study gives valuable data on drugged and drunk driving. Driving under the influence constitutes a major risk in traffic and the relative high frequency of young men driving while taking drugs is worrying. More research in this field is needed also to clarify use of medicinal drugs, i.e., tramadol which was frequently detected, but not included the Danish legal list of narcotic drugs.

Conflict of interest

None.

Ethical approval

Ethical approval was not needed.

Disclaimer and funding

Acknowledgments

References

[1] J. Davey, J. Freeman, A. Lavelle, Screening for drugs in oral fluid: illicit drug use and drug driving in a sample of urban and regional Queensland motorists, Transp. Res. F: Traffic Psychol. Behav. 12 (2009) 311–316.

[2] H. Gjerde, P.T. Normann, B.S. Pettersen, T. Assum, M. Aldrin, U. Johansen, L. Kristoffersen, E.L. Øiestad, A.S. Christophersen, J. Mørland, Prevalence of alcohol and drugs among Norwegian motor vehicle drivers: a roadside survey, Accident Anal. Prev. 40 (2008) 1765–1772.

[3] A. Steentoft, K.W. Simonsen, K. Linnet, The frequency of drugs among Danish drivers before and after the introduction of fixed concentration limits, Traffic Inj. Prev. 11 (2010) 329–333.

[4] O.H. Drummer, D. Gerostamoulos, M. Chu, P. Swann, M. Boorman, I. Cairns, Drugs in oral fluid in randomly selected drivers, Forensic Sci. Int. 170 (2007) 105–110.

[5] I. Behrensdorff, A. Steentoft, Medicinal and illegal drugs among Danish car drivers, Accident Anal. Prev. 35 (2003) 851–860.

[6] F.M. Wylie, H. Torrance, A. Seymour, S. Buttress, J.S. Oliver, Drugs in oral fluid. Part II. Investigation of drugs in drivers, Forensic Sci. Int. 150 (2005) 199–204.

[7] H.P. Krüger, E. Schultz, H. Mageri, The German roadside survey 1992–1994. Saliva analyses from an unselected driver population: illicit and illegal drugs, in: C.N. Kloeden, A.J. McLean (Eds.), Proceeding of the 13th International Conference on Alcohol, Drugs and Traffic Safety, Adelaide, (1995), pp. 55–61, http://casr. adelaide.edu.au/t95/paper/s3p3.html.

[8] J.M. Walsh, J.J. De Gier, A.S. Christopherson, A.G. Verstraete, Drugs and driving, Traffic Inj. Prev. 5 (2004) 241–253.

[9] I.M. Bernhoft, A. Steentoft, S.S. Johansen, N.A. Klitgaard, L.B. Larsen, L.B. Hansen, Drugs in injured drivers in Denmark, Forensic Sci. Int. 150 (2005) 181–189.

[10] J. Mørland, A. Steentoft, K.W. Simonsen, I. Ojanperä, E. Vuori, K. Magnusdottir, J. Kristinsson, G. Ceder, R. Kronstrand, A. Christophersen, Drugs related to motor vehicle crashes in northern European countries: a study of fatally injured drivers, Accident Anal. Prev. 43 (2011) 1920–1926.

[11] P. Mura, P. Kintz, B. Ludes, J.M. Gaulier, P. Marquet, S. Martin-Dupont, F. Vincent, A. Kaddour, J.P. Goullé, J. Nouveau, M. Moulsma, S. Tilhet-Coartet, O. Pourrat, Comparison of the prevalence of alcohol, cannabis and other drugs between 900 injured drivers and 900 control subjects: results of a French collaborative study, Forensic Sci. Int. 133 (2003) 79–85.

[12] O.H. Drummer, I. Kourtis, J. Beyer, P. Tayler, M. Boorman, D. Gerostamoulos, The prevalence of drugs in injured drivers, Forensic Sci. Int. 215 (2012) 14–17.

[13] O.H. Drummer, J. Gerostamoulos, H. Batziris, M. Chu, J. Caplehorn, M.D. Robertson, P. Swann, The involvement of drugs in drivers of motor vehicles killed in Australian road traffic crashes, Accident Anal. Prev. 36 (2004) 239–248.

[14] http://www.statistikbanken.dk/statbank5a/default.asp?w=1920.

[15] www.druid-project.eu.

[16] S. Houwing, M. Hagenzieker, R. Mathijssen, I.M. Bernhoft, T. Hels, K. Janstrup, T. van der Linden, S.A. Legrand, A. Verstraete, Prevalence of alcohol and other psychoactive substances in drivers in general traffic. Part I: general results and part II: country reports, DRUID Deliverable 2.2.3, 2011, www.druid-project.eu.

[17] T. Hels, I.M. Bernhoft, K.W. Simonsen, A. Steentoft, in: S. Houwing, M. Hagenzieker, R. Mathijssen, I.M. Bernhoft, T. Hels, K. Janstrup, T. Van der Linden, S.A. Legrand, A. Verstraete (Eds.), Prevalence of alcohol and other psychoactive substances in drivers in general traffic. Part II: country reports, DRUID Deliverable 2.2.3, 2011, www.druid-project.eu.

[18] H. Gjerde, P.T. Norman, A.S. Christophersen, The prevalence of alcohol and drugs in sampled oral fluid is related to sample volume, J. Anal. Toxicol. 34 (2010) 416–419.

[19] N. Badawi, K.W. Simonsen, A. Steentoft, I.M. Bernhoft, K. Linnet, Simultaneous screening and quantification of 29 drugs of abuse in oral fluid by solid-phase extraction and ultraperformance LC–MS/MS, Clin. Chem. 55 (2009) 2004–2018.

[20] A. Verstraete, A. Knoche, R. Jantos, G. Skopp, H. Gjerde, V. Vindenes, J. Mørland, K. Langel, P. Lillsunde, Per se limits – methods of defining cut off values for zero tolerance, DRUID Deliverable 1.4.2, 2011, www.druid-project.eu.

[21] H. Gjerde, E.L. Øiestad, A.M. Øiestad, M. Langødegård, I. Gustavsen, K. Hjelmeland, J.-P. Bernard, A.S. Christophersen, Comparison of zopiclone concentrations in oral fluid sampled with Intercept® oral specimen collection device and Statsure Saliva Sampler™ and concentrations in blood, J. Anal. Toxicol. 34 (2010) 590–593.

[22] Betænkning om alkohol i udåndningsluft og en nulgrænse for euforiserende stoffer mv. Betænkning nr. 1486, Copenhagen, 2007, http://www.jm.schultzboghandel.dk/ upload/microsites/jm/ebooks/bet1486/pdf/bet1486.pdf (in Danish).

[23] K. Langel, C. Engblom, A. Pehrsson, T. Gunnar, K. Ariniemi, P. Lillsunde, Drug testing in oral fluid- evaluation of sample collection devices, J. Anal. Toxicol. 32 (2008) 393–401.

[24] Danish Medicines Agency, 2008, www.medstat.dk.

[25] E. Cone, M.A. Huestis, Interpretation of oral fluid tests for drugs of abuse, Ann. N. Y. Acad. Sci. 1098 (2007) 51–103.

[26] A. Pehrsson, T. Gunnar, C. Engblom, H. Seppä, A. Jama, P. Lillsunde, Roadside oral fluid testing: comparison of the results of Drugwipe 5 and Drugwipe benzodiazepines on-site tests with laboratory confirmation results of oral fluid and whole blood, Forensic Sci. Int. 175 (2008) 140–148.

[27] http://pro.medicin.dk/Medicin/Praeparater/2516 (in Danish).

[28] R.S. Niedbala, K.W. Kardos, D.F. Fritch, S. Kardos, T. Fries, J. Waga, J. Robb, E.J. Cone, Detection of marijuana use by oral fluid and urine analysis following single-dose administration of smoked and oral marijuana, J. Anal. Toxicol. 25 (2001) 289–303.

[29] M. Huestis, E. Cone, Relationship of delta-9-tetrahydrocannbinol concentrations in oral fluid and plasma after controlled administration of smoked cannabis, J. Anal. Toxicol. 28 (2004) 394–399.

[30] S.M.R. Wille, E. Raes, P. Lillsunde, G. Teemu, M. Laloup, N. Samyn, A.S. Christophersen, M.R. Moeller, K.P. Hammer, A.G. Verstraete, Relationship between oral fluid and blood concentrations of drugs of abuse in drivers suspected of driving under the influence of drugs, Ther. Drug Monit. 31 (2009) 511–519.

[31] J.K.M. Aps, L.C. Martens, Review: the physiology of saliva and transfer of drugs into saliva, Forensic Sci. Int. 150 (2005) 119–131.