Perspective

Drug Testing and Analysis

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A snapshot of workplace drug testing in Italy

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The Italian Decree on Health and Safety at Work (81/08) prescribes mandatory drug tests for jobs which pose safety hazards to others. Workplace drug testing is performed in accordance with the Provision of the Government-Regions Conference, 2008. The aim of our survey was to examine the prevalence of drug use and the main drug findings in a sample of Italian workers performing hazardous jobs. From September 2009 to February 2011, 551 urine samples were collected in 42 Italian companies. Sample collection was carried out at the workplace by qualified laboratory personnel sent from the Institute of Occupational Medicine of the Catholic University (UCSC) of Rome. The workers to be tested were informed the day before, as the law requires. The samples were checked for adulteration, coded, and sent immediately to the laboratory of the UCSC Forensic Toxicology Analytical Unit. The screening test was an immunoassay. The positive samples proceeded to the confirmatory analysis with liquid chromatography-tandem mass spectrometry (LC-MS/MS). The urine samples were analyzed for cannabis, opiates, amphetamines, methamphetamines, cocaine, methadone, and MDMA. Out of 16 samples .9% screened positive; only 4 of them (0.7%) were confirmed with the LC-MS/MS. Confirmed results included cocaine (2 samples), cannabis (1 sample), both cocaine and cannabis (1 sample). The prevalence of positive samples was lower than expected. Such finding cannot be explained by a low reliability of the testing procedure but could be due to test scheduling. More positive cases might be found performing short-notice random testing.

Keywords: workplace drug testing; hazardous jobs; Italian law

Introduction

Drug abuse has adverse effects on workers health and safety. The effects associated with the use of drugs, such as slow reaction time, loss of coordination, impairment of judgement and reasoning, sedation, hyperactivity, and impatience can endanger the safety of employees, co-workers, and the general population. Besides the acute effects, substance use occurring hours before the beginning of the shift can cause spillover effects, such as fatigue and hangovers that may independently increase the injury risk.[1] In fact, it has been shown that hangovers affect cognitive skills, including tasks related to driving or piloting aircrafts, which may influence the risk of injury in a manner similar to the influences of acute alcohol intoxication.[2,3]

In addition, workers using drugs may be more likely to be engaged in behaviour that increases the risk of occupational injury (deviance proneness) such as sensation seeking, increased risk-taking, and non-compliance with workplace safety policies.[4]

According to the 2011 Annual Report on Drug Use, in Italy 5.2% of the general population used cannabis in the preceding 12 months (2010), 0.9% used cocaine, and 0.22% used amphetamines and other stimulants. Higher rates of drug use were found in the student population (age range: 15–19 years) with a prevalence of 18.2% for cannabis, 2.1% for cocaine, and 1.3% for amphetamines and other stimulants.[5]

It has been estimated that in Italy at least 10% of all occupational injuries and 40% of mortal injuries are related to the use of psychoactive substances but, so far, there are no exact statistical data about the relationship between occupational injuries and drug use. In fact, the National Insurance Organization for Occupational Injuries (INAIL), which is also committed to drawing up statistics about injuries at the workplace, has never quantified the impact of drug use on work-related injuries.

Given the extent of drug use and the occupational risks deriving from it, drug tests have been included in employee health surveillance and a specific legislation about workplace drug testing has been developed in Italy.

The first legislative reference on *Drugs and Work* was the Decree 309/90.[6] For the first time in Italy It prescribed 'tests to verify the absence of drug addiction for employees assigned to job duties which pose risks to the security, safety and health of others'.

The recent *Decree on Health and Safety at Work*, entered into effect in April 2008,[7] reasserted the importance of drug testing for jobs which pose safety hazards to others. The critical occupations to be tested are jobs requiring accuracy, reliability, independent judgement, and quick reactions. They include different occupational fields: transport (hauliers, pilots, air traffic controllers, flight attendants, and railway personnel), shipping, petrochemical, nuclear, explosives industry (Table 1).

Workplace drug testing is performed in accordance with the *Provision of the Government-Regions Conference, 18 September 2008*[8] which enacts the agreement reached in October 2007[9] by the same conference.

Workplace drug testing programs aim to detect drug-addicted employees but also occasional and habitual users. Drug testing is usually performed at worker's recruitment and on a periodic basis. Nevertheless it can be required also on a suspicion basis (if the employee is suspected to work under the effect of drugs), after an accident at work, or as a follow up after a period of suspension from the hazardous duty for a previous positive test (Table 2).

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Table 1. Safety sensitive duties

JOB DUTIES WHICH POSE RISKS TO THE SECURITY, SAFETY AND HEALTH OF OTHERS	
JOBS REQUESTING A QUALIFICATION CERTIFICATE TO PERFORM HAZARDOUS TASKS	Use of toxic gases Manufacture and use of fireworks; placement and blasting of mines Technical management of nuclear plants
JOBS RELATED TO TRANSPORT	Drivers with a category C, D, E driving licence; taxi drivers; drivers of vehicles for hire; drivers of vehicles transporting hazardous goods Railway workers Pilots of pleasure boats for hire Security personnel of railways, metro, tramway, funicular railways, air transport and road transport Maritime personnel Flight attendants and air traffic controllers Personnel certified by the Italian aircraft register Testers of sea, air and road means of transport Operators assigned to the 'movements control panels', in the transport field Drivers of cargo transport and ground transportation vehicles
OPERATORS AND MANAGERS OF THE EXPLOSIVES INDUSTRY (PRODUCTION, PACKAGING, POSSESSION, TRANSPORTATION AND SALE)	All the employees belonging to this job category

Table 2. Drug testing scheduling

SITUATIONS WHERE WORKPLACE DRUG TESTING IS PRESCRIBED FOR WORKERS PERFORMING 'HAZARDOUS JOBS'	
AT WORKER'S RECRUITMENT	Before starting to perform his job duty, the worker is subjected to a preventive evaluation of his job fitness. A negative drug test is needed to state that the worker is fit for the hazardous job duty in question.
ON A PERIODICAL BASIS	The worker is subjected to a periodical medical examination (usually on an annual basis) aiming to assess his job fitness. Drug tests are included in the health surveillance program.
ON A SUSPICION BASIS	If the worker is suspected to work under the effect of drugs, he will undergo a medical evaluation, including drug testing, to assess his job fitness. The reports of 'reasonable doubt' are made by the employer or his representative to the occupational health physician who will verify the validity of the 'reasonable doubt' and, if necessary, will subject the worker to specific medical tests.
AFTER AN ACCIDENT AT WORK	After an accident occurred while driving motor vehicles at the workplace, the worker has to be tested to rule out the use of drugs and psychotropic substances.
FOLLOW UP	The worker who was suspended from his hazardous duty, because of a previous positive drug test, has to be checked at regular intervals before being readmitted to his duty (precautionary monitoring). The tests will be performed at least on a monthly basis, granting the test unpredictability. The minimum length of time of the follow up is 6 months.
ON THE WORKER'S RETURN TO THE HAZARDOUS DUTY, AFTER A PERIOD OF SUSPENSION FOR A PREVIOUS POSITIVE TEST	Before being readmitted to his duty, after a period of follow up for a previous positive test, the worker has to undergo a drug test in order to verify the condition of 'non user'. The occupational health physician, as precaution, can decide to extend the follow up period by 6 months, scheduling more frequent tests than ordinarily performed.

So far, published data about workplace drug testing in Italy are limited. In this paper, the prevalence of drug use and the main drug findings in a sample of Italian workers performing hazardous job duties is reported.

Methods

The survey population was constituted of 551 hauliers working in 42 companies located in different northern, central, and southern Italian regions. Such population represents the total number of workers to be tested in these companies according to Italian legislation. The biological matrix used was urine.[8] The samples were collected from September 2009 to February 2011. Observed sample collection was performed at the workplace, as part of the health surveillance programme, by qualified laboratory personnel sent from the Institute of Occupational Medicine of the Catholic University of the Sacred Heart (UCSC) of Rome.

The overall procedure to carry out the test is described in a very detailed way in the *Provision of the Government-Regions Conference*, issued in September 2008.[8] Standardized chain of

Geographical location of the companies participating in the survey.

custody procedures were used to document the integrity and security of the specimen from the time of collection until receipt by the laboratory. In order to grant the test unpredictability required by law, the workers to be tested were informed just the day before the test. The samples were checked for adulteration (temperature, pH, creatinine, nitrites, glutaraldheyde, and oxidants were measured), coded and sent immediately to the laboratory of the UCSC Forensic Toxicology Analytical Unit. The urine samples were analyzed for cannabis, opiates, amphetamines, methamphetamines, cocaine, methadone, and MDMA. The current legislation doesn't allow testing for other substances (barbiturates, benzodiazepines, etc.) in addition to those ones listed in the Procedure.[8] The cut-off limits for the immunological screening and the confirmatory analysis are listed in Table 3.

The screening test was performed by a semi-quantitative assay using Siemens Viva E Drug Analyzer EMIT (Enzyme Multiplied Immunoassay Technique). This method is based on the competitive binding of labelled antigen and unlabelled antigens from the sample with a limited, known amount of an antibody in the reaction mixture. The test uses enzyme-linked antibodies that react only with the particular drug for which the sample is being tested.

If the sample result was positive, it proceeded to the confirmatory analysis with liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Materials, standards and chemicals for LC-MS/MS analysis

The standard solutions of all the studied drugs and internal standards at 100 μg/ml or 1 mg/mg were purchased from Cerilliant (LGC Standards s.r.l., Milan, Italy) and stored at −20°C. Working solutions were also prepared in methanol and stored at the same temperature. All solvents and the formic acid were HPLC or LC-MS grade and were obtained from Sigma-Aldrich-Riedel-de Haen (Steinheim, Germany). The blank samples were from drug-free volunteers from the laboratory staff.

A working solution (MixA) containing amphetamine, methamphetamine, 3,4-methylendioxyamphetamine (MDA), 3,4-methylendioxymethamphetamXXine (MDMA), cocaine, benzoylecgonine (BEG) and methadone, was prepared by appropriate dilution in methanol at a concentration of 10-fold the relative cut-offs. The internal standard solution (MixD) of the abovementioned analytes was prepared in methanol at the same concentration. Working solutions of morphine, morphine-d3, 6-monoacetyl-morphine (MAM), 6-monoacetyl-morphine-d3 (MAM-d3), morphine-3-glucuronide (M-3-G), morphine-3-glucuronide-d3 (M-3-G-d3), morphine-6-glucuronide (M-6-G), codeine, codeine-d3, 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH), 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid d3 (THC-COOH-d3) and THC-COOH-glucuronide at 1 μg/ml were prepared separately in methanol.

Sample preparation

An aliquot of urine sample was centrifuged at 4500 x g for 10 min at 20°C, to pellet large particles. For analysis of amphetaminines, methamphetamines, MDMA, cocaine, and methadone, 200 μl of the centrifuged sample were transferred with a micropipette to a 1.5 ml glass autosampler vial, added with 20 μl of MixD and diluted with 200 μl of methanol and 200 μl of formic acid 0.01%; for the analysis of opiates, the dilution was performed with 400 μl of formic acid 0.01%, avoiding methanol. For analysis of cannabinoids, 500 μl of

Table 3. Cut-off values for the immunological screening and the LC-MS/MS confirmation.

SUBSTANCE	SCREENING TEST CUT-OFF CONCENTRATION	CONFIRMATORY TEST CUT-OFF CONCENTRATION
OPIATES (morphine, MAM, codeine, morphine-glucuronide)	300 ng/ml	100 ng/ml
COCAINE (cocaine, benzoylecgonine)	300 ng/ml	100 ng/ml
CANNABIS (THC, THC-COOH)	50 ng/ml	15 ng/ml
AMPHETAMINES, METHAMPHETAMINES	500 ng/ml	250 ng/ml
MDMA	500 ng/ml	250 ng/ml
METHADONE	300 ng/ml	100 ng/ml

the sample were transferred with a micropipette to a 1.5 ml glass autosampler vial, added with 30 ng of deuterated internal standard and diluted with 500 μl of methanol. Ten μl of the final solution were analyzed with LC-MS/MS.

LC-MS/MS analysis

The LC-MS/MS analyses were performed with a Surveyor MS Pump liquid chromatograph coupled with an ion trap mass spectrometer LXQ (Thermo Fisher, San José, CA, USA). The chromatographic separation was performed on an HPLC column 150 x 2 mm, 4 μm Synergi Polar (Phenomenex) equipped with a guard column. The mobile phase was a mixture of formic acid 0.01% aqueous solution (A), methanol (B) and acetonitrile (C), with a flow rate of 0.250 ml/min. The gradient program of the mobile phase started with A:B:C (70:10:20, *v/v*), maintained for 6 min, then changed to A:B:C (50:10:40, *v/v*) over 1 min, maintained for 5 min, then changed to A:B:C (30:10:60, *v/v*) over 1 min and maintained for 7 min. The column was then set back to the initial mobile phase conditions and let equilibrate for 8 min.

Only for confirmation of opiates, the gradient programme started with A:C (95:5), maintained for 1 min, then changed to A:C (80:20) over 3 min, maintained for 4 min, then the column was set back to the initial mobile phase conditions and let equilibrated for 8 min.

The mass spectrometer was equipped with an ESI probe operating in positive-ion mode. The mass spectra of the standard compounds were first acquired in full-scan mode (150–550 *m/z*) by infusion of a reference solution at 10 μg/ml. From these spectra, the precursor ions were selected and fragmented acquiring the full-scan MS/MS spectra. Fragmentation of the selected precursor ions was performed by collision-induced dissociation (CID) with helium, which fills the ion trap. Finally, the higher product ions were chosen and each compound was analyzed in SRM mode to obtain the best sensitivity and specificity. The limit of detection (LOD) and the limit of quantitation (LOQ) for all analytes are reported in Table 4.

Results

A total of 551 workers (550 male and 1 female) participated in the survey. The age ranged from 18 to 65 years. All the employees to be tested gave a urine sample, nobody refused. According to the Procedure,[8] if the employee refuses to be tested, the occupational health physician will state that it was not possible to perform the test and the worker will be suspended from his or her job duty. No problems in specimen collection, laboratory analysis, and results interpretation were reported. All the samples were valid; neither adulteration nor dilution were detected. The observed samples collection granted that the tests were not beaten.

Of all the urine samples analyzed, 16 (2.9%) tested positive with the screening test but only 4 of them (0.7%) were confirmed with the LC-MS/MS analysis. All the positive workers were men, with an average age of 40.5 years old (range: 26–43).

Confirmed findings included cocaine (2 samples) and cannabis (1 sample). In one sample, both cocaine and cannabis were detected.

Table 4. LOD and LOQ for all analytes.

COMPOUNDS	LOD (ng/ml)	LOQ (ng/ml)
Amphetamine	1,5	4,0
Methamphetamine	5,0	10,0
MDA	10,0	25,0
MDMA	1,5	5,0
BEG	1,0	2,5
Cocaine	6,0	18,0
Methadone	1,5	4,0
Morphine	1,5	4,0
MAM	0,5	1,5
Morphine-3-glucuronide	25,0	100,0
Morphine-6-glucuronide	25,0	100,0
THCCOOH	5,0	15,0
THCCOOH-glucuronide	15,0	25,0

Discussion

This survey represents one of the first experiences in workplace drug testing in Italy. Not much data is available in the literature about the prevalence of drug use among the Italian workers. Our results are in line with the prevalence rates showed by a previous study reporting 1.6% of positive tests in a group of 2745 workers.[10] Similar findings were also found by a recent study of Kazanga *et al.*[11] who tested 43. 535 Italian workers (mainly road vehicle drivers and lift truck drivers), reporting a rate of positive tests equal to 1.9%.

As regards the prevalence of the different drug categories among the workers we tested, cannabis resulted as the most frequently detected substance followed by cocaine. These findings confirm the prevalence data reported in the *Annual Report on Drug Use* showing that cannabis and cocaine are the most commonly used drugs in Italy and their association is frequent even in cases of polyabuse.[5]

Our results are not comparable with the data reported in studies performed in other countries because of the significant differences concerning the job duties tested, the situation where workplace drug testing was performed, the biological matrices used, the notification time, and the substances included in the test panel.[12,13]

The prevalence of positive samples in the worker population we tested was lower than expected. In fact, based on the prevalence data reported in the *Italian Annual Report on Drug Abuse*,[5] we expected to find drug use rates in line with the prevalence rates detected in the general population. Our findings cannot be explained by a low reliability of the testing method, because standardized and secure collection procedures and highly specific and sensitive analytical techniques were applied to grant the maximum reliability of the results.

Two significant aspects related to test scheduling could have affected the results of our survey. The first one is the lack of a real cooperation of the people in charge of sending the workers for the test. According to the Procedure,[8] the donors should be informed with 24 h notice at the most, but this term is not always respected. In fact, for organizational reasons, the people in charge of sending the workers for the test are likely to inform the donors in advance, allowing a more elapsed time between the test notice and the urine collection. This way, drug users are more likely to test negative because of the narrow detection time for drugs in urine (only cannabis remains detectable in urine for a longer time in case of frequent use).

Secondly, the worker has the possibility to avoid the test in case of 'justified reason' such as certified disease or work reasons. In this case as well, because of the narrow detection window of

drugs in urine, it is possible to elude the control, postponing the test even only for a few days, a sufficient time for urine to turn negative.

Moreover, urine is the only biological matrix that currently can be used for the first-level analyses; the use of other biological matrices, such as oral fluid and hair, is allowed only for the second-level tests performed by the Local Drug Addiction Rehabilitation Center (Ser.T.), where the worker testing positive at the first-level analyses is sent, in order to assess if he or she is an occasional user, a habitual user, or a drug addict.[8]

More positive cases might be found if random testing is performed at short notice; our findings suggest that currently all employees with drug addiction problems are detected with the urine test because they can't control the use of substances and consequently they always result positive when tested; on the other hand, because of the abovementioned critical issues, the percentage of occasional and habitual users is probably underestimated.

Conclusion

The development of a specific legislation and a detailed procedure about workplace drug testing in Italy has been a very important goal. The main aim of the legislator in the first years of enforcement of the procedure was to test the effectiveness of the method and to detect any critical issues of the procedure. It's still too early to take stock of the effectiveness of the current legislation. Of course in the future, the drug testing programme will be broadened to include other job categories and the procedure will be improved taking into account the critical aspects which are emerging, in order to promote an effective drug free workplace programme.

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