ABSTRACTS – VORTRÄGE HAUPTSYMPOSIUM

V1 Die Zunahme alter Menschen in der Bevölkerung und ihre Bedeutung für das Fachgebiet der Toxikologie

The increasing number of aged people in our population and its importance in the field of toxicology

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------- Das Abstract zu diesem Hauptvortrag lag zum Redaktionsschluss noch nicht vor. -------

V2 Der ältere Mensch in der forensich-toxikologischen Begutachtung

Aged people in forensic toxicological expertise

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There is only a small amount (1.8 %) of people older than 59 years (104 persons: 79 male, 25 female) in a database with about 6000 forensic cases from the years 1996 - 2002 collected at the institute of forensic medicine in Duesseldorf. A little more than a half (57 persons) was in the age of 65 or older - the regular retirement age.

Traffic offences (89 %) and homicide (6 %) were the main reasons for the toxicological examinations. 72 % of the above mentioned traffic participants were involved in accidents, this is a four times higher ratio than in the group of drivers under 30 years (17 %). The accident ratio rises up to 80 % within the group of the over 64 years old drivers.

Relaxing substances (sedatives/hypnotics, antiepileptics, antidepressants) were found in 44 % of the samples, only one person took illicit drugs (cocaine/cannabis).

V3 Analytische Untersuchungen zu einem Todesfall durch Ersticken in einer Argon-Atmosphäre

Analytical investigations in a death case by suffocation in an argon atmosphere

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A 31 year old engineer was found dead in a reaction vessel (diameter 0.8 m, height 1.8 m) of a bulb factory some minutes after he had entered it for repair work. Normally, the vessel was used in the production of quartz lamps and was filled with argon at the end of a production cycle, but during repair it should have been filled with air from a compressed air reservoir. Resuscitation attempts with artificial respiration were unsuccessful. By autopsy no other cause of death could be found and the usual toxicological analysis for alcohol, illegal and therapeutic drugs and other poisons lead also to negative results. Therefore, the possibility of suffocation in an argon atmosphere had to be investigated. This was rendered more difficult because of the natural content of 0.93 Vol % argon in air and because of the resuscitation attempts, by which the argon could have been removed.

Gas samples from larynx, oesophagus, bronchi and stomach, separated blood samples from both ventricles of the heart and from the vena iliaca externa as well as tissue samples from lung and liver were collected during autopsy into headspace vials in a way that a loss of gas and a dilution by surrounding air was avoided as far as possible. Samples from 4 other corpses were collected in the same way for comparison and calibration. The samples were analysed by headspace GC-MS. The abundance of Ar^+ (m/e = 40) was used for quantification with N_2^+ (m/e = 14) as internal standard. Some of the results obtained from the rather complicated evaluation of the measured data areshown in Table 1.

Table 1. Argon concentrations in samples from a fatality in a bulb factory

Sample	Argon concentration	
	Case under investigation	Comparison
Gas from larynx	1.79 Vol %	0.96 Vol %
Stomach gas	1.58 Vol %	0.89 Vol %
Heart blood (left ventricle)	7.2 µg/mL	2.7 µg/mL
Heart blood (right ventricle)	5.8 µg/mL	2.7 µg/mL
Blood from vena iliaca externa	3.6 µg/mL	2.7 µg/mL

A clearly increased concentration was also found in lung tissue, whereas in liver tissue no significant difference in comparison to other cases was measured. From the results follows that the deceased inhaled an increased amount of argon a short time before death. The concentrations are in agreement with asphyxia and subsequent resuscitation attempts. They cannot be explained by a long-term inhalation of an atmosphere enriched with argon before the incident as it in principle could occur in the factory hall.

V4 Todesfall nach Konsum eines Kava-Getränks in Kombination mit Alkohol und Cannabis

Death after consumption of kava beverage in combination with alcohol and cannabis

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Pacific islanders are no longer drinking kava as a traditional drink, except in rare occasions. A case study is presented concerning the death of a 32 years old Melanesian male after long day alcohol drinking and consumption of kava beverage in a New Caledonian kava-bar. Friends found the intoxicated man lying on the ground being unable to walk alone. He was brought home. He refused to sleep, instead he stayed in the garden where he stumbled against a wall which fell down on him, causing lethal head injuries. No autopsy was performed, however heart blood was sampled around 4 to 5 h after the accident. After addition of fluoride, the sample was stored at -20°C and sent to Duesseldorf for toxicological analysis. Kavain and its main metabolites were detected in blood using a method described recently [1].

The analyses showed the presence of 4000 ng/ml kavain, 60 ng/ml p-hydroxy-7,8-dihydrokavain and 400 ng/ml p-hydroxy-5,6-dehydrokavain. Other kava lactones like yangonin, methysticin, dihydromethysticin, dihydrokavain and tetrahydroyangonin were detected by HPLC-DAD and confirmed by LC-MS-MS. In addition, 1.70 ng/ml THC, 11 ng/ml THC-COOH, and 1.2 ‰ of alcohol were detected in blood by further toxicological analysis.

This is the first report of a deadly accident consecutive to the absorption of kava combined with ethanol and cannabis. In addition, such concentrations of blood kava lactones have never been reported before.

[1] Tarbah F., Mahler H., Kardel B., Weinmann W., Hafner D., Daldrup Th. (2002) Kinetics of kavain and its metabolites after oral application. Journal of chromatography A (in press)

V5 Analyse von Propan/n-Butan/i-Butan in drei Todesfällen

Analysis of propane/n-butane/i-butane in three cases of death

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Light hydrocarbons are easily accessible and rather cheap narcotics which are mainly consumed as "sniffing drugs" by young people. Apart from their narcotic effects desired by consumers they may cause changes in per-

sonality, organic lesions and even death. The paper reports special features of determining these drugs in autopsy samples.

Materials and methods: Three cases of death with suspected intoxication through light hydrocarbons (e.g. lighter gas, aerosol dispensers, liquid gas) were investigated. Parts of organs and body fluids were taken at forensic autopsies at Freiburg, Heidelberg and Magdeburg, and stored under gas-tight conditions. A static Headspace-GC/MS analysis was performed to identify and quantify the hydrocarbons, following a matrix-independent MHE-procedure and an internal standard (1,1,2-trichlorotrifluoroethane) method. To avoid evaporation of the analytes, all organ samples were homogenized in a porcelain mortar at liquid nitrogen temperatures, and weight in. The calibration standards were produced from a test gas mixture consisting of propane (0.483% by volume), n-butane (0.5% by volume) and i-butane (0.486% by volume).

Results: Propane, n-butane and i-butane findings in the blood and all organs under asservation were positive. In case #1 and case #2 the values established in the organs were 0.5 to 12 and 2 to 12 µg/g for the main components i-butane or n-butane, respectively. In case #3 all three hydrocarbons were detectable at comparatively low concentrations of 0.1 to 0.5 µg/g of organ.

Discussion: The applied method facilitates easy determination of light hydrocarbons concentrations in body fluids and organs. As expected, concentrations were highest in the brain. The paper reports special features of the cases of death under consideration, asservation and processing procedures as well as methods of determination, evaluation and interpretation of results.

V6 Post-mortem Verteilung von Amylnitrit (Isopentylnitrit, "Poppers") im Körper
Post Mortem Disposition of Amylnitrite (Isopentylnitrite, "Poppers") in Body

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Amylnitrite belongs to the alkylnitrite compounds (street name "poppers"). Included compounds are amyl-, butyl-, and isobutyl nitrite among several other nitrites. Due to their vasodilatory effects nitrites have a respectable medical pedigree. Since 1867, amylnitrite was used in case of angina pectoris. By inhalating this volatile liquid from broken, or "popped", glass ampules arteries are expanded and thus permit smooth muscles to relax. Since the nineteen seventies the scientists noticed a proliferated abuse among homosexuals because of prolonged orgasm and increased sense of excitement effects in spite of their potential carcinogenic consequences.

In April 2002 a man died after swallowing a liquid called poppers. Heart blood, femoral blood, myocard, gastric fluid, muscle, brain, liver, kidney, lung, bile and urine were measured by headspace gas chromatography after adding the internal standard butylnitrite (about 200 mg tissue or body fluids, filled up to 300 mg by HPLC-water + 50 µl internal standard (0,01 % butylnitrite in DMSO / water) + 0,2 g Na_2SO_4). The concentrations ranged between approximately 1 µg / g (femoral blood) and 5000 µg / g (gastric fluid). In addition cocaine, benzoylecgonine and alcohol were found in blood.

V7 Vergiftung mit einem a_2-Adrenorezeptor-Agonisten nach versehentlicher Einnahme von Alphagan® Augentropfen durch ein Kind
Poisoning with an a_2-Adrenoceptor Agonist after accidental oral ingestion of Alphagan® eye drops in an Infant

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Brimonidine tartrate (Alphagan®), an a_2-agonist, is used in the treatment of chronical elevated intraocular pressure and acute rise of ocular pressure. It acts by decreasing of the aqueous production and increasing of the uveoscleral outflow. The drug belongs to the imidazoline derivatives and is chemically and pharmacologically related to clonidine.

A 2 year old boy showed symptoms of a systemic α_2-adrenergic intoxication following oral ingestion of 2 ml Alphagan® ophthalmic solution. He revealed severe cardio-respirating signs thirty minutes after accidential oral ingestion and became somnolent-lethargic with additional hypotension, bradycardia, bradypnoea, and generalized muscle hypotonus. An intensive supportive therapy was required. Symptoms resolved completely within the the next 12 hours. The largest concentration of brimonidine in plasma was 38 ng/ml measured five hours after ingestion but plasma concentration did not correlate with the severity of the clinical manifestations assuming a

redistribution from CNS into the intravasal compartment. The half-life was calculated with 2,7 hours and is similar to values after topical administration. Elimination half life in urine was calculated with 3,2 hours.

Qualitative and quantitative analysis of brimonidine in plasma and urine were done by gaschromatography-mass spectrometry. The substance was isolated together with clonidine as internal standard by solvent extraction and derivatized with pentafluorobenzylbromide.

The case demonstrates that young children have an unusually sensitivity to CNS depressant effects of a-adrenergic agents. The symptoms reflect CNS dysfunction due to the drug entering systemic circulation and crosssing the blood-brain barrier. Signs of brimonidine intoxication develop rapidly and CNS depression appears within the first hour post ingestion. Since symptoms are severe and plasma concentrations do not correlate to clinical findings we would strongly recommend children to be admitted at PICU. Supportive management, monitoring and maintenance of an adequate airway are the mainstay of therapy.

V8 Diskurs zur Stabilität, Präanalytik und Ergebnisbewertung forensisch relevanter Substanzen
Discourse on stability, preanalytical phase and interpretation of forensically relevant compounds

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Background: The most useful analytical method and sophisticated interpretation based on pharmacokinetic data will be of limited value or even useless if significant changes in the analyte concentration or pattern had occurred during the preanalytical phase. Therefore, the history of a particular blood sample is a problem in forensic toxicology. Quality control assessments have adressed many issues related to the analytical procedure but largely neglected the stability of the measurable quantities within the sample matrix prior to analysis. Even in standard reference material relevant metabolites or degradation products are often not included.

Method: Investigations on the preanalytical stability of amphetamines, cannabinoids, cocaine, LSD and opiates in blood, plasma or serum samples were summarized and up-dated. In addition, the study designs and the procedures estimating time-dependent changes in the analyte concentration were reviewed.

Results: For all analytes, data on their short-term stability are generally available. Mostly, the experimental designs used optimized collection and storage conditions, and studies on authentic material were rare. A further limitation of the issued data was due to the high variation of the study designs. In addition, only a few studies followed the standard definition of the stability of the measurable quantities within the sample matrix, which affords periodical analysis. Results obtained from experimental investigations could not be transferred to authentic samples without restrictions.

Conclusion: Overall, the published data demonstrated an urgent need for an unique definition of the term stability, for the establishment of guidelines for stability studies on experimental as well as on authentic samples and for so-called stability-indicating analytical procedures. In the future the transport and storage of forensic samples must also be part of the quality management.

V9 Metabolismus von Kokain und Anhydroecgoninmethylester – Bedeutung für die in vitro Stabilität
Metabolism of Cocaine and Anhydroecgoninemethylester – Implications for in vitro Stability

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Objectives of the study: Derivatives of ecgonine like cocaine or the “crack” marker anhydroecgoninemethylester (AEME) are subject to in vitro degradation to a very high degree. Therefore it is almost impossible to estimate the original concentrations of the analytes present at the time of blood sampling. Metabolism studies were performed to assess hypothetical differences between in vivo metabolism and in vitro degradation enabling a more conclusive interpretation of analytical data.

Material and methods: Microsomes from rat liver were incubated with cocaine and with several of its metabolites as well as with AEME and some synthesized hypothetical metabolites. In another series of experiments cocaine or AEME were perfused through freshly isolated rat liver. The incubates or the perfusion effluents were analyzed after solid-phase extraction and trimethylsilylation by gas chromatography-mass spectrometry.

Results: Cocaine was metabolized by hydrolysis of the two ester groups, by N-demethylation, N-hydroxylation and aryl hydroxylation. The different pathways exhibited cross sections, however a further metabolism of metabolites with a free carboxylic group was not detected. The perfusion experiment indicated that the main pathway in rat liver was hydrolysis to benzoylecgonine followed by oxidation to hydroxycocaine and hydroxymethoxycocaine. AEME was metabolized by hydrolysis, by N-demethylation and N-oxidation. In the perfusion experiment only 6% of the initial AEME were left after one liver passage.

Discussion: The studies on AEME metabolism showed that the parent compound is of limited interest due to its fast in vivo hydrolysis to anhydroecgonine which is a stable analyte. Cocaine metabolites with a free carboxylic group seem to be end points of in vivo metabolism. Further studies on in vivo concentrations and in vitro stability should be performed to elucidate whether these patterns may serve as an indicator for the post-ingestion interval.

V10 (In)stabilität und mangelnde Reproduzierbarkeit von Nandrolon-Metaboliten im Schweiß
(In)stability and lack of reproducibility of nandrolone metabolites in sweat

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Dealing with sweat as ‘missing link’ between metabolic patterns of nandrolone in blood and hair, which often appeared controversial, the respective biotransformation products were examined in sweat. Nandrolone laurate was administered to four geldings and sweat was collected under controlled physical exercise (moving belt).

In spite of standardised experimental conditions (food, shelter, medical treatment) the metabolic pattern in sweat differed *qualitatively* in between the four horses. The situation was characterised by the dominant occurrence of

- epi-norandrosterone,
- epi-noretiocholanolone,
- norandrostenedione or
- epi-nortestosterone

depending on the individual and the specific collection site. This finding is of particular interest, as the corresponding blood and urine profiles are mainly identical and the samples were immediately extracted on-site. Such a condition is likely to be a result of a swift microbiological transformation of the steroids in the sweat, leading to a further degradation after longer storage periods and is in total contradiction to the situation in other matrices (esp. urine) where the steroid levels remain qualitatively unchanged during comparable cycles.

V11 Die Bedeutung der Pharmakogenetik und metabolischer Interaktionen für die Klinische und Forensische Toxikologie
The relevance of pharmacogenetics and of metabolic interactions for clinical and forensic toxicology

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Individual variations concerning the pharmacological or toxicological effects of the same drug dose may be caused by body mass, age, sex, kidney and liver function, drug-drug (food-drug) interactions, and finally by genetic variations. Over the last years, many studies have been published regarding the impact of pharmacogenetic variations e.g. on drug metabolism resulting in different pharmacokinetic behavior or toxic risks. Drug metabolism is largely determined by relevant genetic enzyme or transporter variants. The most important variants are the following polymorphically expressed proteins: the cytochrome P450 isoenzymes CYP2C9, CYP2C19, and CYP2D6, the alcohol/aldehyde dehydrogenase (ADH2, ALDH2), the phase II enzymes UDP-glucuronyltransferases (UGT1A1), thiopurin methyltransferases (TPMT), arylamine N-acetyltransferases (NAT2) and glutathione S-transferases (GSTM1, GSTT1), and finally transporters like P-glycoprotein.

After the involvement of these particular isoenzymes/proteins in the metabolism of individual xenobiotics has been elucidated, the knowledge of such polymorphisms allows to predict possible variations in pharmacokinetic behavior of those. In case of relevant genetic variations, genotyping and phenotyping of the individuum should be performed.

Knowledge of the involved metabolizing (iso)enzymes further allows to predict possible interactions with other xenobiotics, i.e. inhibition or induction of individual polymorphic and non-polymorphic isoenzymes by e.g. poisons, drugs (of abuse), alcohol, tobacco smoke or food ingredients.

Individual variations in the pharmacokinetic behavior of xenobiotics are therefore of importance in therapeutic drug monitoring as well as in clinical and forensic toxicology, especially if pharmacokinetic calculations are used as basis for interpretation of analytical results.

V12 Beteiligung humaner hepatischer Cytochrom P450 Isoenzyme am Metabolismus des Amphetamin-Precursors Clobenzorex

Involvement of Human Hepatic Cytochrome P450 Isoenzymes in the Metabolism of the Amphetamine Precursor Drug Clobenzorex

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Background: Previous studies on rat and human liver microsomes showed, that polymorphic CYP2D6 was not involved in the N-dealkylation of amphetamine precursor drugs to amphetamine. Studies on Wistar and Dark Agouti rats gave a hint that this isoenzyme might be involved in the hydroxylation of such drugs. The aim of our present study was to identify the human hepatic cytochrome P450 (CYP) isoenzymes involved in the N-dealkylation as well as in the hydroxylation of the amphetamine precursor clobenzorex.

Methods: Activity screenings with nine individual cDNA expressed CYPs for general involvement were performed. Kinetic profiles were established using baculovirus infected insect cell microsomes and pooled human liver microsomes as enzyme sources. Experiments with chemical inhibitors completed the studies. Metabolites were quantified by NICI-GC-MS (HP5-MS column) after aqueous derivatization of the amine group with *S*-(-)-heptafluorobtuyrylprolyl chloride, liquid-liquid extraction and ethylation of the hydroxy groups.

Results: Besides CYP3A4, the most abundant human CYP isoform, CYP2B6 was involved in the N-dealkylation of clobenzorex to amphetamine. Hydroxylation to the different hydroxy-metabolites of the parent compound clobenzorex was catalyzed mainly by CYP1A2, CYP2C19 and also by CYP2B6. Polymorphic CYP2D6 was not involved in the hydroxylation of clobenzorex. The kinetic constants, K_m and V_{max}, were determined for both enzyme sources.

Conclusions: As CYP3A4 is responsible for N-dealkylation of clobenzorex, interactions with inhibitors or inducers of this isoenzyme are possible. CYP2B6, which is also involved in this metabolic step, is expressed polymorphically in humans. Therefore, different pharmacokinetic behaviours are possible in vivo. Concerning the second metabolic pathway, the hydroxylation, different pharmacokinetic behaviours are also possible in vivo, because CYP2C19 and CYP2B6 are expressed polymorphically in humans and CYP1A2 is known to be inducible.

V13 Beteiligung humaner hepatischer Cytochrom P450 Isoenzyme am Metabolismus der Alkaloide Californin und Protopin

Involvement of Human Hepatic Cytochrome P450 Isoenzymes in the Metabolism of the Alkaloids Californine and Protopine

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Background: Californine and protopine represent two of the main benzyltetrahydroisoquinoline alkaloids isolated from *Eschscholtzia californica*, papaveraceae. The genus *Eschscholtzia* is a traditional medicinal plant. Furthermore, misuse as a substitute for marijuana has been described. In a previous investigation, demethylenation of californine and protopine and N-demethylation of californine were found to be the main metabolic steps. The aim of this study was to identify the human hepatic cytochrome P450 (CYP) isoenzymes involved in these metabolic steps, in order to predict possible drug drug interactions.

Methods: General involvement of specific CYP isoforms was determined using nine individual cDNA expressed CYPs. Kinetic profiles were established using these baculovirus infected insect cell microsomes and pooled human liver microsomes as enzyme sources. Inhibition experiments with specific chemical inhibitors completed the studies. Metabolites were determined by HPLC-FLD (Merck LiChroCART column (125 x 2 mm I.D.) with

Superspher 60 RP Select B; ammonium formate buffer pH 3 and acetonitrile as eluents) without further working up of the incubation samples. The metabolites corresponing to the respective peaks were identified by GC-MS after fraction collection and lyophilization.

Results: Mainly CYP2C19, CYP3A4 and CYP2D6 were involved in the demethylenation of californine and protopine with minor involvement of CYP2C9 or CYP1A2, respectively. N-demethylation was mainly catalyzed by CYP3A4. CYP2D6 and CYP2C9 were also involved. The kinetic constants, K_m and V_{max}, were determined for both enzyme sources.

Conclusions: Several CYP isoenzymes are involved in demethylenation of the two alkaloids. This reduces the likelihood of pharmacokinetic interactions *in vivo*. However, because CYP2C19 and CYP2D6 are expressed polymorphically in humans, interindividual variations in kinetic behavior can not be excluded. As CYP3A4 is mainly responsible for N-demethylation of californine, interactions with inhibitors or inducers of this isoenzyme are possible.

V14 Beteiligung humaner hepatischer Cytochrom P450 Isoenzyme am Metabolismus der neuen Pyrrolidinophenon Designerdrogen MPPP, MOPPP, MDPPP und MPHP

Involvement of Human Hepatic Cytochrome P450 Isoenzymes in the Metabolism of the new Pyrrolidinophenone Designer Drugs MPPP, MOPPP, MDPPP and MPHP

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Background: Alpha-pyrrolidinophenones like 4'-methyl-α-pyrrolidinopropiophenone (MPPP), 4'-methoxy-α-pyrrolidinopropiophenone (MOPPP), 3',4'-methylenedioxy-α-pyrrolidinopropiophenone (MDPPP) and 4'-methyl-α-pyrrolidinohexanophenone (MPHP) are new designer drugs which have appeared on the illicit drug market. The aim of our study was to identify the human hepatic cytochrome P450 (CYP) isoenzymes involved in the formation of their main metabolites.

Methods: Activity screenings with nine individual cDNA expressed CYPs for general involvement were performed. Kinetic profiles were established using baculovirus infected insect cell microsomes and pooled human liver microsomes as enzyme sources. Experiments with chemical inhibitors completed the studies. Metabolites were quantified by LC-MS. (Merck LiChroCART column (125 x 2 mm I.D.) with Superspher 60 RP Select B; ammonium formate buffer pH 3 and acetonitrile as gradient eluents) without further working up of the incubation samples.

Results: Only CYP2C19 and CYP2D6 were involved in the biotransformation to the main metabolites of MPPP, MOPPP, and MDPPP. CYP1A2, CYP2B6 and CYP2C9 were also responsible for the formation of the main metabolite of MPHP. The kinetic constants, K_m and V_{max}, were also determined. CYP2D6 was the high affinity enzyme for all pyrrolidinophenones with K_ms in the range from 5-20 µM.

Conclusions: Different pharmacokinetic behaviors of the drugs are possible in vivo, because CYP2C19 and CYP2D6 are expressed polymorphically in humans and other drugs can inhibit CYP2D6. However, this phenomenon does not seem to be of relevance in all cases, since the other enzyme can at least partly take over the role of the missing one.

V15 Untersuchungen zum CYP Isoformen abhängign Metabolismus der neuen piperazinartigen Designer Droge TFMPP mittels LC-MS

Studies on the CYP isoform dependent metabolism of the new piperazine-derived designer drug TFMPP using LC-MS

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Background: Abuse of piperazine-derived designer drugs has increased significantly during the last years. As a result of this development two compounds of this group, N-benzylpiperazine (BZP) and 1-(3-trifluoromethylphenyl)piperazine (TFMPP), have been placed into Schedule I of the Controlled Substance Act (CSA) on September 20, 2002 in the United states of America. The aim of this study was to elucidate the CYP isoform de-

pendent metabolism of the main metabolic reaction of TFMPP, data which are needed for toxicological risk assessment.

Methods: Activity screenings with nine individual cDNA expressed CYPs for general involvement were performed. Kinetic profiles were established using baculovirus infected insect cell microsomes and pooled human liver microsomes as enzyme sources. Experiments with chemical inhibitors completed the studies. Metabolites were quantified by LC-MS. (Merck LiChroCART column (125 x 2 mm I.D.) with Superspher 60 RP Select B; ammonium formate buffer pH 3 and acetonitrile as gradient eluents) without further working up of the incubation samples.

Results: The incubations showed that the formation of hydroxy-TFMPP was catalyzed only by three isoforms (CYP1A2, CYP2D6, CYP3A4). The kinetic constants were also determined. CYP2D6 was the high affinity enzyme for TFMPP with K_m < 10 µM. The involvement of CYP2D6 could further be emphasized by inhibition studies using quinidine

Conclusion: CYP2D6, the main CYP450 responsible for the metabolism of TFMPP to its main metabolite hydroxy-TFMPP, is expressed polymorphically. Thus, different pharmacokinetic behaviors of this drugs are possible in vivo, as well as drug drug interactions with inhibitors of this isoform.

V16 Pharmakokinetik von intravenous und intramuskulär injiziertem Heroin bei Opiatabhängigen

Pharmacokinetics of intravenous and intramuscular heroin in narcotic addicts

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Background: In several countries, medical prescription of intravenous diacetylmorphine is being evaluated as treatment option for heavily dependent narcotic addicts. Because of damaged veins, diacetylmorphine is frequently intramuscularly. Therefore, we characterized the pharmacokinetics of intravenous and intramuscular diacetylmorphine in the high dose range required by opioid addicts.

Methods: Three different intravenous and intramuscular diacetylmorphine doses and one intravenous deuterated morphine dose were administered to 8 heroin-addicted patients. Arterial and venous plasma samples were collected and diacetylmorphine, monoacetylmorphine, morphine, morphine-3-glucuronide, morphine-6-glucuronide and morphine-d3 concentrations measured by LC-MS.

Results: After intravenous application, maximal arterial concentrations of diacetylmorphine, monoacetylmorphine and morphine were 2.4-, 5.4- and 1.4-times higher and occurred 2 to 3 minutes earlier than maximal venous concentrations. Arterial areas under the concentration time curves (AUC) of diacetylmorphine and monoacetylmorphine were 35% and 26% higher than venous AUCs, whereas for morphine arterial AUCs were 15% lower. All AUCs increased linearly with dose. Arterial half-lives for diacetylmorphine and morphine-d3 were 2.4±0.8 and 88±21 min, respectively. Intramuscular diacetylmorphine resulted in unexpectedly high and considerably sustained (arterial) diacetylmorphine exposures with a bioavailability of 380±188% in comparison to intravenous bolus dosing. In addition, also monoacetylmorphine and morphine exposures were increased after intramuscular administration to 120% and 134%. However, peak concentrations especially for monoacetylmorphine and morphine after intramuscular diacetylmorphine administration were considerably lower and delayed as compared to intravenous diacetylmorphine.

Conclusions: These data indicate that arterio-venous differences exist for diacetylmorphine and metabolite kinetics. Not only diacetylmorphine, but also monoacetylmorphine is substantially metabolized peripherally to morphine. Because of lower and delayed peak concentrations, the high bioavailability for the intramuscular route should not increase the risk for adverse effects. Pharmacokinetics were linear for both routes of administrations and thus, intravenous and intramuscular diacetylmorphine is pharmacokinetically safe and can be used under medical prescription as a substitute in narcotic addicts.

V17 Bestimmung von beta-Carbolinalkaloiden und Tryptaminen in Ayahuasca und pflanzlichen Drogen mit MEKC-UV-LIF

Determination of beta-carboline alkaloids and tryptamines in ayahuasca and medicinal plants by MEKC-UV-LIF

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Biogenous drugs are becoming increasingly popular in Germany in recent years. Substance classes that come into the focus of forensic investigation in this context are the ß-carboline and tryptamine alkaloids. The ß-carboline alkaloid harmine is the main alkaloid component of the Amazonian liana Banisteriopsis caapi, one of the crucial ingredients of *ayahuasca*, the famous psychotropic drink and sacramental drug of the Shamans from the Amazon and Orinoco river basins.

Micellar electrokinetic chromatography (MEKC) with UV-laser-induced fluorescence detection is a powerful choice for the analysis of the natively fluorescent ß-carboline alkaloids and tryptamines in complex biological matrices. An MEKC method was developed for seven frequently occurring ß-carboline alkaloids including harmine, harmaline, tetrahydroharmine (THH), harman, norharman, harmol and harmalol. The optimised running buffer consisted of 10 mmol/L borate and 60 mmol/L sodium dodecylsulphate at pH 8.80; 12.5 % (v/v) methanol was added as an organic modifier. Two pyrene carboxylic acids proved to be suitable as internal standards.

Baseline separation of the seven ß-carboline alkaloids and the two internal standards was achieved within 13 minutes using a high voltage of 28.5 kV. After adaptation of the method (15 % methanol, 20 kV) baseline separation of the seven ß-carboline alkaloids, the two internal standards and four naturally occurring tryptamines (tryptamine, bufotenine, N,N-dimethyltryptamine and 5-methoxy-N,N-dimethyltryptamine) was possible within 28 minutes. Two diode-pumped Nd:YAG lasers with emission wavelengths of 266 nm and 355 nm and optical output powers of 0.5 mW were employed as excitation sources. The limits of detection were 0.5 - 5 nanomol/L except for THH and the tryptamines with ca. 50 nanomol/L.

Several samples of seized ayahuasca as well as Banisteriopsis caapi liana parts, Peganum harmala seeds, Psychotria viridis leaves and Mimosa tenuiflora root bark were analysed by UV-LIF-MEKC. The utilisation of two different UV excitation wavelengths increases the versatility of the method significantly and proved to be very helpful with respect to the accurate determination of trace amounts in complex matrices.

V18 Anwendung von ESI-HPLC-MS/MS-Experimenten im Fall einer ungewöhnlichen „General-unknown“-Intoxikation

Application of ESI-HPLC-MS/MS-experiments in an unusual case of a “General-unknown”-Intoxication

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A 64 year old male korean citizen was admitted to the hospital with vertigo, headache, a generalized pruritus and dark coloured urine. A primarily suspected viral hepatitis could be excluded, so that an intoxication was assumed to be the most possible cause. The patient stated, that he had used a “naturopathic” korean antifungal agent recommended by a neighbour as a remedi for onychomycosis.

In a routinely performed GC/MS-screening procedure evidences of methylnaphthalene derivatives in serum samples were found. It was not possible to ascertain the source of these chemicals. Other toxicological screenings (REMEDI, CEDIA, HPLC-DAD) revealed no pathological findings.

For setting up a special design for ESI-HPLC-MS/MS analysis the common fragment of the measured mass spectra (m/z=141) was used to acquire precursor ion scan chromatograms and product ion scans from the identifyed molecular ions. The antifungal agent terbinafine and three metabolites could be identified.

A quantitation method by means of MRM (multiple rection monitoring) in the MS/MS mode after collision induced dissociation appeared to be very sensitive with a LOD of 0,06 ng/ml and a LOQ of 0,25 ng/ml for the active substance terbinafine after a simple protein precipitation with acetonitrile directly injected into the HPLC system.

The analytical procedures, quantitative results of terbinafine in serum and finger nails and the metabolic pathways associated with the assumed pathogenesis are discussed.

V19 The suitability of oral fluid testing in outpatient clinics for treatment of drug addiction

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Oral fluid testing can provide valuable information of drug abuse. In October 2002, a field study with "real" (clinical) oral fluid samples was conducted in Slovenia, addressing the issue of drug consumption of patients in addiction treatment programs, with a potentially high rate of polytoxicomanic drug users.

94 drug addicted patients from outpatient clinics (19 female, 75 male, aged from 15-45 years, 86 of them included in methadone maintenance program) were voluntarily willing to cooperate and gave self-reported data of their legal and illegal drug use. 92 valid findings of the Dräger DrugTest® System, a rapid on-site diagnostic testing system screening for the presence of drugs of abuse in oral fluid samples, were compared with GC-MSMS analyses of the appropriate oral fluid samples. In the table are listed the statistical parameters for THC, Cocaine, and Opiates; no cases were positive for Amphetamine and Methamphetamines.

Table 1. DrugTest® - oral fluid GC-MSMS

	THC	Cocaine	Opiates
Total	92	92	92
TP	20	7	26
FP	7	3	10
FN	1	1	2
TN	64	81	54
Sensivity	95,24	87,50	92,86
Specifity	90,14	96,43	84,38
PPV	74,07	70,00	72,22
NPV	98,64	98,78	96,43
Accuracy	91,30	95,65	86,96

78 urine samples were also collected and screened by MAHSAN® Rapidtest for THC-COOH, Opiates, Cocaine, Amphetamine, Methamphetamine, and analyzed with GC-MS. 76 pairs (urine GC-MS ⇔ oral fluid GC-MSMS) were qualitatively compared:

Table 2. Urine GC-MS ⇔ oral fluid GC-MSMS

	THC	Cocaine	Opiates
Total	76	76	76
U- and S-	34	63	38
U- and S^+	1	0	4
U^+ and S-	23	5	16
U^+ and S^+	18	8	18
Agreement U and S (%)	68,4	93,4	73,7

As a result, the overall accuracy of >90% of the oral fluid screening in comparison with GCMSMS proving ones more the usefulness of the DrugTest collection device for on-site and confirmation analysis.

The results of U+ and S- support the well known difference of the analytical detection window between the two different body fluids screened during this study, as oral fluid reflects recent drug consumption. The evaluation show no interference for the screened drugs with the reported medication and/or polytoxicomanic behavior.

V20 Verbesserung der Sensitivität des GC-MS Suchverfahrens durch Verwendung einer Säule für hohe Temperaturen und Anwendung einer speziellen Säulenreinigungsprozedur

Improvement of the Sensitivity of GC-MS in the Scan Mode by Use of a High Temperature Column and a Special Cleaning Procedure

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Aims: The total analytical sensitivity of a chromatographic test procedure depends on quantity of sample volume, extraction efficiency and the chromatographic method itself. The sensitivity of the chromatographic process is determined by the signal/noise ratio. For quantitative procedures guidelines exist for the determination of the analytical sensitivity, but not for qualitative, i.e. screening tests. Therefore it seems obvious to optimise the chromatographic process with the aim of a low baseline signal noise.

Methods: Since more than 5 years we use 30 m DB 5-HT columns (J&W, Chromatographiehandel Müller) for our GC-MS general unknown screening. Typically, more than 1000 runs can be performed by one column with only a small reduction of sensitivity at the end of the use of an individual column. We inject 1 µl of a liquid/liquid extract in ethylacetate, in most cases after MSTFA-derivatization. The initial temperature is 70°C and held for 2 minutes. Then the temperature programme starts with a rate of 20°C per min and the final temperature is held for 12 min. When the system is not actually used, a cleaning procedure automatically starts in a repeated sequence: 1 µl ethylacetate injected at 250°C (2 min), followed by heating with a rate of 5 °C per min and a final temperature of 300°C held for 18 min. For standby the temperature is set on 120°C.

Results: Crucial for the optimisation of the sensitivity of our STA method were a) clean liquid/liquid extracts by the use of Toxilab extraction tubes and b) high temperature GC columns. The frequent use of cleaning runs at high temperature reduced the baseline signal noise, typically by a factor of 10 and allow very sensitive peak detection by the autointegrator. With the Agilent 5892 detector the baseline abundance could be reduced to as low as 10.000 units. Therefore the full scan detection of many substances was significantly improved. In combination with ultrasonic derivatization (Hallbach 2002) substances like paracetamol, amphetamines, codeine and especially benzodiazepines could be detected with higher sensitivity.

Discussion: The use of a high temperature column in combination with frequent cleaning runs provides a high sensitivity and a long-time stability of our GC-MS STA procedure.

References: Hallbach J: Ultrasonic derivatization procedures: a new rapid and effective method in STA for GC-MS sample preparation. Annales de Toxicologie Analytique, vol. XIV, 3, P52, 2002.

V21 Einfaches Screening-, Library-unterstütztes Identifizierung- und validiertes Quantifizierungverfahren mittels APCI-LC-MS für Anästhetika, Benzodiazepine und niedrig dosierte Opioide in Plasma insbesondere für die Hirntoddiagnostik

Simple APCI-LC-MS method for screening, library-assisted identification and validated quantification of anaesthetics, benzodiazepines and low dosed opioids in plasma often asked for in the context of the diagnosis of brain death

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Background: Monitoring of the brain stem function by recording an electroencephalogram (EEG) is one of the most important prerequisites in the diagnosis of brain death, e.g. prior to organ explantation. Various drugs acting on the central nervous system might have a negative influence on the cerebral activity and thus could falsify the recorded EEG. Therefore, an LC-MS assay was developed for screening, identification and quantification of etomidate, ketamine, clonazepam, diazepam, flunitrazepam and its two metabolites 7-aminoflunitrazepam and norflunitrazepam, midazolam, nordazepam, alfentanil, fentanyl, sufentanil and piritramide in plasma.

Methods: After standard liquid-liquid extraction of 0.5 mL of plasma (*Maurer HH et al., J Mass Spectrom 37:687-692, 2002*), the 13 analytes and four deuterated internal standards (diazepam-D_5, fentanyl-D_5, flunitrazepam-D_7 and ketamine-D_4) were separated on a Superspher 60 RP Select B column (125 x 2 mm I.D.,

guard column: 10 x 2 mm I.D.) using fast gradient elution (ammonium formate buffer/acetonitrile). The compounds were screened for and identified using an APCI-LC-MSD (SL version) in the scan mode with fragmentor voltages of 100, 200 and 300 V, and quantified in the SIM mode at a fragmentor voltage of 100 V using calibration curves.

Results: The presence of the analytes was successfully screened for by mass chromatography with selected ions followed by library search of the underlying APCI mass spectra with our new LC-MS reference library. The assay was found to be selective for the 13 tested compounds. The assay was linear from subtherapeutic to overdose concentrations of each compound (e.g. 0.0025-0.5 mg/L for fentanyl or 0.5-8 mg/L for ketamine). The low and high-level recoveries ranged from 18.7 % (7-Aminoflunitrazepam) to 114.8 % (ketamine). The LODs (*S/N* 3) in the scan mode screening ranged from 0.0025 mg/L (fentanyl) to 0.5 mg/L (ketamine). Intra- and inter-day accuracy and precision were inside the required limits.

Conclusions: The LC-MS assay has proven to be appropriate for screening, identification and quantification of the studied analytes in plasma after application of therapeutic dosages. It was successfully applied to several authentic brain death cases.

V22 Bestimmung von Drogen in Haarproben mittels dynamischer Festphasenextraktion/Gaschromatographie/Tandem-Massenspektrometrie (HS-SPDE/GC/MS-MS)

Determination of drugs of abuse in hair samples by headspace solid-phase dynamic extraction/gas chromatography/tandem mass spectrometry (HS-SPDE/GC/MS-MS)

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A new method combination, headspace solid-phase dynamic extraction coupled with gas chromatography/tandem mass spectrometry (HS-SPDE/GC/MS-MS), is introduced to determine drugs of abuse in hair samples. This highly automated procedure utilizes SPDE for pre-concentration and on-coating derivatization as well as GC and triple quadrupole MS-MS for selective and sensitive detection. All these steps, apart from washing and cutting of the hair samples, are performed without manual intervention on a robot-like autosampler.

SPDE is a solventless extraction technique related to solid-phase microextraction (SPME). The analytes are absorbed from the sample headspace directly into a hollow needle with an internal coating of polydimethylsiloxane by repeated aspirate/dispense cycles.

The HS-SPDE/GC/MS-MS procedure was applied to the analysis of methadone, the trimethylsilyl derivatives of cannabinoids and the trifluoroacetyl derivatives of amphetamines and designer drugs. The method was shown to be sensitive with detection limits between 6 and 52 pg/mg hair matrix and precision between 0.4 and 7.8 % by the use of an internal standard technique. Linearity was obtained from 0.1-20 ng/mg with coefficients of correlation between 0.995 and 0.999.

Compared to conventional GC-MS SIM methods of hair analysis, the sensitivity could be enhanced by factor 8-35. HS-SPDE/GC/MS-MS is easier to use, substantially faster, with the degree of sensitivity and reproducibility demanded in clinical and forensic toxicology.

V23 Screening auf und validierte Quantifizierung von Amphetaminen sowie von Designerdrogen des Amphetamin- und Piperazin-Typs im Blutplasma mittels GC-MS

Screening for and validated quantification of amphetamines as well as of amphetamine- and piperazine-derived designer drugs in blood plasma by GC-MS

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Background: The classical stimulants amphetamine, methamphetamine, ethylamphetamine and the amphetamine-derived designer drugs MDA, MDMA, MDEA, BDB, and MBDB have been abused for a relatively long time. In recent years, several newer designer drugs have entered the illicit drug market. MTA, PMA, and PMMA are also derived from amphetamine. Others are derived from piperazine like BZP, mCPP, MDBP, MeOPP, and TFMPP. A number of severe or even fatal intoxications involving these newer substances, especially PMA, have

been reported. Therefore, methods for the determination, especially of these newer designer drugs, in blood plasma are needed due to the growing importance of this matrix in analytical toxicology. In the presented study, 4-hydroxyamphetamine and 4-hydroxymetamphetamine (pholedrine), the main metabolites of PMA and PMMA, were also included.

Methods: After mixed-mode solid-phase extraction (HCX) of 1 mL of plasma and derivatization with heptafluorobutyric anhydride, the analytes were separated on an HP-5MS column (30 m x 0.25 mm I.D., 250 nm film thickness). They were detected using an HP 5972 MSD operated in the EI-SIM mode. Calibration curves were used for quantification.

Results: The method was fully validated according to international guidelines. It was linear from 5 to 1000 µg/L for all analytes. Extraction efficiencies lay between 30% and 110%. Accuracy data (bias in %) for all analytes ranged from -6.3 to 12.9 near the LOQ and from -14.0 to 5.3 at high concentrations. Repeatability values (RSD in %) ranged from 1.9 to 18.7 near LOQ and from 1.2 to 10.5 at high concentrations. Intermediate precision data (RSD in %) ranged from 3.7 to 20.0 near LOQ and from 1.6 to 17.7 at high concentrations. The acceptance criteria were fulfilled for all analytes with the exception of MDBP. The limit of quantification was 5 µg/L for all analytes. The applicability of the assay was proven by analysis of authentic plasma samples and of a certified reference sample.

Conclusions: The presented GC-MS assay has proven to be applicable for screening and quantification of the studied analytes, with exception of MDBP quantification, in plasma. It should also be suitable for confirmation of immunoassay results positive for amphetamines and/or designer drugs of the ecstasy type.

V24 Die Staatsanwaltschaft zwischen Wissenschaft, Recht und Rechtsprechung
The Prosecution Attorneys between Science, Law and Jurisdiction

H.K. Larcher,

Staatsanwaltschaft Mannheim

§ 316 StGB trägt die vom Gesetzgeber verbindlich vorgegebene Headline „Trunkenheit im Verkehr“. Damit wurde bis vor wenigen Jahren grundsätzlich nur „Alkohol im Straßenverkehr“ assoziiert, obwohl diese Rechtsnorm schon immer den Teilsatz „..oder anderer berauschender Mittel…“enthält.

Bereits das Reichsgericht hatte sich in einer Grundsetzentscheidung mit der Frage der Fahruntauglichkeit bei Alkohol auseinandergesetzt und eine Kernaussage getroffen, die bis heute unverändert Gültigkeit hat. Dem Problem „Drogen im Straßenverkehr“ hat man sich in der Strafverfolgung erst seit wenigen Jahren bewusst angenommen und deren praktische Bedeutung realisiert.

Wie schon zu Zeiten des Reichsgerichts, so erst recht heute hat die Rechtsmedizin mit ihrem komplexen interdisziplinären Tätigkeitsfeld den Strafverfolgungsbehörden Ermittlungshilfen vermittelt, um die Rechtsnorm des § 316 StGB effektiv umsetzen zu können. Während der Bereich Alkohol mittlerweile ein wissenschaftliches ausgereiftes und hoch entwickeltes Instrumentarium anbietet, sind wir im Bereich der „Drogen im Straßenverkehr“ in einer ähnlichen Situation, wie das Reichsgericht seinerseits beim Alkohol.

Wir wissen zwar, dass Drogen grundsätzlich fahruntauglich machen, aber wir haben oftmals immer noch erhebliche Schwierigkeiten dies im konkreten Fall unter Berücksichtigung der Rechtsprechung hinlänglich belegen zu können.

Dies ist für die Staatsanwaltschaft eine Herausforderung, um in Kooperation und wissenschaftlichem Austausch vor allem den vor Ort geforderten Polizeibeamten ein effektives und leicht einsetzbares „Instrumentarium“ zur Aufklärung und Beweissicherung an die Hand geben zu können.

Um dieses „Instrumentarium“ entwickeln zu können bedarf es einer Darstellung der Problematik aus juristischer Sicht unter Berücksichtigung der Rechtsprechungsentwicklung und Darstellung konkreter Fallgestaltungen.

V25 Absolute Fahruntüchtigkeit unter der Wirkung von Cannabis - Vorschlag für einen Grenzwert
Absolute Inability for Driving under the Influence of Cannabis - Proposal for a Threshold Limit

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Data from 2001/2002 on 585 car drivers under the sole influence of cannabis and conspicuous to the police were reevaluated. It was tested, whether from the deficiency symptoms, as described by the police, a threshold value for the absolute driving inability under cannabis can be derived. For this purpose, THC in serum and the Canna-

bis Influence Factor CIF = ([THC] + [THC-OH]) x 100 / [THC-COOH], as proposed by Daldrup, were compared to the frequency of different deficiency symptoms. Statistically significant results were obtained between both factors and the statements " concentration deficiencies", "continuously forgets something", "cannot follow longer sentences", "instructions must be repeated several times", and the tests finger-to-nose and one-leg-stand. There was a continuous increase of the frequency of these deficiencies with THC in serum. This indicates that in principle a toxicological defined threshold limit can be established for the THC concentration. The height of this limit, however, cannot be deducted from our study. Moreover the THC concentration decreases with the space of time between the incident and the bloodletting. In contrast to this, the CIF did not depend from this time difference within the first 120 minutes. With the CIF, the frequencies of the deficiencies are distributed in a different way than with the THC concentration: At a CIF of 10, there is a distinct leap from low to high frequencies, while at higher CIF values a saturation results in a plateau value for the frequencies. The value for the best separation between low and high frequency can be determined at a CIF of 10. It is therefore proposed to assume an absolute driving inability by cannabis at a CIF of 10 or higher.

V26 Benzoylecgonin als Indikator neuropsychologischer Defizite nach akutem und chronischem Kokainmißbrauch: ein Überblick

Benzoylecgonine as an indicator of neuropsychological deficits after acute and chronic cocaine abuse: an overview

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Cocaine abuse, which is generally associated with impairments in road safety, is usually inferred by the detection of cocaine or the cocaine metabolite benzoylecgonine (BZE) in the urine, blood or hair. Cocaine users are either recreational users, who can control their consumption, or cocaine addicts, who consume it in an uncontrolled, binge-like pattern. Studies with recreational users revealed an increased level of aggression, but also showed increased alertness and attention and less fatigue up to 4 hours after consumption. However, in very high doses cocaine can lead to psychotic-like behaviour. Even if BZE can be quantitatively detected in the urine of recreational users, due to strong interindividual differences and a doese-latency problem, there is no reliable relationship between the measured BZE concentration and neuropsychological deficits that are relevant for road safety. Unfortunately, there are no studies with recreational users that assess the effects of cocaine in the post-acute phase (>4 hours after consumption). Cocaine addiction, conversely, results in a wide range of neuropsychological, neurological and psychiatric deficits, even up to 3 month of abstinence, which suggests a potential impairment of road safety. The diagnoses of a person to be an cocaine addict (e.g. by clinical assessment) with an abstinence period of less than 3 month may in itself be sufficient as an indicator of road safety related impairments. If a cocaine addict relapses, the binge phase is followed by a crash, which comprises heavy psychiatric symptoms, like depression, anxiety and paranoia, that can last up to 4 days. After the crash a longer period of neuropsychological deficits follows. Thus, for a known cocaine addict a qualitative BZE or cocaine detection in the urine or blood should be a sufficient indicator for road safety related impairments.

V27 Grenzen der Fahrtüchtigkeitsbegutachtung - aufgezeigt am Beispiel eines Verkehrunfalls einer jungen Fahrerin unter Alkohol und Cannabis.

Limits of Giving an Expert Opinion on Driving Inability as Demonstrated by the Case of a Young Female Driver under Alcohol and Cannabis.

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When she drove home in the morning after a wedding party, a 19 years old female driver was involved into a road traffic accident in an S-bend. According to her report, she was recognising that she will be cut and slowed her car down to nearly standing. However, she could not avoid scraping against the approaching vehicle. Knowing to have consumed cannabis some hours before and later alcohol, too, she did not stop immediately at the scene of accident but parked her car nearby at home in order to first ask her parents for help and advice. Her parents called the police immediately.

Smelling alcohol in her breath, a police officer caused that a blood sample for drugs and alcohol analysis was drawn: Results: BAC: 1.07 ‰ and THC: ~ 0.8 ng/mL Serum, 11-OH THC: negative, THCCOOH 18.3 ng/mL serum. During the medical examination, she sowed no relevant signs of driving impairment.

As concerns the accident encounter, the opposite driver, a young lady, too (24), gave a similar report but disputed any dangerous way of driving. The photographs and a sketch of the accident scene recorded by the police will be shown and discussed. Both reported versions of the way of driving seemed possible, however, the reporting of the driver under influence should have been correct.

Considering the corresponding articles in the German criminal act, the expert's problem is finding true signs of driving inability. In this view, the suspicious facts for the police need to be discussed. Such fact are „road-side" drug testing (1), conspicuous outward appearance or figure which need to be demarcated from impaired mental or physical states (2), mere conspicuous driving (3) and real driving errors and mistakes (4).

In the case demonstrated, however, whether or not she was clearly unfit to drive and the accident should have been inevitable for her was not consequently questioned at a court. In order to avoid any trial including the possibility of a hit-and-run offence, she accepted a summary punishment as ordered by the prosecution attorney.

V28 Verkehrsunfälle unter dem Einfluss von Drogen im Saarland
Accidents under the influence of drugs (AUID) in Saarland

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Objectives of the study: In the recent few years the number of drivers, detected driving under the influence of drugs (DUID) has increased several fold in the state of Saarland. This was due to the new law (§24a STVG) and well trained police officers, who are able to detect signs of drug use and/or signs of impairment roadside. However the number of DUID cases with accidents, accidents with injured or with fatalities (AUID) has remained constant. This was the reason to investigate the accidents, where drivers were suspected to be under the influence of drugs in more detail.

Material and methods: In the years 1998 – 2000 in 308 DUID cases with accidents(=AUID), we received blood samples from the police to be tested for drugs/drugs of abuse. In 195 cases the BAC levels were below 0, 1 %. Only these cases were investigated in more details, because the influence of drugs on driving ability only can be established with no or low alcohol concentrations. Beside the analytical data and the doctor's report (during the blood sampling) we tried to get also the police report and/or the prosecutors files. This was only possible in 126 cases.

Results: In 24 of the 195 cases no alcohol or drugs could be detected by screening procedures.In the remaining 171 cases, which were analyzed by GC/MS for drug/drug of abuse, the following data could be received: in 47 % alcohol was positive, in 42 % cannabis; stimulants were present in 23 % and morphine type drugs in 13 %.

Due to the rather low number, the 171 cases were grouped in 4 categories: control group I (no alcohol, no drugs: 41 cases), alcohol group II (alcohol, no drugs: 41 cases), drug group III (drugs, no alcohol: 50 cases) and alcohol/drug group IV (drugs and alcohol: 39 cases).

The average age was in group I 34 years, in group II 28 years, in group III 27, 5 years and in group IV 25 years. Additional information from the police report and/ or prosecutors files show (group I – IV) that 21 % of the cases were hit and run cases.

Table 1. Kind of accidents

	Group I	Group II	Group III	Group IV
fatalities	16%	4%	2%	8%
injured	34%	31%	35%	23%
damage	50%	65%	63%	69%

Discussion: It seems that only a fraction of AUIDs become known to the police. Therefore, police officers should be trained and sensibilitzed about drug influence in road traffic accidents (AUID).The real amount of danger of drugged driving should became known to public to initiate countermeasures.

V29 Verkehrsunfälle unter Drogen- und Medikamenteinfluss: Beispiele aus Westösterreich

Traffic Accidents and Toxicological affections: Examples from Western Austria

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In the year 2000 in altogether 49 cases of motoring offences toxicological examinations for illicit drugs and medicaments were ordered at our Institute. Blood specimens were available in 23 of these cases. Among them, 18 drivers were routinely controlled, whereas 5 drivers were involved in accidents. The course of these 5 accidents and the results of the toxicological analyses are presented in detail.

Three drivers caused frontal collisions, two because of overtaking (cases 1 and 2), one because of drifting to the left side of the road (case 3). One drove to the right side of the road and against the pavement (case 4), and one damaged two parked cars (case 5). Toxicological examinations found prothipendyl and carbamazepin in therapeutic concentrations in case 1; the driver showed additionally a psychiatric affection.

In case 2 the immunological assays were positive for opiates and benzodiazepines, a quantification could not be done because of lack of specimen. In the urine, benzodiazepines, morphine and methadone were detected. This driver had been controlled routinely some time before, and benzodiazepines and metabolites of cocaine had then been detected in the blood.

In case 3, benzodiazepines, morphine and metabolites of cocaine were quantified in the blood specimen. Driver 4 was affected by benzodiazepines, driver 5 by THC. None of these drivers were alcoholised.

In cases of road accidents, toxicological analyses for illicit drugs and medicaments have seldom been ordered in western Austria up to now. In most cases only a measurement of blood alcohol was performed. It should be possible to tell up to the date of this presentation whether the number of toxicological analyses will rise due to the new law in Austria, legal as of January 2003, which allows taking blood samples from drivers if an affection by illicit drugs is suspected.

V30 Verkehrsicherheit: welche wichtigen Erkenntnisse werden verfehlt wenn nur eine BAK angefordert wird?

Traffic safety: Which relevant information a single BAC request fails to show?

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The negative impact of alcohol consumption on fitness to drive has been recognized for a long time. The behavioral influence of illicit drugs (ID) and medical drugs (MD) was more recently investigated among drivers and becomes a question of growing interest in numerous countries.

This work was performed to assess information missed by blood alcohol concentration (BAC) requests, such as trends in psychoactive substances (PAS) consumption (nature of the ID and MD, polydrug users…), and to establish a profile of subjects (gender, age, chronic alcoholism…) involved in road traffic accidents.

The study was carried out on serum samples from an anonymous population of 198 drivers apprehended in Luxembourg (with the authorization of public prosecutor) and submitted to BAC requests. The screening and quantification for a selection of major ID and MD was performed using GC-MS and HPLC respectively. Alcohol was routinely monitored with the GC-FID and ADH techniques in use for proceedings at law. The quantification of carbohydrate deficient transferrin (CDT) using capillary electrophoresis allowed to establish the proportion of chronic alcoholics (CDT > 2%).

Results analysis revealed that only 9% of drivers were free of any of the substances tested. Alcohol was found as single consumption in 61% of subjects, and associated with ID and/or MD in 8% and 16% respectively. The most used substances were cannabis (9.6%, e.g. THC ranging from 3.1 to 48.7 ng/mL) for ID, and benzodiazepines (10.1%, e.g. Nordiazepam ranging from 0.07 to 8.8 mg/L) for MD. 2 substances were simultaneously found in 18% of the samples, 6% of the subjects had associated 3 substances, and for 1% of the population, 5 different PAS were detectable in blood. 29% of the persons tested were chronic alcohol abusers.

Our study brings to the fore the overrepresentation of chronic alcoholics among apprehended subjects. Furthermore, the rate of ID and MD use, and the frequency of PAS associations highlight the underestimated involvement of these substances in traffic accidents.

V31 Phänomenologie der Akutwirkungen von "Partydrogen" bei Diskothekenbesuchern

Phenomenology of acute effects of "party drugs" on users in discotheques

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In this study, a cohort (n=202) recruited from typical visitors of two well-attended "Techno discotheques" in the Rhein-Main area was investigated. The examinations of the anonymous volunteers were located within these discotheques. The participants had mainly consumed MDMA, amphetamine and cannabis, less often cocaine or LSD. Not often and only modestly they drank alcoholic beverages. With one exception, opiates and sedatives were not abused in our cohort. In order to determine the extent of being influenced by psycho stimulants, tests like straightforward walking with turning around or balancing on one leg with closed eyes can be used partially. The pupil light reaction and the wideness of the pupils determined with the proposed P/I index (diameter of the pupil divided by the iris diameter) proved to be well-suited criterions for diagnosing drug influence. The average P/I ratio of drug-influenced individuals was 0,81 (controls: 0,68); of the probands 68 % had diminished light/dark adaptation. The duration of the postrotatory nystagmus was measured to be longer than 8 seconds in 72 % of the cases (mean: 13.6, median: 12 seconds) and seems to be suitable for detection of drug influence. Unlike the effect of alcohol the postrotatory nystagmus under influence of ecstasy has a lower amplitude and is fading out. Therefore, inexperienced investigators are susceptible to measure incorrect low values. Being aware of the fact that psycho stimulants elicit quite a different appearance of influence compared to alcohol, the documentation of special features is mandatory, particularly since all physical signs reflect a transient state. Therefore, physicians investigating e.g. drivers under influence of drugs should be specially trained and instructed and the documentation forms used should be modified.