ABSTRACTS – VORTRÄGE HAUPTSYMPOSIUM

V1 Feststellung der Todesursache, insbesondere bei Intoxikationsfällen
Determination of cause of death, especially in cases of intoxication

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The determination of the cause of death as a medical scientific basis for answering legal questions – and intoxications are generally followed by legal questions (autointoxication, intoxication by others, accidental intoxication, breach of supervisory duties) - requires a differentiated and multi-stage examination. Postmortem findings (morphological, toxicological, postmortem biochemical) have to be divided according to their duration and dignity into basic ailments, resulting state of health and final cause of death. Postmortem findings should correlate with the medical history reported in lifetime. If anamnesis is missing or anamnestic information is questioned – like in cases of intoxication by other persons – the anamnesis has to be reconstructed by postmortem examinations.

Special difficulties arise from multidimensional pathogenesis and in cases where the cause of death is a summation of individual phenomena. This might also be of importance in cases of intoxication. Thus, an orientation towards the so-called “dying types” (linear dying type, diverging dying type, converging dying type, complex dying type) which are derived from morphological and toxicological findings is advisable. These dying types have also been called a “thanatological bridge” between basic ailments and cause of death. A graduation of postmortem findings (developed by Richter in 1905) according to their dignity (findings that definitely explain death at a given time; findings which are suitable to explain death but not the isolated point of time of death; findings which are doubtful as cause of death) is also helpful. An orientation towards these schemes basically means to identify all findings in detail and then to weigh them up against each other regarding their importance. Thus, in cases of intoxication a complete morphological status (autopsy findings including histology) has to be carried out as well as chemical-toxicological examination of body fluids and tissues - if necessary with identification of metabolites, identification of the way of the intoxication, discussion of chemical-toxicological findings taking into account pre-existing diseases, pharmacodynamics, concentration of the active substance etc.

V2 Intoxication with Convallaria majalis (Lily of the Valley)
Vergiftung mit Convallaria majalis (Maiglöckchen)

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Two women (mother and daughter, 81 and 48 years old) believed to gather leaves of Allium ursinum (Bears garlic) in her own garden and consumed them as a salad. One day later, they were taken to an emergency department, both with similar symptoms of serious intoxication with cardiac glycosides.

Blood of both patients were investigated in the clinical laboratory by immunoassays (CLIA from Bayer Diagnostics) for digoxin and digitoxin. Initial plasma concentrations of digitoxin were 80 / 113 ng/mL, declining during the following days to 62 / 64 ng/mL, 45 / 38 ng/mL and finally 18 / 20 ng/mL (patient 1 / patient 2). No digoxin was detected by immunoassay. As none of the two patients had any medication with digitoxin or other cardiac glycosides, blood samples (collected 18 h after admission into hospital) were sent to our laboratory and a general unknown toxicological analysis was performed on HPLC-DAD (1): digitoxin was not detected (LOD 30 ng/mL). In contrast, the immunological results (FPIA from Abbott) were 45 / 63 ng/mL digitoxin, similar to that measured in the clinical laboratory.

The supposition was, that the patients (because of the great similarity) probably ingested leaves of Convallaria majalis instead of Allium ursinum. Convallaria majalis contains about 40 different cardiotonic glycosides, in particular convallatoxin. In the plasma (collected 18 h after admission to hospital) convallatoxin was measured at concentrations of 17 ng/mL (patient 1) and 13 ng/mL (patient 2) by LC/MS (methanole-precipitation; column: Waters Atlantis (20 x 2.1 mm, 3 µm; mobile phase: methanol/water-gradient, supplemented with 1 ml formic acid; SIM-mode (m/z): 573 (ESI (+), LOD: 1 ng/mL). In our laboratory in spiked plasma samples the cross-reactivity of the antibody of FPIA from Abbott with convallatoxin was 50%.

Serious intoxications with Convallaria majalis are very unusual. Due to the great structural similarity with digitalis – glycosides an overdose can be detected by immunoassays for digitoxin from Bayer Diagnostics (CLIA, chemiluminescent assay) and from Abbott (FPIA, fluorescence polarization immunoassay). The quantification of convallatoxin can be performed with LC/MS. The difference between the concentrations measured by immunoassay compared with those got by LC/MS is possible due to the fact, that much more glycosides than convallatoxin are bound by the antibody.

1. F. Degel, W. Steiner, H.-J. Birkhahn, D. Lampe, U. Demme. In Klinisch-toxikologische Analytik, Herausgeber W. R. Külpmann, WILEY-VCH Verlag, Weinheim 2002.

V3 Sechs Vergiftungsfälle nach intravenösem Konsum von mit Atropin verschnittenem Kokain

Six Intoxication Cases after Intravenous Injection of Cocaine adulterated with Atropine

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Background: Intravenous injection of cocaine is a popular form of application among heavy drug users. Within one week, six patients with hallucinations, blurred visions, and a central anticholinergic syndrome were admitted to various hospitals. All were heavy drug users who concede intravenous abuse of cocaine.

Methods: Blood plasma and urine samples as well as the used syringe from one case were submitted to the authors' laboratory for toxicological analysis. The samples were analyzed according to the authors' standard toxicological analysis procedures (Maurer HH (2000) Methods for GC-MS. In: Pfleger K, Maurer HH, Weber A (eds) Mass spectral and GC data of drugs, poisons, pesticides, pollutants and their metabolites, part 4. Wiley-VCH, Weinheim, pp 3-241). Quantification of benzoylecgonine, cocaine, and atropine in plasma was performed by liquid chromatography-mass spectrometry (LC-MS) after solid phase extraction (according to Maurer HH, Tenberken O, Kratzsch C, Weber AA, Peters FT (2004) J.Chromatogr.A 1058:169) using benzoylecgonine-d_3 and cocaine-d_3 as internal standards.

Results: The screening analysis of urine showed in all cases the presence of large amounts of cocaine (metabolites) and atropine. In five cases, small amounts of morphine, benzodiazepines, paracetamol, salicylic acid, and/or ethanol were also detected. The plasma concentrations of benzoylecgonine were below 100 ng/mL in four cases and in the other two, they were 513 and 1549 ng/mL, respectively. Cocaine concentrations were in all cases below 100 ng/mL. The atropine concentrations ranged from 4 to 31 ng/mL. The syringe contained cocaine, large amounts of atropine as well as small amounts of phenacetin.

Conclusions: The concentrations of atropine were above the therapeutic range in two cases and within the therapeutic range in four cases. Thus, the patients' symptoms were attributable to atropine. The presented cases have shown that atropine was used as adulterant of cocaine and that intravenous injection of this mixture made medical treatment necessary.

V4 Falsch diagnostizierte tödliche Vergiftung mit Herbstzeitlosen bei einem Kleinkind

Misdiagnosed fatal meadow saffron poisoning in a toddler

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Background: In Switzerland meadow saffron (colchicum autumnale) is a widespread plant throughout the country. In springtime its leaves can be mistaken for bear's garlic (allium ursinum), which is collected for alimentary purposes. The mistake can lead to severe and fatal colchicine poisoning.

Case: In august, a 3-year-old boy was admitted to the local hospital with dehydratation, tachycardia, somnolence and confusion. Two days before admission he started vomiting, had severe abdominal cramps and profound diarrhoea. His laboratory results indicated a metabolic acidosis, prerenal kidney insufficiency and liver failure with a tremendously elevated ammonia concentration (800 µmol/l; normal range < 33). The salicylate concentration at entry in the hospital was 0.4 mmol/l (therapeutic range: 0.4 – 2.2). Despite intensive medical treatment, the boy died 5 hours after admission. Based on the symptoms and the detection of salicylates in the patient's blood the diagnosis of Reye syndrome was made. No postmortem examination was performed.

One year later two fatal cases of mistaken meadow saffron leaves were reported in the newspapers. A relative of the child heard the news and remembered that the child was playing on grassland in the mountains and eating leaves, the day before the symptoms started. Meadow saffron was found at that time at the place where the boy was playing and the suspicion aroused that the boy might have eaten leaves of meadow saffron. A serum sample was still available and colchicine was quantified using HPLC mass spectrometry. The colchicine concentration was 7 µg/l (toxic > 5 µg/l).

Conclusion: Colchicine poisoning usually occurs in springtime after intake of mistaken meadow saffron, but in higher altitude nature is behind as compared to lower places and plants regularly existing in spring can also be found in summertime. Colchicine must be considered as a differential diagnosis in cases of unexplained multi organ failure.

V5 Tod nach der Einnahme von Amphetaminen

Death due to an intake of amphetamines

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A drug-party ended after 3 days when a 47 year old man came into the state of extreme agitation after having consumed a high dose of amphetamines. His 2 friends covered him with a blanket fixing it with a cable. They forced him to swallow carbamazepine and finally called the ambulance. But the man could not be saved. The body temperature measured 2½ hours after death was of 38.6 °C. The autopsy revealed a cerebral edema, congestion of the organs and damages of the myocardium.

Toxicological examination of the femoral blood revealed an amphetamine concentration of 510 µg/L, a MDMA concentration of 255 µg/L and a carbamazepine concentration of 1000 µg/L. Ketamine and traces of LSD were detected qualitativeley. The analyses were accomplished by GC-MS, HPLC-PDA and LC-MS. Amphetamine blood levels of the two surviving persons were determined as 230 µg/L and 20 µg/L respectively, the blood samples being taken 6 hours after death of their friend. In both blood samples traces of LSD were detected.

In the flat of one of the surviving persons large amounts of drugs and preparations of drugs were found. Worthy of note is hereby the seizure of Magnesium GHB, a GHB preparation not under control in the Swiss legislation.

In conclusion we suggest that the death occurred due to a hyperthermia and a heart failure caused by an overdose of amphetamine and MDMA. A judgement concerning the behaviour of the 2 persons at the time of death of their friend and concerning the preparation of Magnesium GHB did not occur yet.

V6 Death after accidental gasoline intoxication

Tod durch Benzinintoxikation

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Consequences of gasoline intoxications are slurred speech, disturbance of walk and lose one's bearings in later phases delirium, cramps, the inability to breath and cardiac arrest are observed.

A male accidentally inhaled a high dose of gasoline by repairing a car in a garage. He was found dead sitting in a pit under the car. An oil sump partly filled with gasoline like solvent was also found there. Heart blood, liver, lung, urine, brain, myocardial muscle, gall, kidney and stomach were taken at forensic autopsy and stored under gas-tight conditions. Measuring has been performed by headspace gas chromatography. Validation of the method has been carrying out using commercial available gasoline.

In all organs and body fluids numerous fugitive solvents usually being part of commercial technical gasoline were found. Only low concentrations were found in urine (2 mg/kg), in lung (33 mg/kg) and heart blood (42 mg/kg). High concentrations were found in liver (217 mg/kg) and above all in brain (317 mg/kg). The low concentrations in lung and heart blood can be explained by post mortal evaporation.

Fatal gasoline intoxications with concentrations of 44 mg/kg [1] and 400 mg/kg [2] in brain are described. In consideration of the literature, the autopsy results and the toxicological results death by gasoline intoxication is likely.

[1] Nelms RJ (junior), Davis RL, Bond J, Verification of fatal gasoline intoxication in confined spaces utilizing gas-liquid chromatography. Am J Clin Path 1979, 53, 641-646.

[2] Carnevale A, Chiarotti M, De Giovanni N, Accidental death by gasoline ingestion. Am J For Med Path, 1983, 4, 153-157.

V7 Mord ohne Giftnachweis? Succinylcholin als potentielles Mordgift?

Homicide without detection of the poison? Succinylcholine as potential poison?

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A 35-yr-old woman was found dead by a mobile physician and paramedic rescue team at the bottom of a stairway. Prolonged CPR was unsuccessful. A few minutes earlier, the team has been dispatched to the scene, a couple's home, by the woman's husband, a trained anaesthesiologist. Due to his report he had found his wife unconscious and looking pale, with a slow and weak pulse that eventually faded. Before the alert, he himself had administered CPR, including tracheal intubation and bag ventilation using his own emergency equipment. Furthermore, he presented several electrocardiogram strips showing consecutive sinus bradycardia, asystole, and ventricular fibrillation, recorded before the team's arrival, and he suggested that his wife's blood pressure problems possibly evoked her accident. After the woman was pronounced dead, police officers performed a workup and seized materials. They noted, that the dead woman's husband might have tried to conceal one of the electrocardiogram strips. Furthermore a succinylcholine vial was missing from the anaesthesiologist's emergency case.

On forensic autopsy, some pulmonary edema, a scalp laceration, and findings consistent with intubation and CPR were seen, but no pathology indicating inflicted external force or explaining the woman's death. Especially a lethal fall down stairs could be ruled out. Toxicological analysis revealed inconspicuous results, especially no succinylcholine or its degradation products were found in any specimens by using GC-MS or LC-MS/MS. For further interpretation three experts in toxicology and several anaesthesiologists were involved, only one of them excluded the possibility of an administration of succinylcholine, which is rapidly metabolised and degraded over succinylmonocholine to succinate. Further investigation revealed that electrocardiograms, previously stated from the accused to have been recorded personally from his wife during CPR, were faked, thus implying a criminal act.

Subsequently, the anaesthesiologist admitted to having killed his wife, without elaborating on the circumstances for legal reasons, and was sentenced to prolonged imprisonment. Toxicological problems concerning the analysis of succinylcholine will be discussed.

V8 An Unusual Death Scene of Morphine Poisoning Fatality

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This case report presents a morphine poisoning fatality in which circumstantial evidence made the police suspect homicide. Blood stains all over the scene of death, multiple incised wounds, bondage of the wounded right arm, used razor blades carefully placed on a small table, lack of any blood stains on the left arm and a digital wrist blood pressure measuring device applied to the left forearm. A used syringe was lying on the floor and a needle prick mark was seen on the left arm.

Autopsy revealed multiple rib fractures on the left side of the chest. Toxicological investigation revealed the presence of 1 mg of morphine per liter of blood.

The forensic medical examiner (author) was of the opinion that this was a case of accidental morphine overdose. Based on past experiences of fatalities of similar nature, it was suggested that the wrist wounds had been inflicted by his friends in a futile attempt to rid him of the poison! Rib fractures indicated some energetic, albeit unsuccessful attempts at resuscitation. Many of the previous morphine fatalities encountered by the author showed multiple incised wounds on the sole of the feet. This was the first case of its kind where the "resuscitation wounds" had been inflicted to the wrist rather than the usual place. The deceased's friends were eventually found and confirmed the expert opinion that had already been put forward.

V9 Unexpected toxicological findings after a post mortem interval of almost 20 years

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Interpretation of toxicological findings from exhumation requires carefulness, since poisons may be transferred from or to the surrounding environment or may be to a greater or lesser extent degraded. In January 2003 an exhumation was ordered by the public prosecutor's department of Rostock after an almost 20-year post mortem

interval. The dead person was a well-known theatre manager from the former GDR. In connection with a critical historical review of his life's work suspicions of a non-natural death (homicide) or poisoning were arisen.

As expected the cadaver was completely skeletonized and the casket was heavily degraded whereas clothes and shoes were relatively well preserved. The inside of the cranium contained loamy, amorphous material which was strongly positively tested for benzodiazepines by immunoassay (EMIT). After ultrasonic extraction with a mixture of acetone and methanol (1:1, v/v) diazepam (127 ng/g), traces of nordiazepam and an oxazepam metabolite (6-chloro-2-methyl-4-phenylquinazoline = oxazepam -CO_2) were detected by GC-MS. The latter was clearly dominantly and could be identified by complete mass spectra. The oxazepam metabolite was additionally detected in material inside the pelvis and in material overlaying the bones from different parts of the skeleton.

Due to the lack of availability of a reference substance only estimations of relative concentrations by signal areas were possible. Highest contents of the oxazepam metabolite were found in the inner parts of the pelvis suggesting an ingestion of diazepam (or medazepam) about to death.

Furthermore organic persistent pesticides and their conversation products [1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene = DDE metabolite of DDT; pentachlorophenol, hexachlorobenzol] were present in the layer adherent to bones. It is probably assumed that these compounds are residues of wood preservatives (Hylotox® etc.) which were widely-used in the late GDR for protection of wooden caskets. Inorganic substances detected by AAS were basically in normal ranges.

Although the cause of death could be not clarified, these findings again underline the importance and value of exhumation even after long post mortem periods.

V10 Betäubung mit Benzodiazepinen im Zusammenhang mit einem Tötungsdelikt, eine Fallstudie

Intoxication by benzodiazepines in relation to a homicide, a case report

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A 41 year old woman was found dead lying in the pond of her garden in the evening in august. The suspected husband pretended that they had a nice evening the day before and spoke about their divorce and the sell of the house. The police confiscated the drinking glass with water in the kitchen, on the terrace a drinking-cup filled with a blue liquid and white components and later in the kitchen a mortar with a pestle. The autopsy was performed one day after finding the body. The cause of death was a drowning. The body showed several haematomas caused by violence against the head, the neck, the chest and the shoulders.
Toxicological results:

Femoral blood: alcohol 0,02 ‰, midazolam 40 ng/ml, flurazepam 180 ng/ml, N_1-desalkylflurazepam 30 ng/ml, N_1-hydroxyethylflurazepam 85 ng/ml.

Gastric fluid: alcohol 0 ‰, bromazepam 1,2 mg/750 g, midazolam 5,35 mg/750 g, flurazepam 43 mg/750 g

Water of the drinking glass: midazolam, flurazepam

Liquid of the drinking cup: midazolam, flurazepam, flunitrazepam

Mortar with pestle: midazolam, bromazepam, triazolam, alprazolam, metabolites/artefacts of flurazepam

Considering the toxicological results and the results of the autopsy we presumed a homicide by drowning after intoxication by a mixture of benzodiazepines.

V11 Deadly Fentanyl Patches? – Findings in a Case of Mixed Poisoning with Fentanyl and Tramadol after Long-Term Abuse

Tödliche Fentanyl-Pflaster? – Befunde im Fall einer Mischintoxikation bei Fentanyl- und Tramadolabusus

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A 31-year-old female physician assistant was discovered dead in the morning after she had spent the night with her lover, a married medical practitioner. On the preabdomen two patches "Durogesic 50 µg/h" and "Durogesic 70 µg/h" were found in addition to traces of older remains of adhesive. At the location a bottle of tramadol solu-

tion and "Imeson" tabletts (active ingredient: nitrazepam) were secured among a variety of different pharmaceuticals.

In the peripheral blood 27.9 ng/ml of fentanyl, 3336 ng/ml of tramadol, 230 ng/ml of nitrazepam and 20343 ng/ml of caffeine were determined.

To investigate whether the concentration of fentanyl is likely to have been caused by the applied patches alone, the remaining quantity of fentanyl in the patches was determined. According to our results about 5 mg of fentanyl had been released into the body (weight: 56.7 kg). Blood concentrations in the observed range can be explained either by an accelerated release of fentanyl from the patches (e.g. by warming them) or by accumulation of fentanyl from previously applied patches. An additional oral or intravenous uptake of fentanyl must also be taken into consideration.

Furthermore it had to be clarified if the deceased had been abusing the aforesaid opioids. For this purpose a 25 cm long hair strand cut into 6 segments was analyzed. Using d5-fentanyl and venlafaxin as internal standards concentrations of 0.10 – 0.27 ng/mg fentanyl and 7.2 – 101 ng/mg tramadol were found in the different segments showing an extensive long-term abuse of fentanyl and tramadol. There was no indication of third party fault. Considering all circumstances death was due to an accidental overdose.

V12 Hair analysis for kavalactones and their metabolites after oral consumption of kava beverages using HPLC-DAD, LC-MS/MS and GC/TOF-MS

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A simple, sensitive and reproducible method for the determination of kavalactones in human hair has been developed. Hair samples were collected from nine persons with different genesis (Caucasian, Melanesian, Indonesian and African). The kava consumption varied among those persons (single oral dose and mild, regular and heavy intake of kava beverage). The concentrations of the kavalactones in the human hair samples ranged between 0.2 and 25 ng/mg for kavain, 0.5 and 34 ng/mg for 7,8-dihydrokavain, 0.7 and 8 ng/mg for yangonin, 1 and 14 ng/mg for 5,6-dehydrokavain (= desmethyoxyyangonin) and 0.9 and 6 ng/mg for the metabolite 12-hydroxy-5,6-dehydrokavain (Met. 3). Methysticin, 7,8-dihydromethysticin and the metabolite 11-hydroxy-5,6-dehydrokavain (Met. 4) were detected but not quantified. Additionally 12-hydroxykavain and 12-hydroxy-7,8-dihydrokavain were detected by LC-MS/MS in one case. General unknown screening for other drugs as well as confirmation of the HPLC-DAD results were performed by GC/TOF-MS.

V13 Untersuchungen zum Metabolismus und toxikologischen Nachweis der Designer-Droge 4-MTA in Humanurin mittels GC-MS-Techniken

Studies on the metabolism and toxicological detection of the designer drug 4-MTA in human urine using GC-MS techniques

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Objectives: 4-Methylthioamphetamine (4-MTA), a designer drug of the amphetamine type, plays an increasing role on the illicit drug market. After some fatal poisonings with 4-MTA, it was scheduled e.g. in the German Act of Controlled Substances. For toxicological detection, it is necessary to know its metabolites. Therefore the aim of our study was to identify those in human urine using gas chromatography-mass spectrometry (GC-MS) techniques.

Methods: For the metabolism study, urine samples from poisoning cases were extracted after enzymatic cleavage of conjugates by liquid/liquid extraction (LLE) at pH 8-9 followed by acetylation or pentafluoropropionylation

as well as at pH 4-5 followed by methylation. The metabolites were separated and identified by GC-MS in the electron ionization and in the positive chemical ionization mode. For toxicological detection, the urine samples were extracted by LLE at pH 8-9 after acid hydrolysis of half of the sample followed by microwave-assisted acetylation.

Results and Discussion: 4-MTA is only poorly metabolized in humans. Besides 4-MTA, five metabolites could be identified. The following metabolic steps can be postulated: side-chain β-hydroxylation leading to two diastereomers, ring hydroxylation, oxidative deamination followed by reduction to the corresponding alcohol and partial conjugation, and finally, side-chain degradation to 4-methylthiobenzoic acid.

Using our STA, mainly 4-MTA itself could be detected with a recovery of 69±2% (1000 ng/ml, n=5) and a limit of detection of 30 ng/ml. With the exception of 4-methylthiobenzoic acid, all metabolites could also be detected in the STA. The sensitivity should be sufficient for confirmation of a 4-MTA intake.

V14 Untersuchungen zum Metabolismus und zur toxikologischen Analytik der neuen Designer-Droge 4´-Methyl-α-pyrrolidinobutyrophenon (MPBP) in Rattenurin mittels GC-MS-Techniken

Studies on the metabolism and toxicological analysis of the new designer drug 4´-methyl-α-pyrrolidinobutyrophenone (MPBP) in rat urine using GC-MS techniques

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Objectives: 4´-Methyl-α-pyrrolidinobutyrophenone (MPBP) is a new designer drug, which has appeared on the illicit drug market. The aim of the presented study was to identify the MPBP metabolites in rat urine and to develop a toxicological detection procedure in urine using GC-MS.

Methods: For the metabolism study, urine samples from male Wistar rats, which had been administered a 20 mg/kg BW dose of MPBP nitrate, were extracted either directly or after enzymatic cleavage of conjugates using Isolute Confirm HCX cartridges. After derivatization by acetylation, methylation, ethylation, silylation, combined ethylation/acetylation, or combined methylationed/acetylation, the metabolites were separated and identified by GC–MS in the electron ionisation and in the positive chemical ionisation mode. For toxicological detection, a 1 mg/kg BW dose of MPBP nitrate was administered to rats and urine was collected over a 24 h period. The urine samples were cleaved and extracted as described above followed by silylation. For details see the paper on the metabolism of the related drug MPHP: Springer D, Peters FT, Fritschi G, Maurer HH (2003) J.Chromatogr.B Analyt.Technol.Biomed.Life Sci. 789:79.

Results and Discussion: Besides MPBP, six metabolites could be identified. The following metabolic steps can be postulated: oxidation of the tolyl methyl group to the corresponding carboxy compound, hydroxylation of the pyrrolidine ring followed by dehydrogenation to the corresponding lactam or reduction of the keto group to the 1-dihydro compound. The carboxyoxo and the carboxydesaminooxo metabolites were partially excreted in conjugated form.

The toxicological detection procedure focused on the carboxy metabolite. Assuming similar metabolism and dosages in humans, an intake of MPBP should be detectable via its metabolites in urine.

V15 *Sassafrasöle als Grundstoffe für die Herstellung synthetischer Drogen: Profiling* mittels MEKC-UV/(UV)LIF

Sassafras oils as precursors for the production of synthetic drugs: Profiling via MEKC-UV/(UV)LIF

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The still high demand for Ecstasy among drug users in Germany encourages its clandestine production. The surveillance of the chemicals used for the synthesis of mainly MDMA (3,4-methylendioxymethamphetamine) as active substance is a major issue to break down supply chains and find suppliers. One of the most important

precursors for MDMA is safrole, the major compound (up to 95%) found in the essential oils of *sassafras albidum, cinnamomum camphora* and *ocotea pretiosa*.

A micellar electrokinetic chromatography (MEKC) method was developed for the separation of their hydrophobic constituents, such as safrole, eugenol, methyleugenol, α-asaron and trans-anethol. The run buffer consisted of borate (c = 7.5 mmol L^{-1}), SDS (c = 60 mmol L^{-1}), urea (c = 4 mol L^{-1}), $CaCl_2$ (c = 0.5 mmol L^{-1}) and 20% (v/v) acetonitrile. The ten analytes and two internal standards were baseline separated at a high voltage of 30 kV within 12 minutes. UV- (λ_{abs} = 240 nm) and LIF-detection (λ_{ex} = 266 nm) were used in tandem in order to quantify major and minor compounds simultaneously. The method validation included the determination of the detection limits (0.2-6.9 mg L^{-1}), the linear working range and the repeatability using a mobility axis of migration time (UV: 0.1 - 0.7% RSD, LIF: 0.3 - 0.9% RSD) and peak area (UV: 1.8 - 7.9 % RSD, LIF: 9.5 - 10.5% RSD).

The constituents of several sassafras oils from clandestine laboratories were investigated. The safrole content was found to be 60-90%; minor compounds detected were mainly eugenol and methyleugenol. These as well as traces of non-identified substances afforded a fingerprint region with a clear recognition of two different patterns. The comparison with electropherograms from defined plant material enabled the correlation with their biological sources.

V16 Untersuchungen zum Metabolismus der Designer-Droge PCEPA in Rattenurin mittels GC-MS-Techniken

Studies on the metabolism of the designer drug PCEPA in rat urine using GC-MS techniques

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Objectives: N-(1-Phenylcyclohexyl)-*N*-(3-ethoxypropyl)amine (PCEPA) is a phencyclidine–derived designer drug which has appeared on the illicit drug market and which shows pharmacological effects similar to ketamine. The aim of this study was to identify its metabolites in rat urine and to study their detectability within our standard GC-MS screening procedure (STA).

Methods: For the metabolism study, urine samples from male Wistar rates, which had been administered a 20 mg/kg BW dose of PCEPA, were extracted after enzymatic cleavage of conjugates by solid-phase extraction followed by acetylation or trifluoroacetylation. The metabolites were separated and identified by GC-MS in the electron ionization and in the positive chemical ionization mode. For toxicological detection, a 0.1 mg/kg BW dose of PCEPA was administered to rats and urine was collected over a 24 h period. The urine samples were extracted by liquid/liquid extraction at pH 8-9 after acid hydrolysis of half of the urine sample, followed by microwave-assisted acetylation.

Results and Discussion: Besides PCEPA, 13 metabolites could be identified. The following metabolic steps can be postulated: *N*-dealkylation, *O*-deethylation, hydroxylation of the cyclohexane ring at positions 2, 3 or 4 of PCEPA, *N*-dealkyl PCEPA, or *O*-deethyl PCEPA and finally, aromatic hydroxylation the *O*-deethyl PCEPA partially followed by hydroxylation of the cyclohexane ring. All metabolites were partially excreted in conjugated form.

Using our STA, the main PCEPA metabolites could be detected in rat urine after a common dose. Assuming similar metabolism in humans, the STA should be suitable for proof of an intake of PCEPA in human urine.

V17 Stabilisotopenuntersuchungen an Grundstoffen zur illegalen Herstellung von synthetischen Drogen (ATS). Welchen Mehrwert bringen solche Analysen?

Isotopic characterisation of precursor chemicals used for the clandestine production of amphetamine-type stimulants (ATS)

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The global production of ATS (amphetamine, methamphetamine and ecstasy), which has increased over the last decade, is estimated at around 520 tons in 2002. In regard to the threat posed by ATS the Council of the European Union noted, that since synthetic drugs to a very large extent originate from clandestine laboratories within

the Union, targeted actions in this field should be directed at all aspects of production and distribution of these drugs. One of the efforts should be directed at the illegal diversion of precursor chemicals. Understanding the source of the precursor which have been diverted to the clandestine production of ATS could provide the enforcement agencies with important information for countering that trade.

Determination of the stable isotope content of the bioelements in foods has been established as importent approach among the methodologies used for authenticity assessment. Contrary to drugs derived from plant extracts (e.g. cocaine) isotopic measurements can not provide information on geographical origin for synthetic drugs. In this case, isotopic ratios might be expected to depend on both precursors and synthetic way used. It is hypothesised that inconsistencies in the manufacturing process will produce a specific "fingerprint" for not just an individual product but individual batches of that product.

In this study we present a comprehensive examination of the $\delta^{2}H$, $\delta^{13}C$, $\delta^{18}O$ and $\delta^{15}N$ characteristics of phenylacetone (P2P / BMK), 3,4-methylendioxyphenylacetone (MDP2P / PMK) and ephedrine, three of the main precursor chemicals for the illegal production of ATS today, by isotope ratio mass spectrometry (IRMS) using elemental analysis (EA) in the "combustion" and "pyrolysis" modes.

The study was performed on 30 samples of BMK (different manufacturers and batches), 15 samples of seized PMK and 10 ephedrine hydrochloride purchased from different producers.

The results obtained by multielement isotopie analysis show that such measurement can be an importent parameter for the comparison of different batches /seizures of precursor chemicals. It opens also the way for studies to investigate the evolution of vthe different isotopic abundances during drug synthesis.

V18 Untersuchungen zum Metabolismus und toxikologischen Nachweis der Designer-Droge 2C-I in Rattenurin mittels GC-MS-Techniken

Studies on the metabolism and toxicological detection of the designer drug 2C-I in rat urine using GC-MS techniques

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Objectives: 2,5-dimethoxy-4-iodo-β-phenethylamine (2C-I) is a new scheduled designer drug of the phenethylamine type playing an increasing role on the illicit drug market. The aim of this study was to identify its metabolites in rat urine and to study their detectability within our standard GC-MS screening procedure (STA).

Methods: For the metabolism study, urine samples from male Wistar rats, which had been administered a 20 mg/kg BW dose of 2C-I, were extracted after enzymatic cleavage of conjugates by liquid/liquid extraction (LLE) at pH 8-9 followed by trifluoroacetylation as well as at pH 4-5 followed by methylation and trifluoroacetylation. The metabolites were separated and identified by GC-MS in the electron ionization and in the positive chemical ionization mode. For toxicological detection, a 0.3 mg/kg BW dose of 2C-I was administered to rats and urine was collected over a 24 h period. The urine samples were extracted by LLE at pH 8-9 after acid hydrolysis of half of the urine sample, followed by microwave-assisted acetylation. For details see the paper on the metabolism of the related drug 2C-T-7: Theobald DS, Fehn S, Maurer HH (2005) J.Mass Spectrom. 40:in press.

Results and Discussion: Besides small amounts of 2C-I, ten metabolites could be identified. The following metabolic steps can be postulated: *O*-demethylation in position 2 or 5 of the aromatic ring followed by *N*-acetylation or by deamination to the corresponding aldehyde, followed by oxidation to the corresponding acid or reduction to the corresponding alcohol. Another metabolic step was the deamination of the parent compound to the corresponding aldehyde, followed by oxidation to the corresponding acid or reduction to the corresponding alcohol. Most of the metabolites were excreted in conjugated form.

Using our STA, 2C-I and its main metabolites could be detected in rat urine after a common dose. Assuming similar metabolism in humans, the STA should be suitable for proof of an intake of 2C-I in human urine.

V19 Origin of MDMA precursors: carbon-14 analysis of safrole and piperonylmethylketone

Herkunft von MDMA-Vorläufersubstanzen: Radiocarbonanalyse von Safrol und Piperonylmethylketon

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Today about 95 % of all Ecstasy tablets seized in Germany exclusively contain 3,4-methylenedioxymethamphetamine (MDMA) as the controlled active substance. The most important precursor for the clandestine production of MDMA is piperonylmethylketone (PMK) which can be synthesised from safrole, the main component of sassafras oil. Analytical methods for the elucidation of the origin of PMK, safrole and other precursors for synthetic drugs can deliver valuable information for police intelligence. Besides the so-called ”chemical profiling”, based on chromatographic techniques, and stable isotope analysis (IRMS, SIRA, NMR), carbon-14 analysis is a suitable analytical tool on that score.

Liquid scintillation counting (LSC) procedures were developed for the determination of the carbon-14 content of safrole, PMK and other precursors of synthetic drugs. The main aim of the carbon-14 analyses is the unambiguous discrimination between precursors from artificial sources (produced from substances of petrochemical origin) and precursors from plant sources (like essential oils).

For method development ”natural” and ”artificial” safrole and PMK samples were prepared. Natural safrol was obtained by distillation of essential oil of Sassafras albidum and subsequently converted into natural PMK in two steps. Artificial safrole was synthesised in three steps from pyrocatechol of petrochemical origin and partially converted to artificial PMK. In contrast to the complex sample preparation mostly employed with radiocarbon dating (sample conversion to benzene via CO_2, Li_2C_2 and acetylene) direct LSC analysis of the precursor or an easily accessible derivative was employed for this work. Direct analysis of natural and artificial safrole utilising the Optiphase HiSafe 3 scintillator yielded counting rates in the carbon-14 window of 13,14 cpm and 3,92 cpm (only slightly above the background of 3,63 cpm). PMK was not suitable for direct analysis because of optical and chemical quenching as well as luminescence effects. Therefore, PMK samples were converted to LSC analysable piperonylic acid by phase-transfer catalyst aided oxidation with $KMnO_4$.

V20 Screening auf und validierte Quantifizierung von Designerdrogen des Phenethylamin-Typs und Mescalin in humanem Blutplasma mittels GC-MS

Screening for and validated quantification of phenethylamine-type designer drugs and mescaline in human blood plasma by GC-MS

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Objectives: In recent years, designer drugs of the so-called 2C series such as 2C-D, 2C-E, 2C-P, 2C-B, 2C-I, 2C-T-2 and 2C-T-7 have entered the illicit drug market. Only scare data have been published about analyses of these substances in human blood and/or plasma. This paper describes a method for screening and simultaneous quantification of the above-mentioned compounds and their analogue mescaline in plasma.

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). For details see refs. [1, 2]. They were detected using an HP 5973 MSD operated in the SIM mode.

Results and Discussion: The method was fully validated according to international guidelines. It was linear from 5 to 500 µg/L for all analytes except 2C-T-2 and 2C-T-7. Data for extraction efficiencies (83-103%), accuracy (-3.4-31.7% bias), repeatability (1.2-11.3% RSD), and intermediate precision (1.5-16.7% RSD) were within required limits with the exception of those for 2C-D at low concentrations and those for 2C-T-2 and 2C-T-7. The limit of quantification was 5 µg/L for all analytes. For details see ref. [2]. This assay allows simultaneous screening for all studied phenethylamine designer drugs in plasma as well as their validated quantification with the exception of 2C-T-2 and 2C-T-7.

[1] Peters FT, Schaefer S, Staack RF, Kraemer T, Maurer HH (2003). J.Mass Spectrom. 38:659-676.

[2] Habrdova V, Peters FT, Theobald DS, Maurer HH (2005). J.Mass Spectrom. 40:submitted.

V21 Schwere Intoxikation mit relativ tiefen Diphenhydramin-Konzentrationen
Severe Intoxication with Relatively Low Diphenhydramine-Concentrations

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Case: A 42-year old patient was admitted unconsciously to the intensive care unit of our hospital after having severe tachycardia and ventricular fibrillation. The electro cardiogram showed a prolonged QT-interval. The patient's history revealed a polytoxicomanic behavior and an HIV infection, which were treated with methadone and antiretroviral drugs, respectively.

Blood and urine was sent to the toxicological laboratory, which used drug-screening immunoassays, HPLC-UV (Remedi©) and GC-MS for the analysis. In urine benzodiazepines, methadone and metabolite as well as diphenhydramine could be identified. The quantification of diphenhydramine in the serum resulted in a concentration of 1249 nmol/l (therapeutic range: 98 – 392). This concentration was too low for the explanation of the patient's severe symptoms. A review of the patient's laboratory results revealed an albumin concentration of 16 g/l (normal range: 40 – 50). Therefore the free ("active") diphenhydramine concentration was determined after filtration of the patient's serum sample at 30'000g. The unbound concentration of diphenhydramine was 250 nmol/l, which corresponded to a protein binding of 84% (literature: 98 – 99%). In a patient with a normal albumin concentration this fraction would match to a total diphenhydramine concentration of 12'000 nmol/l, which easily could explain the patient's symptoms.

Conclusions: Usually toxicological laboratories determine the total concentrations of the different drugs and the interpretation is based on this data. As could be shown in this case, in patients with liver failure, the albumin concentration may be tremendously reduced and therefore, the free fraction of a highly protein bound drug may be highly elevated.

V22 Alpha-Liponsäureintoxikation - Kasuistik und analytischer Befund
Intoxikation with alpha-lipoic acid - case reports and analytical results

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Objective: Alpha-lipoic acid (ALA) is an OTC preparation used in the treatment of diabetic polyneuropathy or as antioxidative dietary supplement. Although ALA causes severe and even fatal intoxications only few pharmacokinetic data have been published yet and almost nothing is known about its toxicokinetic properties. In this presentation clinical representation of four patients intoxicated with ALA are analyzed and toxicokinetic parameters are derived from two suicidal poisonings. *Material and Methods:* The clinical courses of four patients, who ingested > 200 mg ALA per kg body weight and who were reported to poison information centres were monitored by follow-up reports. For toxicokinetic analysis plasma concentrations of ALA from a 69 year old male, who ingested ALA twice within four months in a dose of 340 mg/kg and 510 mg/kg respectively, were measured by RP-HPLC before, during and after haemodialysis until 72 hours after ingestion. Oral bioavailability, elimination half-life, volume of distribution and efficacy of haemodialysis were computed based on linear kinetics and compared to pharmacokinetic data published elsewhere.

Results: Quantitfication of ALA in plasma samples obtained from a single patient with an ingested 510 mg ALA per kg allowed estimation of a prolonged elimination half-life ranging from 80 to 160 min., which was not significantly reduced by high-flow haemodialysis. Estimated oral bioavailability in intoxication (26.7% at dose 340 mg/kg) did not significantly differ from that found under therapeutical dose (29.1% at dose 2.7 mg/kg), indicating a nonsaturable absorption of ALA with a substantial hepatic first-pass effect*Discussion:* (a) Occurrence and severity of symptoms are rather related to peak plasma concentration of ALA than to ingested dose. (b) Early gastric emptying and repeated application of charcoal/catharatic appear to be of major importance to reduce peak plasma concentration of ALA and the occurrence of delayed absorption thus reducing severity and duration of symptoms. (c) Haemodialysis or haemofiltration are ineffective in enforced elimination of ALA, but may be lifesaving in ALA poisonings with severe lactate acidosis.

V23 Klinische und toxikologische Daten zu akuten Vergiftungen mit Parathion und Dimethoat

Clinical and Toxicological Data in Acute Parathion and Dimethoate Poisonings

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Organophosphate toxicity is the leading cause of morbidity and death in poisoning by the insecticides class agents. These substances inhibit both the acetylcholinesterase and pseudo-cholinesterase activities. The clinical symptoms of pesticide toxicity vary widely, ranging from the classic cholinergic syndrome to flaccid paralysis and intractable seizures. Intermediate syndrome and delayed neuropathy may occur in some patients. The course could be quite severe and may need intensive care management. The mainstreams of therapy are atropine, obidoxime, benzodiazepines, and supportive care. Patients who receive treatment would most often promptly recover from the acute toxicity. The toxicokinetics vary not only with the extent of exposure, but also with the chemical structure of the agent.

We report six cases of poisoning with parathion-ethyl and dimethoate. Two patients were admitted in the Intensive Care Unit (ICU) few hours after ingestion and survived. Organophosphate blood and urine levels were determined on admission and during hospitalisation. Both pesticides were rapidly distributed and slow elimination rate of the poisons was documented. In the case of parathion-ethyl the distribution half life estimated was $t_{1/2}$=3.1 hours. The terminal half life was $t_{1/2}$= 17.9 hours for parathion and $t_{1/2}$= 30.4 hours for dimethoate. The serum pseudo-cholinesterase activity may be a useful prognostic parameter during the acute phase although it does not always show significant relationship to the severity of the intoxication. In our patients it was not measurable on the admission and recovered very slowly after 10-14 days. Initially, serum creatinine level was increased and declined in the latter course to the normal values. The C reactive protein concentration was markedly increased six days after poisoning and normalised in 14 days. The patients recovered in the ICU after 10-12 days, were transferred to the normal ward, to be discharged after three weeks.

In the four patients who died, the post mortem investigations revealed very high levels of pesticides in the blood, ranging from 2.5-64 µg/ml and in the liver, ranging from 1.2–4.8 µg/ml for dimethoate. In the early stage of poisoning, the autopsies showed destruction of pancreas, hemorrhagic pulmonary oedema, dilatation of the cardiac ventricles and brain oedema.

V24 Determination of dialkyl phosphates as stable degradation products of suspected OP intoxication

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A reliable and sensitive analytical method for the determination of dialkylphsphates consequently was developed. The aim was to search for organophosphates pesticides (OP) instability. 12 different pesticides (azinphos ethyl, azinphos methyl, chlorovinphos, dichlorvos, etrimfos, pyrazophos, phosphamidon, bromophos ethyl, fenthion, sulfotep, malathion and terbufos) were selected for this study. Fresh blood samples were spiked with 12 OP and stored for 5 min at room temperature and 90 h and 3 months at 4° C respectively. Results: No lost was observed on the concentration of dichlorovos and azinphos-methyl but 30-50% concentration reduction of the other OP was observed after 5 min store at room temperature. Dichlorovos was disappeared after 90 h. The other OP showed high degradation rate up to 3 months. On the other hand the following dialkyl phosphates were detected: Dimethylphosphate (DMP) was detected after 5 min stored at room temperature, diethylphosphate (DEP), O,O-dimethylthiophosphate (DMTP), O,O-diethylthiophosphate, O,O-dimethyldithiophosphate (DMDTP), and O,O-diethyldithiophosphate (DEDTP) were detected after 90 h and 3 month storage. For the detection of the dialkylphosphate compounds by GC/MS derivatisation with pentafluorobenzylbromide was required.

V25 Der besondere Fall einer KO-Mittel Beibringung: Bedeutung der Verzahnung von Ermittlung und toxikologischer Analytik

The special case of a sedative administration: importance of the interaction of investigation and toxicological analysis

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Case: In a case of suspected arson with two persons being involved a blood sample of one suspect should be analyzed, who showed signs of central nervous depression. The urine sample was to be stored only.

Material and method: The analysis of the blood sample using Mahsan enzyme immunoassays for drugs of abuse and benzodiazepines yielded negative results, likewise a screening for further psychoactive substances using HPLC-DAD. The responsible investigation officer informed us some weeks later that the suspect was in fact the victim of an extended suicide attempt. He had been "knocked out" by sedatives in a drink a few hours prior to being found. The residues of that drink in the glass had been analyzed by colleagues in the LKA who identified diazepam, flunitrazepam and levomepromazine. Consistently an analysis of the urine sample should be performed.

Results: By urinalysis the excretion of 7-aminoflunitrazepam and levomepromazine was detected confirming the suspicion of the administration of sedatives. Also the blood sample was reanalyzed using liquid-liquid extraction and HPLC with time-of-flight mass spectrometric detection (TOF). It was found, that in the blood sample effective concentrations of flunitrazepam and levomepromazine were present not detected with the HPLC-DAD methodology. Also diazepam was present in traces without its metabolite nordiazepam indicating a recent ingestion. The analysis using HPLC-MSD-TOF was very sensitive (LOD ca. 0.2 µg/l) and specific due to the usage of accurate masses.

Conclusions: This case showed three points: (1) in criminal cases with suspicious or unclear circumstances a comprehensive analysis of all materials available in only one laboratory is recommended; (2) successful investigations in forensic cases nowadays require the availability of sufficiently sensitive and specific methods with modern and expensive analytical equipment; (3) the combination of HPLC with time-of-flight mass spectrometry turned out to be easy-to-use, robust, sensitive and highly selective in biological matrices.

V26 Toxicological relevance of the multidrug resistance protein MDR 1 and related transporters

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The human multidrug resistance protein MDR1 (P-glycoprotein, P-gp, ABCB1) is a member of a subfamily of transport proteins and was originally identified on the basis of its elevated expression in cancer cells. However, it is now established that this transporter is also expressed in normal tissue with excretory function. One of the striking features is the structural diversity of substrates including various therapeutics as well as a number of toxic agents. Because MDR1 is found in the luminal membrane of the endothelial cells in the brain, many peptides, like endorphins, or opiates, like morphine, are transported across the blood-brain barrier by P-gp thereby influencing the analgesic action. The insecticidal avermectin is its substrate and if P-gp is blocked may accumulate in the brain so enhancing neurotoxicity. The organophosphorus insecticide chlorpyrifos stimulates the expression and the efflux of this transporter. Because of the overlapping substrate specifity between P-glycoprotein and cytochrome P 450 enzymes (e.g. CYP 3A4) many drug interactions involve both mechanisms. P-glycoprotein inhibition has greater impact on tissue distribution, particularly with regard to the brain than on plasma concentrations.

The related transporters multidrug resistance protein MRP1 (ABCC1), MRP2 (ABCC2), and MRP3 (ABCC3) have overlapping substrates specificities but different tissue distributions. They transport glutathione, glucuronide, and sulphate conjugated and unconjugated organic anions of toxicological relevance. Substrates include herbicides, tobacco specific nitrosamines, mycotoxins, heavy metals, and natural products. Because MRP´s transport conjugated compounds, co-expression with relevant phase-II enzymes as glutathione-S-transferases and UDP-glucuronyltranferases is assumed to be involved in their action. This transport process is also called phase III metabolism.

The hepatocellular bile acid transporter Ntcp (SLC10A1) is thought to be responsible for transportation of the lethal mushroom toxin α-amanitin into the liver cell. It is accepted that the efflux transporter limits the absorp-

tion of orally administered drugs, promotes drug elimination, and protects various tissues, e.g. brain, against toxic xenobiotics. The active efflux is generally an important aspect of cellular detoxification.

V27 Dioxins and furans determination in post mortem blood by gas chromatography-high resolution mass spectrometry

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Dioxins and related compounds (furans) are persistent environmental contaminants that cause adverse biological effects. Their influence on humans is still unclear, except for the high dose exposure by accident. However, chronic exposure to these compounds seems to be involved in cancer, endocrine disruption and neurobehavioral effects. For several years, a large concern about the potential health risks of dioxins is emerging in Europe and United States.

Dioxin levels in biological specimens are extremely low and require very sensitive and specific methods of analysis. In this study, gas chromatography coupled to high resolution mass spectrometry was used to evaluate dioxin body burden of two women deceased from generalized cancer.

Fat fraction of blood specimens was obtained after precipitation with ethanol and extraction of both liquid and solid phases spiked with labeled $^{13}C_{12}$-dioxin analogs. Organic phases were grouped, washed and evaporated to weigh the lipid content. Lipids were dissolved in hexane, hydrolyzed with concentrated sulfuric acid and discarded during water washes. Dioxins purification was achieved using three successive columns : silica, alumina/sodium sulfate and carbon/Celite columns. Finally, the toluene eluent was evaporated and the extract injected in the analytical system. After chromatographic separation, detection was achieved in single ion monitoring mode using a high resolution mass spectrometer operating in electron impact mode of ionization (40 eV, minimal resolution of 10 000). Dioxin levels were expressed in pg TEQ/g of fat as defined by the World Health Organisation. Quantification limits for each dioxin congener ranged from 2.5 to 12.0 pg/g fat, with a relative extraction recovery always higher than 60%.

Blood samples were obtained from people with cancer pathology, including 2 autopsy cases, 9 forensic cases on living persons and 2 clinical cases. Twelve from 13 blood samples showed dioxin concentrations ranging from 9.3 to 73.4 pg TEQ/g fat. These concentrations were largely lower than those observed after accidental releases, but in the ranges of those observed in the general European population (4.1 to 113.0 pg TEQ/g fat). In only one case, a higher concentration was observed (449.8 pg TEQ/g fat).

Therefore, it was not possible to correlate dioxin body burden of the subjects in the major cases (n=12) as a potential contributor of their cancer pathology. Nevertheless, knowledge of dioxin body burden in the French population would be of interest for an accurate interpretation of these results. In one case, the higher dioxin body burden was certainly a potential contributor in to the development of cancer pathology.

V28 Einfache und empfindliche Bestimmung der Cannabinoide THC, CBD und CBN im Haar durch Silylierung, Headspace-Festphasenmikroextraktion und GC-MS

Simple and sensitive determination of the cannabinoids THC, CBD and CBN in hair samples by silylation, headspace solid phase microextraction and GC-MS

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From a forensic as well as from a clinical point of view the sensitive determination of tetrahydrocannabinol (THC) in human hair samples becomes more and more important. Besides THC, the analytical evidence of the not psychoactive cannabinoids cannabidiol (CBD) and cannabinol (CBN) in human hair samples is an additional proof of exposure to cannabis products.

Therefore, a new, relatively simple and sensitive method for determination of the three compounds was developed, based on alkaline hair hydrolysis, liquid-liquid extraction, combined derivatization and headspace solid-phase microextraction (HS-SPME), and GC-MS-EI. The method was optimised with respect to the extraction solvent, temperature and time of SPME preincubation and extraction as well as type and volume of the derivatization reagent. After addition of D_3-THC as internal standard, about 15 mg hair were dissolved in 0.5 mL 1 N NaOH and the analytes were extracted twice with 2 mL *iso*octane. The solvent of the organic phase was evaporated in a 5 mL headspace-vial and 10 µL of the derivatization reagent BSTFA were added. THC, CBD

and CBN were detected directly from this mixture by HS-SPME and EI-GC-MS in SIM-mode with LOD's of 0.02 ng/mg.

The method was applied to hair samples collected in context of driving ability investigations and compared with the standard procedure based on derivatization by MeI and liquid injection EI-GC-MS. The THC concentrations obtained by both methods were in good agreement. In the HS-SPME method, matrix-effects were minimized leading to a lower LOD. Besides the more sensitive detection of THC, it is possible to determine CBD and CBN in one run together with THC which was not possible with the standard procedure.

Besides the confirmation of cannabis exposure the additional determination of CBD and CBN should also enable to distinguish between predominant marihuana and hashish abuse and from clinical THC ingestion since different cannabis products contain typical amounts and ratios of the three detected cannabinoids.

V29 Forward Estimation of Alcohol Concentration – Comparison of Different Calculation Methods

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Forward projection of alcohol concentration is often performed when the driver that perpetrated the accident escaped from the place of crime and no blood specimen has been taken. In this situation a range of theoretical blood alcohol concentrations (BAC) at the time of the accident is computed as the result of drinking of a given amount of alcohol beverage by the suspect. The first and still the most popular calculation method is Widmark equation. The authors showed that the main source of uncertainty in forward projecton using Widmark formula is inter- and intraindividual variability of *r* coefficient, which is equivalent to the distribution volume used in pharmacokinetics. The disadvantage of Widmark's *r* factor is that it is constant for males (0.7) and females (0.6) and does not take into consideration the differences of the physique.

In the present work, the different methods of calculation of Widmark's *r* factor and the distribution volume were compared. The study covered four empirical equations. Watson's model and Forrest's model are based on total body water volumes (TBW) and the body mass index (BMI), whereas Seidl, Jensen and Alt's (SJA) model and Ulrich, Cramer and Zink's (UCZ) model were developed by multiple regression analysis, where *r* coefficient is a function of body weight and height.

The authors performed experiments with 74 volunteers, 23 females and 51 males, whose consumed alcohol in the form of vodka (0.7 g of alcohol per kg of body weight for males, and 0.6 g/kg b.w. for females). Samples of venous blood were obtained through an indwelling catheter before ingestion of alcohol and then in 15 minutes intervals timed from the end of drinking. Blood alcohol concentrations were determined by means of headspace gas chromatography.

According to the Widmark formula the consumed amount of alcohol should lead to the maximal BAC of 1.0 g/L. The measured maximal BAC was 0.70 ± 0.16 g/L in avarage.The theoretical alcohol concentration at time zero were calculated individually from pharmacokinetic equation assuming zero-order elimination model and it amounted to 0.90 ± 0.13 g/L in avarage. There was significant difference between results for males and females (0.93 ± 0.13 g/L and 0.83 ± 0.12 g/L, respectively). The BACs at time zero were compared with the values calculated according to the empirical models. The results showed very good agreement for UCZ model (mean difference was 0.00 g/L, in 61% of the cases the empirical value was lower) and SJA model (0.01 g/L – 53%). For Watson's and Forrest's models, the results were slightly higher (0.06 g/L – 38% and 0.05 g/L – 41%, respectively) than obtained from the experimental data. The calculated theoretical maximal alcohol concentration were: 0.93 ± 0.06 g/L, 0.90 ± 0.06 g/L, 0.95 ± 0.06 g/L and 0.94 ± 0.06 g/L, respectively for UCZ, SJA, Watson's and Forrest's models. The values of Widmark's *r* factor or the distribition volume, estimated using the mentioned models, were higher than proposed by Widmark, espacially for females, and the differences were statistically significant.

The study showed that the calculations with use of Widmark's constant *r* factors give the broader range of uncertainty compared to the models based on individual height, weight and age, and therefore the authors recomend the use of anthropological data in forward estimations. The results calculated from the models developed by multiple regression analysis were in very good agreement with the experimental data, and from the models based on TBW and BMI – slightly overestimated. Regardless of the model used in calculations, when the results are used for forensic purposes, the uncertainty in the calculation must be known and reported.

V30 Multivariate analysis of volatile congeners in blood and urine samples to determine the brand of consumed spirit drinks

Multivariate Analyse der Begleitstoffgehalte in Blut und Urin zur Bestimmung der Marke der konsumierten Spirituose

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The analysis of volatile congeners of alcoholic beverages is regularly used as an efficient tool for examining claims of drinking after committed offences. In contrast to the relatively simple Widmark formula for calculation of the ethanol concentration backwards to the offence time, the concentrations for every single volatile congener have to be calculated using complex exponential type formulas. To examine the claims of alcohol consumption, the calculated values of the volatile congeners are usually compared with data from the literature. The aim of this study was to evaluate if multivariate statistic techniques could provide a simpler means of checking claims of drinking.

In a drinking trial, five participants consumed an amount of the same brand of German brandy calculated to reach 0.8‰ of blood ethanol concentration. Venous blood was taken at 30 min, 90 min, and 150 min after the drinks. Urine was taken three times after the drinks. In a second trial, the same participants consumed a second brand of German brandy under the same conditions.

The alcoholic beverages, blood and urine samples were analysed using validated standard gas chromatographic methods. Then Principal Component Analysis (PCA) was used to transform the original measurement variables of the alcoholic congeners into new variables called principal components (PC). By plotting the data in a coordinate system defined by the two largest principal components, it is possible to identify key relationships in the data as well as to find similarities and differences.

Using PCA, outlying samples of one participant were identified, which could be attributed to unstated drinking of other alcoholic beverages in non-compliance with the experimental design. A classification between the two brands of brandy was possible in the blood taken at 30 min and the urine taken in a short time interval after drinking. In conclusion, the determination of the brand of consumed spirit is possible if samples are obtained shortly after drinking.

Besides, it was found during the trial that the expected values calculated after Bonte's formula gave a better correlation to the analysed values if current analysis results of the spirit drinks were used rather than data from older standard references.

V31 Benzodiazepine im deutschen Straßenverkehr - Ergebnisse einer bundesweiten Studie

Benzodiazepines in German road traffic - Results of a nationwide study

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Objectives of the study: In the context of a study for the Bundesanstalt für Straßenwesen (BAST) a database was worked out containing results of toxicological blood analysis (TBA) of motoring offences (timeframe 1998-2001). Whereas the frequencies of detection (FOD) increased for most drugs of abuse or stayed constant, the FOD for benzodiazepines decreased from 24% in 1998 to 11,1% in 2001. This investigation was done to clarify the reasons for this development.

Material and methods: The database contains the results of 25 laboratories in Germany doing TBA at motoring offences. 23 of these labs gave information about their methodology of toxicological analysis by questionnaire.

Results: The part of analysis according to § 24a (2) StVG increased excessive but show considerable regional differences (0,7% -98,2% of the general orders). Evaluation of the questionnaires shows 9 labs of 23 not to analyze for benzodiazepines when an order for analysis according to § 24a (2) StVG is given.

Benzodiazepines are the only group of psychoactive substances with a doubled FOD in traffic accidents compared to traffic offences without accident. They rank second in FOD (28,7% benzodiazepines) after

cannabis (41,9% FOD) in this group. The evident decrease of FOD for benzodiazepines in traffic offences can be seen on the majority of the labs in general as not so pronounced at the traffic accidents (34,7% of the positive cases in 1998 to 21,3% in 2001).

Conclusions: Results indicate the decrease in FOD for benzodiazepines to be due to the strong increase of orders for toxicological analysis according to § 24a (2) StVG as well as to epidemiological development. Benzodiazepines are not included in § 24a (2) StVG. The focus on drugs of abuse showed very good results in the detection of drivers under the influence of drugs of abuse but the legal drugs should be kept in mind.

V32 Freies Morphin in Blut nach Mohnsamenverzehr – Bewertung der Ergebnisse einer Studie in Hinblick auf § 24a StVG

Free Morphine in Blood after Poppy Seed Consumption – Appraisal of Results of a Study with Regard to German Street Traffic Law (§ 24a StVG)

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Aim of the study was to check whether the consumption of poppy seeds can result in measurable concentrations of free morphine in serum samples what can be punished on the basis of Street Traffic Law in Germany. Twenty subjects consumed as much poppy seeds as they could, preferably 100 – 200 g, either in form of a poppy seed mash with rice pudding or a poppy seed cake. The poppy seeds had a morphine content of 72.4 mg/kg (5 subjects) and 114.3 mg/kg (15 subjects) which corresponds to high but not the highest contents mentioned in literature (up to 294 mg/kg).

Blood samples were collected 1, 2, 4, 8, and 24 h after the end of the poppy seed consumption and analysed for the presence of free (and total) morphine by GC/MS.

All subjects had morphine-positive serum samples for at least 8 h after consumption. Only one subject having taken just 25 g of the poppy seeds was tested positive for merely 4 h. 1 h after the end of the intake six subjects showed serum concentrations of free morphine of more than 10 ng/mL (10.2 – 17.3 ng/mL), a value which is recommended as a threshold by the "Grenzwertkommission". 2 h after the consumption four subjects, and 4 h after the intake three subjects showed morphine levels of more than 10 ng/mL. These subjects had consumed 100 - 200 g of poppy seeds corresponding to 2 – 4 pieces of commercially available cake. There was no direct proportionality between consumed amounts of poppy seeds and morphine serum levels, even when the amount of consumed morphine was brought into relation to body weight. In eight subjects free morphine was detectable in serum samples taken 24 h after consumption in concentrations between limit of detection (0.74 ng/mL) and limit of quantitation (2.82 ng/mL).

V33 Nachweis von Tabakzusatzstoffen in Zigaretten mittels HS-SPME/GC-MS und inonenselektiver Elektrode

Determination of Tobacco Additives in Cigarettes by HS-SPME/GC-MS and Ionselective Electrode

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In the manufacturing of cigarettes, up to 25% by weight additives are added. These substances affect the smoking behaviour and are intended to increase the attractiveness of the product, especially for young people and children. More than 600 additives are known, which include also complex mixtures like cocoa and licorice. The aim of this investigation was to screen commercial cigarettes for such additives, to quantify the important one of them and to interpret them with respect to toxicological effects.

Compounds with high and medium volatility were detected by a systematic qualitative analysis using headspace solid phase microextraction (HS-SPME) and GC-MS. The cigarette samples were extracted with water at acidic, neutral and basic pH followed by HS-SPME with a 65 µm CW-DVB-fiber and GC/MS. By library search using commercial spectra libraries 58 compounds were identified, between them benzylalcohol, 2-ethyl-1-hexanol, menthol, vanillin, tripropylenglycol, geranylacetone, anisaldehyde, anisalcohol, thymole, but also some amines

such as pyridine, fururylamine, 3-methylbutanamine, phenylethylamine and 1-methylpyrrolidine. Some of these compounds were found in raw tobacco, the reference cigarette 2R4F and in cigarettes, others were only present in the cigarettes and were obviously added.

Altogether 71 different cigarette sorts from 8 countries were analysed. The quantitation was performed by extern calibration with 5 concentrations in tobacco matrix. After testing of 4 common brands from 5-8 different countries, regional differences in the additive profile of the cigarettes could be seen. As an example, the following concentrations were measured in the cigarettes: 2-ethyl-1-hexanol 0.06-12 µg/g, menthol 0.02-13.3 µg/g (in non-menthol-cigarettes) and 0.79 mg/g in a menthol-cigarette, indole 0.16-2.1 µg/g, pyridine 6.4-19.1 µg/g und benzylalcohol 6.6-16.7 µg/g.

Furthermore, it is known, that basic substances are used to alkalise the tobacco smoke in order to increase the amount of the free base form of nicotine in the smoke. Therefore, ammonium ions and urea in the same cigarettes and tobacco samples were determined using an ammonium selective electrode. After an aqueous extraction of the cigarettes the ammonium concentration was directly measured and urea was enzymatically transformed into NH_3 and then measured. As a result, between 0.15 and 3.7 mg/g ammonia and between 0.01 and 0.37 mg/g urea were determined.

From the evaluation of the known toxicological properties follows that the additives themselves as well as their pyrolysis products increase the harmful properties of cigarette smoking.