V-15 Severe intoxications after use of products containing synthetic cannabinoids – analytical findings versus clinical symptoms

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Objective: Since 2008 approximately 15 synthetic cannabinoids of the aminoalkylindole type and CP-47,497-C8 were identified in 'herbal' blends [1]. Most of them show a much higher CB1 binding affinity compared to Δ^9–THC, indicating a significantly higher potency. We report on 15 cases of intoxication with analytically confirmed consumption of at least one synthetic cannabinoid since October 2008.
Material and Methods: Analysis of aminoalkylindoles was performed using 1 mL of serum and alkaline liquid-liquid extraction followed by LC-ESI-MS/MS using a Q-Trap 4000 mass spectrometer (Applied Biosystems) [2]. The method covered 14 synthetic cannabinoids of the aminoalkylindole type. Whenever possible, samples were additionally examined for consumption of other drugs by multi target screening, immunoassays and confirmatory analyses (GC-MS and/or LC-ESI-MS/MS).
Results: Fifteen patients (age: 15-28 years; sex: 13 m, 2 f) had reportedly smoked 'incense' products. LC-ESI-MS/MS and GC-MS analysis confirmed the consumption of CP 47,497-C8 (1), JWH-018 (7), JWH-250 (4), JWH-081 (4) and JWH-122 (6). Reported symptoms were tachycardia, dyspnoea, thoracic pain, shivering, shaking, vomiting, muscle jerking, muscular pain, hypokaliemia as well as changes of perception, hallucination, agitation, somnolence, generalized seizures and acute psychosis.
Discussion: In most cases symptoms were similar to severe cannabis intoxication, but the occurrence of seizures and pronounced hypokaliemia is usually not seen even after high doses of cannabis. These effects may be mediated by receptors other than CB_1 and CB_2 and give rise to the assumption that these compounds may be significantly more toxic than cannabis. However, interaction effects have to be taken into consideration, since in one third of the presented cases at least 2 synthetic cannabinoids were detected and in one case an additional consumption of cannabis and amphetamine was confirmed. Kind and severity of symptoms emphasize the potential of these compounds of being considerably more toxic than cannabis.
References: [1] Kneisel S, Westphal F et al. Cannabinoidmimetika: Massenspektren und IR-ATR-Spektren neuer Verbindungen aus den Jahren 2009/2010. Toxichem Krimtech 78 (2011) 23-35. [2] Dresen S, Kneisel S et al. Development and validation of a liquid chromatography-tandem mass spectrometry method for the quantitation of synthetic cannabinoids of the aminoalkylindole type and methanandamide in serum and its application to forensic samples. JMS, accepted for publication.

V-16 Contactless and direct MS-techniques for the surveillance of clandestine production facilities of synthetic drugs

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Objective: Illicit amphetamine derivatives are clandestinely produced in large-scale facilities (e.g. MDMA for Ecstasy tablets) or in “kitchen laboratories” (typical for methamphetamine or amphetamine). For action forces who firstly have to observe and then to safeguard such labs it is very important to know which kind of chemical reactions are arranged in the concerning objects. The fast, mobile and at best contactless analysis of hazardous materials (hazmat) at trace levels is able to improve the danger estimation to protect action forces and the population.
Materials and methods: Solid phase microextraction in combination with gas chromatography-mass spectrometry (SPME-GC/MS) is a promising method for air sampling and subsequent analysis of drugs, their precursors and process chemicals. In contrast to conventional extraction techniques the integration of sampling, extraction (including matrix removal), reconcentration and sample introduction within only one step is the major advantage of SPME. Vapour phase sampling with SPME-fibres is fast, contactless and not limited to any specified environment. A new and promising alternative to SPME-GC/MS is the direct mass spectrometric assay of the SPME fibre by placing it in an ESI-sprayer needle with surrounding sheath liquid. A further rapid MS technique, desorption electrospray ionization mass spectrometry (DESI-MS), is well suited for the direct mass spectrometric examination of solid materials. It provides near time detection and identification of synthetic drugs on contaminated surfaces and wipe samples.
Results and discussion: In our work we conducted a small-scale amphetamine production via the Leuckart synthesis route with seized chemicals under controlled conditions. We successfully identified amphetamine, its precursors (for example BMK), process chemicals and by-products in the environmental air of the laboratory by conventional SPME-GC/MS, the combination of SPME and DESI-MS and the direct coupling of SMPE with ESI-MS. It was possible to reconstruct details of the arranged synthesis by analysing contaminated laboratory equipment (e.g. magnetic stir bars, glass rods) and laboratory waste (filter paper, gloves) with DESI-MS.

V-17 Development of a systematic toxicological screening method using an automated on-line SPE-LC-QqTOF System (XLC-QqTOF)

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Objective: The aim was to develop a fully automated on-line SPE-HPLC-QqTOF (XLC-QqTOF) screening method for small neutral and basic molecules and the application to a mass spectral reference library for compound identification. To follow this idea, a check-mix (CM) was designed; different SPE types for on-line extraction and analytical columns were tested, mobile phases, data acquisition and data management were optimized.
Materials and Methods: All HPLC-MS analyses were carried out on a hybrid quadrupole time-of-flight (QqTOF) mass spectrometer (QStarElite, ABSciex) coupled to an automated on-line SPE HPLC system (XLC, Symbiosis PICO, Spark Holland). The QStarElite equipped with a electrospray ion source operated in positive ion mode (ESI+) at 400°C. Analyst QS 2.0 (incl. IDA, DBS), Symbiosis Pico in Analyst 1.0.1.0 and SmileMS (Genebio) were used for instrument control and data

management. CM: Seven substances (histamine, MDMA, rocuronium, haloperidol, carbamazepine, diazepam and amiodarone) covering a wide range of polarities and monoisotopic masses were selected for testing the integrity and proper function of the complete system. Solid phase extraction (SPE or X): Nine different weak cation exchangers (WCX) and reversed phase materials were tested. Chromatographic separation (LC): Nine different analytical columns (e.g. PFP propyl, PFP, biphenyl) were investigated.
Results and Discussion: Best SPE performance was found using Oasis WCX (pH=6) in terms of peak symmetries and recoveries (CM-analytes). A HPLC column containing pentafluorophenyl functional groups (LunaPFP 150x2 mm, 5µm, Phenomenex) showed best performance. Gradient elution was optimized and the final mobile phase consisted of formic acid in water (1%; v/v) and methanol at a flow rate of 0.3 mL/min. The total runtime was 17 min.

V-18 Development of a Metabolite-based LC-MS^n Screening Procedure for Detection of Drugs of Abuse and Their Metabolites in Urine

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Objectives: For a broad screening procedure, immunoassays and several chromatographic methods are in use. For completion of the authors' new metabolite-based LC-MS^n screening (Wissenbach et al., PMID: 21079926), the detectability of drugs of abuse and their metabolites within the new LC-MS^n screening approach was studied and the corresponding data added to the LC-MS^n library.
Materials and Methods: The library was built up with MS² and MS³ wideband spectra using a ThermoFisher (TF) LXQ linear ion trap with electrospray ionization in the positive mode and full scan information-dependent acquisition. Metabolite spectra were recorded after protein precipitation of urine from rats after administration of the corresponding drugs for toxicological diagnostic reasons. After identification, the metabolite spectra were added to the library. Recovery, process efficiency, matrix effects, and limits of detection for selected drugs of abuse were determined using spiked human urine. Automatic data evaluation was performed using TF ToxID and Genebio SmileMS software (Wissenbach et al., PMID: 21079926).
Results and Discussion: After protein precipitation of the rat urine samples, the studied drugs of abuse and their phase I and II metabolites could be detected after sufficient LC separation. The data of the corresponding drugs and of the identified metabolites were added to the LC-MS^n library. This consists now of data of over 800 parent compounds, including over 80 drugs of abuse, and of over 2,000 metabolites and artifacts, among which over 300 were formed by drugs of abuse. The validation data were acceptable, so that the LC-MS^n screening was suitable for urine screening for over 80 amphetamines, designer drugs, synthetic cannabinoids, cocaine, opioids. This was confirmed by comparing the LC-MS results with those obtained using routine GC-MS screening (Maurer et al., 2007). THC-COOH and buprenorphine could only be detected in concentrations above 400 µg/l and 100 µg/l, respectively.

V-19 Semi-quantitative estimation of concentrations in systematic toxicological analysis by liquid chromatography-hybrid quadrupole time-of-flight mass spectrometry (LC-QTOF-MS)

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Objectives: Liquid chromatography-hybrid quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) was shown to be a very efficient method for substance identification in systematic toxicological analysis [1]. However, besides the unambiguous identification, the concentration is an important prerequisite for toxicological interpretation. Therefore, a procedure for semi-quantitative estimation of concentrations from the LC-QTOF-MS areas of identified peaks was developed and examined by application to blood, urine and hair samples.
Material and methods: The retention times and peak areas of more than 2000 toxic substances were measured by LC-QTOF-MS using an Agilent 6530 instrument under standardized conditions (column Poroshell 120 EC-C18, 2.1 x 100mm, 2.7 µm, gradient elution A NH4Ac/H2O B methanol, flow rate 0.4 mL/min) by injection of 100 pg substance together with each 100 pg of 32 deuterated standards with retention times evenly spread over the run time. The linearity of the calibration was shown for selected substances. For each substance five nearby eluting standards were selected and the peak area quotients were entered in a database. In practical application the sample preparation (protein precipitation of blood, dilution of urine or extraction of hair) was performed after addition of all 32 standards. The mixture of the standards without matrix was injected in each measurement series for control of ion suppression (acceptance criterion > 50%). A software tool was developed for automatic calculation of concentrations after peak identification from the analyte peak area and the peak area ratios of the selected standards.
Results and discussion: The application to LC-QTOF-MS files from post-mortem blood, urine or hair samples measured in data dependent acquisition mode and comparison of the results with concentrations obtained by calibrated and validated methods with the same instrument, GC-MS or HPLC-DAD showed that this procedure can successfully be used for approximate estimation of concentrations also if the reference substance is not available.
[1] S. Broecker, S. Herre, B. Wüst, J. Zweigenbaum, F. Pragst, Anal Bioanal Chem 2010, DOI 10.1007/s00216-010-4450-9.

V-20 Modified Multi Target Screening (MTS) with QTrap 3200 and methanol as eluent with a Luna PFP column

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Objectives: Until 2009, a Multi Target Screening (MTS) had been developed with LC-Qtrap-MS/MS with library searching for compound identification using a Restek Allure PFP column and gradient elution with acetonitrile. Due to high costs of acetonitrile we wanted to switch to methanol for elution and therefore searched for an alternative column for Multi Target Screening (MTS) to be used for a broad range of compounds from very polar (methylecgonine, morphine glucuronides etc.) to very lipophilic (amiodarone, THC) and determine retention times for approximately 700 compounds included in our previous screening procedure. The transfer of the procedure to this new column is described.
Materials and Methods: Different batches of Phenomenex Luna PFP columns (2) (150 x 2 mm, 5 µm) were used; two gradient HPLC systems (Agilent 1100 or Shimadzu) and a Qtrap 3200 mass spectrometer were available for determination of retention times. Gradient elution was performed using formic acid (0.2 %, solvent A) and methanol with 0.2 % formic acid (solvent B). Overall run-time was 18 min.
Results: The retention times of 700 compounds were determined and the MTS procedure as used before with Restek Allure PPF column was set-up with a new scheduled MRM catalogue (new retention times for the MRM-survey scans with scheduled MRM) with a time window of 60 s for each compound. In previous experiments different batches of the column showed good reproducibility of retention times for a system suitability test mixture applied. Furthermore, peak shapes were better than with Allure PFP (Restek), especially with the highly lipophilic compounds, which had shown peak broadening with the former system. A new internal standard mixture was applied for quality control of the procedure.
Discussion: The MTS with QTrap 3200 has been adapted to a new column and methanol as eluent with advantages over the previously published method. The reproducibility of retention times has been tested using one type of column from different batches, and was found to be better than with Restek Allure PFP columns. Costs per sample were reduced by use of methanol instead of acetonitrile for gradient elution and solvent flow could be kept constant, whereas in the previous procedure it was raised from 0.3 to 1 mL/min flow rate. The integration of a new mixture of internal standards made it possible to control the efficiency of sample preparation steps (precipitation/dilution, extraction) for use with biological samples. Application of the new MTS procedure to urine, serum and blood samples showed similar results as obtained previously with the Restek Allure PFP column, examples and limits of identification for selected compounds are given with use of different standardised sample preparation procedures.

V-21 MSforID: applications of a tandem mass spectral library in forensic toxicology

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Objectives: The MSforID library (www.msforid.com) represents a robust and transferable tandem mass spectral library [1,2]. At the current stage of development, the library contains reference spectra of more than 1000 compounds, mainly pharmaceuticals as well as illicit drugs. Fields of application include forensic toxicology, metabolomics, pharmaceutical research, toxicology and environmental analysis. Important applications in forensic toxicology are presented.

Materials and methods: The MSforID project (www.msforid.com) relies on the combination of a highly efficient search algorithm with a comprehensive mass spectral library established on a high-resolution mass spectrometer [1-3]. The developed search algorithm is based on peak matching and exhibits a high tolerance towards changes within the intensity distribution among different fragmentation pathways. The reference library was established on a quadrupole-quadrupole-time of flight instrument (QqTOF) using ten different collision energies for acquiring compound-specific reference spectra.
Results and discussion: In forensic toxicology the library is particularly useful for:
(1) Systematic toxicological analysis (STA): Since the outcome of STA can have pivotal judicial, social, personal and/or economic consequences, a number of analytical procedures should be used to minimize the probability of false results. In this context, LC/MS(/MS) under data-dependent acquisition control with automated library search can complement existing screening approaches.
(2) Identification of illicit and counterfeit drugs[3,4]: Fast and efficient identification of potentially toxic compounds present within illicit and counterfeit drugs can be accomplished via automated matching of corresponding tandem mass spectra.
(3) Development of quantitative methods [5]: Single and multiple reaction monitoring are integral parts of quantitative LC/MS/MS approaches. The library is very useful to choose appropriate precursor-to-product ion transitions. Furthermore, their selectivity can easily be checked via search within the library.
(4) Metabolite profiling[6]: Library search is very helpful to verify the structural relatedness of putative metabolites to the precursor drug.
Literature: [1] Oberacher et al. J Mass Spectrom, 2009, 44, 485. [2] Oberacher et al. J Mass Spectrom, 2009, 44, 494. [3] Pavlic et al. Anal Bioanal Chem, 2006, 386, 69. [4] Pavlic et al. Forensic Sci Int, 2010, 197, 40. [5] Beer et al. Anal Bioanal Chem, 2010, 398, 1791. [6] Schubert et al. Anal Bioanal Chem, 2008 , 393, 1299.

V-22 An artificial amino acid derivative in a case involving administration via a drink? – an analytical interesting and surprising result of a substance's identification

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Objective: A woman ingested a few gulps of an ice tea that has been left unattended for some time in her unlocked camping mobile. At once, she felt a bitter taste and told about dizziness. The drink was sent in for toxicological analysis.
Materials and Methods: GC-MS analysis revealed the following components: ethanol*, caffeine, various terpenes such as ß-ionone*, lilial*, methyljasmonate* and two unknown compounds* (* = no actual components of the drink). Library research of the mass spectrum of compound 1 gave N-BOC-piperidine-carboxylic acid as the first result with the best fit. However, the spectrum did not fit perfectly and the result would not really have made sense. The artificial amino acid derivative (used in protein synthesis) was bought and measured by GC-MS.
Results and Discussion: The retention index differed slightly from compound 1. Nevertheless, compound 1 had to have a very similar structure concerning the similar fragmentation pattern. After determination of the molecular weight (229) by CI-

GC/MS, compound 1 was enriched by preparative thin layer chromatography, reextracted and subjected to NMR analysis. However, the interpretation of the ^{1}H-, ^{13}C-, COSY- and HSQC-NMR spectra firstly provided contradictory results. The ^{1}H-NMR showed 23 protons with complex signal structures and a confluence of 10 protons in a huge multiplet signal. The ^{13}C-NMR consisted of 12 signals from which three were broadened and nine were possibly doubled. A molecular formula of $C_{12}H_{23}NO_3$ was assigned using molecular mass, ^{1}H-, and HSQC-NMR data but the complex coupling pattern remained suspicious. Finally, an internet research for compounds with $C_{12}H_{23}NO_3$was carried out yielding many artificial amino acid derivatives. Many of them could be ruled out but one remained interesting because of it's usage as an insect repellent. Knowing its structure total assignment interpretation of NMR data was possible due to the presence of two chiral centers leading to diastereoisomerism.

V-23 Automated Quantification of Doxylamine and Diphenhydramine in Human Plasma using on-line extraction-HPLC-DAD (TOX.I.S.)

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Objective/ Case history: Case 1: A 28-year-old man (185 cm, 85 kg) presented to an emergency department with clinical symptoms of ethanol intoxication. Additionally, the intake of an unknown medication was supposed.
Case 2: The second case involves a 41-year-old female (170 cm, 65 kg) with a history of drug abuse and depression. She was admitted to the hospital showing confusion and uncontrolled movements. An on-site rapid urine test (test strip) was positive for tricyclic antidepressant drugs.
Material and Method: Following a toxicology screening, the method for the quantification of doxylamine (DA) and diphenhydramine (DPH) in human plasma was based on a methanolic extraction of plasma (0.2 ml) and basic (pH=9) automated on-line extraction (Strata X, 25 µm, 20 x 2 mm) followed by HPLC-DAD (TOX.I.S). Analytical separation was carried out on a Gemini NX column (150 x 4.6 mm, 3µm) using gradient elution. The mobile phase consisted of 0.05 M potassium dihydrogen phosphate buffer (pH=2.3) and acetonitrile/water (90/10, v/v). Peak identification was carried out by chromatographic data and spectra comparison with > 500 UV-spectra of weak basic, weak acidic and neutral compounds. Criteria for positive peak identification were a 95% agreement between the obtained and the library spectrum (similarity ≥ 0.995) and a maximum deviation of the relative retention time of ± 5%. The LLOQ of DA and DPH were 0.25 mg/L and 0.15 mg/L.
Results: In case 1, initial toxicological analysis using HS-GC did not reveal any involvement of alcohol, but DPH was detected in toxic concentration (3.24 mg/L); dinordiphenhydramine and nordiphenhydramine were determined qualitatively. In case 2, sub-therapeutic concentrations of DPH (0.29 mg/L) and of DA (<0.25 mg/L) were found. Moreover, amitriptyline (0.084 mg/L) and nortriptyline (0.158 mg/L) and other metabolites of amitriptyline were quantitated in the same analytical run. Concentrations of amitriptyline and of nortriptyline were within therapeutic ranges.
Discussion: The TOX.I.S. on-line HPLC-DAD was able to identify and quantify DA and DPH automatically in acute intoxications. Sample preparation appeared to be

feasible and economical. The on-line extraction offers high comfort and reproducibility and makes it possible to quantify xenobiotics in plasma samples automatically.

V-24 Assisted suicide by application of transdermal fentanyl patches: Tissue distribution of fentanyl and norfentanyl

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Background: Fentanyl is a potent synthetic narcotic analgesic frequently administered in the form of a transdermal patch for the management of chronic pain. A 46-year old woman with a history of cancer was found dead by the emergency physician. The external examination revealed 34 transdermal fentanyl patches (from 25 up to 100 µg/h fentanyl) on the body which were provided by her husband but attached by the woman herself.
Objective: To report the casuistic of an uncommon suicide and to document the level of fentanyl and norfentanyl in different organs resulting from the intake of a lethal dosage.
Material and Methods: Quantitation of fentanyl and its major metabolite norfentanyl in 10 rsp. 7 different post-mortem samples was performed by gas chromatography–mass spectrometry (GC–MS) using selected ion monitoring following liquid-liquid extraction rsp. solid phase extraction (SPE). Determination of benzodiazepines was performed by HPLC.
Results: Fentanyl (norfentanyl) concentrations in the post-mortem samples: 84.6 ng/mL (41.2 ng/mL) in heart blood and 87.8 ng/mL (not determined) in femoral blood, 164.6 ng/mL (118.4 ng/mL) in urine, 498.5 ng/mL (n. d.) in bile, 283.2 ng/g (35.4 ng/g) in lung tissue, 72.3 ng/g (6.8 ng/g) in brain, 134.6 ng/g /(134.4 ng/g) in kidney, 202.1 ng/g (139.2 ng/g) in liver and 60.3 ng/g (22.6 ng/g) in iliopsoas. The total amount of fentanyl in gastric contents was approx. 18.5 µg. Additionally, a level of 874 ng/mL of bromazepam was detected in femoral blood.
Discussion: Overdose situations due to excessive administered transdermal fentanyl patches are not only indicated by blood levels but also mirrored by the concentrations of fentanyl and norfentanyl in different organs and body fluids, especially in liver, lung and bile. In the case at issue, we concluded that the woman's death was caused by combined intoxication with fentanyl and bromazepam.

V-25 Lidocaine – a drug of abuse?

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Introduction: Lidocaine is a local anaesthetic and antiarrhythmic drug that is often detected in postmortem blood either due to medical treatment during resuscitation or as a by-product of cocaine ("cutting agent"). In one case of a known cocaine user where resuscitation failed, lidocaine was at first not quantified but later it turned out to be an intoxication with lidocaine. Police investigations revealed that lidocaine (ca. 90%) was sold to the deceased as a substitute for cocaine.

Method and Results: The lidocaine concentration in heart blood (LC-TOF MS) was 11 mg/l (brain 12 and lungs 9.1 mg/kg) and metabolites (hydroxylidocaine, MEGX, cyclic MEGX) were detected in blood but not in urine. A qualitative hair screening exhibited lidocaine, hydroxylidocaine, MEGX, GX, xylidine and hydroxyxylidine in addition to cocaine (7.86 ng/mg), heroin and methadone.
Discussion: The pharmacologic effect of lidocaine is the inhibition of sodium channels, it does not directly interfere with neurotransmitter release as almost all abused drugs. Apart from acute toxic effects due to inhibition of neuronal or cardiac activity studies demonstrated euphoria and stimulation after intravenous injection or nasal insufflation. The “flush” following lidocain application is reported to be shorter but more intensive compared to cocaine. Effective doses range from 400 to 700 mg (injection) and are above therapeutic doses (infiltration anaesthesia < 300 mg).
In the present case a toxic lidocaine concentration was found, a dosage of 800-2200 mg was estimated. The time to death was obviously very fast as no lidocaine metabolites were detected in urine. The origin of lidocaine in hair from abuse of lidocaine or cocaine (impurity) cannot be differentiated at present. The present case highlights that in cases of suspected death due to drugs of abuse lidocaine might play a major role.

V-26 A highly concentrated metabolite of cyclamate as the only finding in the general unknown screening of urine from a 6 year old girl

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Casuistic: A previously healthy 6 year old girl presented in April 2008 the first time with abdominal pain, intractable vomiting, polyuria, hypertension, severe dehydration, impaired consciousness and aggressive behaviour. Laboratory findings included hyponatremia, hypokalemia and metabolic acidosis requiring massive fluid and sodium supplementation. Within one week, the girl recovered spontaneously. Until May 2009, she sustained 8 further similar episodes and a transient renal Fanconi syndrome was diagnosed. Work-up excluded all known metabolic causes; mitochondrial genome analysis was normal, and there was no exposition to Chinese herbs or heavy metals. During the 10th episode a general unknown screening was requested.
Methods: The urine sample was worked-up and analyzed by GC-MS for a general unknown screening.
Results: The only suspicious finding was a high amount of cyclohexylamine, a metabolite of the artificial sweetener cyclamate.
Discussion: In July 2009, dietary advice was given to omit all potential sources of cyclamate. Until March 2010, the girl has not experienced any further episode. Repeated urine analyses revealed either traces or no cyclohexylamine. In March 2010 the girl sustained the 11th identical episode and the analysis of the urine revealed a high amount of cyclohexylamine. The source was a beverage, artificially sweetened with cyclamate, offered to the girl at school 36 hours before onset of symptoms. The long-term remission after omission of cyclohexylamine and the relapse after the intake of the artificially sweetened beverage suggest a causal relationship as reported once in The lancet in 1969.

Conclusions: The ingestion of cyclamate can lead to acute renal Fanconi syndrome in a susceptible individual. Given the fact that cyclamate is ingested daily by millions worldwide, the observed life-threatening adverse effect is very rare. Nevertheless, it can be speculated, that at least a few individual patients with the cerebral/renal salt wasting syndrome of unknown cause might suffer from cyclamate intoxication.

V-27 Systematic toxicological analysis revealing a rare case of captan ingestion

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Objectives: A case of suicide by intoxication with various pharmaceuticals, especially anticonvulsants, combined with the fungicide captan is presented. Based on the autopsy no cause of death was ascertained; a systematic toxicological analysis, which also included a screening via solid-phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS) for (semi)volatile organic compounds, generated important evidence.
The significance of a complete systematic toxicological analysis should be emphasised. The effect of captan on the human organism, its metabolism and distribution will be discussed.
Material and methods: The screening method by means of SPME and GC-MS and the determination and quantification of the captan metabolite tetrahydrophthalimide (THPI) by GC-MS will be presented in detail.
Results and discussion: Due to the fact that captan degrades by contact with thiols, it was found exclusively in gastric contents. The total amount of THPI in gastric contents was1.5 mg; concentrations decreased in the following order: heart blood (0.35 µg/ml), bile (0.30 µg/ml), liver (0.24 µg/ml), femoral blood (0.22 µg/ml), kidney (0.14 µg/ml) and cerebrum (0.06 µg/ml).
The metabolite THPI would have been missed without previous determination of captan. From a scientific point of view, a rare case like this captan ingestion would have been disguised. And last but not least this fatality could not be investigated satisfactorily.

V-28 Brain doping with modafinil: a well documented self-experiment

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Objective: The possibilities of neuro-enhancement are of great interest in psychiatric circles. Alarmed by high prevalence rates in students and teachers at US universities, a controversial debate on the legitimacy has arisen. Independent of this development, a reliable analytical tool is needed to observe use and misuse of these compounds. The aim of this study was to elucidate the suitability of hair analysis for monitoring the use of modafinil, the most widely used smart drug.
Methods: In a self-experiment, modafinil was consumed during 45 days with controlled dosing of 50 mg for five days (start) followed by 100 mg for 25 days and

again 50 mg for five days (cessation). Head and chest hair was collected before and after this period. Modafinil was analyzed after washing and two step-extraction of pulverized hair (MeOH and MeOH/formic acid). Analyses were done with a Dionex UltiMate 3000 AB Sciex 5500 QTRAP LC-MS system. Separation column, mobile phase and MS mode were: Phenomenex Kinetex, 2.6 µm, 50/2.1; 5 mM ammonium formate buffer pH3/methanol, total flow 0.75 mL/min; ESI, MRM.
Results: Apperception and observations during and after this period were: Reduced sleep duration from 7 to 5-6 h, no diurnal-fatigue, higher motivation for unpleasant tasks (decision making) in work and social life, a general sensation of improved alertness and vigilance, suppressed appetite (weight-loss of 4kg) and an elevated mood. The standard urine-test (Drug-Screen-Multi 12A; performed on days 4 and 14) was negative. Modafinil could be detected and quantified in head and chest hair (highest concentration: 1,900 pg/mg). The drop of the modafinil concentration in segmental analysis reflected the end of intake. The distal segment – actually representing the time before the self-experiment - exhibited an elevated modafinil concentration due to sweat transportation towards the hair tips.
Discussion: This individual case study could proof that a long-term and conclusive detection of modafinil via hair analysis can be done. Most of the noticed effects could also have been a placebo-effect. Further studies with different design will be necessary to monitor the actual “brain-doping” effects.

V-29 Comparison of drug analysis in whole blood and dried blood spots

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Objective: Analysis of dried blood spots (DBS) is an increasingly accepted method in therapeutic drug monitoring whereas its application by analogy to forensic samples has not been further studied. Contrary to whole blood, DBS sampling is easier, allows storage without additional cooling and decreases the risk of infections with blood borne viruses.
The aim of our study was to investigate whether determination of alprazolam, risperidone, 9-hydroxyrisperidone, zopiclone, 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA) and dexamphetamine from DBS is as reliable as from whole blood.
Material and Methods: DBS and whole blood analysis was performed using 100 µL. Analysis was by LC/MS/MS following liquid-liquid extraction. The results were compared using Bland-Altman difference plots.
Results: The number of corresponding specimens (n), the mean concentration ratio (r), the mean difference between the 2 methods (d) and the limits of agreement (l, mean difference ± 1.96 SD) for each analyte were as follows:
Alprazolam: n=22, r=0.99, d= 0.09 ng/mL, l: 1.11 and 0.92 ng/mL; risperidone: n=10, r=1.07, d=0.83 ng/mL, l: 0.67 and 2,32 ng/mL; 9-hydroxyrisperidone: n=14, r=1.04, d=0.64 ng/mL, l: 1.13 and 2.40 ng/mL; zopiclone: n=45, r=0.86, d=3.99 ng/mL, l: 3.62 and 11.59 ng/mL; MDMA: n=35, r=0.99, d=-3.55 ng/mL, l: 14.34 and 7.25 ng/mL; MDA: n=30, r=0.99, d=0.02 ng/mL, l: 1.36 and 1.40 ng/mL; dexamphetamine: n=29, r=0.95, d= 1.03 ng/mL, l: 3.32 and 1.25 ng/mL. Variability of differences between

methods was fairly constant across the range of measurement for all analytes. At least 95% of all differences were within the limits of agreement.
Conclusions: For all analytes except zopiclone results from DBS exactly matched those from whole blood. The blood/DBS-ratio of zopiclone significantly differed from 1.00; the Bland-Altman difference plot showed 3 outliers, 2 of them were close to the limits of agreement. This may be due to zopiclone's lability, which is currently under investigation.
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V-30 Percentages and ratios of N- and O-desmethyl metabolites of tramadol in hair – Use for assessment of tramadol intake vs. external contamination

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Objective: Tramadol was found in a hair sample within the context of abstinence control to regain driving license. The individual denied having taken tramadol. He claimed that external contamination must be the reason for the positive result, because he was working in the production of tramadol medicaments in a pharmaceutical company. Detection of tramadol metabolites should prove the actual tramadol intake. However, only trace amounts of O-desmethyltramadol were found in hair. To interprete the results in this case, the ratios of N- and O-desmethyltramadol vs. the parent compound in other routine tramadol cases were assessed.
Methods: N- and O-desmethyl metabolites of tramadol together with the parent drug were determined in hair of tramadol positive cases (n=10) after washing and a two step extraction procedure (methanolic and aqueous) of pulverized hair. The analytes were separated and detected using a Shimadzu Prominence LC-system coupled to an AB Sciex 3200 QTRAP. Separation column, mobile phase and MS mode were: Phenomenex Kinetex, 2.6 µm, 50/2.1; 1mM ammonium formate buffer pH3/acetonitrile with ammonium formate, total flow 0.5 mL/min; ESI, MRM-IDA-EPI.
Results: Concentrations of tramadol in hair ranged from 800 to 190'000 pg/mg. Only five of the samples showed results below 5'000 pg/mg. Percentages of metabolites were from 4.5 to 22.5 % for O-demethyltramadol and from 10 to 84 % for the N-desmethyltramadol metabolite.
Discussion: Interindividual differences in the metabolite vs. parent drug ratios in hair were huge for tramadol. It has to be taken into account that the O-desmethyl metabolite is formed via the polymorphically expressed CYP2D6. Poor metabolizers for this isoenzyme may produce the O-desmethyl metabolite only to a little extent, as in the suspect case. Since the ratio for the N-desmethyl metabolite had also been in the lowest range (only 10%), a combination of contamination and consumption might be responsible for the results in this case.

V-31 MALDI-Mass Spectrometric Imaging – Analysis of cocaine and metabolites in a single hair

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Objective: Matrix-assisted laser desorption/ionization mass spectrometric imaging (MALDI-MSI) was used to monitor abuse of cocaine by detection of the parent compound and its metabolites in intact single hair samples.
Methods: Acquisitions were performed on a prototype MALDI triple quadrupole linear ion trap fitted with a high repetition rate laser (1 kHz). In contrast to standard hair analysis, sample preparation is simple (washing, fixing the single hair on a MALDI plate and spraying with α-cyano-4-hydroxycinnamic acid (CHCA) or 4-chloro-α-cyano-cinnamic acid (Cl-CCA) as MALDI matrix). Screening and relative quantitation was performed in the selected reaction monitoring (SRM) mode. Sensitive confirmation was achieved with both MS/MS (enhanced product ion) and MS^3 experiments (n=8). Results were compared with routine LC-MS results.
Results: A simple and sensitive method for the simultaneous screening and relative quantitation of cocaine and its main metabolites (benzoylecgonine, cocaethylene and norcocaine) in intact single hair has been developed. MALDI-MSI keeps intact spatial distribution of analytes and thus chronological information about cocaine consumption over several months can be obtained. High sensitivity of MALDI-MSI (SRM) allows the detection of drugs in pg amounts.
Conclusion: MALDI-MSI has proven to be a technique for analysis of cocaine and metabolites in a single hair. More studies are necessary to show its usefulness for other drugs.

V-32 Diagnostic Performance of Ethyl Glucuronide in Hair for the Investigation of Alcohol Drinking Behavior: A Comparison with Hepatic Biomarkers

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Background: Ethyl glucuronide (EtG) in hair has emerged as a useful biomarker for detecting alcohol abuse and monitoring abstinence. However, there is a need to establish a reliable cutoff value for the detection of chronic and excessive alcohol consumption.
Methods: One hundred and twenty-five subjects were classified as teetotalers, low-risk drinkers, at-risk drinkers or heavy drinkers. The gold standard for subjects' classifications was based on a prospective daily alcohol self-monitoring log. Subjects were followed for a 3 month period. Twenty-one alcohol dependents were used as a positive control. The EtG diagnostic performance was evaluated and compared with ASAT, ALAT, γGT and CDT.

Results: A cutoff of >9 pg/mg EtG in hair, suggesting an alcohol consumption of >20/30 g (at-risk drinkers) and a cutoff of >25 pg/mg, suggesting a consumption of >60 g (heavy drinkers) per day were applied. The EtG diagnostic performance was significantly better than any of the hepatic biomarkers alone. EtG, as a single biomarker, yielded a stronger or similar diagnostic performance in detecting at-risk or heavy drinkers, respectively, than the best combination of hepatic biomarkers (CDT and γGT). The combination of EtG with hepatic biomarkers did not improve the diagnostic performance of EtG alone. EtG demonstrated a strong potential to identify heavy alcohol consumption, whereas the hepatic biomarkers failed to do so. EtG was not significantly influenced by gender, body mass index or age.
Conclusion: Hair EtG definitively provides an accurate and reliable diagnostic test for detecting chronic and excessive alcohol consumption. The proposed cutoffs are recommended for clinical and forensic use.

V-33 Immunological Detection of Ricin and Castor Seeds in Beverages, Food and Consumer Products

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The use of ricin or castor seeds in the context of criminal or terrorist attacks poses a significant threat. Two to eight of the readily available seeds can be lethal to adults. Sensory perception of ricin in food is unremarkable, and it has a delayed toxic effect. After metabolism ricin is no longer detectable in the body. When a suspicion has been raised, an investigation should be made retrospectively to the environment of the injured person. Food, objects or vessels have to be analyzed for the presence of the toxin. The unmodified toxin can be detected with sufficient sensitivity using immunological rapid tests. A forensic confirmation procedure can be performed with peptide analysis.
We tested two immunochromatographic lateral flow assays (LFA) from different companies. Both tolerated turbid samples and showed the same sensitivity. Visual and photometric quantification was carried out after a maximum of 20 minutes running time.
For drinking water there is a detection limit of 50 µg/L, in food of about 0.5 mg/kg. The visual and photometric quantification allows only an estimation of the ricin content. Therefore, the ricin-band is compared in intensity with the control band and evaluated by an external calibration series.

V-34 Studies on the Metabolism and Toxicological Detection of Glaucine, an Aporphin Alkaloid from Glaucium flavum (Papaveraceae), in Rat urine using GC-MS and LC-MS^n

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Objectives: Glaucine ((S)-5,6,6a,7-tetrahydro-1,2,9,10-tetramethoxy-6-methyl-4H-dibenzo [de,g]quinoline) is an aporphine alkaloid and the main ingredient of Glaucium flavum (Papaveraceae). It was described as legal high alone or in combination with

other drugs (Dragan et al., EJCP, 2008), whereas in Bulgaria, it is still in use as an antitussive. Therefore, the aim of this paper was to identify the metabolites of glaucine and to study its detectability in urine using the authors' GC-MS and LC-MS^n screening approaches.
Materials and Methods: Urine samples were collected over a 24 h period from male Wistar rats, which had been administered by gastric intubation for toxicological diagnostic reasons a 20 mg/kg body weight (BW) dose of glaucine for the metabolism study or a 2 mg/kg BW dose for the detectability studies. The phase I and II metabolites were identified directly or after enzymatic cleavage of conjugates, solid-phase extraction (HCX), and acetylation by GC-MS (EI) according to Maurer et al. (Wiley-VCH, 2007) or after protein precipitation by LC-linear ion trap-MS^n according to Wissenbach et al. (ABC, 2011). For confirmation of the postulated isomeric metabolite structures, possible candidates were synthesized and identified by H-NMR. For toxicological detection, the urine samples were analyzed either after acidic hydrolysis, liquid-liquid extraction (LLE), and acetylation by full-scan GC-MS (STA) or after protein precipitation by LC-MS^n.
Results and Discussion: Besides glaucine, six mono- or bis-demethylated metabolites could be identified in rat urine. The phase-I metabolites were partly excreted as glucuronides and/or sulfates. Toxicological detection should be focused on the O-desmethyl and bis-O-desmethyl metabolites. Using GC-MS (STA approach) as well as the LC-MS^n approach, these metabolites could be detected after a 2 mg/kg BW dose corresponding to a higher user's common dose. Assuming similar metabolism and kinetics, an intake of glaucine should be detectable via its metabolites in urine by using GC-MS or LC-MS^n.

V-35 Automation of Solid-Phase Extraction in Forensic Toxicology

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Objective: The aim of this study was to evaluate different devices for the automation of solid-phase extraction.
Introduction: In forensic toxicology, sample preparation is a crucial pre-requisite for the successful application of high-tech analytical instruments for qualitative and quantitative substance determination.
Because of its high extraction efficiency and its possibility for miniaturization and automation, solid-phase extraction (SPE) is rapidly gaining importance in this field.
SPE is also driven by the need for laboratory accreditation: Automation reduces the possibility of systematic errors, costs, and time spent per case; in addition it improves the level of technicians' safety.
Method: 0.5g of tissue or 1.0mL of body fluid were homogenized with an IKA ULTRA-TURRAX Tube Drive (IKA) and diluted with 5mL of phosphate buffer (0.05 M, pH 7.4). After centrifugation the supernatant was used for SPE, which was automated using the following devices: RapidTrace (Zymark), ASPEC XL (Gilson), MultiPurposeSampler MPS (Gerstel).
Results and discussion: When developing an automated SPE procedure, appropriate pre-treatment of body fluids and tissues as well as proper choice of the extraction device is essential.

The following will be discussed: Sample pre-treatment, viscosity of the sample, speed of sample application and flow-control, flexibility of the different extraction devices (in respect to maximal amount of samples, cartridge sizes, and speed of extraction). Moreover, the control software and issues like contamination and possibilities for error handling (clogging of cartridges) are important as well.
Automation of SPE results in better recovery rates, and improves consistency and reproducibility of the results.

V-36 Studies on the metabolism of five model drugs by fungi colonizing cadavers using LC-MS/MS and GC-MS analysis

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Objectives: It is well-known that cadavers may be colonized by bacteria and fungi but there is no information if or to what extent these microbes are capable of metabolizing drugs or poisons and thus to change concentrations and metabolic pattern of such compounds in postmortem samples. Drug metabolism by fungi known to colonize cadavers was studied using five model drugs and two fungi species.
Materials and methods: Each model drug (mirtazapine, amitriptyline, zolpidem, metoprolol, and promethazine) was incubated with 10 ml cultures of each of the two model fungi known to colonize cadavers (*Absidia repens* and *Mortierella polycephala*) and the positive control fungus *Cunninghamella elegans*. The drug concentration in the incubation mixtures was 1 mM and the incubations were carried out for 96 h at 20°C (*A. repens* and *M. polycephala*) or 30°C (*C. elegans*). Samples (800 µl) were taken from the incubation mixtures at 24, 48, 72 and 96 h. After centrifugation, one part of the supernatant (50 µl) was analyzed by LC-ESI-MS/MS in the product ion scanning mode after dilution with mobile phase. The rest of the supernatant was analyzed by GC-MS after liquid-liquid extraction and acetylation.
Results and discussion: All model drugs were metabolized by *C. elegans* resulting in two (metoprolol) to five (amitriptyline) metabolites per compound. O-demethylation followed by side chain oxidation of metoprolol to the respective carboxylic acid as well as sulfoxidation of promethazine was observed in incubations of both *A. repens* and *M. polycephala*. In incubations with the latter, N-demethylation of amitriptyline also occurred. Mirtazapine and zolpidem were not metabolized under the given conditions. These results suggest that fungi colonizing cadavers may change concentrations and metabolic patterns in postmortem samples, at least of certain drugs. Further studies are needed to assess whether or not this may be important in case interpretation.

V-37 Fully Validated Liquid Chromatographic-Tandem Mass Spectrometric Procedure for Identification and Quantification of Antidepressants and Benzodiazepines in Human Blood Plasma

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Objectives: Multi-analyte procedures make the analytical process much simpler, faster, and cheaper, and allow monitoring of analytes of different drug classes in a single body sample. The aim of the present study was to validate a multi-analyte procedure for fast target screening, reliable identification, and quantification exemplified here on 62 tested antidepressants (AD) and benzodiazepines (BZ).
Materials and Methods: Analyte identification and quantification was performed after liquid-liquid-extraction (Remane et al., ABC, 2010) using a ThermoFisher (TF) TSQ Quantum Access (APCI) with gradient elution on a TF Hypersil GOLD Phenyl column (100 x 2.1 mm, 1.9 µm) in the timed multiple-reaction monitoring mode. The method was validated with respect to selectivity, cross-talk, ion suppression/enhancement of matrix compounds (matrix effects), co-eluting analytes and internal standards, recovery, process efficiency, accuracy and precision, stabilities, and limits of quantification and detection. For accuracy and precision, full as well as one point calibration was performed.
Results and Discussion: During validation, no severe selectivity problems could be detected; cross talk was seen for amitriptyline caused by maprotiline but was distinguishable according to their retention times. Ion suppression/enhancement was already monitored in detail (Remane et al., RCM, 2010 a+b; ABC, 2010). Severe matrix effects could be detected for bupropion and hydroxybupropion. Instability during freeze/thaw cycles was shown for bupropion, hydroxybupropion, and norfluoxetine. The lower limit of quantification was set at the lowest calibrator concentration and was at least at the lower therapeutic concentration with exception for cyclobenzaprine and reboxetine. One point calibration was shown to be an acceptable calibration model for 21 AD and 17 BZ.
This multi-analyte procedure allowed selective detection as well as accurate and precise quantification of 28 AD and 21 BZ, zaleplon, zolpidem, and zopiclon in plasma as part of a multi-analyte method of 136 analytes of different drug classes using the accepted validation criteria.

V-38 No evidence of increased time intervals between offense and blood sampling or decreased drug concentrations in THC positive DUID samples after Jena Higher Regional Court order regarding § 81a of the German Code of Criminal Procedure

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Objective: After Higher Regional Courts (OLG) stressing the requirement of a judge's order for blood sampling in DUID cases (§ 81a of the German Code of Criminal Procedure), there has been concern that the delay to obtain such an order may negatively influence drug concentrations in DUID samples. The aim of this study was therefore to look for objective evidence for increased time intervals between offense and blood sampling or negative influences on drug concentrations exemplified for THC after the Jena OLG order in 09/2008.
Methods: From the institute's database, the following parameters were extracted for all serum samples with confirmed positive THC results analyzed from 01/2008 to 11/2010: serum concentrations of THC, dates and times of the offence and blood sampling. Incomplete and inconclusive datasets as well as those with time intervals between offenses and sampling exceeding 24 h were excluded. After sorting THC

concentrations into annual bins as well as interval bins of 0.0-0.5 h, 0.5-1.0 h, 1.0-1.5 h, 1.5-2.0 h, 2.0-3.0 h, and >3.0 h, the medians of the bins were compared. Moreover, time intervals were sorted into monthly and annual bins and compared accordingly.
Results and Discussion: The study included 1931 datasets. Median THC concentrations (µg/l) increased from 3.0 (2008, n=578) over 3.5 (2009, n=557) to 4.0 (2010, n=796). They showed a steady increase with shorter time intervals between offense and sampling (with exception of those in the 0.0-0.5 h being lower than those in the 0.5-1.0 h bin). The median time intervals between offense and sampling were similar over the years lying at 1.17 h in 2009 and at 1.08 h in 2008 and 2010, while they varied considerably over the months albeit with no particular pattern. In conclusion, there was no increase of time intervals between offense and sampling while THC concentrations increased rather than decreased.

V-39 Ethyl glucuronide and ethyl sulfate for detection of alcohol abuse in drunken drivers

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Objectives: To correlate EtG and EtS concentrations in blood of drunken drivers with blood alcohol concentrations to use as markers for alcohol addiction and as prerequisite for withdrawal of driving license due to suspicion of alcohol misuse.
Materials and Methods: A validated LC-ESI-MS/MS has been used for ethyl glucuronide (EtG) determination in heparinised blood (whole blood) from traffic cases of drunken drivers. Blood alcohol has been determined with two different GC-FID methods according to Swiss regulations using whole blood. Statistical regarding sensitivity and specificity of the markers was performed.
Results and Discussion: Comparison of blood alcohol and EtG and EtS concentrations showed a good correlation. For blood alcohol levels higher than 1.6 permille, linear regression of all data gave thresholds of 1300 ng/mL for EtG and 1000 ng/mL for EtS. Sensitivity and specificity was tested for different thresholds. If EtG and EtS concentrations in addition to the 1.6 permille threshold would be used as a prerequisite, a larger number of cases would be submitted to programs for control of abstinence, and detection of uncovered alcohol misuse or alcoholism would be possible.
Conclusions: According to German (and presumably also to future Swiss) laws blood alcohol concentrations (BAC) in drunken drivers lead to the withdrawal of the driving licence if higher than 1.6 permille (currently 2.5 permille in Switzerland) and subsequent ethanol withdrawal or abstinence maintenance therapy and monitoring of abstinence by analysis for EtG in hair or in urine samples. Our study shows, that EtG and EtS can be used for the same purpose after setting a threshold for these markers, and can detect consumption habits typical for alcoholism or alcohol misuse (regular consumption of high amounts of alcohol or consumption of very high single doses).
