XXIV. GTFCh-Symposium - Vorträge

V01 The importance of large case cohorts to investigate time-dependent postmortem redistribution of various drugs (of abuse): A comparison study

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Aims: Postmortem redistribution (PMR) can lead to artificial drug concentration changes over time, which pose issues for forensic toxicological case interpretation. Systematic studies on the extent of time-dependent PMR are sparse and often comprised of small study-cohorts. The aim of the current study was to evaluate the relevance and importance of large case-cohorts for the study of time-dependent PMR of various drugs (of abuse). **Methods:** The results of previously published opioid- (n=23) and antidepressant/neuroleptics (n=37) cases (Brockbals et al. 2018, 2021, JAT) on time-dependent PMR from the Zurich Institute of Forensic Medicine (ZIFM, Switzerland) were compared to a not yet published case-cohort comprised of femoral blood samples (two sample collection time-points) of 477 routine toxicological cases from the Victorian Institute of Forensic Medicine (VIFM, Australia). Xenobiotics (n=83) were quantified using a targeted LC-MS/MS method. Results (median concentration changes across cases) were compared between the two datasets. **Results and Discussion:** In the ZIFM-cohort, median time-dependent concentration changes indicated a weak/moderate potential for PMR for oxycodone (+12 %) and tramadol (+21 %) (n=4 each). This interpretation was confirmed with increased case numbers (+9 %, n=57 and +17 %, n=18). For venlafaxine, initial data showed negligible concentration changes (+1.9 %, n=7), however in the bigger VIFM-cohort (n=19), median concentration increases of +53 % were observed. Similarly, for methadone (+7 %, n=12) and quetiapine (+9 %, n=9), with larger case numbers (n=29 and n=27) higher median concentration changes were found (+23 % and +28 %). Overall, the variability of observed changes increased for all xenobiotics with larger sample sizes, likely caused by a broader range of postmortem intervals across cases. **Conclusion:** The comparative results emphasize that large case-cohorts are crucial for the evaluation of time-dependent PMR, which could be misinterpreted when only looking at a small number of cases. More studies are needed to come closer to a generalized substance-specific interpretation of PMR.

V02 The ring must be destroyed - Mass-spectrometric identification of two unusually formed main metabolites of the novel synthetic opioid dipyanone involved in two fatal cases

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Aims: The emergence of novel synthetic opioids (NSOs) poses substantial challenges to public health and law enforcement. Following the global scheduling of fentanyl analogues, several non-fentanyl-based NSOs have appeared on the illicit drug market, increasing the risks of addiction and fatal overdoses. Dipyanone, a methadone analogue first detected in drug seizures in 2021, was associated with four fatalities in Germany between 2022 and 2023. This study aimed to characterize the two main metabolites of dipyanone in human samples using mass spectrometry. **Methods:** The metabolism of dipyanone was investigated using *in silico* predictions, *in vitro* incubations with human hepatocytes, and authentic urine specimens from two of the four dipyanone-positive fatal cases. Analyses were performed using an UltiMate 3000 liquid chromatography system coupled to a Q-Exactive Quadrupole-Orbitrap hybrid high-resolution mass spectrometer operating in full scan and MS/MS mode. Separation was achieved using a Kinetex biphenyl column. **Results and Discussion:** The two main metabolites (m/z 336.2303, M12, and 350.2119, M13) exhibited notably different fragmentation patterns compared to the parent compound, although M12 suggested the same sum formula as the parent ($C_{23}H_{29}NO$). *In silico* predictions failed to anticipate these metabolites. The proposed mechanism of dipyanone metabolism indicates alpha-hydroxylation at the pyrrolidine ring, leading to a labile hemiaminal with subsequent ring opening. The resulting aldehyde could be metabolized to either the corresponding N-butan-4-ol or N-butanoic acid derivatives. Analogous to the EDDP formation from methadone, subsequent cyclization could form the two main metabolites, 4'-[2-ethylidene-5-methyl-3,3-diphenylpyrrolidin-1-yl]butan-1'-ol (EMDPB, M12) and 4'-[2-ethylidene-5-methyl-3,3-diphenylpyrrolidin-1-yl]butanoic acid (EMDPBA, M13). This pathway has also been described for other substances, such as daridorexant. **Conclusion:** We propose that EMDPB and EMDPBA serve as specific biomarkers of dipyanone consumption. The unexpected formation of these metabolites, which were not accurately predicted by *in silico* models, should be considered when investigating the metabolism of structurally similar substances, including other methadone analogues.

V03 A case of fatal intoxication with the new synthetic opioid *N*-pyrrolidino protonitazene (protonitazepyne)

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Aims: The availability of 2-benzylbenzimidazole opioids (nitazenes) on the drug market has increased steadily in recent years. Nitazenes are highly potent synthetic opioids whose abuse can result in severe, potentially lethal, adverse effects (e.g. respiratory depression). This case report describes a fatal intoxication following vaping of the novel synthetic opioid *N*-pyrrolidino protonitazene (protonitazepyne). Protonitazepyne was quantified in a serum sample obtained 10 hours after death and various biological specimens collected during autopsy three days postmortem. **Methods:** Protonitazepyne concentrations in postmortem serum, heart blood, femoral blood, liver, bile and stomach contents were quantified by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using a standard addition method. Quantification in urine and serum was performed by LC-MS/MS and external matrix calibration. **Results and Discussion:** Protonitazepyne concentrations were 3.8 ng/mL in postmortem serum, 1.7 ng/mL in heart blood, 0.6 ng/mL in femoral blood, 34.5 pg/100 mg in liver, 32.3 ng/mL in bile and 19.7 ng/mL in stomach contents. In postmortem urine, 8.8 ng/mL of protonitazepyne was measured. Additionally, pregabalin (7270 ng/mL), sertraline (66 ng/mL), naloxone (10.2 ng/mL), mitragynine (11.5 ng/mL) and traces of quetiapine were identified in postmortem blood. The combination and concentrations of these substances alone could not account for the fatal out-

come. As protonitazepyne has an *in vitro* potency approximately 25 times greater than fentanyl, even low doses can be lethal. The elevated concentrations of protonitazepyne in heart blood compared to femoral blood suggest that postmortem redistribution may need to be considered for this substance. The declining concentrations over time may indicate stability issues. **Conclusion:** Due to its high potency, protonitazepyne is considered the primary cause of death in this case. Given the fatal outcome associated with the consumption of protonitazepyne, a toxicological significance score of 3 is suggested for this novel synthetic opioid.

V04 Metabolic characterization and toxicological analysis of *N*-ethylpentedrone: *In vitro* and human *in vivo* data

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Aims: *N*-Ethylpentedrone, a cathinone designer stimulant, might cause adverse effects such as restlessness, anxiety, psychosis, tachycardia, and hyperthermia. This report presents clinical toxicological investigations of two suspected *N*-ethylpentedrone ingestions. Data on the *in vitro* and *in vivo* metabolism and ante mortem *N*-ethylpentedrone plasma concentrations are described. **Methods:** Pooled human liver microsomes (pHLM) and eleven recombinant monooxygenases were incubated with *N*-ethylpentedrone using standard workflows for *in vitro* metabolism. Urine and plasma samples received after suspected intake of unknown drugs of abuse were prepared by organic precipitation or liquid-liquid extraction, respectively. *N*-Ethylpentedrone was quantified in plasma samples via standard addition. All samples were analyzed by liquid chromatography using an Accucore Phenyl-Hexyl column (Thermo Fisher, TF) coupled to high-resolution mass spectrometry (HRMS, TF Q Exactive Plus) using full scan and data-dependent acquisition after heated electrospray ionization. **Results and Discussion:** A total of four phase I metabolites were identified *in vitro*, which were formed after *N*-dealkylation, hydroxylation, ketone formation, and/or reduction. The initial activity screening revealed that CYP2D6 and CYP2C19 were involved in hydroxylation and *N*-dealkylation, amongst others. Three out of four phase I *in vitro* metabolites could be confirmed after analysis of human plasma and urine. In addition, a metabolite formed via glucuronidation after hydroxylation could be detected in human urine. Plasma concentrations of *N*-ethylpentedrone were 3 and 25 ng/mL, respectively. One individual was initially comatose and died approximately two days after samples were collected most likely due to ingestion of several compounds. **Conclusion:** Intoxications with synthetic cathinones continue to be highly prevalent and should remain in the focus of analytical toxicology. The current study provides insights into the *in vitro* and *in vivo* toxicokinetics of *N*-ethylpentedrone, including ante mortem plasma concentrations. The results suggest potential screening targets for *N*-ethylpentedrone in plasma and urine samples.

V05 Deadly confusion: A fatal case of ADB-BUTINACA mislabeled as 'Monkey Dust' - The dark side of novel psychoactive substances

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Aims: A young man was found dead in his apartment alongside a plastic bag containing a white powder, originating from the Netherlands and labeled as the designer stimulant MDPHP (3,4-methylenedioxy-α-pyrrolidinohexanophenone). No suicide note was found. The powder, which was probably smoked using a crack pipe, was later identified as the highly potent and prevalent synthetic cannabinoid ADB-BUTINACA (other names: ADB-BINACA, ADMB-BINACA, IUPAC name: *N*-(1-amino-3,3-dimethyl-1-oxobutan-2-yl)-1-butyl-1*H*-indazole-3-carboxamide). An autopsy was conducted 10 days post-mortem, and concentrations of ADB-BUTINACA were determined in various biological specimens. **Methods:** The powder was analyzed using GC-MS, LC-MS/MS and NMR spectroscopy. A comprehensive toxicological screening, including new psychoactive substances (NPS), was performed on urine and blood. To quantify ADB-BUTINACA in femoral blood, heart blood, urine, stomach contents, bile fluid, and liver tissue, a standard addition method (SAM) was used with d9-AB-PINACA as an internal standard. Additionally, scalp hair (0 – 4.5 cm) was analyzed to assess prior drug use. **Results and Discussion:** The powder was highly pure (> 98 %, as determined by NMR) and free of MDPHP, although trace amounts of MDMB-BUTINACA were detected. The SAM revealed relatively high concentrations of ADB-BUTINACA, approximately 33.2 ng/mL in femoral blood and 101 ng/mL in heart blood. Urine (creatine: 35 mg/dL) analysis showed 3.1 ng/mL of the parent compound and low levels of metabolites. A toxicological significance score (TSS) of 3 was assigned, indicating ADB-BUTINACA monointoxication as the primary cause of death. MDPHP was detected in blood and urine at low concentrations (< 2 ng/mL), likely due to prior consumption. Additional findings proved ibuprofen, caffeine and nicotine uptake. **Conclusion:** Mislabeled drugs, especially NPS from online shops, continue to pose an enormous risk to health and life. This case highlights the critical importance of drug-checking services in preventing fatal poisonings and overdoses. Prior analysis of the substance before consumption could have potentially saved this individual's life.

V06 Case report: A fatal intoxication involving the novel benzodiazepine pro-drug clonazafone and fluoroetonitazene

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Aims: A case of suspected drug poisoning involving clonazafone, a benzodiazepine pro-drug, and fluoroetonitazene was investigated at the Zurich Institute of Forensic Medicine to determine the cause of death. The deceased had consumed a combination of drugs of abuse in the evening and was found dead the following day. **Methods:** Standard toxicological analysis, including immunoassay for common drugs and untargeted liquid chromatography (LC) ion trap mass spectrometry (MS) screening, was performed in urine. Further, the blood alcohol concentration was determined. Quantitative analyses of amphetamine, clonazepam, and 7-aminoclonazepam in peripheral blood were conducted using a previously validated targeted LC-MS/MS method. A high-resolution untargeted screening was carried out using the Sciex ZenoTOF™ 7600 System. Due to the discovery of a powder labeled as "clonazafone" at the scene and an e-liquid, later identified as fluoro-etonitazene, clonazafone was quantified in-house, and fluoro-etonitazene was quantified at the Institute of Forensic Medicine Freiburg, Germany. **Results and Discussion:** Clonazafone was (semi-)quantified in urine (38.6 ng/mL), muscle tissue (3 ng/mL), and the stomach content (75,692 ng/mL, total volume of 150 ml), but could not be detected in peripheral blood, heart blood, and vitreous fluid (LOQ: 0.1 ng/mL). Additionally, clonazepam (1.5 ng/mL) and its metabolite 7-aminoclonazepam (140 ng/mL), as well as

amphetamine (110 ng/mL) and the designer-opioid fluoro-etonitazene (3.3 ng/mL) were found in blood. Within the high-resolution screening, desglycylclonazafone, the intermediate of clonazafone that can be further converted into clonazepam, was detected in the stomach content and urine. **Conclusion:** A combined drug intoxication with fluoro-etonitazene, clonazepam, and amphetamine was reported as the cause of death. It was possible to detect clonazafone in urine, which may indicate that the detected clonazepam probably resulted from clonazafone intake. The intermediate desglycylclonazafon, on the other hand, cannot serve as clear evidence of clonazafone ingestion, since it can also be formed in an equilibrium reaction from clonazepam at acidic pH.

V07 Drug-induced psychosis associated with Kratom? – A case report

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Background: Kratom (*Mitragyna speciosa*) is widely used in Southeast Asia for its ethnomedicinal properties and has gained popularity in Western countries for recreational purposes. Mitragynine and 7-hydroxymitragynine are major alkaloids with affinity to opioid receptors, causing dose-dependent stimulant and sedative effects. Reported adverse effects include gastrointestinal symptoms, tachycardia, confusion, seizures and agitation, with some studies also reporting psychosis. **Analytical methods:** Analytical screening of serum samples was performed by LC-QToF MS in "All Ions" mode, with quantification carried out by LC-MS/MS. **Case presentation:** A 39-year old man was reported to the police due to confusion and psychologically abnormal behaviour. Upon arrival, he was aggressive, unresponsive and had to be restrained by the police. They admitted him to psychiatric care, where he was administered benperidol and the doctor stated him being a polytoxicomaniac. According to the police, the subject had previously required psychiatric treatment for drug-induced psychosis. Analytical results in a blood sample obtained 6 hours and 40 minutes afterwards showed THC and metabolites in low concentrations, haloperidol in sub-therapeutic, and benperidol in therapeutic concentrations. In addition, mitragyna alkaloids and metabolites were detected and mitragynine quantification yielded 0.088 mg/L. **Discussion/Conclusion:** Considering its pharmacokinetic profile, it is assumed that mitragynine concentrations were considerably higher at the time of the incident, almost 7 h before blood sampling. Data on the dose-effect-relationship is scarce, however, an acute mitragynine overdose may be responsible for the psychotic symptoms observed in the present case, yet, a pre-existing psychotic state cannot be ruled out.

V08 Prohibited yet persistent: A clinical toxicology analysis of two pesticide poisoning cases

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Aims: The term "pesticide" describes compounds primarily used in an agricultural setting. They include the alkyl phosphate parathion, also known as E605 and the quaternary ammonium compound paraquat, both known for their high toxicity. Despite the EU ban of parathion (2002) and paraquat (2007) on their supply, import, use, and authorization, remnants are still circulating. This study presents two suspected cases possibly involving parathion or paraquat, respectively. **Methods:** Blood plasma and urine samples received after suspected ingestion of parathion or

paraquat were analyzed using standard in-house screening workflows. Plasma samples and urine samples were liquid-liquid extracted (samples A and B), with the urine being hydrolyzed and acetylated. Urine was additionally prepared using an organic precipitation step (sample C). Samples A and C were analyzed by liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) and samples A and B using gas chromatography coupled to mass spectrometry (GC-MS). In addition, sodium dithionite was used to test specifically for paraquat in plasma and urine. **Results and Discussion:** Analysis of the urine sample following suspected parathion ingestion revealed the presence of the parathion degradation product 4-nitrophenol using GC-MS. Additionally, 4-nitrophenolglucuronide was detected by LC-HRMS. The clinical presentation of reduced cholinesterase activity, and the identified metabolites, strongly supported the parathion ingestion. In the suspected paraquat case the patient showed severe vomiting and reported drinking accidentally from a bottle used for gardening purposes. An initial colorimetric paraquat test was negative and an LC-HRMS method confirmed the absence of paraquat in these samples. This was most likely due to the delayed collection of patient material following ingestion. **Conclusion:** The two cases of suspected parathion and paraquat ingestion demonstrated that despite their long-standing ban, remnants of these pesticides still pose a poisoning risk. Analytical methods within a clinical toxicology setting should remain available to identify such rare analytes.

V09 Suicide with sodium nitrite? Detectability of nitrite and nitrate in postmortem blood

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Aims: Overdose with sodium nitrite has become a common method of suicide. A method for the quantitative determination of nitrate and nitrite in postmortem blood via HPLC-DAD was developed and validated. **Methods:** After using potassium ferricyanide for nitrite stabilization, precipitation, filtration and liquid-liquid extraction were performed, followed by direct nitrate measurement. For nitrite, a subsequent Griess-reaction was conducted. Eleven fatalities of suspected nitrite intoxications without a known competitive cause of death were investigated. Heart and femoral blood concentrations of both analytes were compared. **Results and Discussion:** Nitrite concentrations ranged from 1.0-445 µg/mL in femoral and 1.3-76 µg/mL in heart blood in 10 out of 11 cases. Nitrite was not detected in one case, and either only in heart or femoral blood in two cases. Nitrate was detected in all cases ranging from 58-997 µg/mL in femoral, and 54-829 µg/mL in heart blood. Physiological nitrate concentrations of max. 74 µg/mL were determined in postmortem blood (n=5), whereas physiological nitrite levels were not detectable (LOD/LOQ: 1 µg/mL). Concentrations of both analytes were sometimes highly variable in both matrices of the same fatality. Nevertheless, an intoxication with sodium nitrite was either concluded if: a) nitrite was detected and nitrate concentrations were above physiological level (5/11); b) physiological nitrate levels were measured, but nitrite was detectable (3/11) or c) high nitrate concentrations (above physiological level) were measured if nitrite was not or not constantly detected in both matrices (3/11). The high variability of the measured concentrations may depend on the instability of the analytes (especially nitrite), environmental factors (e.g. putrefaction) and matrix effects. **Conclusion:** Case history in combination with nitrate/nitrate concentrations allowed the assumption of an intoxication in all cases. An analysis

of both, femoral and heart blood is recommended, as well as chemical stabilization of nitrite to minimize degradation. For interpretation, considering the physiological nitrate levels is crucial.

V10 Orbitrap-based untargeted screening or triple quadrupole-based targeted screening for drug testing in oral fluid?

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Aims: We evaluated the performance of a liquid chromatography (LC)-high-resolution tandem mass spectrometry (HRMS/MS)-based untargeted screening for comprehensive drug testing in oral fluid (OF) and compared results to those obtained using a LC-triple quadrupole (QqQ)-MS/MS-based targeted screening. **Methods:** OF extracts obtained after sampling with the Greiner Bio-One device followed by solid-phase extraction (SPE) using mixed-mode SPE cartridges (Biotage ISOLUTE HCX-3) were analyzed. For the untargeted screening, extracts were separated on an Accucore PhenylHexyl column (100 mm x 2.1 mm, 2.6 µm) within 13.5 min total runtime. Detection was done using an Orbitrap Exploris 120 in full scan mode with data-dependent MS^2 after positive or negative heated electrospray ionization. For the targeted screening, a Raptor Biphenyl column (50 mm x 2.1 mm, 2.7 µm) with gradient elution for 5.55 min was used prior to QqQ-MS/MS-based analysis on an Agilent 6470 operating in multiple reaction monitoring mode after positive electrospray ionization. A total of 59 analytes including analgesics, benzodiazepines, cannabinoids, opiates, and stimulants were part of the targeted screening. **Results and Discussion:** In total, 212 OF extracts provided by Synlab MVZ Weiden were analyzed using the untargeted and targeted screening. In addition to the compounds included in the targeted analysis, alkaloids, antihistamines, cardiovascular drugs, (tricyclic) antidepressants, further analgesics, and neuroleptics could be detected using the untargeted screening. Limitations of the untargeted screening were particularly the detection of pregabalin due to the co-elution of matrix-dependent compounds and amphetamine most likely due to the co-elution of a tartrazine adduct not being removed after SPE, leading to ion suppression, and requiring improved chromatographic conditions to sufficiently detect these compounds. **Conclusion:** The targeted screening allowed a more sensitive and reliable detection of analytes included in its panel, while the untargeted screening allowed the detection of a wider range of compounds. Therefore, a combination of both approaches may be feasible in certain situations where a more comprehensive analysis is needed.

V11 Utilizing Glu-C digestion for sensitive LC-MS/MS analysis of insulin variants and C-peptide in postmortem vitreous humor and cerebrospinal fluid

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Aims: There is a lack of sufficiently sensitive, MS-based methods for the postmortem confirmation of insulin variants. Proceeding from a previously presented SPE- and LC-MS/MS-based method for the analysis of eleven intact insulins and C-peptide, we therefore investigated whether the sensitivity of the method can be further increased by protein digestion. For this purpose, the enzyme Glu-C was chosen in order to obtain four or five peptides each that can be unambiguously traced back to their respective parent insulin. **Methods:** Samples of vitreous humor or CSF were spiked with insulin variants and C-peptide. Samples were either first mixed

with Glu-C solution, incubated for 16 hours and then concentrated using Oasis MAX µElution SPE plates, or the samples were first subjected to SPE and then digestion was carried out. Both approaches were also tested with reduction of the disulfide bridges using tris(2-carboxyethyl)phosphine and iodoacetamide. Finally, for LC-MS/MS analysis, a Waters Acquity Premier Peptide CSH C18 column and an Agilent 6495 instrument were used. **Results and Discussion:** Digestion was observed to be slower for non-reduced insulins as well as for the side chain-containing insulins detemir, degludec and icodec. In addition, without reduction, the insulins are preferentially cleaved at position 21^{B}. The resulting peptides can be used to distinguish the majority of insulin variants; however, a second peptide needs to be considered to differentiate human insulin from glargine metabolite M1 as well as bovine from porcine insulin. In contrast to the intact proteins, the chromatographic separation of the peptides worked without additional priming procedures for the LC system. The method currently exhibits a sensitivity of 100 pg/mL in synthetic vitreous and cerebrospinal fluid with promising opportunities for further optimization. **Conclusion:** This novel LC-MS/MS based method shows that digestion of insulins with Glu-C represents a useful alternative approach to the analysis of intact proteins.

V12 Evaluation of the oxidative melanin markers PTCA and PTeCA in hair: LC-MS/MS method development and application in authentic hair samples

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Aims: A major challenge for forensic hair analysis is the adulteration of hair by cosmetic (oxidative) treatments which reduces incorporated xenobiotics in hair resulting in false negative results. The aim of this study was to develop a liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the quantitative analysis of the melanin oxidation markers PTCA (1*H*-pyrrole-2,3,5-tricarboxylic acid) and PTeCA (1*H*-pyrrole-2,3,4,5-tetracarboxylic acid) in hair to detect cosmetic hair adulteration. **Methods:** The method was validated for both compounds according to the guidelines of the Society for Toxicological and Forensic Chemistry (GTFCh) including the following parameters: linearity of calibration, accuracy and precision, recovery and matrix effect, freeze and thaw stability. The method was applied to quantify PTCA and PTeCA in a cohort (n = 75) of authentic samples from routine casework of the Center for Forensic Hair Analysis. Furthermore, the influence of different cosmetic treatments on PTCA and PTeCA was investigated *in vitro* including bleaching, permanent dyeing and tinting. **Results and Discussion:** The developed method allowed detecting and measuring reliable concentrations of PTCA (median concentration 2.1 ng/mg) and PTeCA (median concentration 4.4 ng/mg) in authentic hair samples. Concentration levels of PTCA and PTeCA could be detected and evaluated depending on hair color, hair segment and hair adulteration method. From these results, a concentration for both PTCA and PTeCA in hair exceeding 10 ng/mg is suggested as an indication for oxidative treated hair samples in forensic casework. **Conclusion:** With the validated LC-MS/MS method, a quantitative detection method could be developed to analyze oxidative treated hair samples for PTCA and PTeCA and thus improve interpretation of forensic casework in forensic hair analysis.

V13 Evaluation of a new automated assay for indirect identification of synthetic urine

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Aims: Aim of the study was to test a new commercial automated assay targeting urinary tract proteins for detection of synthetic urine and to compare the obtained results (diagnostic sensitivity/specificity) to already established methods. **Methods:** Temperature was measured during urine sample collection. Urinary creatinine, urea, and uric acid were determined by automated assays on a Beckman Coulter AU480. Additionally, "long duration" (LD) and "short duration" (SD) markers were determined using the new Validity Diagnostics true urine LD and SD kit (Validity Diagnostics, Orlando, USA) according to the manufacturer's manual. Endogenous biomolecule pattern as well as direct synthetic urine markers were detected by an established LC-MS approach. The urine specimens (n=1188) were classified as suspicious (set 1, n=47) or unsuspicious (set 2, n=1141) based on temperature and/or uric acid. In addition, all set 1-specimens were evaluated by LC-MS for confirmation. **Results and Discussion:** Automated uric acid analysis showed once again congruent results to the applied LC-MS/MS approach. In nine set 1 specimens uric acid/LC-MS/MS endogenous biomolecules, in 38 specimens none of these compounds were detected. Additionally, 35 specimens contained direct synthetic urine markers (polypropylene glycol/PPG+16). The LD test was congruent with uric acid/LC-MS/MS results with one false-positive (sensitivity: 100 %, specificity: 88.9 %) and a false-positive rate (FPR) of 11.1 %. The SD test had six false-negatives and five false-positives (sensitivity: 84.2 %, specificity: 44.4 %), and a FPR of 56.6 %. Unusual SD results were also obtained in 414 set 2 samples reducing the FPR to 36.2 %. Based on the small number of urine specimens in set 1, SD's FPR may be overestimated. According to the manufacturer, the SD marker is stable for up to 5 days (2-8°C). All tests were performed within 48h after collection. **Conclusion:** Overall good performance was found for the new LD marker targeting urinary tract proteins in comparison to established automated and chromatographic methods.

V14 Detection of phosphatidylethanol after ethanol intake with targeted blood alcohol concentrations of 0.6 g/kg and 0.75 g/kg

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Aims: Phosphatidylethanol (PEth) can be used to detect alcohol consumption, and is also increasingly being used in different applications such as driving aptitude assessment or abstinence control. However, the minimum amount of alcohol needed to result in a quantifiable blood concentration is still not fully known. This study aimed to address this issue in a drinking study with twelve volunteers under controlled conditions including four weeks of abstinence prior to the study. **Methods:** Participants were recruited at the University of Freiburg. After an abstinence period of four weeks resulting in PEth (16:0/18:1) concentrations reaching values below detection limit, the participants consumed alcohol to reach a blood alcohol concentration of 0.6 g/kg or 0.75 g/kg. Blood was collected as dried blood spot samples on each day of drinking as well on the following three days. The samples were analysed for PEth by LC-MS/MS using a validated method. **Results and Discussion:** After the abstinence period, all participants had initial PEth concentrations below the limit of detection. In the first drinking trial, PEth concentrations in nine of twelve participants reached a maximum of more than 20 ng/mL at a concentration of 28.8 ng/mL, whereas in the second trial, PEth concentrations in 7/12 participants reached a maximum above 20 ng/mL at 44.9 ng/mL. Most of the maximum concentrations (19/24) were achieved during the day of drinking. PEth concentrations then rapidly decreased leading to values < 20 ng/mL within one to three days in most of the samples (21/24).

Conclusion: We observed formation of PEth with concentrations exceeding the quantification limit of 10 ng/mL after ingestion of alcohol leading to blood alcohol concentrations of 0.47 and 0.68 g/kg. This makes PEth a promising marker to monitor controlled moderate consumption of alcohol as well as for abstinence control, with limitations regarding the short detection window.

V15 Calibration transferability between dried blood spot sampling devices for accurate quantification of phosphatidylethanol

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Aims: The direct alcohol marker phosphatidylethanol (PEth) is commonly analysed using dried blood spots (DBS) due to the increased stability compared to whole blood. There are many different commercially available sampling devices for DBS but data on their comparability is scarce. The aim of this study was to develop and validate a LC-MS/MS method for the quantification of PEth 16:0/18:1 and PEth 16:0/18:2 for three different DBS sampling systems: Whatman® filter paper (903™ Protein Saver Cards), Mitra® and Capitainer®B Vanadate. The transferability of calibration curves and their applicability across these devices should be assessed. **Methods:** PEth was extracted from DBS using a water/2-propanol mixture and n-hexane for liquid-liquid extraction. Samples were analysed by LC-MS/MS. Validation of the method was carried out according to the GTFCh guidelines. External and in-house quality controls (QCs) were applied to the sampling devices and analysed using the device-specific calibration curves. Each QC was also quantified using the calibration curves of the other devices to assess the cross-applicability of calibrations across the DBS sampling systems. **Results and Discussion:** DBS of all devices could be prepared and analysed using the same procedure. Validation was successful for the devices. Accuracy of QCs was best if quantified using the matching DBS sampling device calibration curve and thus met the criteria of the GTFCh guideline (bias < 15 %). Higher but still acceptable bias values were observed when using a Whatman® calibration curve to quantify DBS of the Capitainer®B Vanadate system and vice versa. Mitra® QCs should only be quantified using device-specific calibration curves as in all other cases, accuracy exceeded the limit of 15 %. **Conclusion:** We recommend analysing samples using device-specific calibrations at all times as determined PEth concentrations might be crucial for patients and should not be subjected to any avoidable uncertainty.

V16 Identification of recently emerging (semi-)synthetic cannabinoids and related products sold in shops and vending machines

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Aims: Increasing numbers of (semi-)synthetic cannabinoids (mostly derived from THC) have recently been recorded in the EU project NETZWEK ADEBAR. Corresponding new products like flowers or vapes/liquids are marketed under fictitious or unintuitive names via online shops or even public vending machines, yielding no information on the active compounds. Within the project, the structures of new substances were elucidated, the composition of several of the aforementioned products has been clarified so far and the data provided. **Methods:** Analysis and characterization of samples included mainly GC-MS, LC-MS, GC-sIR and NMR. About

ten products have been analysed so far. **Results and Discussion:** The identified substances derived from the THC structure showed modifications like the variation of the alkyl side chain and new substitutions on the cycloaliphatic ring or the resorcinolic hydroxy group. For example, THC-methylcarbonate was identified in vapes labelled as "THP 420". Hydroxylated HHC and HHCP derivatives were also identified, usually sold under the according labels. However, further samples labelled as 10-OH-HHC(P) have been sent in which contained "THP420" or other, yet unidentified substances instead. In other products, we could identify halogenated cannabinoids such as Br-HHC-O and Cl-HHC-O. While we found Br-HHC-O in a vape labelled "CBG9", another "CBG9" flower product contained a yet to be identified compound, but no halogenated HHC-O at all. Another common cannabinoid product is "CB9" (in our case flowers) in which we could identify a mix of H4CBD, HHCP and THC-C8. It remains yet to be seen if other "CB9" products will have a different composition. Additional products labelled "TRC-5" or "CBH" are current subjects to investigation. **Conclusion:** With the elucidation of structures and compositions as well as complementation of databases, we contribute to a better knowledge of new cannabinoid products. This is of great impact on the legislative efforts against drug marketing. An increasing number of products is sold not only via online shops, but also in vending machines in public areas. These products not only contain new substances which circumvent the New Psychoactive Substances Act, but also substances already covered by the law with false labels to conceal their presence in the products, making it important to know the composition and therefore the danger of these products.

V17 Shining light on "semi-finished" ("DIY") kits for synthetic cannabinoids: Investigation of a Swiss clandestine production site, seized samples from casework and prisons, and the CB_1 activity of the precursors and final products

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Aims: Recent observations indicate that potent synthetic cannabinoid receptor agonists (SCRAs) prevalent prior to the Chinese class-wide ban in 2021 have remained on the market, despite being covered by the new legislation. This is likely the result of a new production route, where uncontrolled tail-less precursors are shipped from China and converted into the desired controlled SCRA via a one-pot synthesis. This study aimed to shine light on this new trend by reporting on a Swiss clandestine laboratory, replicating the synthesis using varying reaction conditions, presenting seizure data from the US and Scotland, and investigating the activity of precursors and final products at the cannabinoid receptor 1 (CB_1). **Methods:** Seizures were characterized using GC-MS and ATR-FTIR. The seized chemicals at the clandestine laboratory and a handwritten note found on-site were compared to online instructions. The one-pot synthesis was replicated for MDMB-4en-PINACA (MDMB-INACA precursor) and ADB-BUTINACA (ADB-INACA precursor) at varying temperatures (room temperature vs. 70 °C) and reaction times (5 h vs. 10 h). The activity at CB_1 was assessed using two *in vitro* bioassays. **Results and Discussion:** The equipment and chemicals found at the clandestine laboratory are

consistent with online instructions. In the US and Scotland, various SCRAs have been detected with remaining precursors, likely resulting from incomplete reactions from this production method. The CB_1 activity of the precursors is limited compared to the final products. The presence of precursors does not compromise the activity of the mixture at CB_1, where ultimately the final SCRA determines the activity. Replications of the syntheses were successful but resulted in differing end product characteristics (oils, waxy materials, powders). **Conclusion:** Semi-finished kits have become a popular choice for SCRA synthesis, leading to the continued presence and re-emergence of previously prevalent potent SCRAs despite the Chinese ban.

V18 Synthetic cannabinoids and their transesterification products in e-liquids – An investigation of their formation and kinetics

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Aims: A relatively sparsely investigated consumption form of synthetic cannabinoids (SC) are vape- or e-liquids for electronic cigarettes or vaping devices. During GC-MS analysis of an e-liquid, a number of potentially "new" SCs were detected. Upon LC-HRMS analysis, the "new" SCs turned out to be the transesterification products of a number of known SCs present in the sample, with 1,2-propanediol, the carrier material of said sample. Aim of this project was the elucidation of the conditions for the formation of SC-transesterification products and the underlying kinetics. **Methods:** Solutions of the SCs MDMB-4F-BINACA, ADB-4en-PINACA, MDMB-4en-PINACA, ADB-CHMINACA and MDMB-CHMICA in 1,2-propanediol were prepared and incubated under different conditions: Room temperature and 45 °C, in glass vials or authentic plastic bottles, and with or without addition of nicotine benzoate or sodium hydroxide. For kinetic evaluation, samples were collected at different time-points, analysed using LC-MS/MS and the reaction constants calculated. **Results and Discussion:** Comparing the formation of SC-transesterification products, dubbed "1,2-propanediol-dimethyl-butyric acid"-SCs or "PGDMB"-SCs, the reaction constants varied under the conditions observed: A two-fold increase in reaction constant was observed at increased temperature, the addition of basic additives led to a ten- to one million-fold higher reaction constant. However, the storage in authentic plastic bottles lowered the reaction constant. Additionally, the formation of PGDMB-SCs was approximately a thousand-fold faster in SCs with methyl ester-containing linked groups (MDMB-type SCs) compared to SCs with amide-containing liked groups (ADB-type SCs). **Conclusion:** SC-transesterification products can easily and quickly be formed in e-liquids if the correct conditions are met, e.g. MDMB-type SCs, basic additives and elevated temperature. Comparing different SCs, the more hydrolysis-prone MDMB-type SCs also undergo the transesterification faster than the ADB-type SCs.

V19 Chemical analysis of recreational products containing semi-synthetic cannabinoids in Germany

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Aims: Since the first identification of hexahydrocannabinol (HHC), various products have emerged, stating HHC and its structural analogs as the main active ingredients. The quality and content of these products are not regulated, leading to potential discrepancies between advertised and actual content, as well as the presence of impurities from synthesis. This study aimed to characterize semi-synthetic cannabinoid (SSC) products available in Germany to determine their major, minor, and trace chemical components. **Methods:** In 2023, 42 test purchases were conducted in online and local stores, and the products were qualitatively screened for the main active ingredients and other components using GC-EI-MS. The HHC content was quantified, and ICP-OES was applied to test for the presence of heavy metals. **Results and Discussion:** In products labeled as containing HHC (n=14) or H4CBD (n=10), the main compound was analytically confirmed in 86 % and 90 % of cases, respectively. One HHCP product contained primarily HHCP, while most HHCO products contained only trace amounts of the advertised content. Diastereomeric ratios of SSCs varied significantly across all products, likely due to different synthesis procedures. Many unidentified impurities were found to be structurally related to phytocannabinoids or the SSCs present as the main component of each sample, indicating they are likely synthesis byproducts. Besides triethyl citrate and 2-isopropyl-N,2,3-trimethylbutanamide, trioctanoin, no unexpected or potentially harmful chemicals, nor elevated levels of heavy metals, were detected during the screening. **Conclusion:** Widely differing formulations and discrepancies between advertised and actual content were observed. Although some samples contained unidentified impurities, particularly harmful chemicals or heavy metals were not detected. HHCP and HHCO products were frequently mislabeled (86 %). A false declaration might result in unexpectedly strong psychoactive effects and adverse events.

V20 Development of a novel risk score reflecting the relative harm potential of synthetic cannabinoids (SCRAs), based on prevalence estimates, well documented intoxication cases and basic pharmacological data

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Aims: This study aimed to develop a risk score to assess the relative harm of twelve different SCRAs. The score was formulated based on SCRA prevalence estimates in Germany and thoroughly documented intoxication cases. Furthermore, calculated scores for each SCRA were analysed in relation to their pharmacological data (affinity and activity). **Methods:** The risk score links prevalence data from routine case samples (blood/serum and urine) analysed between 2013 and 2021, clinical data (symptoms and their severity) from a prospective study and the Poison Severity Score (PSS) and Toxicological Significance Score (TSS). The human cannabinoid receptor 1 agonist affinities and activities of the SCRAs were determined using a competitive radioligand binding assay with [^{3}H]CP-55,940 and the functional [^{35}S]GTPγS assay. **Results and Discussion:** In the study period from 2013 to 2021, 9,929 serum/blood and 45,464 urine samples were analysed for SCRAs, of which 1,633 serum/blood and 8,030 urine samples were positive for one or more SCRA. 50 non-fatal intoxication cases with clinical symptoms were included in this study. The developed risk score consisted of three parts, with the first part considering their prevalence in routine case samples in relation to the occurrence of intoxication cases in a certain time-frame. Part 2 and 3 of the score are the PSS and TSS, to account for the extent to which each substance contributed to the observed clinical symptoms and consequently to the overall intoxications. The risk score ranged from 9.9 (5F-PB-22) to 3.1 (5F-Cumyl-PEGCACLONE). No correlation could be established between the risk score value

and the receptor affinity or activity of a SCRA. **Conclusion:** Our findings confirm that *in vitro* data cannot be directly extrapolated to *in vivo* scenarios, highlighting the complexity of xenobiotic absorption, distribution, and metabolism. The newly developed risk score offers an innovative approach to evaluate the risks of emerging SCRAs using routine forensic analysis and intoxication case data.

V21 Analysis of nitrous oxide in blood by HS-GC/MS

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Aims: Abuse of nitrous oxide (N_2O) is associated with serious health hazards including road traffic accidents. Paraphernalia (cartridges, bulbs, balloons) are increasingly found in cars and suggest that driving under the influence of "laughing gas" takes place. A validated methodology was introduced to document exposure to N_2O by blood analysis. **Methods:** 1.0 mL of whole blood was sampled in a 20 mL HS-Vial, spiked with internal standard (n-pentane) and tempered to 60°C. A HS-GC/MS system equipped with a 15 m x 0.32 mm PLOT column was used for gas analysis. After separation from isobaric carbon dioxide, N_2O was detected by mass spectrometry and quantified in SIM mode. Calibration of the assay ranged from 0.01 to 10 mL/L. Blood samples taken from suspected (n=55) and non-suspected (n=100) drivers were analyzed by the procedure. **Results and Discussion:** Validation parameters including accuracy, interferences, linearity of calibration, in-process stability and matrix effects complied with international standards. Interday and intraday CV was < 10 %. This method enables detection of N_2O at atmospheric level with a lower limit of quantification (LLOQ) of 0.01 mL/L in whole blood. N_2O concentrations in blood samples from non-suspected drivers were below the LLOQ without exception. Choosing a reporting threshold of 0.1 mL/L, the majority (n= 36 / 65 %) of suspected drivers tested positive and blood concentrations ranged from 0.1 to > 10 mL/L (median: 1.2 mL/L). The individual time periods from incident to blood sampling in positive, informed cases (n = 15) averaged 75 minutes (range: 35-133 min) and were not related to individual N_2O blood concentrations. **Conclusion:** Detection of N_2O in blood samples is possible by a simple HS-GC/MS procedure with a minimum of sample preparation. Despite an elimination half-life of a few minutes as often reported in literature, the gas can be found in blood samples up to hours after consumption, so the presented methodology is suitable to document exposure in cases of driving under the influence of "laughing gas".

V22 Individual application patterns of Cannabis-based medicines in Germany – Descriptive evaluation of a patient survey and discussion from a forensic perspective

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Aims: Recently, an increasing number of countries have legalised cannabis for medical purposes. This can lead to problems with other legal regulations. For example, some countries grant exemptions for cannabis patients from restrictions on driving under the influence of drugs. However, this requires specific medical and legal assessments regarding the participation of cannabis patients in road traffic. Statistics on medical cannabis patients would be helpful to

assess related problems, but these are very incomplete in Germany. **Methods:** A cross-sectional, anonymous patient survey was carried out nationwide in the first quarter of 2022 using an online questionnaire. The overall collective (n = 1030) was evaluated with regard to application patterns of cannabis-based medicines. **Results and discussion:** Taking into account patients with health insurance prescription and, for the first time, self-payers, a high proportion of cannabis flower patients was observed (89.9 %). On average, the intake of flowers is associated with substantially higher daily THC doses (336 mg) compared to the usage of other cannabis-based medicines (≤ 17 mg). Additionally, 16.2 % of patients reported complex usage patterns consisting of combinations of different types of cannabis-based medicines. Over a quarter (28.4 %) of respondents stated smoking of cannabis flowers. A significant proportion of these patients had been in treatment for over 5 years, which could indicate a pre-experience with cannabis and a lack of interest in the medically recommended use of a vaporiser. **Conclusions:** Application patterns, predominantly high-dose inhalation of cannabis flowers, are in substantial contrast to current medical guidelines, advising a low-dose intake of oral preparations. In some cases, doubts may arise relating to an 'intended use' and misuse may be suspected. Descriptive information on individual application patterns of cannabis-based medicines provide a valuable source of information for medical and legal statements as well as a basis for further research projects.

V23 The influence of cannabis legalization: A retrospective study of THC-related traffic accidents

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Aims: The aim of this retrospective study was to assess if the number of Δ9-tetrahydrocannabinol (THC)-associated traffic accidents changes following introduction of the German cannabis legalization in April 2024. **Methods:** The in-lab database was filtered to include only police cases from selected regions of North Rhine Westfalia with regard to “traffic accidents” and either “§ 315c StGB” or “§ 316 StGB”. Cases involving cars, bikes, and small electric vehicles were all considered. Only accidents from April 1st to December 31st in 2023 and 2024, were included, with the final dataset being completed by December 31st 2024. In addition to the toxicological findings including legal and illegal drugs, information from police and medical reports as well as the cause of the accident were evaluated. **Results and Discussion:** So far, a total of 332 traffic accidents in 2024 have been submitted for drug and alcohol analysis (270 cases in 2023). In 13 of these cases, neither alcohol nor drugs were detected (12 cases in 2023), whereas in 193 cases, consumption of cannabis and either other drugs or alcohol was confirmed (148 cases in 2023). In 58 accidents during the same period in 2024, only cannabinoids were detected (38 cases in 2023). Compared to 2023, the mean THC concentration in these incidents increased from 4.4 to 6.4 ng/mL, while THC-COOH levels remained stable at approximately 73 to 81 ng/mL. Since the time between the accident and blood sampling did not change, this may indicate a trend toward a decreased ability to separate consumption and driving. **Conclusion:** Following cannabis legalization, there seems to be a trend towards more accidents under cannabis alone with an increase in blood THC concentration. The current observation period is still very short and further developments should be monitored critically.

V24 Cannabis legalisation “à la Luxembourgeoise”: First lessons after our one-year experience

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Aims: With the law enforced on 21st July 2023 in Luxembourg, the growth of up to 4 cannabis plants per household with no restrictions regarding THC content has been permitted and the consumption of cannabis for adults has been allowed in their residence only. However, possession and consumption of cannabis in the public domain are still prosecuted and no changes in the legal limits for driving under the influence (DUI) of THC (1 ng/mL serum) were implemented. This study aimed at determining the effectiveness of these legalisation measures by evaluating quality and quantity of products found on the market and consumption trends in DUI cases before and after legalisation. **Methods:** Data from one year before (21 July 2022-20 July 2023) and one year after the law came into force (21 July 2023-20 July 2024) was collected from the Forensic Medicine Department in Luxembourg. Investigated parameters included quality and quantity of seized illegal cannabis and hashish samples, the cannabis biomarker THC-COOH in wastewater, the number of THC-related DUI cases and THC and THC-COOH concentrations in respective blood samples. **Results and Discussion:** A decrease of 11 % was observed in seized drug samples, while a slight increase in THC content of flowers (+1,3 %) was shown. In wastewater, THC-COOH was present in all samples analysed, with a small decrease of THC-COOH content (not statistically significant). A general increase in THC-related DUI cases was observed (+22,0 %), with no statistically significant changes in THC serum concentrations but a significant increase in THC-COOH serum concentrations (+23,7 %). **Conclusion:** Although the decline in the illegal drug market reflects the anticipated effects of decriminalisation, the rise in DUI cases indicates increased cultivation and consumption of cannabis. The elevated THC-COOH levels, in connection with unchanged THC concentrations, suggest an increase in the number of regular cannabis users, but not in the quantity consumed. Monitoring cannabis seizures, DUI cases and THC metabolites in wastewater over longer periods is necessary to better understand the effect of cannabis legalisation.

V25 Reliability of roadside pre-tests for the new Δ^9-tetrahydrocannabinol serum cut-off in Germany

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Aims: The legalization of cannabis in Germany, which came into force in June 2024, also led to an increase in the THC serum cut-off for road traffic offences under Section 24a. Hamburg police carries out an annually large-scale check for drugs and alcohol in road traffic (DiS days), using urine tests to identify drivers under the influence of drugs (DUID). In order to detect acute cannabis consumption, the interdisciplinary cannabis expert group recommends the use of highly sensitive oral fluid tests. **Methods:** For this reason, as part of the DiS-days Hamburg in 2024, people suspected of being DUID were offered voluntary participation in oral fluid tests in addition to a preliminary urine test and subsequent blood sample. The THC results in the serum were compared with pre-tests from oral fluid and urine. **Results and Discussion:** A total of 41 people consented to the additional oral fluid tests. All three oral fluid tests were positive in 18 cases. Of these cases, the THC values in the blood serum were ≥ 3.5 ng/mL in 12 cases,

THC ≥ 1 ng/mL and < 3,5 ng/mL in two cases and THC ≤ 1 ng/mL in one case. The THC serum tests were negative in two cases, i.e. the oral fluid tests were false-positive here. In total, THC levels in the blood serum ≥ 3.5 ng/mL were determined in 15 cases for whom oral fluid tests were carried out. However, the oral fluid tests were negative in 3 cases and thus produced a false-negative pre-test result. **Conclusion:** With regard to the new THC serum cut-off, all three oral fluid tests examined in this study have a sensitivity of 80 %. Due to the impact on road safety, the choice of a suitable roadside oral fluid test device must be made carefully.

V26 Sweat analysis of medical opioids and metabolites in critically ill pediatric patients

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Aims: Sweat is a promising, non-invasive matrix in forensic toxicology and arguably contributes to the contamination of hair, posing a challenge for hair analysis. The objective of this study was to investigate sweat samples from a cohort of 112 children (up to 13 years) who were treated with opioids in a clinical setting. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) will be employed for this purpose, targeting fentanyl and analogues (alfentanil, sufentanil, remifentanil), traditional opioids (e.g., morphine), and selected metabolites (norfentanyl, β-hydroxyfentanyl, 4-ANPP, norsufentanil, hydromorphone). **Methods:** This non-interventional study was performed in collaboration with the University Children's Hospital Zurich. Sweat was sampled using forensic swabs and consecutively extracted using a two-step methanol-based protocol. An LC-MS/MS (QTRAP® 7500) method in MRM, ESI positive mode was developed, validated and applied to the sweat samples. **Results and Discussion:** The validated method showed good sensitivity (LLOQ: 1 to 10 pg/swab) for the analytes in focus. All targeted opioids and metabolite were detected, which represents a premiere in sweat analysis for children. Fentanyl was found in 95 (98 %) of 97 treatment involving cases (1.0 - 3595 pg/swab) and sufentanil in 64 % (2.2 - 73.9 pg/swab). The fentanyl analogues alfentanil and remifentanil were only occasionally detected, at concentrations below the LLOQ. The highest concentrations (ranging from 11.9 - 1169 pg/swab) were measured for morphine, which was detected in 97 % of cases. The measured opioid concentrations in the sweat samples appear to follow a trend in correlation with the administered opioid doses. **Conclusion:** A sensitive and specific LC-MS/MS method was developed and validated for simultaneous quantification of medical opioids in sweat and applied to samples of a clinical study. This study enhances understanding of sweat's contribution to hair contamination thus supporting applications in forensic and clinical contexts, including therapeutic drug monitoring.

V27 Frequency of positive drug findings in pregabalin positive hair samples

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Aims: An increase in detections of pregabalin has recently been observed in routine work. Therefore, the frequency of positive drug findings in pregabalin positive hair samples analyzed

in criminal proceedings was evaluated. **Methods:** A total of 5840 hair samples from January 2020 - October 2024 that have segmentally been analyzed for drugs of abuse (cocaine, opiates, opioids [methadone, buprenorphine, tilidine, tramadol, fentanyl, oxycodone], ketamine, anticonvulsants [gabapentin, levetiracetam, pregabalin], amphetamine and methamphetamine, ecstasy, methylphenidate, benzodiazepines [diazepam, nordazepam, oxazepam, bromazepam, alprazolam, lorazepam, flunitrazepam], z-drugs [zolpidem, zopiclone] and cannabis) were selected if positive for pregabalin (≥ 0.01 ng/mg). A total of 1134 hair segments belonging to 641 cases met the criteria. The positivity rate, the concentration range and the combination with other drugs were evaluated over the years. **Results and Discussion:** The frequency of pregabalin rose from 8.5 % in 2020 to 19.5 % in 2024 whereas its median concentration remained constant (ca. 0.1-0.2 ng pregabalin/mg hair. Most cases were also positive for other drug classes: cocaine 62 %, opiates 41 %, opioids 65 %, ketamine 18 %, other anticonvulsants 24 %, amphetamines 36 %, ecstasy 37 %, methylphenidate 11 %, benzodiazepines 40 %, z-drugs 13 % and cannabis 60 %. About 75 % of the segments analyzed were positive for 2-6 additional substances with the majority at 4 substances (18 %). Less than 15 % had more than 6 additional substances. Only 21 segments belonging to 16 individual cases were solely positive for pregabalin, whereas in two of these cases additional substances were found in segments negative for pregabalin. **Conclusion:** In most pregabalin-positive cases, a combination with multiple other drugs was observed whereas only in about 2 % of the analyzed cases solely pregabalin was present. Among multidrug users, not only opiates/opioids as sometimes expected were combined with pregabalin but esp. also cocaine, amphetamines, benzodiazepines and cannabis.

V28 Frequency of new psychoactive substances (NPS) in hair samples analyzed in criminal proceedings

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Aims: Contrary to popular belief that new psychoactive substances (NPS) are difficult or impossible to detect analytically, comprehensive screening strategies can now be offered in appropriately equipped forensic toxicology laboratories. Previously, analyses for NPS were generally only requested in cases of reasonable suspicion. However, now that investigating authorities are aware of the possibilities, orders are regularly issued in criminal cases, primarily for hair as test material. The aim of the study was to determine the frequency of NPS in hair samples analyzed in criminal proceedings between January 2023 and September 2024. **Methods:** After suitable segmentation of the hair samples, they were extracted with methanol in an ultrasonic bath. Thereafter, an aliquot of the solution was evaporated to dryness under nitrogen and reconstituted in buffer solution. For the analysis of designer drugs, new synthetic opioids and designer benzodiazepines, liquid chromatography-quadrupole-time-of-flight mass spectrometry (LC-QTOF-MS) was used. To test for synthetic cannabinoids, liquid chromatography-tandem mass spectrometry (LC-MS/MS) was performed. **Results and Discussion:** Of the 405 cases being ordered to test for NPS between January 2023 and September 2024, a total of 171 cases (42.2 %) screened positive for NPS. Synthetic cannabinoids tested positive in 153 out of 390 cases (39.2 %), whereas designer drugs were positive in 31 out of 236 cases (13.1 %). In 16 cases, additional testing for new synthetic opioids and designer benzodiazepines was requested with 2 cases (12.5 %) being positive for designer benzodiazepines. **Conclusion:** The results of this evaluation indicate that NPS are frequently detected in hair samples analyzed in criminal proceedings. Besides the analyses for common illegal drugs and pharmaceutical substances, additional testing for NPS provides further helpful information for examining the consumption behavior of the accused. Such an examination is not only important for deter-

mining the sentence but is particularly interesting when the question of placement in an addiction treatment facility according to § 64 of the German Criminal Code arises.

V29 Metabolic fate of the MDMA prodrug MDMA-tryptophan – A multi-model approach

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Aims: 3,4-Methylenedioxymethamphetamine (MDMA) has not yet been globally approved for therapeutic use due to concerns about safety and abuse potential. However, prodrugs of MDMA may offer a solution to these concerns. This study investigated the metabolic fate of MDMA-tryptophan (MDMA-Trp) in five different *in vitro* and *in vivo* models including pooled human liver S9 fraction (pHLS9), pooled human liver microsomes (pHLM), zebrafish embryos (ZE), fresh human plasma (FHP), and one human subject after microdosing (HMD). **Methods:** MDMA-Trp metabolism was investigated by incubating 2.5 µM MDMA-Trp for 60 and 360 min in pHLS9 and 0.5 µM for 60 min in pHLM. ZE were exposed to 100 µM via medium for 24 h, FHP was incubated at 0.5 µM for 240 min, and HMD was done by oral application of a dose equivalent to 100 µg of MDMA. Urine was collected for 24 h and prepared using solid-phase extraction with and without conjugate cleavage. All samples were analyzed by reversed phase liquid chromatography coupled to high resolution tandem mass spectrometry. **Results and Discussion:** Cleavage of MDMA-Trp to MDMA could be observed for pHLS9, ZE, and HMD but not for pHLM and FHP. This might be explained by a reduced pattern of metabolic enzymes in pHLM/FHP compared to pHLS9. Several further metabolites of MDMA-Trp and/or MDMA were identified throughout all experiments, including those after hydroxylation, N-demethylation, and demethylenyl+methylation. Compared to metabolic pathways of MDMA, neither sulfonation nor glucuronidation could be detected. **Conclusion:** This study demonstrated that MDMA-Trp undergoes cleavage and further metabolism but also direct metabolism, suggesting that MDMA-Trp might be considered as alternative to MDMA for its therapeutic use. Data also showed that an ingestion of MDMA-Trp can be analytically distinguished from a MDMA intake using MDMA-Trp specific metabolites.

V30 Hepatic metabolism of MDMA analogues: Toward safer therapeutic alternatives?

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Aims: 3,4-Methylenedioxymethamphetamine (MDMA) has demonstrated considerable therapeutic potential for the treatment of depression and post-traumatic stress disorder. However, its abuse and cytotoxic effects (mostly by formation of a catechol after metabolic O-demethylenation) present considerable limitations, necessitating the investigation of structurally related analogues with probably enhanced safety profiles. This study investigated the *in vitro* hepatic metabolism of the MDMA and 3,4-methylendioxyamphetamine (MDA) analogues SDMA (1-(1,3-benzoxathiol-5-yl)-*N*-methylpropan-2-amine) and SDA (1-(1,3-benzoxathiol-5-yl)propan-2-amine), focusing on metabolic stability, isozyme-mapping, and phase I/II metabolism.

Methods: *In vitro* metabolic stability was assessed by incubating 0.5 µM SDMA, SDA, MDMA, or MDA in pooled human liver microsomes at different time intervals. Qualitative hepatic metabolism was analyzed by incubating 25 µM SDMA or SDA with pooled human liver S9 fraction for one and six hours. Monooxygenase activity screening was conducted at 25 µM SDMA and SDA using eleven recombinant isoenzymes. Samples were analyzed by liquid-chromatography coupled to high-resolution mass spectrometry (Thermo Fisher Q Exactive). **Results and Discussion:** SDA exhibited the shortest *in vitro* half-life (61 min), followed by SDMA (71 min), while half-lives of MDMA and MDA exceeded 150 min. Phase I reactions for SDMA and SDA included hydroxylation, N-oxygenation, and S/O-demethylenation. Methylation and *N*-acetylation were observed as phase II reactions for both analogues. Furthermore, sulfation was observed for SDMA. These pathways exhibited similarities to those known for MDMA. Monooxygenase activity screening revealed that CYP1A2, CYP2C19, CYP2D6, and CYP3A4 are the predominant isoenzymes involved in biotransformation of both analogues, which again exhibited similar patterns to those for MDMA. **Conclusion:** Based on *in vitro* half-lives, SDMA and SDA should undergo enhanced *in vivo* clearance compared to MDMA and MDA. Consequently, these analogues may be considered as alternatives to MDMA in future studies. Furthermore, their metabolites could serve as analytical targets for drug screening in case of abuse. Isoenzyme polymorphisms and drug interactions need to be considered.

V31 Metabolic fate of methoxy- and methylthiocathinones - Studies by means of HepaRG cells and hyphenated mass spectrometry

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Aims: Stimulants are the largest group of new psychoactive substances, with synthetic cathinones accounting for the largest proportion. In this study, the in vitro phase I and II metabolism in HepaRG cells of three methoxycathinones (MO group) and three complementary methylthiocathinones (MT group) should be elucidated. Data should allow the recommendation of urine screening targets in analytical toxicology. The MO group included 4′-methoxy-N-ethylbuphedrone (4-MeO-NE-BP), 4′-methoxy-α-pyrrolidinobutiophenone (4MeO-αP-BP), and 4′-methoxy-αP-valerophenone (4MeO-αP-VP) and the MT group 4′-methylthio-N-ethylbuphedrone (4-MeS-NE-BP), 4′-methylthio-α-pyrrolidinobutiophenone (4MeS-αP-BP), and 4′-methylthio-2-morpholinopropiophenone (4MeS-αMor-PrP), respectively. **Methods:** Cell incubations were performed as monolayer assay. After 4 hours of cell preincubation, growth medium containing 25 µM of each compound and 0.5 % dimethyl sulfoxide was added (n=3 each) and incubated for 24 hours. The medium supernatant was precipitated, centrifuged and the supernatant analyzed by reversed-phase liquid chromatography coupled to high-resolution tandem mass spectrometry using full scan and subsequent data-dependent acquisition. **Results and Discussion:** Phase I reactions of all MO compounds primarily included O- and N-dealkylation, hydroxylation, oxidation, and combinations thereof. Similar phase I reactions were detected in the MT group, but with S-demethylation instead of O-demethylation. In total, 20 metabolites were identified in the MO group and 22 metabolites in the MT group. Formation of phase II metabolites (glucuronide and sulfate) were only observed after O-demethylation of 4MeO-αP-VP. Most abundant signals after incubation were besides the parent compounds O- or S-demethyl and hydroxy metabolites. The results for 4MeO-αP-VP were consistent with literature data.

Conclusion: Apart from the parent compound, the abundant phase I metabolites of both compound groups can be recommended as analytical urine screening targets, especially if sample preparation contains conjugate cleavage. Replacement of the 4′-substituents had no major effect on phase I metabolism. As glucuronidation and sulfation were only observed for 4MeO-αP-VP, the structural features that facilitate phase II metabolism should be further investigated.

V32 Characterization of synthesis intermediates and side-products in Δ^9-tetrahydrocannabiphorol and hexahydrocannabiphorol isolated from one product each

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Aims: The aim of the presented study was to identify the synthetic precursors used for the production of commercially available products containing hexahydrocannabiphorol (HHCP) and Δ^9-tetrahydrocannabiphorol (Δ^9-THCP). Additionally, the enantiopurity of the main compounds was analysed. A vape pen liquid containing 90 % Δ^9-THCP according to the label and a wax containing HHCP were analysed for this purpose. **Methods:** The samples were analysed by GC-MS to identify declared and undeclared compounds. For the isolation of the declared and undeclared compounds, the samples were fractionated by flash column chromatography. Structure elucidation was performed using various 1D- and 2D-NMR experiments. The enantiopurity of the isolated compounds was determined by GC-MS after derivatization with *R*- and *S*-Mosher acid chloride, respectively. **Results and Discussion:** Six compounds were isolated from the HHCP wax. Besides the labelled (9*R*)-HHCP, the side-products *iso*-HHCP, *cis*-(9*R*)-HHCP, *abn*-(9*R*)-HHCP and the synthetic intermediates to (9*R*)-HHCP and *cis*-*abn*-(9*R*)-HHCP were identified by NMR. The side-products were regio- and stereoisomers of (9*R*)-HHCP. Both of the synthetic intermediates were α,β-unsaturated ketones indicating that the precursor for the synthesis of this HHCP sample was also not aromatic. From the Δ^9-THCP sample Δ^9-THCP and 5-heptyl-1,3-resorcinol were isolated. The latter is a homolog of olivetol, which is a known precursor for Δ^9-THC. The chiral isolated compounds were enantiopure indicating a stereospecific synthesis. Additionally, compounds were detected, which probably result from overalkylation. **Conclusion:** Semi-synthetic cannabinoids with a side-chain other than pentyl have been synthesized from scratch and not from cannabidiol as many distributors claim as shown from the analysis of a Δ^9-THCP vape pen and a HHCP wax.

V33 Drug profiling of synthetic cocaine derivatives: Unveiling the risks and impurity profiles of research chemicals

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Aims: Synthetic derivatives of cocaine, originally designed to treat cocaine use disorders, are now emerging substances of abuse. This diverse class includes phenyltropanes, 1,4-dialkylpiperazines, and dimethocaine, with over 200 documented substances, often marketed as research chemicals. Troparil and dichloropane are prominent phenyltropanes, with their (-)-2β,3β-stereoisomers exhibiting the highest psychoactive potential. Synthesis involves stereoisomer mixtures and by-products depending on methods such as Grignard reactions or Suzuki coupling. This study develops an LC-MS/MS method to identify troparil and dichloropane stereoisomers and analyzes samples acquired online to determine their identity, purity, and concentration of

the respective substance, as well as impurities such as solvents. **Methods:** The (-)-2β,3β-, 2α,3β-, and 2β,3α-stereoisomers of troparil and dichloropane were synthesized and characterized as reference standards. So far, 12 authentic samples ordered online have been analyzed for identity, purity, content, and impurities using NMR, IR, HPLC-UV, and GC-FID. A novel LC-MS/MS method was developed to establish stereoisomeric profiles, utilizing the synthesized stereoisomers as references. Method development involved optimization of columns, solvents, and gradients to achieve baseline separation of stereoisomers. **Results and Discussion:** Half of the samples purchased online did not match the ordered substance; dichloropane was often delivered instead of troparil and vice versa. One sample contained an unidentified substance. Quantities specified frequently deviated from orders. Purity, determined by HPLC-UV, ranged from 82 % to 98 %, with all samples containing solvent residues, including methanol, isopropanol, acetonitrile, ethanol, and benzyl alcohol. The LC-MS/MS method could successfully be applied to separate the (-)-2β,3β-, 2α,3β-, and 2β,3α-stereoisomers of troparil and dichloropane. While troparil stereoisomers were resolved using a biphenyl column, dichloropane stereoisomers required a C18 column for baseline separation. **Conclusion:** Synthetic cocaine derivatives marketed as "research chemicals" pose public health and forensic challenges due to mislabeling, variable purity, and solvent residues. Our LC-MS/MS method offers a reliable tool for stereoisomeric profiling and will be used to analyze authentic samples in future.

V34 Analytical challenges and pitfalls in the identification of rilmazafone

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Aims: Increasing numbers of benzodiazepine prodrugs have been recorded in the EU project NETZWERK ADEBAR. Beside newly emerging prodrugs, cases of rilmazafone and difficulties in its identification accumulate. The analysis poses challenges and pitfalls when using GC-based methods as the degradation and formation of artifacts, including the corresponding benzodiazepine rilmazolam, while rilmazafone is not detected at all. We want to highlight these pitfalls and provide strategies to avoid false interpretation. **Methods:** Analysis and characterization of samples included mainly GC-MS, LC-MS, neat FT-IR and NMR. Eight samples, both tablets and powder, have been analysed so far. GC injection system: Thermo Trace 1310 SSL injector, 280 °C liner temperature, Trajan SGE inlet liners. **Results and Discussion:** We found that rilmazafone was not detectable by GC-MS, while the corresponding benzodiazepine rilmazolam as well as an artifact with the same molecular weight were, by a ratio differing from the workup conditions or instrument parameters. Under alkaline extraction conditions, no benzodiazepine or artifacts were detected at all. When preparing the sample in methanol (especially when handling the hydrochloride), notable peaks of rilmazolam and artifact could be found. The concentration of both rilmazolam and artifact might as well change depending on instrumental setup and parameters (e.g. injector system, see above). A strategy to verify the presence of rilmazafone requires the use of LC-MS, as no formation of rilmazolam was observed in this case. A second method to complement validation, especially for tablets, is neat FT-IR spectroscopy from an alkaline extract. **Conclusion:** The detection of rilmazolam or artifacts can cause false interpretation of data obtained from suspected rilmazafone samples which leads to false conclusions. The seamless identification of samples is of great importance for forensic reports and assessments as well as for legislation. We greatly incentivise getting familiar with cases like rilmazafone and rilmazolam to avoid false reporting and being attentive for the utilisation of alternative analytical methods for the successful and unambiguous identification of rilmazafone.

V35 Clinical toxicological follow-up analysis of a suicide attempt using amisulpride

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Aims: A patient was admitted to the emergency department after suspected intentional ingestion of a high dose of amisulpride. Observed cardiac complications included QT-prolongation, bradycardia, and subsequent torsade de pointes tachycardia. A gastroscopy including gastric lavage and activated charcoal therapy was performed due to the critically unstable conditions and remains of pills in the nasogastric tube. Five days after admission to the intensive care unit, the patient was extubated and transferred to a psychiatric hospital few days later. We report the results of the emergency toxicology screening and those of the follow-up analyses. **Methods:** The emergency toxicology screening consisted of the authors` systematic toxicological analysis (STA) approaches for plasma and urine based on gas-chromatography and liquid chromatography-high-resolution tandem mass spectrometry (LC-HRMS/MS). The stomach content was also analyzed for amisulpride later. Amisulpride concentrations in the initial and follow-up plasma samples (six in total) were determined using three-point calibration. Sample preparation for quantitation included dilution and liquid-liquid extraction followed by LC-HRMS/MS analysis. **Results and Discussion:** STA confirmed the assumed amisulpride intake by revealing the presence of amisulpride in patient`s blood and urine. Amisulpride was also detectable in the stomach content. The initial plasma concentration was determined to be 18 mg/L (therapeutic range is up to 0.4 mg/L) and was shown to be still increasing during hospitalization, most likely due to formation of pharmacobezoars. One hour after the gastroscopy and gastric lavage, the plasma concentration decreased, and a concentration of 1.0 mg/L was determined two days later. **Conclusion:** This report presents an acute amisulpride intoxication with severe cardiac and central nervous complications and a clinical toxicological follow-up over a period of two days covering six analyses in total. The successful clinical management included gastroscopy with gastric lavage and activated charcoal therapy based on a judicious individual case choice.

V36 2-Methyl-2-butanol – A new substance of abuse?

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Aims: The aim was to investigate and clarify the cause of death of a 26-year-old male found dead in his apartment. It was known that the individual used drugs of abuse. In the apartment, the police found a "brown powder" and a glass bottle labelled with "2-methyl-2-butanol" (2m2b). **Methods:** Autopsy und chemical analyses were performed. Headspace gas chromatography with flame ionization detection (HS-GC-FID) was used to determine 2m2b and ethanol. **Results and Discussion:** The autopsy revealed unspecific morphological signs of an intoxication (cerebral swelling and cyanosis, pulmonary edema, full urine bladder). Concentrations of 245 mg/L and 225 mg/L 2m2b could be detected in the femoral blood specimen and urine, respectively. Ethanol was detected below the level of quantification (0,2 g/L) in both matrices. The detected blood concentration of 2m2b was more than twice as high as the 2m2b concentration as

assigned as comatose in a previous published case report. However, in the presented case further analysis revealed toxic concentrations of methamphetamine and venlafaxine and also identified the intake of heroine. Thus, a combined drug intoxication was assumed as the cause of death. **Conclusion:** Little is known about the abuse of 2m2b as an alternative to ethanol. Since 2m2b is widely available, cheap, more potent than ethanol, while lacking its hangover effects and is not detected by routine HS-GC-FID methods for the detection of blood ethanol concentrations, it may be of forensic relevance.

V37 „Off-label use“ of the veterinary drug xylazine

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Aims: Xylazine is a veterinary drug used for sedation, analgesia and muscle relaxion, and is not approved for human use. However, xylazine has become a drug of abuse, particularly for people working in a veterinary context. Side effects of xylazine can be bradycardia, hypotension, hyperglycemia, central nervous system depression and respiratory depression. A case is presented in which xylazine was used to sedate someone. **Case history:** A 54-year-old woman caused a traffic accident by veering off the road. During police questioning she showed an uncertain orientation, anxiety and slowed coordination and speech. She was charged with driving under the influence of drugs. In the hospital, the woman voiced her suspicion that she had been administered a sedative drug, possibly xylazine. **Methods:** Blood and urine samples were toxicologically screened by GC-MS and results quantified with LC-MS/MS. **Results and Discussion:** Xylazine and its metabolites were identified in both specimens. The xylazine concentration in the serum sample was approximately 0.3 mg/L. The symptoms described were attributed to the effect of xylazine. However, it was neither possible to classify the xylazine concentration found in the serum sample with regard to the absorption route, the time of absorption and the dosage, nor to differentiate between an abusive self-intake or an external administration. Further criminal investigations proved an external administration of xylazine by the partner of the woman who worked as a veterinarian. By sedating her, he supposedly wanted to gain time to meet other women. **Conclusion:** Although xylazine is usually used as a veterinary drug, it can be administered orally to humans without being noticed due to its odorless and nearly tasteless nature. The effects correspond to those of other sedatives, making a clear diagnosis difficult without toxicological testing. In low concentrations, xylazine can be administered unnoticed for a long time, as another case has shown.

V38 A rare case of aconite poisoning via injection: Comparison of body fluid and organ tissue concentrations with oral ingestion

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Aims: Aconite, also known as monkshood, is a highly toxic plant in the *Aconitum* genus, with the blue monkshood (*A. napellus*) being common in Central Europe. All parts of the plant contain the diterpene alkaloid aconitine, one of the most potent plant toxins. In 2024, a middle-aged woman with a history of psychiatric issues was found deceased outside her apartment with injection marks. The police investigation raised suspicions that the injected substance may have been derived from the roots of blue monkshood. To this date, no comparable cases have been reported in the scientific literature. This study aimed to analyse the distribution of the alkaloid aconitine in body fluids and organ tissues following parenteral administration of monkshood