17TH MEETING OF
THE SOCIETY OF HAIR TESTING

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The Hospital for Sick Children,
Toronto, Ontario

TUESDAY, JUNE 26, 2012
SYMPOSIUM: THE USE OF HAIR ANALYSIS TO DETECT DRUG-RELATED RISK IN CHILDREN

1

NEONATAL HAIR ANALYSIS: THE CLINICIAN'S PERSPECTIVE
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BACKGROUND: This presentation will discuss the rationale for developing neonatal hair analysis as a tool for establishing prenatal exposure to drugs. The determination of clinical risk in neonates carries multiple challenges. The assessment of whether a drug may cause physical malformations or impact neurodevelopmental outcomes is complex and requires valid methods to qualify and quantify chronic exposure to xenobiotics. Additional consideration must be given to drugs of abuse and their impact on neonates due to the profound influence of the maternal-child relationship on early child development and the secondary maternal risks associated with abuse of illicit drugs.

2

HAIR ANALYSIS OF NEWBORNS AND CHILDREN TO DETERMINE MEDICAL AND SOCIAL RISK: THE EXPERIENCE OF BARCELONA HOSPITAL DEL MAR
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BACKGROUND: The Barcelona Hospital del Mar (the Sea Hospital) is located between Barceloneta and La Mina, two city areas with low socioeconomic status and a high percentage (more than 40%) of immigrants and local gypsies. Since 2001, the Paediatrics Unit decided to perform hair testing as complimentary analysis to urine and blood testing in newborns and children to disclose, verify or confirm prenatal and postnatal exposure to psychoactive drugs.

METHODS: We used hair testing to investigate the prevalence of prenatal exposure to maternal tobacco and we associated the obtained results to newborn neurobehaviour, with particular attention to nicotine withdrawal syndrome. We also associated hair nicotine in toddlers with incidence of lower respiratory tract illnesses during their infancy. With respect to drugs of abuse, we experimented with a protocol at the Emergency Ward of the hospital which included hair analysis all the times we had suspicion of intoxication by psychoactive drugs in babies, children and adolescents. Applying this protocol, we discovered chronic intoxication in babies and children acutely intoxicated by cocaine, methadone and amphetamines. Finally, we used hair testing to investigate the prevalence of unsuspected exposure to cocaine in a group of 90 children between 18 months and 5 years of age presenting to our paediatric emergency department without signs or symptoms suggestive of exposure. In 85 cases, hair samples from the accompanying parent were also provided.

RESULTS: Hair samples from twenty-one children (23.3%) were positive for cocaine with one sample also positive for MDMA and another for opiates. In 88% of the positive cases, cocaine was also found in the hair of the accompanying parent (15 out of 17 matched parent-child hair samples). The behavioural patterns with potential harmful effects for the child's health (e.g. tobacco smoking, cannabis, benzodiazepines and/or antidepressants use, shorter breastfeeding time) were significantly higher in the parents of exposed children,

CONCLUSION: In the light of these results, we advocated general hair screening to disclose exposure to drugs of abuse in children from risky environments attending our and other hospitals, which could provide the basis for specific social and health interventions.

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METHADONE AND ILLEGAL DRUGS IN CHILDREN HAIR – A MIRROR OF ENDANGERING DRUG USE IN CHILDREN'S ENVIRONMENT
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BACKGROUND: Children living with drug addicted parents are in steady danger of poisoning. In order to examine the exposure to drugs, hair samples from 134 children (age 1-12 years) living with parents substituted by methadone and/or suspected for illegal drug abuse, and from 96 of their parents in a German community were investigated.

METHODS: The hair samples were analysed by liquid chromatography-hybrid quadrupole time-of flight mass spectrometry (LC-QTOF-MS) for methadone, opiates, cocaine, amphetamines and benzodiazepines and by GC-MS for cannabinoids with limits of quantification LOQ of 10 pg/mg or lower.

RESULTS: From 134 children, only in 28 samples no drugs were detected. Cannabinoids were found in 48 samples, in 19 as the only drug. Methadone was identified 32times with additional exposure to hard drugs in 24 cases. Drug use in the children's environment was obvious for heroin in 39 cases, cocaine in 63 cases, amphetamines in 5 cases and benzodiazepines in 7 cases. The concentrations varied from LOQ to 2.16ng/mg methadone, 11.1ng/mg 6-acetylmorphine, 8.3ng/mg cocaine and 0.62ng/mg THC. Generally, hair from younger children contained higher concentrations than from elder children. Systemic incorporation of methadone and of cocaine appeared likely from detection of EDDP (11 cases) and nor-cocaine (15 cases). Within families, hair samples of children and parents provided often a similar pattern of drug exposure.

CONCLUSIONS: Investigation of children's hair proved to be a useful way to detect endangering drug use in their environment. The results lead to a more thorough inspection, and measures were taken to improve the situation of the children in many of the cases.

4

THE CROSSROADS OF MEDICINE AND CHILD ENDANGERMENT: EXPERIENCES IN CHILD HAIR ANALYSIS

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BACKGROUND: The use of hair as a toxicological matrix has historically been reported primarily in a forensic toxicology context. Drug exposure in children is a major topic of concern; in child endangerment cases, it is challenging to assess the level of risk due to passive exposures. In cases of pediatric overdose involving drugs of abuse, consideration is warranted to determine whether drug exposure was acute and isolated or chronic and possibly intentional. This presentation will explore the characteristics of drug detection in child hair analysis and highlight the investigation of several cases of pediatric overdose augmented by the use of hair analysis.

METHODS: Urinalysis was performed by LC-MS/MS and/or immunoassay. Hair samples were sectioned and analyzed by ELISA and/or GC-MS following headspace solid-phase microextraction where applicable. Aggregate data showing frequency of detected drugs in child protection cases will be reviewed as well as clinical cases involving children aged 5 to 20 months of age.

DISCUSSION/CONCLUSIONS: Cases involving exposure to cocaine, opioids, and cannabis will be discussed. In all cases, toxicological analysis of hair samples provided valuable supplementary information to clinicians or child welfare workers.

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<u>ORAL SESSION:</u> HAIR TESTING IN PAEDIATRIC POPULATIONS

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DETECTION OF MIDAZOLAM IN CHILDREN'S HAIR AFTER ADMINISTRATION OF A SINGLE DOSE

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BACKGROUND: In cases of child maltreatment or of Munchhausen by proxy, hair testing can be an important tool for retrospective monitoring of the ingestion or administration of drugs. The aim of this study was to determine midazolam concentration in children`s hair after single doses of midazolam.

METHODS: Participants of this study were volunteers of a group of children who had to undergo a medical intervention at Children's Hospital including benzodiazepine sedation by midazolam at a level of 0,5 mg/kg. After having

obtained informed consent of the parents, hair samples of 12 children were collected before (sample A) and few days after the intervention (sample B). In 7 cases, an additional sample C was collected between 6 to 10 weeks later by the parents. Segmental analysis of these hair strands was performed by our standard procedure: decontamination of the hair, pulverization and extraction, analysis by LC-MS/MS (Shimadzu prominence XR, ABSciex 5500QTrap, Phenomenex Kinetex C18, 2.6μm, 50/2.1, 5M Formate Buffer/5M NH4-Formate MeoH).

RESULTS: All samples A were negative. In 7 of the 12 B-samples midazolam was detectable. Two B-samples exhibited elevated values indicating that incorporation of midazolam into hair might have been mediated via sweat. In 6 out of the 7 C-samples traces of midazolam were detectable in the hair segments corresponding to the time window of the medical intervention. All concentration levels were below 3 pg/mg.

CONCLUSIONS/DISCUSSION: In children's hair, midazolam is detectable even after single administration. The concentrations levels are very low. Additionally incorporation from sweat can be traceable few days after the administration.

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MATERNAL HAIR ANALYSIS FOR THE DETECTION OF ILLICIT DRUGS, MEDICINES AND ALCOHOL EXPOSURE DURING PREGNANCY

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BACKGROUND: Drugs of abuse consumption throughout pregnancy is a serious public health problem, and an important economic cost to health system. The aim of this work was to compare maternal interview and hair analysis to determine drug consumption throughout pregnancy, and to study relations between maternal interview, hair results and neonatal outcomes.

METHODS: 209 mothers agreed to participate. After delivery, they were interviewed and a hair sample collected. Hair samples were segmented in trimesters, and analyzed for 35 drugs (opioids, cocaine, amphetamines, THC, ketamine, methadone, antidepressants, benzodiazepines and hypnotics; LOQ 5-100pg/mg) and for ethyl-glucuronide (LOQ 10pg/mg) by LC-MS/MS. Statistical analysis was performed with Chi-square Test and T-Test (SPSS software).

RESULTS: In the interview, 4.3% mothers declared using illicit drugs during pregnancy (cocaine 1.4%, THC 2.9%, opioids 1%), 3.3% medicines (methadone 1.9%, benzodiazepines 1.9%, antidepressants 0.5%), 21.5% tobacco and 9.1% alcohol. Hair analysis showed 15.4% prevalence in illicit drugs (cocaine 12.4%, THC 3.8%, opioids 1%, ketamine 1%), 22.5% in medicines (methadone 3.3%, benzodiazepines 11%, antidepressants 9.1%, zopiclone 1%, fentanyl 1.4%), and 3.9% in alcohol. Neonatal abstinence syndrome was developed in 8.1% newborns, all of them from mothers with high methadone positive results (>926.2pg/mg). Statistically significant lower newborn weight and length were only found in neonates from declared smokers compared with non-smokers (p<0.05).

CONCLUSION: Maternal hair analysis showed to be more sensitive than maternal interview to detect drug use during pregnancy, except alcohol. In this preliminary study, no statistically significant differences were found between exposed and non-exposed newborns, except for tobacco consumption.

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ALTERNATIVE MATRICES FOR COCAINE, OPIOIDS AND METHADONE IN UTERO DRUG-EXPOSURE DETECTION

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BACKGROUND: Drug determination in biological matrices from mother and newborn is an objective measure of maternal and fetal drug-exposure. The aim of this study was to compare mother-hair, meconium, umbilical cord and placenta for detecting in utero drug-exposure to cocaine, opioids, methadone and amphetamines.

METHODS: Mother-hair, meconium, umbilical cord and placenta were collected from 82 mother-newborn dyads. Mother-hair (segmented in trimesters) and meconium specimens were analyzed for cocaine, opioids, methadone and amphetamines. If either mother-hair or meconium had tested positive, umbilical cord and placenta specimens were analyzed. Analyses were performed by LC-MSMS.

RESULTS: In hair, 13 out of 82 participants tested positive; 11 to cocaine (cocaine 12-50,605pg/mg; benzoylecgonine 9-46,668pg/mg), 4 to methadone (89-26,845pg/mg), 1 to opioids (morphine 1,275-2,398pg/mg; codeine 261-914pg/mg; 6-acetylmorphine 6,061-15657pg/mg). In meconium, 3 out of 82 were positive; 3 to methadone (methadone 326-3,752ng/g; EDDP 5,957-25,179ng/g), 1 to cocaine (cocaine 7ng/g; benzoylecgonine 79ng/g; hydroxi-benzoylecgonine 135ng/g; ecgonine-methylester 56ng/g) and 1 to opioids (morphine 1,025ng/g; morphine-3-glucuronide 22ng/g; codeine 34ng/g). Placenta and umbilical cord were positive 3 out of 13 specimens; 3 to methadone in placenta (methadone 7-543ng/g; EDDP 10-51ng/g) and cord (methadone 17-183ng/g; EDDP 6-109ng/g); 1 to cocaine in placenta (benzoylecgonine 1ng/g; hydroxi-benzoylecgonine 2ng/g) and cord (benzoylecgonine 6ng/g); and 1 to opioids in cord (morphine-3-glucuronide 15ng/g; morphine-6-glucuronide 1ng/g). Meconium, placenta and umbilical cord only tested positive if hair concentrations were >SoHT cutoffs.

CONCLUSIONS: Mother-hair is the most sensitive specimen to detect drug consumption during pregnancy. Placenta and umbilical cord could be alternative to meconium to detect high in utero drug-exposure.

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<u>ORAL SESSION:</u> ALCOHOL BIOMARKERS

8

ETHYL GLUCURONIDE CONCENTRATIONS IN BEARD HAIR AFTER A SINGLE HIGH ALCOHOL DOSE: EVIDENCE FOR INCORPORATION IN HAIR ROOT

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BACKGROUND: Despite the growing importance of EtG in hair for detection of chronic excessive alcohol consumption, the mechanism of incorporation is not yet clear. Deposition from sweat is believed to be the main route. In order to get more information, EtG was determined in daily shaved beard hair after single higher alcohol doses.

METHODS: Three volunteers drunk within 5.5h 153, 165 and 200g ethanol followed by 30 days abstinence. Daily shaved beard hair was analyzed for EtG using a validated LC-MS/MS method with a limit of quantification LOQ of 2pg/mg.

RESULTS: For all three volunteers, small concentrations of EtG were already detected on the first day (9 h after end of drinking). The concentration increased to maxima of 182, 242 and 74pg/mg on days 2 to 4, and then gradually decreased to LOQ on days 8 to 10.

DISCUSSION: The time course of EtG is discussed based on data about the anatomic dimensions of the hair root, the physiology of hair growth, the kinetics of EtG formation and elimination in blood, and in comparison to literature data about drugs in beard hair. Deposition from sweat into the residual hair stubble after shaving is only possible in the infundibulum down to the sebaceous gland mouth and can lead to positive results only for 2-3 days after end of drinking. Therefore, for beard hair, the predominant part of EtG is incorporated within the hair root between papilla and isthmus leading to a positive zone of about 3 mm (8-9 days) after a single drinking event.

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A NOVEL ELISA METHOD FOR DETECTION OF THE ALCOHOL BIOMARKER ETHYL GLUCURONIDE IN HAIR

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BACKGROUND: Ethanol can be measured by means of various biomarkers in the body, the most direct of which is ethyl glucuronide (EtG). Monitoring sobriety for purposes of driver license reinstatement necessitates screening for EtG in hair, since it offers

a significantly longer half life than that afforded by either urine or blood.

METHODS: A proprietary hair extraction buffer (1 mL) was added to hair specimens (20 mg) and then sonicated at 60°C for 2 hours. Specimens (125 µL) were then pipetted in duplicate onto the microplate and incubated 30 min, followed by enzyme conjugate (60 min incubation). The plates were washed, then incubated with substrate (30 min) and then stopped with 1N HCl and read at 450 nm.

RESULTS: The cutoff of the assay was 30 pg/mg. The intra and inter-day precisions were <10%. The assay is EtG specific and shows no cross-reactivity with ethyl sulfate (EtS), ethanol, other alcohols or glucuronides. The assay was further validated with 18 hair specimens obtained from a clinical laboratory. One false negative was observed at the assay cutoff of 30 pg/mg, compared with LC-MS confirmation.

CONCLUSION: The described method is the first immunoassay screen developed for EtG in hair. The assay is sensitive, specific and precise and complies with the Society of Hair Testing cutoff of 30 pg/mg for chronic alcohol consumption.

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ETHYL GLUCURONIDE IN SCALP AND NON-HEAD HAIR: AN INTRA-INDIVIDUAL COMPARISON

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BACKGROUND: The quantitative determination of ethyl glucuronide (EtG) in scalp hair has become a useful aid in the retrospective assessment of alcohol consumption, providing a specific window of detection that depends on the length of the hair sample. The aim of this study was to assess the suitability of secondary hair for consumption-monitoring by intra-individual comparison of EtG concentrations.

METHODS: Scalp and secondary hair samples were obtained from 68 subjects, aged 21-68 years. Drinking behaviour over the preceding months was assessed with the AUDIT questionnaire. Based on the EtG concentration, scalp and secondary hair samples were allocated to one of three drinking categories using two threshold values: A cut-off of 5

pg/mg (>LOQ) separated "social drinking" from "negative", while the interpretation limit of 30 pg/mg recommended by the SoHT (2009 and 2011) was used to distinguish "chronic excessive consumption" from "social drinking". EtG-quantification was done by standard method (GC-NCI-MS/MS).

RESULTS: Ethanol daily intake (EDI) values ranged from 0.3 to 120 g alcohol per day. 11 cases were classified as negative, 30 as social drinking and 27 as chronic excessive consumption. Sensitivity and specificity of the drinking categories classification was calculated for both threshold values for the non-head hair specimens. These values are e.g. for chest hair 75%/100% (Sens./Spec., 5 pg/mg), and 86%/88% (30 pg/mg).

DISCUSSION/CONCLUSIONS: Taken overall, determining the EtG concentration in hair samples from different localizations (chest, arms, legs) represents a useful alternative in cases where scalp hair is not available. Chest hair is to be preferred as the alternative matrix for assessing alcohol consumption or abstinence.

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COCAETHYLENE AS A BIOMARKER IN HUMAN HAIR OF CONCOMITANT ALCOHOL AND COCAINE USE IN A HIGH-RISK POPULATION

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BACKGROUND: Cocaethlyene (CE) is formed during cocaine and alcohol co-consumption. CE is pharmacologically active, prolonging cocaine-related effects, and is found in human hair. To our knowledge, this is the first study to look at ability of using CE as a biomarker of heavy drinking in a suspected population.

OBJECTIVES: Determine if CE can identify chronic alcohol abusers.

METHODS: Participants were referred for hair testing by social workers, lawyers, and other professionals. Since September 1 2010, 588 samples were tested for alcohol and cocaine abuse. Cocaine, CE, benzoylecgonine, and fatty acid ethyl esters (FAEE), a biomarker of alcohol consumption were analyzed using GC-MS. Logistic Regression, Mann-Whitney Test, and a Chi-Square Test was performed on the data.

RESULTS: Out of 588 tests, 353 tests were FAEE negative. Of these, 103 were positive for cocaine use, representing 29.2% of the FAEE negative population. Of 235 FAEE positive tests, 99 were positive for cocaine use, representing 42.1% of FAEE positive results. The proportion of positive cocaine use was associated chronic alcohol consumption (P<0.05, OR 1.767). FAEE positive (P<0.05, OR 2.44) results were 2.44 times more likely to have a positive CE result, suggesting only a proportion of alcohol consumption occurred with cocaine. Additionally, negative FAEE (<0.5 ng/mg) results identified negative CE results 95.18% of the time with a positive predictive value of 0.66, indicating a positive CE will more than likely be positive for FAEE, with a low rate of false positivity.

CONCLUSIONS: Although only 42% of FAEE positive results were positive for cocaine use, the association was still significant as should be expected. This study demonstrated that a positive CE more than likely results in a positive FAEE result, the first to do so in a suspected population.

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COMPARATIVE EVALUATION OF ETHYL GLUCURONIDE AND FATTY ACID ETHYL ESTERS DATA IN HAIR UNDER THE ASPECTS OF GENDER, HAIR COSMETICS AND ABSTINENCE ASSESSMENT

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BACKGROUND: Hair cosmetic is one of the most serious obstacles of a reliable use of hair alcohol markers. In this study, data from routine analysis of ethyl glucuronide (EtG) and fatty acid ethyl esters (FAEE), mostly in child custody cases, were evaluated with respect to cut-offs in abstinence assessment and under the particular aspect of different kinds of hair cosmetics.

METHODS: Concentrations of EtG and FAEE were measured in more than 1600 hair samples and statistically evaluated in context of self-reported data about alcohol consumption and hair cosmetics. Particular emphasis was led on the verification of the cut-offs of 7pg/mg EtG and 200pg/mg (3cm) or 400pg/mg (6cm) FAEE proposed for the consensus about abstinence assessment.

RESULTS: Comparison of EtG and FAEE results from hair samples without cosmetic treatment showed that the FAEE cut-offs 200pg/mg (3 cm, n=107) and 400 pg (6cm, n=403) lead to a higher number of positive results than the EtG cut-off 7pg/mg and would better suit to 3 or 4pg/mg. This discrepancy was increased by bleaching or dyeing (false negative EtG) and use of hair spray (false positive FAEE). Since women and men treat their hair differently, hair alcohol results are biased by gender if hair cosmetic is ignored.

CONCLUSIONS: From the results follows that the proposed cut-offs for abstinence assessment are less restrictive for EtG than for FAEE and should be revised after further experience with both markers. Cosmetic hair treatment may decisively affect the outcome of the test and, therefore, must be ascertained during sampling and considered in interpretation of the result.

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APPLICATION OF BAYES THEOREM AND LIKELIHOOD RATIOS TO THE REPORTING OF RESULTS IN ETG/FAEE HAIR TESTING: CREATION OF A SCIENTIFICALLY ROBUST REPORTING SYSTEM

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BACKGROUND: The analysis of the markers EtG and FAEEs is well documented in hair and can be used to demonstrate alcoholism. The interpretation of the analytical data is however complicated, and presentation of such evidence in court has the potential to be misleading. The current consensus relies on the application of cut off values to the concentrations of alcohol markers in hair, and above said concentrations an analytical result is indicative of alcohol abuse.

METHODS: This research presents a statistical evaluation of the evidence utilizing the Bayesian Theory and Likelihood ratio (LR) approach. For this, the frequency of a value within a concentration range of an alcohol marker was calculated under two conditions: given the person is an alcoholic or a non-alcoholic.

RESULTS: It was shown that the LR in favour of alcoholism increases with higher concentrations of alcohol metabolites. Also, a higher LR was calculated for the combined detection of EtG and FAEE compared to the analysis of one of the markers.

DISCUSSION/CONCLUSION: Likelihood ratios can be used to determine the value of the evidence and can indicate a trend. The application of this statistical interpretation method to data from alcohol hair tests could provide a very robust and scientifically valid system to aid in the understanding and reporting of alcohol hair test evidence

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A NOVEL METHOD FOR THE COMBINED DETECTION OF THE ALCOHOL MARKERS: ETHYL GLUCURONIDE (ETG) AND THE FATTY ACID ETHYL ESTERS (FAEE)

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BACKGROUND: The combined use of FAEE and EtG in hair as markers for alcoholism increases the accuracy of the interpretation compared to the analysis of one of the markers as it helps to avoid false-positives and false-negatives. A method where both markers are extracted and measured may be commercially more viable than two separate analyses.

METHODS: The newly developed method was validated for the combined detection of EtG and FAEE in 20 mg hair. A GCMS system was used. First extraction with methanol, then solid phase extraction (OasisMAX), nitrogen evaporation, BSTFA derivatisation and head space solid phase micro-extraction (PDMS/DVB) were applied.

RESULTS: The extraction with methanol was similar to that of hexane for FAEE and water for EtG. The calibration curve was linear (R2 > 0.99) for all analytes. Furthermore, quality control samples were measured at a low, medium and high level and detected 90-111% of the actual value with a coefficient of variation of 7-23%. The quantification limits were the same as the lowest calibrator that are currently used for the detection of one marker.

DISCUSSION/CONCLUSION: The combined extraction and measurement of FAEE and EtG is possible. This can save time and money compared to the separate analysis of EtG and FAEEs and may make it commercially more interesting.

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EXAMINATION OF GENDER BIAS IN FAEE V. ETG HAIR ANALYSIS

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BACKGROUND: Fatty acid ethyl esters (FAEE) and ethyl glucuronide (EtG) are both validated biomarkers of chronic excessive alcohol consumption in hair. One of the most promising clinical applications of alcohol hair analysis is to identify infants as risk for Fetal Alcohol Spectrum Disorder (FASD) by assessing maternal ethanol consumption; however, studies on FAEE and EtG hair analysis to determine alcohol abuse have been primarily conducted in male populations. Previous studies have indicated that the use of ethanol containing hair-care products may elevate FAEE concentrations in hair, providing a potential source of bias in the use of this biomarker in female populations. This study examines individuals undergoing hair analysis under suspicion of chronic alcohol abuse in child safety investigations.

METHODS: A total of 207 hair specimens were analyzed for both FAEE and EtG, approximately 70% of all samples were from female donors. One-hundred fifty-eight samples were blindly assessed for both biomarkers; n = 49 additional samples were analyzed for EtG following positive FAEE analysis. Cut-off values of 0.50 ng/mg-FAEE and 25 pg/mg-EtG were applied to determine chronic excessive alcohol consumption. Hair samples were pre-washed to remove external contamination and analyzed for FAEE and EtG by GC-MS using deuterated internal standards.

RESULTS: A higher rate of correspondence was found between FAEE and EtG levels amongst male (71%) over female (28%) subjects.

DISCUSSION/CONCLUSIONS: These data suggest a gender bias exists making determination of chronic excessive alcohol use challenging in female subjects. The source of this bias is likely cosmetic hair treatments; with ethanol-containing hair products producing false-positive FAEE findings and hair bleaching/colouring possibly producing false-negative EtG results contributing to a greater discordance amongst samples of female origin.

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TESTING ETHYLGLUCURONIDE IN MATERNAL HAIR AND NAILS FOR THE ASSESSMENT OF FETAL EXPOSURE TO ALCOHOL: COMPARISON WITH MECONIUM TESTING

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BACKGROUND: The deleterious effects exerted by prenatal ethanol exposure include physical, mental, behavioural and/or learning disabilities that are included in the term fetal alcohol spectrum disorder (FASD). The measurement of ethylglucuronide (EtG) in alternative biological matrices, including neonatal and maternal hair, neonatal meconium, and maternal nails is receiving increasing interest for the accurate evaluation of the utero exposure to alcohol. The aim of the present study was to evaluate the correlation between EtG in maternal hair and nails with EtG in neonatal meconium to further explore the suitability of these biomarkers in disclosing prenatal exposure to ethanol.

METHODS: A total of 130 maternal hair strands (0-6 cm), nail clips (2-6 mm) and corresponding neonatal meconium and nails samples were obtained from neonatal wards of 4 Mediterranean public hospitals: Rome, Florence and Belluno in Italy and Barcelona in Spain. Hair, nails and meconium were analyzed for the presence of EtG by validated liquid chromatography mass spectrometry assay. Meconium was also analyzed for the presence of fatty acid ethyl esters (FAEEs) as a complementary biomarker of eventual in utero exposure to alcohol.

RESULTS: On the basis of the accepted cut-off for EtG and FAEEs in neonatal meconium, 16 newborns resulted in utero exposed to maternal alcohol consumption. Unfortunately, none of these cases were confirmed by the presence of EtG in maternal hair and nails, which resulted all negative to this biomarker. Conversely, one high positive result in nails (24 pg EtG/mg nail) was obtained in Pakistan woman, whose hair resulted negative to EtG likewise the meconium of her newborn.

DISCUSSION/CONCLUSIONS: Obtained results confirm that EtG and FAEEs in meconium are the best biomarker to assess in utero exposure to maternal alcohol. The 16 newborns exposed to maternal ethanol came from mothers who declared alcohol consumption through the entire pregnancy, but not on daily basis and with no more than 1 drink per day. This fact led us to suppose that firstly EtG in hair and nails is not a good biomarker to disclose an alcohol consumption lower than on daily basis and lower than one alcoholic unit per day. Secondly that both in case of hair and in that of nails, the amount of collected sample is crucial to obtain a positive result, since available methods are yet not enough sensitive for less than 20 mg keratin matrices.

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ORAL SESSION: ADVANCEMENTS IN ANALYSIS OF DRUGS OF ABUSE

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IDENTIFICATION OF NEW PSYCHOACTIVE SUBSTANCES AND THEIR DETECTION IN HUMAN HAIR

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BACKGROUND: A central task of many German forensic labs is the identification and quantitative analysis of conventional illicit drugs in seized material like heroin, cocaine, cannabis, amphetamines and many others that are listed in the German Narcotics Law. Seizures of dangerous – insufficiently studied – designer drugs by the Bavarian police have increased substantially within the last few years. Designer drugs, usually termed "research chemicals" and mixtures as so-called "legal highs" are marketed in headshops and via the Internet. These drugs are subject to the German Medicinal Products Act.

METHODS: The labs of the Bavarian State Criminal Police Office have been nearly overloaded with different preparations, products and compounds; about 25,000 single packages and an enormous variety of different products have had to be analyzed since 2009.

RESULTS: The analyses of "legal highs" and "research chemicals" showed that most of the samples contained pharmacologically active substances from the groups of 14 cathinones, 23 synthetic

cannabinoids, 3 tryptamines, 5 piperazines, 7 phenethylamines and amphetamines and 10 others. A special challenge is the identification and quantification of novel drugs of abuse in human hair. On a routine basis the detection of mephedrone, m-CPP, methylone, methylenedioxypyrovalerone (MDPV), and butylone is performed by using GC/MSMS. In case of m-CPP the concentration ranged between 0.3 to 5.7 ng/mg hair, the concentrations for methylone and MDPV were lower than 1.1 ng/mg hair.In case of need, special designer drugs, e.g. MDAI, metamfepremone, naphyrone, 4-MEC, 5-MeO-DALT and some important synthetic cannabinoids abused, e.g. JWH-018, JWH-019, JWH-073, JWH-081, JWH-122, JWH-200, JWH-210, JWH-250, AM-2201 and RCS-4, could be analyzed by using LC/MSMS. More than just one new psychoactive substance was detectable in a hair sample from a suspected rapist: 4-MEC, 5-MeO-DALT, JWH-210, JWH-122, and MDPV.

DISCUSSION/CONCLUSIONS: The diversity of new psychoactive substances is increasing rapidly and forensic labs are forced to find analytical solutions promptly. Some selected analytical findings of seized material and hair samples will be presented.

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MICROPULVERIZED EXTRACTION – A FAST AND SIMPLE SAMPLE PREPARATION FOR GENERAL UNKNOWN SCREENING IN HAIR BY LC-QTOF-MS

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BACKGROUND: Hair extraction is the most time-consuming part of hair analysis. For a general unknown screening it should extract toxic substances of quite different structure with high yield, avoid substance decomposition and exclude matrix components as far as possible. Based on the method of Miyaguchi, in this study, the advantages of micropulverized extraction, that means ball milling in presence of the extraction solvent, for hair analysis by LC-QTOF-MS were tested.

METHODS: After optimization for all essential parameters, the following procedure appeared most favourable: After washing with water and acetone, drying, and cutting to pieces, 20mg hair, 200µl extraction solvent (methanol/acetonitrile/2mM $HCOONH_4/H_2O$, 25:25:50 v/v/v) and 5µl deuterated standard solution were placed in a 1.5ml Eppendorf vial together with 3 stainless steel balls (∅ 3mm). The vial was tightly closed and treated for 20min at 30Hz on a Retsch mixer mill MM400. Up to 10 samples could be simultaneously extracted. After centrifugation, the supernatant was separated from the hair powder and 5µl were injected for measurement by LC-QTOF-MS in data dependent acquisition mode.

RESULTS: The method was applied to 5 post-mortem hair samples with 51 different drugs. In comparison to the current routine procedure (2x16 h incubation on a thermomixer) similar or even higher extraction yields were obtained. From exhaustive extraction by repeated application follows that for almost all drugs between 90 and 100 % were extracted in 20 min.

CONCLUSIONS: In routine application, micropulverized extraction proved to be a fast, efficient and simple sample preparation for general unknown analysis in hair by LC-QTOF-MS.

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DEVELOPMENT OF A SIMULTANEOUS ANALYTICAL METHOD FOR SELECTED ANORECTICS, METHAMPHETAMINE, MDMA AND THEIR METABOLITES IN HAIR USING LC-MS/MS TO PROVE ANORECTICS ABUSE

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BACKGROUND: Due to the tight control of methamphetamine, it is presumed that phentermine, an amphetamine-type anorectic, is considered as a supplement to methamphetamine abusers in Korea recently. In addition, the abuse of other anorectics obtained by inappropriate ways has become another social issue. Therefore, a simultaneous analytical method for the detection in hair of phentermine, phendimetrazine, amfepramone, fenfluramine, mazindol, MDMA, as well as their metabolites was established and validated using LC-MS/MS.

METHODS: The drugs and their metabolites in hair were extracted using 1% HCl in methanol, filtered and analyzed using the LC-MS/MS with electrospray ionization in positive mode.

RESULTS: The validation results of selectivity, linearity, matrix effect, recovery, process efficiency, intra- and inter-assay precision and accuracy and

processed sample stability were satisfactory. The limits of detection ranged from 0.025 to 1 ng/10 mg hair and the limits of quantification were 0.25 ng/10 mg hair for every analyte except mazindol and phentermine, for which they were 10 ng/10 mg hair. **CONCLUSION**: The method was successfully applied for the segmental determination of selected anorectics, methamphetamine, MDMA and their metabolites in hair from 38 drug suspects. Among the anorectics, phentermine and/or phendimetrazine were identified with or without methamphetamine in the hair samples. Closer supervision of the inappropriate use of anorectics is necessary. Also, hair analysis is useful for monitoring abuse potential of unnoticed drugs.

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SIGNIFICANT ACCELERATION OF SAMPLE PREPARATION FOR SEVERAL PSYCHOACTIVE DRUGS IN HUMAN HAIR BY MICROPULVERIZED EXTRACTION
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BACKGROUND: The aim of this study is to extract psychoactive drugs in human hair rapidly and completely.
METHODS: A black hair specimen was obtained from a donor who ingested seven psychoactive drugs daily. A washed hair (10 mg) was micropulverized for 10 min at 2500 strokes/min in a polypropylene tube with a stainless-steel bullet, 5 ?L of an internal standards mixture (1 ng each), and 200 ?L of 45% (w/v) ammonium phosphate (pH 8.4). Liquid–liquid extraction was carried out in the tube using 100 ?L of acetonitrile, and the organic layer was filtrated and analyzed by liquid chromatography/triple-quadrupole mass spectrometry in selected reaction monitoring. Conventional methods were also employed, and the relative efficiency of extracting zolpidem, amitriptyline, nortriptyline, mianserin, flunitrazepam, 7-aminoflunitrazepam, and desalkylflurazepam were calculated from the ratio of the peak areas to the corresponding deuterium analogues.
RESULTS: For all analytes, the extraction efficiencies determined by the method described above were higher than the efficiencies determined using the other methods, despite the fact that it took only 10 min for extraction. Among the conventional

methods, solid–liquid extraction in the ammonium phosphate buffer (38°C, 18 h) had the highest extraction efficiency, especially when the hair sample was pulverized before extraction (70–103% in comparison with the method described above). On the other hand, extraction in methanol (38°C, 16 h) was the least efficient method for extracting most analytes (9.2–79%).
DISCUSSION: These results demonstrate that the mechanical pulverization of hair in an aqueous medium facilitates rapid and intensive extraction of the psychoactive drugs.

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Hair analysis for the emerging new drugs: tramadol and mephedrone
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BACKGROUND: Although extensive information on detection of drugs in hair is available for many drugs, previous emphasis has been on the most common drugs with less focus on misuse of new emerging psychoactive compounds, such as tramadol and mephedrone. Tramadol is an analgesic opioid available on prescription, used to treat moderate to severe pain. Mephedrone is a designer stimulant, and has been outlawed in the UK since 2010. Up to now only a few papers have reported the detection of tramadol and mephedrone in hair samples.
METHODS: Hair samples (N=1205) were sectioned, washed, submitted to alkaline digestion and purification using solid-phase extraction and analysed by LC-MS/MS in ESI positive ion with multiple reaction monitoring. Calibration curves for all analytes were from 0.1 to 2.5 ng/mg with cut-offs at 0.2 ng/mg. The intra-day precision for all analytes was less then 8% and inter-day precision less then 10% for mid and high levels and 23-35% at cut-off levels. Tramadol was detected in 107 samples, (range: 0.3-57.1ng/mg; median: 1.3ng/mg and mean: 5.6ng/mg) and its main metabolite, desmethyltramadol was detected in 52 samples (range 0.2-8.1ng/mg, median=0.7ng/mg and mean=1.3 ng/mg). Use of tramadol was declared in 46 cases. Mephedrone was detected in 39 samples (range: 0.2 to 36.6 ng/mg; median: 0.8ng/mg and mean: 3.5ng/mg)

RESULTS: The results show that tramadol use and misuse is more common in comparison to mephedrone and confirm the usefulness of hair analysis as an aid in monitoring use of these drugs over a longer period of time. Data presented will include details on other drugs that were simultaneously detected.

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INTRAINDIVIDUAL VARIATION OF LEVELS OF ENDOGENOUS GHB IN HAIR

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BACKGROUND: Gamma hydroxybutyrate (GHB) belongs to a group of substances that may be used for drug-facilitated sexual assaults (DFSA). It can also be found in hair as an endogenous molecule. A method for the detection of GHB in hair segments was developed and validated. Analytical data of 80 volunteers (49 females/ 31 males) not claiming any exposure are presented and the results are discussed in the context of the identification of a potential single exposure in cases of DFSA. CUSUM (cumulative sum control chart) is typically used for monitoring changes in time series. In this study, its applicability to the detection of changes of levels of GHB in hair due to a single administration was investigated.

METHODS: The hair samples were solubilized, segmented, and GHB was extracted from each segment and analyzed using a liquid chromatography-tandem mass spectrometry (LC/MS/MS) method. In this paper CUSUM charts were used to analyze the sequential data as a self-controlled case series.

RESULTS: For all the samples that gave results greater than the limit of quantification (LOQ) of 0.1 ng/mg the intra-individual variation range is 7% to 44 % expressed as relative standard deviation (RSD). GHB levels ranged from below LOQ to 3.0 ng/mg on average. Preliminary results of one-sided CUSUM analyses did not indicate evidence against the null-hypothesis in >90% of the profiles.

DISCUSSION/CONCLUSIONS: The intra-individual variation range of this study is consistent with previously published results that are based on only a few cases. Statistical evaluation of our data indicates that CUSUM might help in establishing a standardized approach for the interpretation of analytical results in DFSA cases concerning a single exposure to GHB.

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INFLUENCE OF CHEMICAL STRAIGHTENING ON THE STABILITY OF DRUGS OF ABUSE IN HAIR

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BACKGROUND: Hair analysis has become a popular method used in forensic and workplace settings to determine a subject's illicit drug use. While it tends to be complementary to urine or blood analysis, it offers the advantage of a longer detection window and can be used to distinguish between intermittent and chronic usage. However, factors such as cosmetic treatments can alter the measured content thus leading to false negative or false positive results. This study examines the effect of chemical straightening, a technique commonly employed by African American women to obtain straighter hair, on the stability of drugs of abuse in hair.

METHODS: Commercially available "Lye" or "No-Lye" chemical straightening products (Silk Elements™) were applied *in vitro* to drug fortified hair (standard reference materials(SRM) 2379 and 2380) and hairs clipped from authentic drug users. Target analytes (cocaine, benzoylecogonine, cocaethylene, phencyclidine, and tetrahydrocannabinol) were isolated using solid-phase extraction then analyzed with isotope dilution gas chromatography mass spectrometry with selected ion monitoring.

RESULTS: After either treatment, drug concentrations were significantly ($P<0.05$) reduced in both the SRM sample and the hair from authentic abusers. In the SRM groups, 6-67% of the original concentration remained after a single chemical treatment. Similarly, only 5-30% of the original concentration remained in authentic drug hairs that had formerly tested positive for cocaine, benzoylecoginine, and cocaethylene.

CONCLUSION: The results of this study demonstrate that after a single application of a chemical straightening product, the measured illicit drug concentration can be drastically reduced which could lead to false negative results.

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A DOSE-CONTROLLED STUDY WITH COCAINE USERS: CONSUMPTION MONITORING USING A VALIDATED LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY METHOD FOR THE QUANTIFICATION OF COCAINE AND METABOLITES IN HAIR AND TOENAILS

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BACKGROUND: Hair is being widely used as a matrix to control abstinence from drugs of abuse. This work aims to establish a method for the analysis of cocaine and metabolites in toenails by a validated LC-MS/MS method, a dose-response correlation of cocaine concentrations in hair and toenail samples and assessment of the suitability of toenails as an alternative matrix for consumption monitoring of cocaine use.

METHODS: Ten hair samples, 14 toenail clippings, and 11 toenail scrapings were obtained from cocaine users consuming a constant dose of cocaine per week by nasal application. Data on consumption behaviour was obtained during an interview about psychotropic drug consumption. Hair and toenail samples were washed and analysed for cocaine and its metabolites benzoylecgonine, norcocaine, cocaethylene, ecgonine methyl ester, and anhydroecgonine methyl ester using a standard routine procedure for hair samples and a validated method for toenail samples. Validation of the latter method was performed according to Society of Toxicological and Forensic Chemistry Guidelines.

RESULTS: Cocaine concentrations ranged from 170-4500 pg/mg of hair and from 4-588 pg/mg of toenail clippings. Higher concentrations were found in toenail scrapings ranging from 26-1960 pg/mg. All hair and toenail samples were positive for norcocaine, benzoylecgonine and cocaethylene.

Metabolite to parent drug ratios were by trend higher in toenail scrapings compared to toenail clippings.

CONCLUSIONS: All toenail samples of cocaine users were positive for cocaine. Cocaine concentrations found in hair samples were greater than in toenail samples. A dose-response correlation could not be observed for hair neither for toenail samples.

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VALUE OF EXTERNAL QUALITY CONTROL IN HAIR ANALYSIS AND ITS IMPACT FOR RESULT INTERPRETATION

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BACKGROUND: The SoHT promotes external quality controls as one primary goal according to its constitution. Thus, for several years, proficiency tests have been organized including the most important drugs of abuse and newly, marker of alcohol abuse. Nevertheless, the quantitative results for these compounds varied largely depending on the analyte. Only for the last two years, quantitative results have been used as criteria for a successful participation. The measurement uncertainty has not yet played a big role in hair analysis probably related to the limited importance of a quantitative result as consumption criteria for illicit drugs where a zero tolerance is generally asked by law. But when it comes to the consumption of legal drugs like ethanol, a fixed cutoff needs an analytical certainty as evidence for the consumption of a certain amount of the substance (e.g. > 60 g/ethanol/day).

METHODS: Basis for the argumentation are the SoHT proficiency test results of 2011.

RESULTS: Using classical statistical parameters for quality controls like the 2xZ-score, error margins of more than 70% (for amphetamines, cocaine and EtG) and more than 100 % for opiates and THC have been obtained.

DISCUSSION: Hair is especially affected by matrix inhomogeneities and varying extraction efficiencies caused by different solvents. These errors sources come along with the known systematical bioanalytical errors. In food, but even in blood analysis, the use of harmonized methods has been a routine for many years.

CONCLUSION: In order to decrease the total error and to keep hair analysis credible, the authors would

encourage steps towards a standardization of the analytical methods and the use of measurement uncertainty for result interpretation

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THE EFFECTS OF SPECIALISED 'TOXIN REMOVAL' SHAMPOOS ON THE DETECTION OF DRUGS IN HAIR
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BACKGROUND: The objective of this study was to investigate the effect of the 'toxin removal' shampoos Spectrum Labs Get Clean, Zydot Ultra Clean and Folli-Kleen on drugs in hair. The analytes amphetamine, diazepam, desmethyldiazepam, cocaine, benzoylecgonine, methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), morphine, codeine and 6 acetylmorphine were studied.
METHODS: Head hair samples known to contain the analytes were divided into two portions; one was treated with shampoo and one was left untreated. The samples were cut into sections, digested and analytes extracted using liquid-liquid extraction. Samples were analysed using enzyme linked immunosorbent assay (ELISA) followed by solid phase extraction (SPE) prior to gas chromatography with mass spectrometry (GC-MS) or liquid chromatography with tandem mass spectrometry (LC-MS/MS). ANOVA was used to determine whether the difference between the results of the treated and untreated sections were significant.
RESULTS: Overall the results of the ANOVA statistical analysis show no significant difference between the results of the hair sections that had been treated with shampoo and those which were left untreated. The results also show that the use of the shampoos don't interfere with the ELISA analysis or with the chromatography of the LC-MS/MS or GC-MS analysis. The overall results show that the toxin removal shampoos Spectrum Labs Get Clean, Zydot Ultra Clean and Folli-Kleen do not interfere with the analysis of amphetamine, diazepam, desmethyldiazepam, cocaine, benzoylecgonine, methadone, EDDP, morphine, codeine or 6 acetylmorphine using ELISA and GC-MS or LC-MS/MS. In addition, the levels of these analytes in hair are not significantly reduced by the use of the shampoos.

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ISSUES ABOUT AXIAL DIFFUSION DURING SEGMENTAL HAIR ANALYSIS
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BACKGROUND: The detection of a single drug exposure in hair (doping offence, drug-facilitated crime) is based on the presence of the compound of interest in the segment corresponding to the period of the alleged event. However, in some cases, the drug was detected in consecutive segments. As a consequence, interpretation of the results is a challenge that deserves particular attention. Our strategy will be reviewed in this presentation.
METHODS: Literature evaluation and data obtained from our 20 years experience in drug testing in hair are the basis to establish a theory to validate the concept of single exposure in situations where the drug is detected in 2 or 3 segments, both in controlled studies in volunteers and authentic forensic cases.
RESULTS: This laboratory recommends to wait for 4-5 weeks after the alleged event and then to collect strands of hair. Assuming normal hair growth rate (1 cm/month), it is our opinion to cut the strand into 3 segments of 2 cm in order to document such cases. Administration of a single dose would be confirmed by the presence of the drug in the proximal segment (root) while not detected in the other segments. However, in our daily experience, we have noticed that sometimes, the drug can be detected in 2 or 3 consecutive segments. The following case is an example: a young woman was victim of a sexual assault in a highway station. She declared that the perpetrator forced her to absorb a white tablet before abusing her. Blood sample, collected 18 hours after the offence, revealed the presence of 151 ng/mL of bromazepam. A strand of hair was collected 3 weeks after the event and the proximal segment (0-2 cm) was positive for bromazepam at 5.7 pg/mg, the consecutive segment (2-4 cm) was positive at 0.9 pg/mg and the last segment remained bromazepam-free. Such a disposition was even observed in volunteers experiment, receiving a single dose of estazolam, clonazepam, zolpidem or zopiclone.
DISCUSSION: As it was also described for cocaine in 1996, there is considerable variability in the area over which incorporated drug can be distributed in the hair shaft and in the rate of axial distribution of

drug along the hair shaft. This can explain why a small amount of bromazepam, as compared with the concentration in the proximal segment, was measured in the second segment, as a result of an irregular movement. Another explanation for broadening the band of positive hair from a single dose is that drugs and metabolites are incorporated into hair during formation of the hair shaft via diffusion from sweat and other secretions. The presence of confounding interferences in the hair matrix, or changes in the hair structure due to cosmetic treatments might mislead the final result of hair analysis.

CONCLUSION: To qualify for a single exposure, we propose to consider that the highest drug concentration must be detected in the segment corresponding to the period of the alleged event, and that the measured concentration be at least 3 times higher than those measured in the consecutive segments.

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COLOURING, BLEACHING AND PERMING: INFLUENCE ON ETG CONTENT IN HAIR
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BACKGROUND: Hair analysis of ethyl glucuronide (EtG has become beside fatty acid ethyl ester a valuable marker for the detection of moderate and chronic excessive alcohol consumption. So far only few studies exist about the influence of cosmetic treatment on EtG content in hair. The aim of this study was to evaluate the effect of colouring, bleaching and perming on the concentration of this alcohol marker in hair. Studies were also performed to evaluate the chemical stability of EtG in presence of hydrogen peroxide and ammonium thioglycolate.

METHOD: Hair samples were treated *in vitro* by the different commercial cosmetics following the suppliers' instructions. After washing, pulverization, incubation in ultrasonic bath and solid phase extraction, EtG was determined by GC/MS-NICI after solid phase extraction and heptafluorobutyric anhydride derivatization.

RESULTS: The results showed that samples (n =10) treated with the colouring product didn't show any important change in the EtG results. In the bleaching study (n=23) a mean decrease of 68 % was observed. After incubation of a solution of EtG with hydrogen

peroxide (15%), a decrease of 85 % was shown supporting the hypothesis of a chemical degradation of EtG. In the perm treatment study (n=23) a mean decrease of 93 % of EtG was found. Incubation of a solution of EtG with ammonium thioglycolate (5%) showed a total decrease of EtG.

CONCLUSION: In conclusion, colouring treatment did not importantly influence EtG content in hair. However, an important decrease of EtG in hair could be found after bleaching and permanent wave treatment. This decrease seems to be due in both treatments more to a chemical degradation of EtG than a leaching out effect from the hair matrix. This data has to be considered for the correct interpretation of EtG results in hair.

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HAIR ANALYSIS IN WORKPLACE DRUG TESTING AND DRIVING LICENCE REGRANTING AS A TOOL TO DISCLOSE PATTERNS OF DRUG USE
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BACKGROUND: In Italy, hair analysis is used in the second level-tests of workplace drug testing (WDT) protocol and for driving licence regranting/renewal (DLR). The WDT National Protocol includes two-level tests. The second level of the protocol is required when in the first level-tests urine sample results positive. In this case, the worker is obliged to go to the Public Drug Treatment Unit to undergo an eventual diagnosis of drug dependence. The second level-tests consist of analysing both urine and hair for opiates, cocaine, amphetamines, cannabinoids, methadone and buprenorphine. As far as driving licence regranting/renewal is concerned, subjects are instead always obliged to undergo toxicological analyses if they have either a documented past or a suspect present history of drug abuse, (art. 187 "Driving under influence of drugs"), or if they are forced by the prefecture or by the traffic control authority. In some Regions such as Lombardy, hair analysis is used to rule out illicit drug use. This study presents the data obtained from hair analysis performed in the Laboratory of Forensic Toxicology of the University of Pavia in the last two years.

METHODS: A total of 1017 hair samples were analysed for WDT (322) and DLR (695). Analyses

were carried out by GC-MS using the method routinely applied on hair samples.

RESULTS: The positive rate for the WDT second level test was 45% versus the 10% for the DLR. The different percentage of positive results demonstrate how hair analysis is useful both for the diagnosis of drug dependence and as deterrent of drug consumption. As regard to positive results distribution, a cocaine abuse emerged in 74.6% of workers, while drivers resulted positive mainly for cannabinoids (41%), and cocaine (39%). On the contrary, the second main consumption in WDT analysis resulted the polydrug abuse (16,5%). Opiates percentage was 1.4% in both cases while positive samples for methadone were 9 in the DLR analysis versus 3 in the WDT one. MDMA was present only in two workers and in association with cocaine.

WEDNESDAY, JUNE 27, 2012
SYMPOSIUM: DEVELOPMENT AND APPLICATIONS OF CORTISOL ANALYSIS IN HAIR

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DEVELOPMENT AND VALIDATION OF HAIR CORTISOL AS A BIOMARKER FOR CHRONIC STRESS

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BACKGROUND: Chronic stress is omnipresent in our society and is related to the development of many health issues. Acute stress activates the Hypothalamus-Pituitary-Adrenal (HPA)-axis resulting in increased cortisol secretion. Traditionally, this is measured in serum, saliva and urine. Less information is available on long term cortisol secretion in relation to chronic stress. During the last several years, the measurement of cortisol in scalp hair has been developed as a tool to assess chronic cortisol secretion, and studies started to demonstrate its potential use as a biomarker for chronic stress.

METHODS: Here we briefly review the HPA axis, and present several validation studies on the measurement of hair cortisol in patient populations. These include patients with known cortisol overproduction (Cushing's syndrome), with insufficient cortisol production (adrenal insufficiency) receiving replacement treatment, and with severe chronic pain. For evaluation of long term stability, we measured hair cortisol in mummies buried several centuries ago. To study the role of stress in developing myocardial infarcts, we measured hair cortisol in patients admitted with chest pain.

RESULTS: Hair cortisol content is elevated in patients with hypercortisolism, and is correlated to hydrocortisone dose in patients with adrenal insufficiency. Hair cortisol is present and elevated in hair samples from mummies that are several centuries old. Hair cortisol content is increased in patients with severe chronic pain. Stress, as measured by hair cortisol, is higher in patients who develop a myocardial infarct than in a control group.

CONCLUSIONS: These studies suggest that measurement of cortisol in hair cortisol can be used for assessment of chronic systemic cortisol exposure. It is stable over time and responds to variation in cortisol exposure. Hair cortisol content is a promising biomarker for prolonged physical and mental stress.

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METHODOLOGICAL ASPECTS OF MEASUREMENTS OF STEROIDS IN HAIR

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BACKGROUND: For the past twenty years, hair analysis has been proposed for identifying drug abusers in forensic science. However, new applications have been developed, including clinical toxicology, experimental toxicology and doping control. Beside the classic drugs of abuse testing, only few laboratories have paid attention to steroids, including anabolics and corticoids. The analytical aspects of steroids testing in hair will be reviewed in this presentation.

METHOD: Literature evaluation and data obtained from our 20 years experience in drug testing in hair are the basis to present the best analytical approaches to test for these compounds.

RESULTS: As it is the case for drugs of abuse, hair preparation for steroids involves decontamination, pulverization, incubation and extraction. In most cases, the amount of hair is at least 50 mg. Incubation is mostly achieved under mild conditions. Complicated multiple extractions are necessary to purify the extracts before chromatography. In most papers, liquid chromatography coupled to tandem mass spectrometry has been used. Gas chromatography can be an alternative for anabolics, after derivatization. Sometimes, ELISA tests have been used. The concentrations of endogenous steroids in hair are low, in the pg/mg range. For example, physiological concentrations of testosterone and DHEA are lower than 15 and 50 pg/mg, respectively. Cortisol endogenous concentrations in hair are in the range 5 to 100 pg/mg, with a mean value at about 20 pg/mg. Cortisone endogenous concentrations are generally in the range 10 to 200 pg/mg, with a mean value at about 70 pg/mg.

DISCUSSION: The first identification of prednisone in human hair was published in 1999. Anabolics have been detected in hair some years earlier, mostly for documenting doping offences. It is obvious that these pioneer studies have opened new possibilities, particularly when using endogenous compounds as markers.

CONCLUSION: Although very few laboratories are testing for steroids in hair, there are numerous applications that deserve attention.

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GLUCOCORTICOIDS IN HAIR IN RELATION TO CARDIOMETABOLIC RISK MARKERS

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BACKGROUND: Altered long-term secretion of glucocorticoid hormones is believed to play a pivotal role in linking chronic stress to cardiometabolic risk. Despite experimental data supporting this link, previous epidemiological field studies have often yielded inconsistent results. Amongst other things, this is likely to be related to methodological limitations in the assessment of glucocorticoid secretion over prolonged periods of time.

METHODS: The measurement of glucocorticoids in hair may constitute a major advancement here, enabling the assessment of cumulative hormone levels over periods of up to six months. Here, we will present first data from a large industry-funded cohort study investigating links between work-related stress, long-term glucocorticoid secretion and cardiometabolic risk factors. Hair samples were obtained from 1315 employees of the airline manufacturing industry and assayed for cortisol (F) and cortisone (E) concentrations using liquid chromatography with tandem mass spectrometry detection (LC-MS/MS). In addition, relevant anthropometric, psychosocial and physiological biomarkers of cardiometabolic risk were assessed. Results reveal positive associations of hair F and E concentrations with measures of central obesity (body-mass-index, waist-to-hip ratio), resting systolic and diastolic blood pressure as well as fasting morning blood levels of glucose, glycated haemoglobin (HbA1c), C-reactive protein and high density lipoprotein (negative).

RESULTS: Significant positive associations with low-density lipoprotein and triglyceride levels were only seen for hair E but not for hair F. These findings are in line with current conceptions suggesting an important role of aberrant glucocorticoid secretion in the development of cardiometabolic risk.

DISCUSSION/CONCLUSIONS: Implications of these data for hair analysis as an important future tool in epidemiological field research will be discussed.

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CLINICAL APPLICATIONS OF HAIR CORTISOL ANALYSIS

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BACKGROUND: Diagnosis of Cushing's Syndrome (CS) and in particular cyclic CS can be difficult using standard tests as measurement of cortisol in 24-hours urine and midnight saliva. In case of cyclic CS, results of these tests can be normal in between periods of hypercortisolism. Measurement of cortisol in scalp hair provides the opportunity to

investigate historical cortisol levels of months to years ago, with each centimetre of hair corresponding to approximately one month. In patients with Addison's Disease (AD), measurement of historical cortisol levels could provide useful information concerning disease course and effect of hydrocortisone replacement therapy. Our aims were to study whether hair cortisol levels 1. can be used as a diagnostic tool and 2. corresponded with clinical course, in (cyclic) CS and AD.

METHODS: Hair samples were collected from 16 CS patients, 6 cyclic CS patients and 3 AD patients. Cortisol was extracted with methanol and cortisol levels were measured using an ELISA. We used a control group of 195 healthy individuals.

RESULTS: Cortisol levels were significantly elevated in CS patients ($p<0.0001$) and decreased in AD patients ($p=0.002$) compared to controls. Hair cortisol timelines of patients with CS, cyclic CS and AD corresponded well with clinical course.

CONCLUSION: Scalp hair can be used to evaluate clinical course in patients with (cyclic) CS and AD and provides valuable historic information about cortisol exposure which can contribute greatly in diagnosing these diseases. In addition, in this presentation applications of hair cortisol in shift work, obesity, and cardiovascular diseases will be addressed as well.

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HAIR AND FEATHER STEROIDS ARE ASSOCIATED WITH FITNESS IN WILDLIFE
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BACKGROUND: Fitness, or the ability to survive and reproduce, is enabled through an organism's adaptations to its environment. Hormones are coordinators, adjusting behaviours to circumstances and contexts, and can therefore provide information about the individual's physiological, as well as social, condition. An inverse relationship between fitness and stress or sex hormone concentrations has been widely assumed, although empirical evidence is scarce.

METHODS: Using hair and feathers, which are relatively easy to obtain in a non-invasive manner, I study the relationship between baseline steroid hormones and fitness-related parameters in wildlife. I wish to share findings from two long-term wildlife

studies. The rock hyrax (*Procavia capensis*) is a long-lived African social mammal. Following almost a decade of study of two hundred hyraxes belonging to five social groups, I found that hair steroid levels were associated with social status and behaviour. In the future, I plan to experimentally manipulate steroid levels in this system in order to assess the costs and benefits of steroids hormones. In the house sparrows (*Passer domesticus*) on Lundy Island, UK, we had a unique opportunity to assess the costs of steroids. On this island all individuals are marked and monitored, and survival data is accurate and reliable. Using LC-MS/MS, I measured testosterone, corticosterone and cortisol in sparrow feathers, and found that all three steroids were significantly higher in birds that subsequently died over the following winter than in birds that survived. Thus, hair and feather steroids may be useful in evaluating the risks associated with steroid hormones, and to predict the future survival of individuals in the wild.

WEDNESDAY, JUNE 27, 2012
ORAL SESSION: CORTISOL ANALYSIS IN HAIR

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HAIR ANALYSIS IN THE CLINICAL LABORATORY, ACCREDITATION & QUALITY MANAGEMENT
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BACKGROUND: Accreditation and quality management in the clinical laboratory setting is rapidly developing worldwide. Quality management refers to all the activities used by organizations to ensure product or service consistency. The Motherisk laboratory is licensed under the Hospital for Sick Children. The lab is performing both research and clinical toxicology tests in hair and meconium, most of the samples are involved in chain of custody cases. Establishing a Quality Management System (QMS) and achieving accreditation is mandatory in all Ontario clinical labs by legislation since 2003. Ontario Laboratory Accreditation (OLA) program is based on "ISO 15189 - Medical laboratories - Particular requirements for quality and competence".

METHODS: Implementation of a QMS involves planning and staff education, documentation of the system, validation of the processes, and assessment against the requirements.

CONCLUSION: Maintenance of a QMS requires control and monitoring of the laboratory's entire path of workflow. The process of transformation of a research/clinical lab into an accredited lab, as well as the benefits of maintaining an effective QMS are presented.

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THE DETECTION OF CORTISOL IN HUMAN SWEAT: IMPLICATIONS FOR MEASUREMENT OF CORTISOL IN HAIR

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BACKGROUND: Hair cortisol analysis is an effective measure of chronic stress. Cortisol is assumed to enter the hair via blood, sebum, and sweat, however the extent to which sweat contributes to hair cortisol content is unknown.

OBJECTIVES: The primary objective of this study was to determine if cortisol is present in sweat. A secondary objective was to determine if exposures to a hydrocortisone solution with a sweat-like cortisol concentration would affect hair cortisol concentrations, and if this could be normalized with washing.

METHODS: Sweat and saliva samples were collected from 17 subjects, and analyzed by salivary ELISA. Subsequently, an *in vitro* test on hydrocortisone exposure was conducted. Residual hair samples were immersed in a 50ng/ml hydrocortisone solution for periods lasting 15 minutes to 24 hours, followed by a wash (isopropanol) or no-wash condition. Hair cortisol content was determined using a modified salivary cortisol ELISA protocol.

RESULTS: Sweat cortisol concentrations were 74.62±41.51ng/ml (mean±SD) and ranged from 8.16- 141.7ng/ml. Sweat cortisol was significantly correlated with salivary cortisol (r^2=0.30, P<0.05), and was significantly negatively correlated with the log-transformed time of day (r^2=0.43, P<0.01). Hair exposure to a 50ng/ml hydrocortisone solution for 60 minutes or more resulted in significantly increased hair cortisol concentrations (P<0.01). Washing with isopropanol did not affect immersion-increased hair cortisol concentration.

DISCUSSION: Human sweat contains cortisol that likely contributes to hair cortisol content. Subjects with prolonged sweating at the time of hair collection may have increased hair cortisol concentrations that cannot be decreased with conventional washing procedures.

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HAIR CORTISOL CONCENTRATIONS IN PATIENTS WITH OBSTRUCTIVE SLEEP APNEA

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BACKGROUND: Obstructive sleep apnea (OSA) is a common sleep disorder with serious cardiovascular and metabolic co-morbidities that may be mediated by increased cortisol secretion. Recent studies have focused on the ability of continuous positive airway pressure (CPAP) to reduce cortisol secretion in OSA patients, but the results have been mixed and only point measures of cortisol measurement have been used. Hair cortisol analysis presents a means of non-invasively and retrospectively examining cortisol production in these patients.

OBJECTIVES: This study examined whether hair cortisol concentrations are increased in OSA patients. Further, the effect of CPAP on hair cortisol concentrations was examined.

METHODS: Patients were recruited after undergoing a polysomnogram. Physical exam information and medical history were recorded. Polysomnogram data

including the apnea-hypopnea index (AHI), total hypoxemic time, and arousals per hour were recorded before and after CPAP. Additionally, a hair sample and Perceived Stress Scale (PSS) were collected before and after CPAP. Hair cortisol concentrations were determined using our modified salivary cortisol ELISA protocol.

RESULTS: Ninety-two patients were enrolled in the study, of which 31 returned after 3 months of CPAP therapy. Hair cortisol concentrations were negatively associated with total hypoxemic time (r^2=0.06, P<0.05) and trended toward a negative association with AHI. Hair cortisol concentrations were not significantly changed after placement on CPAP, but perceived stress was significantly reduced (P<0.001).

DISCUSSION: Cortisol secretion may be down-regulated in severe cases of OSA. The psychological stress of OSA may be reduced with CPAP, however physiological stress may remain.

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COMPARISON OF CONCENTRATION PROFILES OF GLUCOCORTICOSTEROIDS AND ENDOCANNABINOIDS IN HAIR

Detlef Thieme, Patricia Anielski, Aniko Krumbholz

BACKGROUND: Hair concentrations in general -and concentration profiles in particular- are potentially affected by contamination processes and washout and any quantitative interpretation, e.g. its correlation to drug abuse or biological effects- requires special care. Corticosteroids (e.g. cortisol) and endocannabinoids [arachidonoyl- (AEA), palmitoyl- (PEA), stearoylethanolamine (SEA) and 2-arachidonylglycerol (2-AG)] are agonists of corticosteroid or cannabinoid receptors and may potentially inhibit anti-inflammation or stress. Although both classes are biochemically independent, a positive correlation of respective hair concentration might be assumed.

METHODS: Hair profiles of these compounds were examined by LC-MS/MS -extra to other reference steroids, e.g. anrostendione, testosterone, cortisone, progesteron and 17-hydroxyprogesterone. All detection limits were better than 6pg/mg and permitted positive identification of relevant endogenous concentrations.

RESULTS: Hair concentration profiles were examined in a number of cohorts, e.g. pregnant vs. non-pregnant females or congenital adrenal hyperplasia (CAH) –patients. In contrast to our expectations, hair profiles exhibited little correlation between glucoccorticosteroids and endocannabinoids. This may be due to more complex biochemical mechanisms –e.g. the additional involvement of endocannabinoids in the regulation of appetite, anxiety and pain- and may additionally result from different decomposition and washout kinetics of the analytes.

THURSDAY, JUNE 28, 2012
SYMPOSIUM: ENVIRONMENTAL APPLICATIONS OF HAIR ANALYSIS

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DETECTION OF FLAME RETARDANTS IN HAIR AS BIOMARKERS OF SYSTEMIC EXPOSURE IN HUMANS
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BACKGROUND: Over the last 20 years, hair has moved from being a highly questionable biological matrix to mainstream and acceptable biomarker in forensic sciences where it is primarily used to determine past and present exposure to illicit drugs. In contrast, the use of hair to assess exposure to pesticides and persistent environmental pollutants is still not common. The applicability of this matrix to assess an individual's body burden of chemicals such as polybrominated diethyl ethers (PBDEs) can provide critical insight into current, but also to past exposure levels, which is not possible with more conventional matrices such as blood and urine. Furthermore, as PBDEs cross the placenta and since the hair the fetus is born with begins to grow during the third trimester, this matrix can be used to assess *in utero* exposure. These features of hair may therefore be used to determine the potential roles of chemicals such as PBDEs in mediating physiological or anatomical abnormalities in infants, children or adults.

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STATE OF THE ART IN HAIR ANALYSIS FOR THE BIOMONITORING OF HUMAN EXPOSURE TO ORGANIC POLLUTANTS
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BACKGROUND: We present here an overview of the different works dealing with the use of human hair for the detection of organic pollutants after environmental or occupational exposure. This state of the art covers both "classical" compounds whose detection in human hair was first reported more than ten years ago and that are still investigated in current studies, and newcomers that were only recently analyzed in hair. The variations in pollutants concentration in hair depending on the different populations and the different countries are also presented. A focus is set on the analytical progresses that were observed during the last decade and the associated improvement in method sensitivity and increase in rates of positive detection reported in the different works. We also highlight here the recent development of multi-residue methods to investigate cumulative exposure.

CONCLUSIONS: Finally, we underline the specificities associated with the field of environmental exposure biomonitoring regarding analytical aspects and result interpretation.

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STABLE ISOTOPES IN HAIR
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BACKGROUND: The use of stable isotopes as an application research tool has been around for over 60 years. The tools were almost exclusively used by geological scientists for the better part of the 60s and 70s. Biologists began using stable isotopes in the late 60s but it wasn't until the advent of continuous-flow technology in the mid-80s to early 90s that various fields of research, from geology to medicine, really expanded where isotopes of ^{2}H (D), ^{13}C, ^{15}N, ^{18}O and ^{34}S are used for various fields of applications. Surprisingly, studies using stable isotopes in hair began with anthropology looking for clues of mummies' diets before modern applications for medicine or forensic was used. A Pan-Canadian project is looking at stable isotopes and elemental analysis of hair. The relationship of hair with drinking water and food consumption is culminating in a multi-dimensional database of Canadian hair for geo-location purposes. Along with an advanced custom-made database search engine and GIS modeling, it is expected to be used for tracing the movements of cold case human remains where hair is available. Two case studies will be presented: The Mme Victoria case (Montréal, Québec) and Ms. Napanee (Napanee, Ontario).

THURSDAY, JUNE 28, 2012
ORAL SESSION: NOVEL APPLICATIONS OF HAIR ANALYSIS

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METABOLIC RATIO OF OPIOIDS IN HAIR: A NOVEL METHOD TO STUDY POPULATION GENETIC POLYMORPHISMS

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BACKGROUND: Codeine, still widely prescribed for its analgesic effects, is subject to CYP2D6 polymorphisms affecting its metabolism. Metabolic ratio (MR) of morphine to codeine represents the extent of codeine metabolism to its active metabolite. MR has previously been studied in blood and urine. However, these compounds can be found in hair, which has not been used as a matrix to study MR. Studying MR in hair can provide a simple method to evaluate population variability obviating the need for blood.

METHODS: Hair samples were collected from the Motherisk Laboratory for testing as per request by social workers and other agencies. From July 2010 to December 2011, 1966 samples were tested for codeine and morphine through GC-MS analysis. All codeine positive samples and respective morphine results were used to calculate metabolite-to-parent MR.

RESULTS: Of the 239 codeine positive samples, 192 had an MR of 0, indicating no morphine detection. Nineteen samples were found to have an MR <1. Fifteen samples with an MR between 1 and 2 were

found. In addition, 5 samples with an MR between 2 and 3 were detected. Finally, 8 samples were found to have an MR >3. Contrasting these data to blood MRs will allow for validation of this method.

CONCLUSIONS: From these results, wide differential incorporation of codeine and morphine into human hair has been found. With better understanding of codeine and morphine incorporation into human hair, polymorphisms can be studied in hair using MR. Hair collection is non-invasive and shows past use, unlike blood.

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DOSE DEPENDENT PHARMACOKINETICS OF POLYBROMINATED DIPHENYL ETHERS (PBDE) IN RAT HAIR?

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BACKGROUND: The ability to detect xenobiotics in biological samples (*e.g.*, blood or urine) has been long established allowing for relatively noninvasive estimations of exposure in individuals. There are times when obtaining traditional matrices can be difficult (*e.g.*, newborns), and there is a need for an alternate matrix that can reflect long-term exposure. Hair offers a wider window for retrospective detection of chronic and past exposure/consumption of xenobiotics. Such information is lacking in the case of environmental toxins such as polybrominated diphenyl ethers (PBDE) which are implicated as endocrine-disrupters. To determine if hair levels of PBDEs are of use as biomarkers of systemic exposure, hair from rats fed a PBDE laden diet was collected and analyzed.

METHODS: Adult Sprague Dawley rats were divided into 5 groups (n=10/group) and fed a diet containing PBDEs at concentrations of 0, 0.25, 2.5, 25 mg/kg diet for 70 days. Thirty mg of hair was analyzed by GC/MS for BDE-28, 47, 99, 100, 153, 154, 183 and 209 by GC/MS.

RESULTS: PBDEs were detected in all treatment groups and there was a clear dose response curve for BDE-47, 99, 100, 153, 154, and 209, with levels being statistically significant (p<0.001) for 2.5 and 25 mg/kg/day. The ΣPBDE was 0.169±0.157, 0.265±0.089, 1.706±.918 and 7.504±2.595 ng/mg hair for 0.025, 0.25, 2.5 and 25 mg/kg/day treatments, respectively. The levels were statistically significant (p < 0.001) from control for each group. BDE-28 was not detected in any treatment group; BDE-183 was present in only the 25 mg/kg/day group.

CONCLUSION: PBDE accumulates in a dose response manner. Variations may be due to differences in individual congener metabolism. Although not all congeners showed a dose response, further work to determine the *in vitro* metabolic pathway is needed to determine why these differences are present.

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RETROSPECTIVE HISTORY OF PBDE EXPOSURE MEASURED IN HAIR

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BACKGROUND: Polybrominated diphenyl ethers (PBDEs) are chemicals that are added to a variety of consumer products as flame-retardants, and have been classified as emerging endocrine disruptors. They are persistent in the environment and have been detected in humans. Previous studies have suggested hair is a suitable matrix for examining human exposure to organic pollutants, as moderate-strong correlations were observed between hair and blood levels. It is believed that the majority of exposure is from contaminated dust in our environment. The aim of this study is to measure the intra-individual stability of PBDE levels detected in hair of individuals who remained in the same home environment for one year.

METHODS: Questionnaires and hair samples from 29 females were collected at the Hospital for Sick Children as part of another study. To assess long-term stability of PBDEs, hair samples were separated into four 3 cm segments representing a one-year period. Hair segments were analyzed for levels of eight PBDE congeners, BDE-28,-47,-99,-100,-153,-154,-183 and -209 GC-MS. A repeated measures

ANOVA was used to detect differences in exposure among segments ($p<0.05$).

RESULTS: Smaller concentrations of ΣPBDEs (pg/mg) were observed in more proximal segments (mean±SD), first (42.0±24.3), second (62.6±40.6), third (86.0±48.6), and fourth (108.7±74.2). A significant increase in total ΣPBDEs was seen overtime ($p<0.0001$). BDE-47 and 99 were dominant congeners that contributed to 60% of total PBDEs in hair.

CONCLUSION: Variations of PBDE levels detected overtime may be suggestive of multiple sources and pathways of exposure. Environmental adsorption may contribute to the increases in PBDE levels observed in distal hair segments. Additional data is needed to determine the primary route of PBDE incorporation into hair.

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ANALYSIS OF CYCLOSPORIN A IN HAIR SAMPLES FROM LIVER TRANSPLANTED PATIENTS

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BACKGROUND: Cyclosporin A (CsA) is one of the most important immunosuppressants used to prevent organ rejection after liver transplantation. Therapeutic benefit and adverse toxicity are associated with only small differences in CsA blood concentration. Because of large interindividual differences in CsA pharmacokinetics, correct individual dosage and compliance are essential for successful therapy. An analytical method for the determination of CsA in hair samples was developed. Hair samples from patients treated with CsA after liver transplantation are analysed, to investigate correlations between hair concentrations, blood concentrations and CsA doses and to evaluate if hair analysis could be useful in immunosupressive therapy.

METHODS: Hair samples from patients were segmented and decontaminated. After alkaline hydrolysis and liquid-liquid extraction, CsA was analysed by liquid chromatography/ tandem mass spectrometry. Method development and validation parameters are presented.

RESULTS: CsA was detected in all hair samples from patients in concentrations between 315 pg/mg and 1317 pg/mg. Correlation between CsA hair concentrations and CsA-doses was poor, as well as the correlation between hair concentrations and trough blood concentrations. Segmental hair analysis in patients with long term CsA-treatment did not reflect minor changes in CsA-dosage, but a pattern in segmental CsA hair concentration could be observed with major modifications of dosage.

CONCLUSIONS: CsA can be detected in hair samples. Because relations of CsA-doses and hair concentrations in between patients are different, it seems to be not possible to verify the amount of CsA intake by hair analysis. Future studies will show whether segmental analysis could be useful in the detection of substantial noncompliance.

POSTER PRESENTATION ABSTRACTS

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SCREENING OF DRUGS IN HAIR USING LIQUID CHROMATOGRAPHY TIME-OF-FLIGHT TECHNOLOGY

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BACKGROUND: The objective of this study was to develop and validate a qualitative screening method that met the new SoHT guideline criteria.

METHODS: Extraction of 20 mg hair was performed by a previously validated procedure using overnight incubation in a mixture of methanol:acetonitrile:formiate buffer pH 3 (10:10:80). Analysis was performed on an Agilent 6540 quadrupole time-of-flight mass spectrometer in combination with an Agilent 1290 Infinity UHPLC system. Separation was achieved within 12 minutes by linear gradient chromatography on a HSS T3 column at acidic conditions. An in-house database containing 30 compounds from the groups amphetamines, opiates, opioids, cocaine, benzodiazepines and other sedatives including 6 deuterated internal standards was built by analyzing solutions from certified standards. Data were extracted using mass accuracy of ± 10 ppm, retention time deviation of ± 0.15 min and area of ≥ 30 000 counts. Identification was based on scoring of

retention time, accurate mass measurement and isotopic pattern. Validation included matrix effects, selectivity, imprecision of the scoring parameters at the proposed cut-offs and two additional levels at 10 times and 25 times the cut-off and a method comparison with the present LC-MS-MS method using 50 authentic hair samples. A daily cut-off calibrator was used to identify positive samples.

RESULTS: All cut-offs could be met with imprecisions of less than 5% for most parameters and analytes. Hair from 12 drug free subjects did not produce any positive results. There was a good concentration-response relationship up to 25 times the cut-off and the method comparison agreed in more than 90% of the cases.

CONCLUSIONS: We conclude that the developed method meets the criteria of the new SoHT guidelines for screening cut-offs.

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PHARMACOPSYCHOSIS OR SCHIZOPHRENIA AMONG CONSUMERS OF CANNABIS? AID IN DIAGNOSIS WITH HAIR SAMPLING

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BACKGROUND: Among consumers of cannabis, a brief psychotic disorder (BPD) can be at best, the clinical manifestation of a pharmacopsychosis, at worst the harbinger of schizophrenia's onset. We measured THC and CBD concentrations in hair among different groups of patients to evaluate the possible protective role of CBD in the onset of non-affective psychotic disorders (NAPD) (delusional disorder and Schizophrenia).

METHODS: Four groups of cannabis users were determined.
Group 1: 16 patients hospitalized for surgical reasons (Witnesses).
Group 2: 26 patients undergoing psychiatric follow-up for the treatment of NAPD.
Group 3: 15 patients with a pharmacopsychosis.
Group 4: 11 patients with other psychiatric disorders (borderline personality disorder, mood disorder...).

After sampling of head or axillary hair, THC and CBD were assayed by GC-MS (LOQ: 0.05 ng/mg) on their whole length for axillary hair and with a maximum head hair length of 6 centimeters. The results were analyzed statistically by the nonparametric Kruskal-Wallis test.

RESULTS: The NAPD group presented the highest concentrations of cannabinoids in hair, and the ratio THC/CBD was also higher reflecting the "imbalance" between psychoactive THC and "protective" CBD. Pharmacopsychosis group had the lowest concentrations and ratio. The differences were significant between the pharmacopsychosis group and the other groups (Witnesses, NAPD, Others) for THC and CBD (THC: p = 0,0004; 0,0006; 0,0353 and CBD: p = 0,0008; 0,0087; 0,1703; respectively).

CONCLUSIONS: This study shows that the ratio of THC/CBD is higher among patients with NAPD. It also shows that the cannabis pharmacopsychosis occurs in "low" consumers, who might present an individual susceptibility. Among consumers of cannabis who exhibit a BPD, the inclusion of three values (capillary THC, CBD and THC/CBD ratio) provides help for the psychiatric diagnosis and would, in some cases, retrospectively reclassify a psychiatric disorder.

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FINDINGS OF COCAINE AND METABOLITES IN HAIR OF YOUNG CHILDREN AFTER PASSIVE COCAINE EXPOSURE: A CASE REPORT

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BACKGROUND: We report a case of passive cocaine exposure of two children, proved by hair analysis after a positive immunochemical test in the urine of the youngest at the hospital.

METHODS: Hair was decontaminated prior to analysis. Hair specimens (20 mg) were extracted with deuterated(-D3) cocainics (cocaine (COC), benzoylecgonine (BZE), ecgoninemethylester (EME), and cocaethylene (CE)) as internal standards. Liquid-liquid extraction was performed before injection into a LC-MS/MS TSQ Vantage (ThermoFisher Scientific). Separation was achieved on a C_{18}-column. The detection was performed in positive ESI / SRM mode and allowed for the simultaneous detection of COC, BZE, EME, CE, and anhydroecgonine methylester

(AEME). Quantification limits in hair were 25 pg/mg for COC and BZE, 10 pg/mg for EME and AEME.

RESULTS: Children #1 and #2 were 2 and 5 year-old. Segmental hair analysis results for child #1 on seven segments (1-2cm length) ranged 0.203-0.692 ng/mg for COC and 0.041-0.256 ng/mg for BZE. EME and AEME were not detected. For child #2 the detected concentrations on four segments (2-4cm length) ranged 0.450-2.40 ng/mg for COC, 0.05-0.250 ng/mg for BZE, 0.15-0.20 ng/mg for EME. AEME was detected on one segment at 0.01 ng/mg.

DISCUSSION/CONCLUSION: Segmental hair analysis provides very helpful retrospective information to prove drug exposure in children. The parent's hair were also submitted to segmental analysis and proved that the father had used cocaine in the lasts months (> 14 ng/mg) and that the mother was probably also passively exposed to the drug (< 0.5 ng/mg). This case that has arisen in an unsuspected population underlines the potential risk of passive cocaine exposure to children living in household environments where cocaine use occurs.

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COMPARISON OF SOLID PHASE EXTRACTION PROCEDURES FOR THE SEGMENTAL ANALYSIS OF ETHYL GLUCURONIDE IN HAIR BY UPLC-MS/MS

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BACKGROUND: Ethyl glucuronide (EtG) detection in hair offers the opportunity to evaluate alcohol consumption over a long period in time and is of particular interest for driver licence regranting. In this context, a solid phase extraction (SPE) procedure for EtG extraction in segments of hair (30 mg) was developed as part of a sensitive UPLC-MS/MS method. Several SPE cartridges were evaluated concerning their extraction efficiency and matrix effects in the final MS method.

METHODS: Nine cartridges were tested and can be divided in five categories. Strong anion exchangers studied were Bond Elut SAX (Agilent), Strata SAX (Phenomenex), Isolute SAX and Isolute PE-AX (Biotage). Ion-exchange polymeric sorbents consisted on Oasis MAX (Waters) and Strata X-AW (Phenomenex). Bond Elut NH_2 (Agilent) was a mixed mode combining anion exchange with polar properties. Strata Screen A (Phenomenex) was evaluated as mixed mode combining anion exchange properties with C8 polymer. Clean Screen EtG (UCT) was also evaluated. Pulverised hair samples were spiked with EtG at 500 pg/mg hair. Water (1.5 mL) was added before incubation at 45°C during 2 hours. These hair samples were loaded onto the conditioned SPE cartridges. Loading, washing and eluting solutions were collected separately and analyzed to detect EtG. Cartridges with poor retention during loading and washing or with a low ability to elute EtG were not used for further experiments. Several wash and elution solvents were tested for each cartridge.

RESULTS: Recovery and ion suppression were 83% and 27% respectively for Clean Screen EtG. For Bond Elut SAX a recovery of 33% and no matrix effect were observed.

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QUANTIFICATION OF ETHYL GLUCURONIDE (ETG) IN HAIR BY LC/MS/MS AS A MARKER FOR CHRONIC EXCESSIVE ALCOHOL CONSUMPTION

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BACKGROUND: (For Research Use Only. Not for Use in Diagnostic Procedures.) The traditional approach to assess the extent of alcohol consumption is through blood and urine analysis but unless the samples are stored in appropriate specimen containers, with preservatives, ethanol can be produced as an artifact of bacterial fermentation post collection. This can result in elevated and inaccurate ethanol concentrations in these samples. In this poster, we present a sensitive LC-MS/MS method to detect and quantify ETG in hair.

METHOD: Hair samples were incubated in water, extracted using an anion exchange SPE cartridge with ETG-D5 as the internal standard and then reconstituted. The extracts were run over a 7 minute gradient using an Agilent HILIC+ 3.5 µM 100 x 2.1 mm column, at 40 ºC using gradient conditions from 95% to 60% organic with the flow changing from 400 µL/min at the beginning of the run to 1100 µL/min at the end of the run.

RESULTS/CONCLUSION: An advantage of the use of ETG in hair is that it is not subject to the effect of bacteria and also offers the opportunity to extend

the window of detection compared to blood and urine. As ethanol metabolism is independent of dose, blood and urine concentrations can vary widely between individuals, however with hair analysis the use of a 30 pg/mg cut off, as proposed by the Society of Hair Testing (SoHT), can be applied as standard and the developed LC-MS/MS achieved this level of detection with a LOQ of 8 pg/mg and linear over the range tested; 8-50 pg/mg.

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THE DISTRIBUTION OF DRUGS OF ABUSE FOUND IN HAIR IN THE EASTERN PART OF SWITZERLAND

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BACKGROUND: Hair analysis is well accepted to provide valuable information for testing the driving ability for re-granting the driving licence. In many cases drug abstinence or teetotalism is to complied with for a certain period of time in the range from one to four years.

METHODS: In the present work, drugs of abuse are extracted from pulverized hair overnight in the presence of deuterated standards and a mixture containing methanol/acetonitrile/phosphate buffer (pH 6) = 1/1/2 (v/v). After solid phase extraction, the measurement is performed by LC-MS/MS using the MRM mode for quantification.

RESULTS: Since July 2011, a number of 665 analyses for drugs of abuse and some medicaments was analyzed in our institute yielding several positive cases e.g.: 81 for Cocain and metabolites; 46 for Methadon and EDDP; 32 for MDMA and MDA with an average relative amount of 5% of MDA; 20 for Amphetamin; 11 for Buprenorphin and Norbuprenorphin with an average relative amount of 11% of Buprenorphin; 11 for Methylphenidat and 5 for Monoacetylmorphine (MAM) displaying a ratio of MAM/Morphin > 1.3.

CONCLUSIONS: In Switzerland, the role of hair analyses for testing the driving ability has been well established and is meanwhile performed in all Swiss institutes of forensic medicine. In the case of a change of law due to the currently discussed « Via Sicura », the number of cases for which abstinence has to be proven over a certain period of time will be further increasing.

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CAFFEINE AND PARAXANTHINE IN HUMAN HAIR TO ASSESS CYP1A2 ACTIVITY: DEVELOPMENT AND VALIDATION OF AN LC-MS/MS-BASED PROCEDURE

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BACKGROUND: Phenotyping is the proper method to assess CYP1A2 activity, because inter-individual differences in the activity are caused by genetic and environmental factors. The standard approach is determination of the paraxanthine/caffeine ratio in plasma, after intake of caffeine. Since CYP1A2 is important in the metabolism of antipsychotics, it may be advantageous for e.g. psychiatric patients to develop an alternative, non-invasive procedure, in which intake of caffeine and the preceding caffeine-free period are no longer required.

METHODS: All experiments were performed on a UPLC™-API 4000 system. Isotopically labeled internal standards were used. An optimized hair extraction protocol was developed.

RESULTS: Separation is achieved on a HSS-T3 column, followed by detection in MRM-mode after positive ESI. The optimized sample preparation comprises double decontamination, manual grinding, proteolytic digestion, and clean-up using RP-SPE. Linear calibration curves for caffeine and paraxanthine range from 0.02 to 0.50 ng/mg. Precision (within-run and between-run) was below 12% (%CV) and accuracy below 5% (bias) for all QC's (0.02, 0.06, 0.20 and 0.40 ng/mg). Ion suppression is compensated by the IS for both caffeine and paraxanthine. No interferences with both compounds were seen in the chromatogram. Stock solutions, incurred and processed samples were stable under the evaluated conditions.

CONCLUSION: A reliable LC-MS/MS method for caffeine and paraxanthine analysis in human hair was successfully optimized and validated.

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HUMAN HAIR CORTISOL ANALYSIS USING AN ELISA: A COMPARISION OF THE DIFFERENT REPORTED METHODS

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BACKGROUND: Recently, hair cortisol analysis has been a topic of global interest among researchers. Therefore, the need for critical examination of the analytical methods has to be done in order to standardize the method and allow uniform interpretation.
OBJECTIVE: To assess the similarities and differences among methods published to date.
METHODS: This study compares among four published laboratories procedures: Drs.Van Rossum, Kirschbaum, Laudenslager, and Dr.Koren - Van Uum. We examined several common dimensions in their procedures.
RESULTS: A major difference was the ELISA kit used. Alpco diagnostics (Salem, NH, USA) is used by Koren-Van Uum group, Van Rossum uses DRG International, (USA) or DRG Instruments GmbH, (Marburg, Germany), Kirschbaum uses IBL(Hamburg-Germany), and Laudenslager uses Salimetrics, (LC). Koren and Van Rossum appear to have nearly the same mass of hair (10-15mg), do not wash the hair samples, have the same pulverization method which is mincing with surgical scissors, and the same amounts of the extraction and reconstituting solvents. In contrast, the other two groups use 50 mg of powdered hair and wash hair samples 2-3 times/3 minutes each with 2.5 ml isopropanol. Considerable other similarities were found. The cortisol range determined for Koren was; 83.1 pg/mg compared to the control group 46.1 pg/mg. Whereas for Van Rossum, cortisol levels were 47.32 pg/mg versus 29.72 pg/mg. Kirschbaum cortisol range was ~10 - 45 pg/mg, and Laudenslager ~25 - 45 pg/mg. We found that due to the variability in the groups' methods, some technical issues occurred and caused differences in the cortisol range.
CONCLUSIONS: Consensus toward developing one method that is comprehensive, convenient and appropriate should be aimed.

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EVIDENCE OF CATHINONES USE THROUGH HAIR ANALYSIS USING LC-MS/MS

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BACKGROUND: (For Research Use Only. Not for use in diagnostic procedures). We have developed a novel method for cathinones in hair by LC-MS/MS to include cathinone, methcathinone, methylethcathinone, mephedrone (4-methylmethcathinone), methedrone/bk-PMMA (methoxymethcathinone), MDPV (methylenedioxypyrovalerone), butylone/bk-MBDB (methylamino-1-(3,4-methylenedioxyphenyl) butan-1-one) and methylone/bk-MDMA (2-Methylamino-1-(3,4-methylenedioxyphenyl) propan-1-one.
METHODS: After decontamination of the hair strand in methylene chloride, segments were cut into small pieces with scissors, weighed and incubated in methanol overnight at 40°C in the presence of internal standard. After sonication and centrifugation, the supernatant was recovered and evaporated to dryness. After reconstitution in 1 mL of acetonitrile/0.1% formic acid in 2mM ammonium formate buffer (5:95), 10 μL were injected. Chromatographic separation was achieved on a Kinetex™ XB-C18 (2.1 x 100mm, 2.6μm) column. The LC-MS/MS system consisted of a Shimadzu™ UFLC-XR coupled to an AB SCIEX™ QTRAP 5500® using Positive Electrospray Ionisation. The acquisition was done in Scheduled™ MRM™ mode.
RESULTS: Linearity was from 50 pg/mg to 20 ng/mg. Inter and intra-day variability (%CV) were less than 10% at 0.5 and 2 ng/mg. Sixty one hair specimens were screened for cathinones. Twenty one were found positive for mephedrone with concentrations ranging from 0.1 to 36 ng/mg with a median concentration of 0.42 ng/mg. These concentrations correlated with results previously obtained by GCMS. MDPV and methedrone were also detected in two cases at trace levels.
DISCUSSION/CONCLUSION: The cathinones seem to be well incorporated in hair and the method is sensitive enough to be able to detect cathinones in subjects where occasional or regular use is suspected.

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AN ASSESSMENT OF HAIR DRUG FINDINGS IN THE PEDIATRIC POPULATION OVER A FOUR-YEAR PERIOD

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BACKGROUND: Clinicians rely upon test results to make decisions concerning the medical treatment of minors. This presentation's objective is to categorize the drugs found in hair submitted over a four-year period when the request to test for "abused and therapeutic drugs" was made.

METHODS Samples were screened by ELISA and full-scan GC/MS. Confirmation testing was performed by GC-NPD, SIM- and full-scan GC/MS. Hairs were rinsed and pulverized before undergoing methanolic extraction for 2-hours at 45°C. The extracts and rinses were then analyzed.

RESULTS Findings from 100 positive cases were categorized: *Neonate* (≤ 28 days), *Infant* (< 1 year), *Toddler* (1-3 years), *Pre-school* (4-5 years), *School-age* (6-12 years) and *Teen* (13-16 years). Of 64 drugs/metabolites identified, nicotine, diphenhydramine and chlorpheniramine were the most frequent. The most prevalent drugs by group excluding nicotine were: *Neonate* - caffeine, *Infant* - benzocaine, *Toddler*, *Pre-School*, *School-age* and *Teen* - diphenhydramine. One *Toddler* had 12 drugs present including cold medicines, a narcotic analgesic and a muscle relaxant. Methamphetamine and cocaine were also commonly found. Our first levamisole case was in 2009, the year SAMHSA warned about levamisole-cocaine mixtures. MDPV was identified in a 2011 case.

DISCUSSION/CONCLUSIONS: The pediatric population should not be exposed to illicit drugs or unnecessary medications. This analysis substantiates the need to test beyond illicit drugs. Cold medications were prevalent, but also identified were pain relievers, antidepressants and sleep-aids. Comprehensive hair testing may be utilized to evaluate past or on-going exposure.

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INCREASED HAIR CORTISOL CONTENT IN A CANADIAN FIRST NATION

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BACKGROUND: Cortisol content in hair is increasingly being used as a biomarker of chronic stress. Many members of Canadian First Nation communities are experiencing stress related to a higher incidence of chronic diseases, socio-economic factors, the state of the environment, and particularly about the health of their children and grandchildren. This study aims to investigate the hypothesis that members of a First Nation community exhibit stress levels above those of an urban, non First Nation community.

METHODS: Hair samples were collected from the posterior vertex of 40 Walpole Island First Nation (WIFN) volunteers and from 32 Caucasian volunteers living in and around London, Ontario. An enzyme immunoassay technique was used to measure cortisol content in 1 cm of hair, considered to represent 1 month of exposure.

RESULTS: Mean hair cortisol content (+/- SEM) in WIFN volunteers was 180.7 (+/- 9.5) ng/g. This is significantly higher than the mean hair cortisol content in the Caucasian population of 112.5 (+/- 54.4) ng/g (p <0.0001 , Mann-Whitney U-test).

DISCUSSION: We demonstrated increased hair cortisol concentrations in volunteers from Walpole Island First Nation compared to volunteers from a urban, non First Nation community. This suggests that chronic stress, as assessed by increased hair cortisol is greater in this Canadian First Nation community. The reasons for this apparent increased stress are likely to be socio-economic and are worthy of further evaluation.