

A review Hair tissue analysis: An analytical method for determining essential elements, toxic elements, hormones and drug use and abuse

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ABSTRACT: Hair is formed from a cluster of matrix cells that make up the follicle. During the growth phase of the hair, metabolic activity is greatly increased, exposing the hair to the internal metabolic environment; extracellular fluids, circulating blood and lymph. As the hair reaches the surface, its outer layers harden, locking in the metabolic products accumulated during this period of hair formation, providing a permanent record of metabolic activity. Determining the levels of the elements in the hair is a highly sophisticated analytical technique, when performed to exacting standards and interpreted correctly, it may be used as a screening aid for mineral deficiencies, excesses, and/or biochemical imbalances. Tissue mineral analysis (TMA) provides the veterinarian and trainer with a sensitive indicator of the long term effects of diet, stress, and toxic metal exposure. Most deficiencies in animals are brought about by altered relationships of minerals within the body. It has become evident that either the retention or loss of minerals by the animal is equally important as the nutrients consumed from the feed itself. Both appearance and performance can be greatly influenced by adequate tissue levels of essential nutrients due to their effect upon cellular function. Minerals are necessary for several important functions in the growing and mature horse, such as, formation of structural components, enzymatic co-factors, and energy transfer. They are also used in the production of hormones, vitamins and amino acids. Tissue mineral testing can aid in measuring mineral retention; it may also help to determine which supplements and feeds are required and more importantly, what is not required in order to avoid nutritionally-induced deficiencies or imbalances.

Keywords: Hair analysis, Mineral, Essential elements, Drug, Hormone

INTRODUCTION

Monitoring the nutritional status of essential elements and assessing exposure of individuals to toxic elements is of great importance for human health. Thus, the appropriate selection and measurement of biomarkers of internal dose is of critical importance. Due to their many advantages, hair samples have been widely used to assess human exposure to different contaminants (Rodrigues et al. 2008).

Hair mineral analysis (HMA) is a safe, non-invasive test that measures the levels of nutrients and toxic metals found in hair. Hair mineral analysis can detect whether there is an excess or deficiency of vital nutrient minerals such as calcium, potassium, zinc and iron. It can also identify over-exposure to toxic metals such as aluminum, lead, arsenic and mercury. HMA is an invaluable screening tool in both every day and preventive health care. This test provides a reading of the minerals deposited in the cells and interstitial spaces of the hair over a 2-3 month period. It does not provide an assessment of the mineral content of other tissues of the body. However, testing the hair can allow one to infer what is occurring in other tissues. The use of human hair analysis technique has proved to be a well-suited biological marker of occupational and environmental exposure of man (Bencko 1995).

A hair analysis can be done anywhere and in a short amount of time. Unlike other biopsies, hair mineral analysis is painless. Urine, sweat and saliva measure only the components that are excreted from the body. Blood

measures the components that are absorbed and temporarily circulating in the body before excretion and/or storage. Hair can be used to measure the components stored in the body. Hair tissue mineral analysis reveals the body's mineral imbalances and metal toxicities. Toxic elements found in the hair produce a permanent record of exposure. The concentrations are 10-15 times higher in the hair than in the blood or urine. The body produces over 3,000 enzyme reactions that control our body's functioning and are completely dependent upon sufficient and balanced amounts of minerals. Recent scientific research has shown that almost all non-viral diseases are related to mineral imbalances in the body. Early detection of mineral imbalances and metal toxicities has been found to be predictive of health impairments and therefore essential in the prevention of advanced metabolic disease conditions (PNHC 2013).

REFERENCE REVIEW

Hair differs from other materials used for toxicological analysis because of its unique ability to serve as a long-term storage of foreign substances with respect to the temporal appearance in blood. Over the last 20 years, hair testing has gained increasing attention and recognition for the retrospective investigation of chronic drug abuse as well as intentional or unintentional poisoning. In this paper, we review the physiological basics of hair growth, mechanisms of substance incorporation, analytical methods, result interpretation and practical applications of hair analysis for drugs and other organic substances. Improved chromatographic and mass spectrometric techniques with increased selectivity and sensitivity and new methods of sample preparation have improved detection limits from the ng/mg range to below pg/mg. These technical advances have substantially enhanced the ability to detect numerous drugs and other poisons in hair. For example, it was possible to detect previous administration of a single very low dose in drug-facilitated crimes. In addition to its potential application in large scale workplace drug testing and driving ability examination, hair analysis is also used for detection of gestational drug exposure, cases of criminal liability of drug addicts, diagnosis of chronic intoxication and in postmortem toxicology. Hair has only limited relevance in therapy compliance control. Fatty acid ethyl esters and ethyl glucuronide in hair have proven to be suitable markers for alcohol abuse. Hair analysis for drugs is, however, not a simple routine procedure and needs substantial guidelines throughout the testing process, i.e., from sample collection to results interpretation (Pragst and Balikova 2006).

To demonstrate the utility of hair for the diagnosis of a drug-addiction. Because of its peculiar characteristics, hair analysis provides a way of obtaining information that cannot be acquired by other commonly used biological matrices, mainly because of the wide diagnostic window of detection allowed by this specimen and the possibility of establishing the chronological profile and the severity of drug consumption. In this paper some factors that have to be considered during the interpretation of the results, such as dose/concentration relationship, influence of cosmetic treatment and other limitations, will be discussed. An important point will be devoted to the applicability of these analyses to establish chronic and heavy alcohol consumption. Material and methods A revision of the published papers related to the different aspects of these analyses of drugs of abuse in hair samples has been performed, emphasizing the experience of the author in this field. Results Hair analysis for medical purposes was initiated some decades ago. In the 1960s and 1970s, hair analysis was used to evaluate exposure to toxic heavy metals. At the beginning of the 1980s hair analysis for drugs of abuse was initiated. Currently, these analyses are routinely used in many laboratories from all over the world. They are applied in forensic, clinic or criminal toxicology, in workplace testing, in revocation or restoration of a driving license, etc (Jurado Montoro 2007). An overview of the most common sectioning patterns utilised in the analysis of hair for drug use; report on the major user groups (sectors) that currently make use of hair analysis in the United Kingdom (UK); present the results for the different drug groups analysed in samples of hair samples analysed at TrichoTech between 2001 and 2005. The chances of identifying people on drugs in the workplace by testing hair samples are twice as likely than urine samples (Tsanaclis and Wicks 2007).

Concentrations of minor and trace elements (Mg, Ca, Fe, Ba, Cu, Zn, Cd, Ni, Al, Mn, Cr, Ti, and V) in the hair of three races of dogs (fox terrier, schnauzer, and mini schnauzer) were analyzed by the inductively coupled plasma-atomic emission spectrometry method. The influence of five washing solutions, deionized water, acetone, methanol, EDTA, and Triton X-100, on the concentrations measured in hair was investigated. Triton X-100 was found to be suitable to use for the removal of exogenous elements in multielemental hair analysis. Additionally, the results indicated that the concentration of the elements measured in the dogs' hair were similar to those reported for human hair. The relation between the element content in the dog hair and its color were similar to those found for human hair (Chyla and Zyrnicki 2000).

Background Zinc is essential for all life forms and plays a vital role in human nutrition and biochemical functions. Epidemiologic studies suggest that zinc deficiency may be associated with increased risk of cancer.

There were measured the concentration of Zn in whole blood and scalp hair of female patients with breast and ovarian cancers from different cities of Pakistan. There was a significant decrease in mean total of Zn in whole blood and scalp hair samples of both cancer groups of patients compared to a controlled healthy female group. There was an association of Zn with biological samples in different types of cancer in females (Memon et al. 2007).

A sample of 573 infants (aged 0 to 12 months) from the Moroccan city of Marrakech was studied in order to determine the level of Pb and Al contaminations. Age, gender, and parents' occupation influenced significantly Pb content but not Al content. Larger mean values were measured for Al compared with Pb. This finding can be explained by a higher level of Al available in both the infant diet (complementary feeding) and the local environmental factors (soil and drinking water). During weaning, beverages like tea, widely used in Morocco, represent an important source of Pb and Al contamination. Al content in drinking water was above the international standard (Souad et al. 2006).

The concentrations of essential trace elements (copper, zinc, selenium, manganese, chromium, molybdenum, cobalt, and iodine) in the scalp hair of 21 patients with severe motor disabilities receiving enteral nutrition were measured using inductively coupled plasma-mass spectrometry. Preliminary results show that copper, selenium, and molybdenum concentrations in the patients' hair were significantly lower than those in an age-matched control group ($p < 0.01$). This suggests that intake of these elements may be reduced in patients receiving restricted enteral nutrition, although the clinical significance of these results should be discussed (Munakata et al. 2006).

The causes of night blindness in children are multifactorial and particular consideration has been given to childhood nutritional deficiency, which is the most common problem found in underdeveloped countries. Such deficiency can result in physiological and pathological processes that in turn influence hair composition. Method An ultrasonic-assisted acid leaching procedure was developed as a sample pretreatment for the determination of Zn, Cu, Cd, As and Pb in human scalp hair samples of night blindness male children with age between 5 to 15 y and compared with the children without vision anomalies that lived in the same localities. The effects of different factors on acid leaching of metals, such as preintensification time (without ultrasonic stirring) after treatment of acid mixture, exposure time to ultrasound and temperature of the ultrasonic bath have been investigated. The mean values of Zn and Cu in scalp hair samples of children having night blindness were significantly lower as compared to normal healthy children, while the level of toxic metals As, Cd and Pb were significantly higher in children having ocular problems as related to normal children. These data present guidance to clinicians and other professional investigating deficiency of essential trace metals and excessive level of toxic metals in biological samples (Kazi et al. 2006).

Hair samples were contaminated by rubbing with cocaine (COC) followed by sweat application, multiple shampoo treatments and storage. (Hill et al. 2008).

Short-term changes in activity of the hypothalamic-pituitary-adrenal (HPA) system are routinely assessed by measuring glucocorticoid or metabolite concentrations in plasma, saliva, urine, or feces. However, there are no current methods for determining long-term (i.e., weeks or months) activity of this system were described the development and validation of a simple procedure for measuring cortisol concentrations in the hair of rhesus macaques. This procedure involves two brief isopropanol washes of the hair strands to remove surface contaminants, subsequent powdering of the washed and dried hair, a 24-h methanol extraction followed by evaporation of the solvent and reconstitution of the extract in assay buffer, and finally analysis of the extracted cortisol by a sensitive and specific enzyme immunoassay. Our results confirm the specificity of the procedure for cortisol, show that proximal and distal segments of hair do not differ in their cortisol concentration, and demonstrate that a significant and prolonged stressful experience produces a significant increase in hair cortisol. This new procedure should be valuable for assessing baseline HPA activity in nonhuman primates (and, with appropriate validation, in other species as well) over relatively long periods of time, and also for monitoring chronic stress that might be associated with various experimental manipulations (Davenport et al. 2006).

A study was performed on 5 subjects (N=28) that lived together or were family related. The elemental composition of hair of the studied subjects was compared with the average content of the population living in the same urban area (Wroclaw city, south-west Poland), with the population of a non-industrialized area in Poland (Silesian Beskid), as well as with the population of north-east Sweden and Rio de Janeiro. When comparing the composition of hair from the studied subjects with the people living in the same city, it was found that the differences resulted mainly from different living habits (Na, Si, Co, Fe, Mn, Zn) and local exposure (Pb, Cd, Al). When comparing with the reference material for unexposed population, it was found that the studied subjects were exposed to Al. Time profile of element contents in hair of a given person showed that the level changed significantly (even several fold) with changes of living habits or environmental exposure. Also, it was found that

there were similar tendencies in the accumulation of the majority of elements by people that lived together. The effect of living habits on the level of a given element was found to be stronger than the influence of either sex or family relationship. The paper also discusses inter-element interactions within the studied group. Statistically significant ($p < 0.05$) correlations were found between elements that occur together: Ca-Mg, Fe-Mn, Na-K, Co-K, Au-Pt, Cd-Pb. In order to determine the influence of various elements on the content of another element, linear multiple regression was performed that revealed the following relationships: $Ca=f(Mn, Sr)$, $Na=f(K, Mn)$, $K=f(V, Ti)$ (Chojnacka et al. 2006).

To evaluate mercury and selenium concentrations in hair samples of reproductive age women from riverside communities, 19 pregnant and 21 non-pregnant women, 13 to 45 years old, living in the region were studied for at least 2 years, and having a diet rich in fish. It was concluded that Hg exposure levels in reproductive age women were only slightly higher than a provisional tolerable weekly intake of MeHg would provide, that Hg concentration in maternal hair samples was independent of gestational age, and that low Se concentration in pregnant women indicates high mineral consumption by fetal organism to satisfy their metabolic requirements raised during pregnancy, including as a protective mechanism for Hg cytotoxic effects (Pinheiro et al. 2005).

The etiology of Parkinson's disease (PD) is still unknown, but some hypotheses have focused on the imbalances in body levels of metals as co-factors of risk. To assess whether hair could be a reliable marker of possible changes, calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), silicon (Si) and zinc (Zn) were determined in hair from 81 patients affected by PD and 17 age-matched controls. Care was taken to eliminate external contamination of the hair by thorough washing. Digestion of the matrix was achieved by an acid-assisted microwave procedure. Quantification of the elements was performed by inductively coupled plasma atomic emission spectrometry. Results indicated significantly lower levels of Fe in the hair of patients ($p=0.018$) compared with controls. Ca and Mg levels were slightly lower while Zn levels were higher in patients, although these differences were not significant; neither were variations in Cu and Si. Ca and Mg were at least 1.5 times higher in females than in males in both controls and patients. In addition, Ca correlated positively with Mg in both groups and in both sexes ($p < 0.03$), and negatively with age in patients ($p < 0.01$). Finally, element levels did not correlate with either the duration or the severity of the disease or with anti-Parkinson treatment (Forte et al. 2005).

Reindeer, as terrestrial herbivores, generally have low levels of Hg, but monitoring Hg levels can help in understanding ecological toxicity related to a changing environment. In a study, Alaskan reindeer were analyzed for total mercury (THg) in their hair. Both free-ranging reindeer from the Seward Peninsula, Alaska and reindeer fed a pollock-based fishmeal diet were surveyed. Free ranging reindeer had mean THg levels of (55.3 ng/g; $n=5$). The mean MeHg level in the free ranging reindeer was 45.5 (ng/g; $n=5$) or 79% of the THg level. The mean level for THg in the fishmeal fed reindeer was 19 ng/g ($n=10$). Younger reindeer (2 years of age or less) showed lower levels (0.8 ng/g, $n=2$) compared to adult reindeer (30.8 ng/g, $n=6$) (Duffy et al. 2005).

Human hair and blood samples from persons living in the town of Wanshan, a mercury mine area in Guizhou Province of China, were collected and the quantitative speciation and structural information of Hg and S in hair samples and of Hg in erythrocyte and serum samples were studied using X-ray absorption spectroscopy. Least-squares fitting of the X-ray absorption near-edge spectra found that inorganic mercury is the major mercury species in hair samples (91.74%), while inorganic and methyl mercury are both about 50% of total mercury in RBC and serum samples, which is in agreement with the data obtained by acidic extraction, fractionation of Hg^{2+} and CH_3Hg^+ and quantification by ICP-MS. The techniques for speciation, structural and binding information described in this study will find the potential application in similar studies of other elements (Li et al. 2008a).

Background Mercury is a global pollutant that affects neurodevelopment of children. To measure and evaluate mercury concentration of children and mothers, and its association with exposure. Methods A cross-sectional assessment was done using questionnaires and hair mercury were analysed by Cold Vapor Atomic Absorption Spectrometry in the National Institute for Minamata Disease in Japan. Results A total of 112 children and 111 mothers were included; mean age was 34 months and 32 years, respectively. 17.9 % of children and 34.2 % of mothers had concentrations greater than 1 parts per million (ppm) as reference level. Body weight at birth, feeding methods, maternal age, and maternal education level were significantly different in each group ($p < .05$). Mean maternal hair mercury level (0.91 ppm) was higher than children (0.74 ppm), and has a positive correlation between them ($p < .05$). 68.1% of children, 75% of pregnant period, 63.4% of lactating period, and 78.6% of last six months have been consuming fish. With multiple regression analysis, hair mercury levels in children aged less than 6 months had a linear relationship with body weight at birth, gestational weeks, feeding methods (breast- or bottle- feeding) and maternal educational level. While children aged over 6 months significantly differed with gender, frequency of fish servings per week, and frequency of maternal fish consumption in lactation period. And hair mercury levels had inverse linear relationship with maternal monthly income in this age group. Maternal mercury levels had linear relationship with maternal age. Conclusion Mercury levels in children may be affected by

their mothers due to similar dietary patterns. Further long-term large-scale and follow-up studies are needed (Kim et al. 2008).

For most people the main route of exposure to the toxic elements is through the diet. Consequently, information concerning dietary intake is of the utmost importance in being able to assess risks to human health. A study was to intend to assess the usefulness of hair as a biomonitor of the mineral status in young adults. Daily intakes of selected toxic and essential mineral elements were evaluated using a food frequency questionnaire. In addition, the levels of these same elements in hair samples were measured by inductively coupled plasma mass spectrometry and inductively coupled plasma atomic emission spectrometry. The contents of the essential elements in the study population were all well above Spanish recommendations for adult males and females. The estimated intakes of toxic elements were appreciably below the respective PTWIs, indicating that these intake levels do not pose a health concern for this group. Significant differences in hair metal levels were observed between the men and the women, who were in the same age group. Interestingly, no correlation was found between trace element intakes and the corresponding levels in the hair. In conclusion, hair is only limited usefulness as a means of estimating the nutritional status of the essential and toxic elements considered (Gonzalez-Muaoz et al. 2008).

The health implications of the consumption of high arsenic groundwater in Bangladesh and West Bengal were well-documented, however, little is known about the level of arsenic exposure elsewhere in Southeast Asia, where widespread exploitation of groundwater resources is less well established. To measure the arsenic concentrations of nail and hair samples collected from residents of Kandal province, Cambodia, an area recently identified to host arsenic-rich groundwaters, in order to evaluate the extent of arsenic exposure. On the basis of results, the good correlation with the groundwater arsenic concentration, allied with the relative ease of sampling such tissues, indicate that the arsenic content of hair and nail samples may be used as an effective biomarker of arsenic intake in this relatively recently exposed population (Gault et al. 2008).

A quantitative analytical procedure for the determination of cocaine, benzoylecgonine and cocaethylene and norcocaine in hair has been developed and validated. The hair samples were washed, incubated, and any drugs present were quantified using mixed mode solid-phase extraction and liquid chromatography with tandem mass spectrometric detection in positive atmospheric pressure chemical ionization mode. For confirmation, two transitions were monitored and one ion ratio was determined, which was within 20% of that of the known calibration standards. The monitoring of the qualifying transition and requirement for its presence within a specific ratio to the primary ion limited the sensitivity of the assay, particularly for benzoylecgonine, however, the additional confidence in the final result as well as forensic defensibility were considered to be of greater importance. Even with simultaneous monitoring, the concentrations proposed by the United States Federal guidelines for hair analysis were achieved. The limits of quantitation were 50 pg/mg; the limit of detection was 25 pg/mg. The intra-day precision of the assays at 100 pg/mg ($n = 5$) was 1.3%, 8.1%, 0.8% and 0.4%; inter-day precision 4.8%, 9.2%, 15.7% and 12.6% ($n = 10$) for cocaine, benzoylecgonine, cocaethylene and norcocaine, respectively. The methods were applied to both proficiency specimens and to samples obtained during research studies in the USA (Moore et al. 2007).

With aim of using in vitro human skin sandwich system in order to quantify the influence of formulation variables on intrafollicular hydrocortisone permeation. The investigated variables were the pH and the viscosity of the topical formulation as well as the presence of chemical enhancers (carvone, menthone, oleic acid and sodium lauryl sulphate). Furthermore, skin sandwich hydration was also varied in order to determine if the method itself can be run using only partially hydrated skin tissues. It was determined that the follicular contribution to hydrocortisone flux decreased marginally with increasing alkalinity in the pH range 3 to 8.8. Intrafollicular penetration was markedly reduced when HPMC gels were used instead of an aqueous solution. Pretreating the skin with chemical enhancers also reduced the follicular contribution to flux, probably due to permeabilisation of the continuous stratum corneum. Furthermore, it was not possible to satisfactorily modify the skin sandwich method so that it could be deployed using less hydrated skin (Frum et al. 2008).

In order to assess the contamination burden of infants from the city of Marrakech (Morocco), hair lead and aluminium concentrations were studied in a sample of 573 infants, aged 0 to 12 months, and correlated with the infants descriptors such as age, gender and the parents occupations. Moreover, the two metals were measured in the local environment (soil, drinking water) and in the food commonly used during weaning. The higher value for aluminium compared with lead can be explained by the higher levels of aluminium available in both the infant food and the environment. Age, gender, and the parents' occupations influenced significantly lead but not aluminium contents (Zaida et al. 2007).

Specific elements are bioconcentrated in human hair and nails, which have unique advantages of application in population monitoring studies thereby, recognized as biological tools for disease diagnosis and

prevention. However, investigations are meager for relative element profile in hair and nails of same subjects. In this study, hair and nails were analyzed to find effects of age, sex, smoking habit, diet, urban and rural exposure gradients, occupation, and health on element levels. Scalp hair and fingernails were sampled along with a questionnaire from urban and rural subjects of New Delhi; patients of hypertension, coronary heart disease, and diabetes were identified clinically. In comparison to controls, Cr and Zn levels were lower respectively in female hypertensive and total hypertensive subjects, whereas, Zn and Cu were lower respectively in total diabetic subjects, and hypertensive urban subjects. Cd concentrations were higher in both the samples of tobacco smoking rural subjects than that of non-smokers. Farmers had lower Pb than rural businessmen did. Cr, Cu, Ni, and Zn concentrations were different due to rural and urban gradient but not to the influence of age, sex, and diet. Pb value was alone correlated between the paired samples. Thus, higher Cd levels in the smokers and lower Cr, Cu and Zn levels in the patients were observed (Sukumar and Subramanian 2007).

Protein is one of the limiting factors in animal production, and the knowledge of protein requirements by livestock is crucial for the success of a commercial animal raising enterprise. Thirty-four castrated lambs with homogeneous initial BW, were used in the experiment. Under the conditions of this experiment, it is concluded that hair lambs showed a higher concentration of protein in the body, more efficient use of the ingested protein and a consequent additional BW gain when fed isoproteic diets as compared to F1 Ideal France wool lambs (Silva et al. 2007).

An ultrasound-assisted extraction method is proposed for the determination of trace elements in hair samples by inductively coupled plasma-mass spectrometry (ICP-MS) for forensic investigation. Prior to analysis, 25 mg of hair samples were accurately weighed into (15 mL) conical tubes. Then, 2 mL of 20% HNO₃ is added to the samples, sonicated at 2 min (50 W, 100% amplitude), and then further diluted to 10 mL with Milli-Q water. Resulted diluted slurries are centrifuged and the analytes are directly determined in the supernatant. Calibrations against aqueous solutions were carried out with rhodium as internal standard. The method was successfully applied for the extraction of Al, As, Ba, Be, Cd, Co, Cr, Cu, Mn, Pb, Tl, U, V and Zn with a method detection limit (3 s, n = 20) of 0.1, 0.4, 0.2, 0.09, 0.08, 0.04, 0.1, 2.9, 1.0, 0.9, 0.04, 0.05, 0.1 and 4.2 ng/g, respectively. Method accuracy is traceable to Certified Reference Materials (CRMs) 85 and 86 human hair from the International Atomic Energy Agency (IAEA). Additional validation data are provided based on the analysis of hair samples from the trace elements intercomparison program operated by the Institut National de Sante Publique du Quebec, Canada. The proposed method is very simple and can be applied for forensic purposes with the elimination of sample digestion step prior to analysis. Then, a considerable improvement in the sample throughput is archived with the use of the proposed method (Batista et al. 2009).

Urine as well as head and pubic hair samples from drug abusers were analysed for opiates, cocaine and its metabolites, amphetamines, methadone and cannabinoids. Urine immunoassay results and the results of hair tests by means of gas chromatography-mass spectrometry were compared to the self-reported data of the patients in an interview protocol. With regard to the study group, opiate abuse was claimed from the majority in self-reports (89%), followed by cannabinoids (55%), cocaine (38%), and methadone (32%). Except for opiates the comparison between self-reported drug use and urinalysis at admission showed a low correlation. In contrast to urinalysis, hair tests revealed consumption in more cases. There was also a good agreement between self-reports of patients taking part in an official methadone maintenance program and urine test results concerning methadone. However, hair test results demonstrated that methadone abuse in general was under-reported by people who did not participate in a substitution program. Comparing self-reports and the results of hair analyses drug use was dramatically under-reported, especially cocaine. Cocaine hair tests appeared to be highly sensitive and specific in identifying past cocaine use even in settings of negative urine tests. In contrast to cocaine, hair lacks sensitivity as a detection agent for cannabinoids and a proof of cannabis use by means of hair analysis should include the sensitive detection of the metabolite THC carboxylic acid in the lower picogram range (Musshoff et al. 2006).

Another paper reviews the work that has been done in the field of pesticide and persistent organic pollutants (POPs) hair analysis during the last 15 years. It summarizes the compounds of interest, the methods of analyte extraction from the hair matrix, the analytical techniques employed and the results obtained. The most widely studied POPs are the polychlorinated dibenzodioxins (PCDDs), the dibenzofurans (PCDFs) the co-planar biphenyls (co-PCBs) and total biphenyls (PCBs). The most widely studied pesticides are the organochlorine ones, like the hexachlorocyclohexanes and the DDTs, which nowadays are only found as environmental pollutants, some organophosphates, selected pyrethroids and the carbamate methomyl. The most widely applied technique was gas chromatography (GC) coupled to mass spectrometry (MS). Other detectors like the ECD in the case of organochlorine analysis and the NPD in the case of organophosphate analysis were also used. The presented data concern human and animal studies. The levels of DDTs detected in hair were between 19 and 400 ng/g, of co-

PCBs 0.27 and 0.45 ng/g, These results strongly support the possibility of using hair as a suitable indicator for the assessment of long-term exposure to POPs and pesticides (Tsatsakis and Tutudaki 2004).

To evaluate intra- and inter-laboratory agreement concerning hair mineral analysis and interpretation of results, hair samples from 2 volunteers were sent to seven laboratories, which commercially offer hair mineral analysis in Germany. 6 weeks later, another identical part from the hair sample of volunteer 1 was sent to all seven labs. Altogether, 50 elements were analyzed, 23 by all seven labs. For comparability, only the results for these 23 elements were assessed. The intra-laboratory reproducibility was evaluated by the 2 identical hair samples from volunteer 1. On the average, the reproducibility seems to be sufficient, but for individual elements there were unacceptable out-rulers up to 100%. Only one lab classified all elements of the first and the second analysis of the identical hair sample in the same category (below, within, or above normal range). The others grouped 4 to 7 elements different. This is not tolerable. The inter-laboratory comparability was assessed by the results of the hair samples of both volunteers. conclusions, drawn from these results, we must recommend to refrain from using such analysis to assess individual nutritional status or suspected environmental exposure (Drasch and Roeder 2002).

Fatty acid ethyl esters (FAEE) are products of the nonoxidative ethanol metabolism, which are known to be detectable in blood only about 24 h after the last alcohol intake. After deposition in hair they should be suitable long-term markers of chronically elevated alcohol consumption. Therefore, a method for the analysis of ethyl myristate, ethyl palmitate, ethyl oleate and ethyl stearate from hair was developed based on the extraction of the hair sample by a dimethylsulphoxide (DMSO)/n-hexane mixture, separation and evaporation of the n-hexane phase and application of headspace solid-phase microextraction (HS-SPME) in combination with gas chromatography-mass spectrometry (GC-MS) to the extract. For use as internal standards, the corresponding D5-ethyl esters were prepared. The HS-SPME/GC-MS measurements were automatically performed using a multi-purpose sampler. There is no strong difference in their concentrations between pubic, chest and scalp hair, and that they are detectable in hair segments after a 2 months period of abstinence. From the results follows that the measurement of FAEE concentrations in hair is a useful way for a retrospective detection of alcohol abuse (Pragst et al. 2001).

A review focuses on basic aspects and recent studies of hair analysis for abused and therapeutic drugs and is discussed with 164 references. Firstly, biology of hair and sampling of hair specimens have been commented for the sake of correct interpretation of the results from hair analysis. Then the usual washing methods of hair samples and the extraction methods for drugs in hair have been shown and commented on. Analytical methods for each drug have been discussed by the grouping of three analytical methods, namely immunoassay, HPLC-CE and GC-MS. The outcomes of hair analysis studies have been reviewed by dividing into six groups; morphine and related, cocaine and related, amphetamines, cannabinoids, the other abused drugs and therapeutic drugs. In addition, reports on stability of drugs in the living hair and studies on drug incorporation into hair and dose-hair concentration relationships have been reviewed. Applications of hair analysis to the estimation of drug history, discrimination between OTC drug use and illegal drug use, drug testing for acute poisoning, gestational drug exposure and drug compliance have also been reviewed. Finally, the promising prospects of hair analysis have been described (Nakahara 1999).

To investigate comprehensively some relationships between cancer risk and minerals, including essential and toxic metals. METHODS: Twenty-four minerals including essential and toxic metals in scalp hair samples from 124 solid-cancer patients and 86 control subjects were measured with inductively coupled plasma mass spectrometry (ICP-MS), and the association of cancer with minerals was statistically analyzed with multiple logistic regression analysis. There were resulted by multiple logistic regression analysis demonstrated that several minerals are significantly correlated to cancer, positively or inversely. The most cancer-correlated mineral was iodine (I) with the highest correlation coefficient of $r = 0.301$, followed by arsenic (As; $r = 0.267$), zinc (Zn; $r = 0.261$), and sodium (Na; $r = 0.190$), with $p < 0.01$ for each case. In contrast, selenium (Se) was inversely correlated to cancer ($r = -0.161$, $p < 0.05$), followed by vanadium (V) ($r = -0.128$). Multiple linear regression value was highly significantly correlated with probability of cancer ($R^2 = 0.437$, $p < 0.0001$), and the area under the receiver-operating characteristic (ROC) curve was calculated to be 0.918. In addition, using contingency table analysis and the chi-square test, the precision of discrimination for cancer was estimated to be 0.871 (chi-square = 99.1, $p < 0.0001$), (Yasuda et al. 2009).

To investigate the association between hair mineral levels and nutrient intakes, age, and BMI in female adults who visited a woman's clinic located in Seoul. Dietary intakes were assessed by food frequency questionnaire and mineral levels were measured in collected hairs, and the relationship between these was examined. The average daily nutrient intakes of subjects were compared to those of the KDRIs, and the energy intake status was fair. The average intake of calcium in women of 50 years and over was 91.35% of KDRIs and the potassium intake was greatly below the recommended levels in all age groups. In the average hair mineral contents in subjects, calcium and copper exceeded far more than the reference range while selenium was very low

with 85.19% of subjects being lower than the reference value. In addition, the concentrations of sodium, potassium, iron, and manganese in the hair were below the reference ranges in over 15% of subjects. The concentrations of sodium, chromium, sulfur, and cadmium in the hair showed positive correlations ($P < 0.05$) with age, but the hair zinc level showed a negative correlation ($P < 0.05$) with age. The concentrations of sodium, potassium, chromium, and cadmium in the hair showed positive correlations ($P < 0.05$) with BMI. Some mineral levels in subjects of this study showed significant correlations with nutrient intakes, but it seems that the hair mineral content is not directly influenced by each mineral intake. As described above, some hair mineral levels in female adults deviated from the normal range, and it is considered that nutritional intervention to control the imbalance of mineral nutrition is required. Also, as some correlations were shown between hair mineral levels and age, BMI, and nutrient intakes, the possibility of utilizing hair mineral analysis for specific purposes in the future is suggested (Hong et al. 2009). A hybrid progressive algorithm to recognize type II diabetic based on hair mineral element levels is proposed by Huang(2005). So samples of 244 cases (Table 1) are collected from 51 healthy persons (one case each person), 47 unchecked diabetics (one case each person) and 73 checked diabetics (two cases each person). 8 hair elements (Mg, Ca, Fe, Cu, Zn, Se, Cr and Mn) are measured. The hybrid progressive algorithm is used to form a scalar quantity (dynamic diagnosis index (DDI)) based hair element levels. The result show that hair may be a good symptom index to judge whether a person affected by diabetes mellitus if appropriate sampling and measuring procedure adopted and proper algorithm to retrieve information from multi-elements levels in hair. Because the non-invasive characteristics of hair analysis, this procedure and algorithm is very suitable at least to large population screening of early diabetes (Huang et al. 2005).

The advantages of obtaining samples for hair mineral analysis (HMA) as well as the possibility to measure former exposures and in addition to carry out segment analysis make it desirable to work with HMA in order to find a diagnosis. The laboratories which offer HMA differ distinctly in their procedure of analysis and quality control. HMA is generally not usable for individual diagnostic with two exceptions (arsenic and methylmercury) because of the large number of factors of individual and environmental influences and sources of error of the method of analysis. HMA can be used for cadmium, lead, and zinc in order to compare a single person with a larger population. Further research into the relationship of content of elements in hair and other tissues of the body is desirable (Hamilton and Schweinsberg 2004).

The variance of testing was compared between the College of American Pathologists clinical survey and that of a recent review about hair mineral testing. The review suggested that the accuracy of hair mineral testing was unreliable. In general, there was a greater range of variance in the College of American Pathologists testing results. These latter results are based on laboratory testing and are used as a "yardstick" to determine if a laboratory passes or fails that analyte and are considered a "gold standard." An extract, which resulted from a method that avoided the washing step, was compared among five laboratories. Very good precision resulted, indicating that the varied washing steps used by the laboratories in a recent review were probably the source of much variance. Analysis of hair analysis seemed to yield important information in several historical or forensic cases involving Ludwig von Beethoven, Napoleon Bonaparte, ex-US-presidents Zachary Taylor and Andrew Jackson, and Charles Hall, an Arctic explorer. Several elements that were reviewed, including arsenic, cadmium, cobalt, germanium, lead, lithium, manganese, mercury, nickel, and thallium, showed relationships between body burden, dosage, and exposure or toxicity. Evidence of toxicity could not be found by measuring hair aluminum or vanadium. Chromium, selenium, and zinc seemed to have nutritional value. Ratios of hair elements with clinical importance could not be found (Shamberger 2002).

Hair mineral analysis is being used by health care practitioners and promoted by laboratories as a clinical assessment tool and to identify toxic exposures, despite a 1985 study that found poor reliability for this test. To assess whether the reliability of data from commercial laboratories advertising multimineral hair analyses for nutritional or toxicity assessment has improved since the 1985 study. Variations were found in laboratory sample preparation methods and calibration standards. Laboratory designations of normal reference ranges varied greatly, resulting in conflicting classifications (high, normal, or low) of nearly all analyzed minerals. Laboratories also provided conflicting dietary and nutritional supplement recommendations based on their results. Hair mineral analysis from these laboratories was unreliable, and we recommend that health care practitioners refrain from using such analyses to assess individual nutritional status or suspected environmental exposures. Problems with the regulation and certification of these laboratories also should be addressed (Seidel et al. 2001).

Associations of hair mineral (Cu, Zn, Na, K, Ca, Mg, Pb, Cd, and Cr) concentrations with blood pressure in a young normotensive population were studied (N=74). Other factors that may be associated with blood pressure such as sex, age, and weight/height index were evaluated. Age and weight/height index were positively correlated with systolic and diastolic pressures in males. Weight/height index was positively correlated with diastolic pressures in females. Hair sodium/potassium ratios were negatively correlated with diastolic pressures in males. In females,

systolic and pulse pressures were negatively correlated with hair sodium, hair copper, and hair copper/zinc ratios. Hair calcium/magnesium ratios were positively correlated with pulse pressures in females. Hair lead, chromium, and cadmium did not correlate with blood pressure. Partial regression coefficients indicated similar relationships for age, weight/height index, sex, and hair mineral concentrations with blood pressure. This suggested hair mineral concentration associations were independent of other factors studied. Standardized partial regression coefficients suggested in some cases that hair mineral concentrations had an equivalent or a stronger association with blood pressure than age, sex, or weight/height index. The negative relationship of hair copper and copper/zinc ratios with blood pressure is discussed in relation to the hypothesis concerning copper and zinc imbalances in the etiology of coronary heart disease (Medeiros et al. 1983).

Effects of 2 dietary Mg concentrations (deficient and adequate: 0.04 and 0.12 g of Mg/100 g of dry matter, respectively) on body fluid and tissue Mg concentrations and performance of wether lambs were evaluated in a 28-day trial. Nine blood and 6 urine samples were collected from each wether. After 28 days, CSF and wool samples were collected, and diet, body fluids, and tissues were analyzed for mineral concentration. Diet effects on serum and urine Mg concentrations were noticed after day 3 (P less than 0.01; P less than 0.05, respectively). Mean serum and urine Mg concentrations for 6 sampling periods were correlated ($r = 0.83$, P less than 0.001; No. of samples = 12). The effect of dietary Mg on CSF Mg concentrations approached significance (P less than 0.10). Effects of diet on cardiac muscle, liver, and 3rd metatarsal bone Mg contents or hematologic criteria were not observed. Diet affected wool and kidney cortex Mg contents (P less than 0.02). Individual mean 28-day serum Mg concentration was correlated with wool Mg content ($r = 0.73$, P less than 0.05; $n = 8$) and with kidney cortex Mg content ($r = 0.75$, P less than 0.05; $n = 8$). Wethers fed low Mg diet excreted less urine Ca (P less than 0.001) and had slightly lower serum Ca and K values (P less than 0.10) than did wethers fed high Mg. Significant differences in cardiac muscle, liver, spleen, or kidney cortex Ca contents were not observed. Wethers fed low Mg diet consumed less dry matter and gained less weight (P less than 0.001) than did wethers fed high Mg diet. Body fluid and tissue macromineral concentrations of wether with hypomagnesemic tetany are presented for prognostic and diagnostic purposes (Fisher et al. 1985a).

Samples of switch hair, blood, and urine were obtained periodically over 5.5 months from 11 Angus and 13 Angus-Charolais cows grazing either all-grass or grass-legume swards. Liver samples were obtained at the end of the study. Hair growth rate and mineral concentrations in switch hair (magnesium [Mg], copper [Cu]), blood serum (Mg, Cu), urine (Mg), and liver (Cu) were determined. Significant (P less than 0.05) hair-growth rate differences were observed among sampling periods (daily mean = 0.58 ± 0.01 mm). Angus black-pigmented switch hair contained more (P less than 0.001) Mg than did the light-pigmented Angus-Charolais hair. The effect of season was observed on hair Mg and Cu and on serum Mg (P less than 0.01). Serum and hair Mg concentration correlated in both breed groups after removal of individual cow treatment effects (Angus: $r = 0.58$, P less than 0.001, $n = 64$; Angus-Charolais: $r = 0.46$, P less than 0.001, $n = 76$). Likewise, urine Mg and hair Mg concentrations correlated (Angus: $r = 0.35$, P less than 0.05, $n = 53$; Angus-Charolais: $r = 0.26$, P less than 0.05, $n = 63$). Sward type had a pronounced effect on serum and urine Mg concentrations and a slight effect on hair Mg concentrations (P less than 0.10) only during midsummer. Cattle with switch hair Mg values less than 25 to 30 mg (light pigmentation) and 100 to 125 mg (black pigmentation)/kg of dry matter (DM) may be hypomagnesemic (Fisher et al. 1985b).

The measurement of testosterone (T) is essential for the diagnosis of male hypogonadism and for monitoring treatment. Samples need to be obtained at specific times in relation to the diurnal rhythm and therapeutic T injections. There were explored the measurement of T in hair as an alternative method to assess gonadal status and long-term T exposure in men. Thirty-six male subjects comprising 17 healthy volunteers, 10 untreated hypogonadal men, and nine hypogonadal men receiving T injections were studied. T was measured in serum and in hair. T in hair was measured using a commercially available salivary T enzyme immunoassay kit adapted for this use. The T concentration in the hair of hypogonadal men receiving T injections was significantly higher than that in untreated hypogonadal volunteers, but not eugonadal men. Median T concentrations were 3.66 (range, 0.82-15.00), 0.94 (range, 0.33-3.68), and 1.85 (range, 0.58-3.06) pg/g hair, respectively. On the basis of results, T in hair reflects gonadal status in men and may be useful for monitoring T therapy over several weeks to months in hypogonadal men (Thomson et al. 2009).

Doping with endogenous anabolic steroids is one of the most serious issues in sports today. The measurement of anabolic steroid levels in human hair is necessary in order to distinguish between pharmaceutical steroids and natural steroids. This is the first investigation into the physiological concentrations of anabolic steroids in human hair in Chinese subjects. A gas chromatography tandem mass spectrometry (GC/MS/MS) method was developed for the simultaneous identification and quantitation of five endogenous anabolic steroids (testosterone, epitestosterone, androsterone, etiocholanolone and dehydroepiandrosterone) in hair. After basic hydrolysis, hair samples were extracted with diethyl ether, derivatized and then detected using GC/MS/MS in the multiple-reaction

monitoring mode (MRM). The one precursor/two product ion transitions for each anabolic steroid were monitored. The limits of detection for the five endogenous anabolic steroids were in the 0.1 to 0.2 pg/mg range. All analytes showed good linearity and the extraction recoveries were 74.6 to 104.5%. Within-day and between-day precisions were less than 20%. This method was applied to the analysis of testosterone, epitestosterone, androsterone, etiocholanolone, and dehydroepiandrosterone in human hair. Full-length hair samples were taken at the skin surface from the vertex of 39 males, 30 females and 11 children from China. None of the subjects were professional athletes. Testosterone and dehydroepiandrosterone were detected in all the hair segments. The physiological concentrations of testosterone were in the range 0.8 to 24.2 pg/mg, 0.1 to 16.8 pg/mg and 0.2 to 11.5 pg/mg in males, females and children, respectively, however, the mean values of dehydroepiandrosterone were much higher than the concentrations of testosterone. These data are suitable reference values and are the basis for the interpretation of results from investigations into the abuse of endogenous anabolic steroids (Shen et al. 2009).

Methylmercury is an environmental pollutant that can cause irreversible effects on the development of children. Although there is no doubt that high exposure can cause neurodevelopmental deficits, the threshold that will adversely affect the developing fetus is not well defined. There was systematically review the evidence of neurodevelopmental risks of methylmercury to the unborn child from maternal fish consumption to define the lowest observable adverse effect hair concentration (LOAEHC). A systematic review was conducted of all original research reporting on the effects of methylmercury on the human fetus. A literature search was undertaken using SCOPUS, Medline-Ovid, PubMed, Google Scholar, and EMBASE. Papers were selected based on the following inclusion criteria: 1) child neurodevelopmental outcome; 2) comparison groups; and 3) methylmercury exposure through fish consumption. Forty-eight publications met these inclusion criteria. Thirty articles reported on longitudinal studies and 18 were cross-sectional studies. Variations in study design precluded formal meta-analysis. Based on an evaluation of these studies, we defined the LOAEHC at 0.3 microg/g of maternal hair mercury. The longitudinal studies yielded a LOAEHC of 0.5 microg/g. CONCLUSION: In the clinical context, the majority of pregnant women consume mercury-containing fish in amounts that are lower than the LOAEHC defined in this study. However, the LOAEHC is in the same order of magnitude of mercury exposure that occurs in significant numbers of women. Hence, although it appears safe to suggest that eating the recommended types and amounts of fish poses no measurable risks for neurodevelopmental deficits, analysis of hair mercury content before pregnancy might be suggested because dietary modification can decrease body content and risk (Schoeman et al. 2009).

Acute promyelocytic leukaemia (APL) is a distinctive subtype of acute myeloid leukaemias. Even through this human disease can be treated by the intravenous administration of all-trans retinoic acid (ATRA), 25% of patients typically relapse after the first treatment. In a research, the intravenous administration of APL patients with an aqueous solution of arsenic trioxide has also been demonstrated to be successful despite the established mammalian toxicity of this arsenic compound. Accordingly, the administration of a therapeutic dose of arsenic trioxide has resulted in an improved patient survival in both relapsing as well newly diagnosed APL patients. We present here a mini-review of the medicinal use of arsenite, its mammalian metabolism (with an emphasis on biomethylation pathways), its elimination and pharmacokinetics and the novel application of hair analysis as a biomonitoring material. This mini-review also introduces our own results on the analysis of hair of patients receiving arsenic trioxide therapy. In this work, instead of quantifying arsenic content in bulk hair, we performed longitudinal analysis in order to use hair as a marker of arsenic exposure correlated to a time scale. Taking into account the hair growth rate, the longitudinal analysis of hair is demonstrated to provide a chronological record of the treatment of patients with arsenic trioxide. The small quantity of material to be analysed required the use of Synchrotron radiation based X-ray fluorescence (SXRF) spectroscopy. The hair arsenic content was well correlated with the clinical background of patients and reflected the intake of arsenic trioxide. In particular, the onset of arsenic trioxide therapy and interruptions during therapy were reflected by total arsenic content, which suggested rapid elimination. Another type of experiment, micro-XRF cartography on thin hair slices, allowed us to obtain distribution maps of arsenic, which demonstrated that arsenic is located at the periphery of hair. Micro-XANES spectra recorded at the periphery of hair, suggest that inorganic arsenic is incorporated in hair in its trivalent oxidation state, in agreement with previous results (Nicolis et al. 2009).

To identify and evaluate determinants of hair nicotine concentrations in nonsmoking women and children exposed to secondhand tobacco smoke at home. Hair samples were collected from nonsmoking women (n = 852) and from children (n = 1,017) <11 years of age living in households (n = 1,095) with smokers from 31 countries from July 2005 to October 2006. Participants' ages, activity patterns and socioeconomic characteristics including education and employment status, and hair treatment information were collected. Multilevel linear regression modeling was used to identify the main determinants of hair nicotine concentration measured by gas

chromatography coupled with mass spectrometry. Increased indoor air nicotine concentration at home were associated with increased hair nicotine concentrations in nonsmoking women and children. This association was not changed after controlling for other explanatory variables. After controlling for age, length of exposure, and socioeconomic characteristics, hair nicotine concentrations in nonsmoking children and women were estimated to be increased by 3% and 1%, respectively, for a 1 microg/m³ increase in air nicotine concentration. The association between children's exposure to secondhand tobacco smoke at home and hair nicotine concentration was stronger among younger children and children with longer exposure at home (Kim et al. 2009).

Hair is a well-established and widely used matrix for measuring mercury exposure of an individual. Although a variety of washing procedures to remove external mercury contamination have been proposed, no standardized procedures are available yet. However, hair still can be used as an indicator for methyl mercury exposure because, generally, there is almost no exogenous contamination of methyl mercury in hair (Li et al. 2008b).

Methylmercury (MeHg) is one of the most hazardous substances that affects the fetus through fish consumption. There was evaluated the changes in the level of exposure to MeHg by assessing the mercury (Hg) concentrations of the segmental hair at parturition and 3 months after parturition, and to study their correlations with the total Hg concentrations of maternal and cord red blood cells (RBCs) and neonatal hair as biomarkers of fetal exposure to MeHg at parturition. In total, 40 paired samples of maternal hair from the scalp, maternal and cord RBCs, and 21 samples of neonatal hair from the scalp were collected at parturition. In addition, 19 samples of maternal hair from the scalp were collected at 3 months after parturition. The maternal hair samples were cut into 1cm segments from the scalp end toward the tip. The geometric mean of the Hg concentrations in cord RBCs was approximately 1.6 times higher than that in the maternal RBCs, and a strong correlation coefficient ($r=0.91$) was found between them. The increase or decrease in the Hg concentrations of the segmental hair during gestation differed largely among individuals. The correlation coefficients between the Hg concentrations of the segmental hair and cord RBCs were the strongest ($r=0.90$) in the hair segment 1cm from the scalp and decreased gradually with the distance from the scalp. The correlation coefficients between the Hg concentrations of the segmental hair collected at 3 months after parturition and maternal RBCs were over 0.9 in the hair segments 5 and 6 cm from the scalp, suggesting that the time required for the incorporation of Hg from the blood into a growing hair was very short. The geometric mean of Hg concentrations in the neonatal hair at parturition was similar to that in the maternal hair 1cm from the scalp at parturition, and they exhibited a strong correlation ($r=0.95$). The findings of this study indicate that maternal hair close to the scalp at parturition and neonatal hair are useful biomarkers of fetal exposure to MeHg at parturition. In addition, the segmental maternal hair throughout gestation is essential to obtain important information on MeHg exposure during the different sensitive windows or bolus MeHg exposure during gestation (Sakamoto et al. 2008).

There were reviewed focuses on basic aspects of method development and validation of hair testing procedures. Quality assurance is a major issue in drug testing in hair resulting in new recommendations, validation procedures and inter-laboratory comparisons. Furthermore recent trends in research concerning hair analysis are discussed, namely mechanisms of drug incorporation and retention, novel analytical procedures (especially ones using liquid chromatography-mass spectrometry (LC-MS) and alternative sample preparation techniques like solid-phase microextraction (SPME)), the determination of THC-COOH in hair samples, hair testing in drug-facilitated crimes, enantioselective hair testing procedures and the importance of hair analysis in clinical trials. Hair testing in analytical toxicology is still an area in need of further research (Musshoff and Madea 2007).

An analytical method for determining essential elements (Zn, Fe and Cu) and toxic elements (Cr, Pb and U) on single hair strands by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-SFMS) using a double focusing sector field mass spectrometer was developed. Results obtained directly using LA-ICP-SFMS of hair were compared with those measured by inductively coupled plasma quadrupole mass spectrometry (ICP-QMS) of solutions of digested hair samples and the analytical methods were found to agree well. Different quantification strategies for trace element determination in hair samples such as external calibration, standard addition and isotope dilution were compared and demonstrated for uranium. For uranium determination in powdered hair by LA-ICP-MS solution-based calibration was applied by coupling the laser ablation chamber to an ultrasonic nebulizer. The significance of single hair analysis by LA-ICP-SFMS was demonstrated by a case study of a person who changed living environment. Differences in the uranium content observed along the single hair strand correlated with the changes in the level of uranium in drinking water. Measurements of uranium isotope ratios showed a natural isotopic composition throughout the whole period in the drinking water, as well as in the hair samples. This paper demonstrates the potential use of laser ablation ICP-MS to provide measurements on a single hair strand and its potential to become a very powerful tool in hair analysis for biological monitoring (Sela et al. 2007).

An HPLC method with diode array detection (DAD) is proposed for the detection of sulphamethazine (SMZ) residues in pig and cattle hair. Hair samples were extracted under alkaline conditions (NH₄OH 0.2 M for calf samples and NaOH 0.1 M for piglet samples) and purified with a dual solid-phase extraction (SPE) cartridge system (reverse phase/strong-cation exchange). Recovery of SMZ in fortified samples varied from 70 to 85%, with a limit of quantification of 0.155 ng/mg. Residues of SMZ (7.2-59.2 ng/mg) were detected both in calf and piglet hairs after a therapeutic treatment with SMZ, while no interfering peak was observed in samples from untreated animals (Gratacas-Cubarsa et al. 2006).

CONCLUSIONS AND RECOMMENDATIONS

There are thousands of biochemical reactions that ultimately control your metabolism, digestion and the regeneration of body tissues. The vast majority of these reactions depends on certain trace minerals for their activity. If these minute amounts of essential minerals are not there to fuel the processes then your ability to regenerate, metabolize or breakdown noxious substances is compromised. While blood values inform the health professional what is in the blood, hair analysis provides a record of how the body stores and disposes of elements. The choice of hair as a testing medium is based on the fact that the blood chemistries change kaleidoscopically from day to day while the hair values give a more stable view of the overall mineral nutrition.

One of the essential conditions for ensuring the realistic evaluation of excessive population exposure is the examination of sufficiently large population groups and the use of group diagnostics methodology in environmental epidemiology studies. The method of hair analysis appears to be ideally suited for use in pilot prospective studies. If an excessive exposure is detected it is recommendable that the epidemiological examination be completed by analyses of other biological materials, most often blood and urine, in order to obtain a closer specification of the degree of exposure in the respective population. Hair is the biological matrix of choice for the diagnosis of chronic drug-addiction or drug consumption at an earlier time. Hair can also be used to establish the chronological profile and the severity of drug consumption.

Tissue mineral analysis is a test that measures the mineral content of the hair. Mineral content of the Hair accurately reflects the mineral content of the body's tissue. If a mineral deficiency or excess exists in the hair profile it usually indicates a mineral deficiency or excess within the body. Minerals are essential in numerous functions for all phases of metabolism. Various mineral imbalances, as revealed by hair analysis can indicate metabolic dysfunctions before any symptoms occur. (Stress, Medications, Pollution, Imbalanced Rations, Feeds or Supplements).

Also these findings suggest that some minerals such as arsenic, selenium, and probably iodine, zinc, sodium, and vanadium contribute to regulation of cancer and also that metallomics study using multiple logistic regression analysis is a useful tool for estimating cancer risk.

Hair analysis of minerals is used not only for diagnostic purposes but also to monitor the nutritional state of the patient until treatment benefits are achieved and the effects of the program have been stabilized.

The combination of feed ration and hair analysis is an invaluable screening tool to determine the correct program of diet and supplementation for each individual's specific needs. Never before has there been available such an accurate, scientifically valid guide to metabolic function and balance. Hair tissue mineral analysis is responsive not only to trace mineral levels in the diet but to all other factors which influence their metabolism including stress, exercise, endocrine and gastro-intestinal function.

REFERENCES

- Batista BL, Rodrigues JL, de Oliveira Souza VC, Barbosa Jr F. 2009. A fast ultrasound-assisted extraction procedure for trace elements determination in hair samples by ICP-MS for forensic analysis. *Forensic Science International* 192:88-93.
- Bencko Vr. 1995. Use of human hair as a biomarker in the assessment of exposure to pollutants in occupational and environmental settings. *Toxicology* 101:29-39.
- Chojnacka K, Gorecka H, Gorecki H. 2006. The influence of living habits and family relationships on element concentrations in human hair. *Sci Total Environ* 366:612-20.
- Chyla MA, Zyrnicki W. 2000. Determination of metal concentrations in animal hair by the ICP method: comparison of various washing procedures. *Biol Trace Elem Res* 75:187-94.
- Davenport MD, Tiefenbacher S, Lutz CK, Novak MA, Meyer JS. 2006. Analysis of endogenous cortisol concentrations in the hair of rhesus macaques. *General and Comparative Endocrinology* 147:255-261.
- Drasch G, Roider G. 2002. Assessment of hair mineral analysis commercially offered in Germany. *Journal of Trace Elements in Medicine and Biology* 16:27-31.
- Duffy LK, Duffy RS, Finstad G, Gerlach C. 2005. A note on mercury levels in the hair of Alaskan reindeer. *Science of The Total Environment* 339:273-276.

- Fisher DD, Wilson LL, Leach RM, Scholz RW. 1985a. Effects of deficient and adequate dietary magnesium concentrations on body magnesium concentrations in wethers. *Am J Vet Res* 46:1777-80.
- Fisher DD, Wilson LL, Leach RM, Scholz RW. 1985b. Switch hair as an indicator of magnesium and copper status of beef cows. *Am J Vet Res* 46:2235-40.
- Forte G, Alimonti A, Violante N, Di Gregorio M, Senofonte O, Petrucci F, Sancesario G, Bocca B. 2005. Calcium, copper, iron, magnesium, silicon and zinc content of hair in Parkinson's disease. *Journal of Trace Elements in Medicine and Biology* 19:195-201.
- Frum Y, Eccleston GM, Meidan VM. 2008. Factors influencing hydrocortisone permeation into human hair follicles: Use of the skin sandwich system. *International Journal of Pharmaceutics* 358:144-150.
- Gault AG, Rowland HAL, Charnock JM, Wogelius RA, Gomez-Morilla I, Vong S, Leng M, Samreth S, Sampson ML, Polya DA. 2008. Arsenic in hair and nails of individuals exposed to arsenic-rich groundwaters in Kandal province, Cambodia. *Science of The Total Environment* 393:168-176.
- Gonzalez-Muaoz MJ, Pea A, Meseguer I. 2008. Monitoring heavy metal contents in food and hair in a sample of young Spanish subjects. *Food and Chemical Toxicology* 46:3048-3052.
- Gratacas-Cubarsa M, Castellari M, Garca-Regueiro JA. 2006. Detection of sulphamethazine residues in cattle and pig hair by HPLC-DAD. *Journal of Chromatography B* 832:121-126.
- Hamilton T, Schweinsberg F. 2004. [The reliability of conclusions based on hair mineral analysis in individual diagnostic]. *Versicherungsmedizin* 56:136-40.
- Hill V, Cairns T, Schaffer M. 2008. Hair analysis for cocaine: Factors in laboratory contamination studies and their relevance to proficiency sample preparation and hair testing practices. *Forensic Science International* 176:23-33.
- Hong SR, Lee SM, Lim NR, Chung HW, Ahn HS. 2009. Association between hair mineral and age, BMI and nutrient intakes among Korean female adults. In: *Nutr Res Pract*. pp. 212-9.
- Huang H, Hu W, Han Z, Ye H. 2005. Hybrid Progressive Algorithm to Recognize Type II Diabetic Based on Hair Mineral Element Contents. *Conf Proc IEEE Eng Med Biol Soc* 5:4716-8.
- Jurado Montoro C. 2007. Análisis de drogas de abuso en muestras de pelo. Diagnóstico del consumo crónico. *Trastornos Adictivos* 9:172-183.
- Kazi TG, Afridi HI, Kazi GH, Jamali MK, Arain MB, Jalbani N. 2006. Evaluation of essential and toxic metals by ultrasound-assisted acid leaching from scalp hair samples of children with macular degeneration patients. *Clinica Chimica Acta* 369:52-60.
- Kim S, Wipfli H, Navas-Acien A, Dominici F, Avila-Tang E, Onicescu G, Breyse P, Samet JM. 2009. Determinants of hair nicotine concentrations in nonsmoking women and children: a multicountry study of secondhand smoke exposure in homes. *Cancer Epidemiol Biomarkers Prev* 18:3407-14.
- Kim SA, Jeon CK, Paek DM. 2008. Hair mercury concentrations of children and mothers in Korea: Implication for exposure and evaluation. *Science of The Total Environment* 402:36-42.
- Li Y-F, Chen C, Li B, Li W, Qu L, Dong Z, Nomura M, Gao Y, Zhao J, Hu W, Zhao Y, Chai Z. 2008a. Mercury in human hair and blood samples from people living in Wanshan mercury mine area, Guizhou, China: An XAS study. *Journal of Inorganic Biochemistry* 102:500-506.
- Li Y-F, Chen C, Li B, Wang J, Gao Y, Zhao Y, Chai Z. 2008b. Scalp hair as a biomarker in environmental and occupational mercury exposed populations: Suitable or not? *Environmental Research* 107:39-44.
- Medeiros DM, Pellum LK, Brown BJ. 1983. The association of selected hair minerals and anthropometric factors with blood pressure in a normotensive adult population. *Nutrition Research* 3:51-60.
- Memon A-u-R, Kazi TG, Afridi HI, Jamali MK, Arain MB, Jalbani N, Syed N. 2007. Evaluation of zinc status in whole blood and scalp hair of female cancer patients. *Clinica Chimica Acta* 379:66-70.
- Moore C, Coulter C, Crompton K. 2007. Determination of cocaine, benzoylecgonine, cocaethylene and norcocaine in human hair using solid-phase extraction and liquid chromatography with tandem mass spectrometric detection. *Journal of Chromatography B* 859:208-212.
- Munakata M, Onuma A, Kobayashi Y, Haginoya K, Yokoyama H, Fujiwara I, Yasuda H, Tsutsui T, Iinuma K. 2006. A preliminary analysis of trace elements in the scalp hair of patients with severe motor disabilities receiving enteral nutrition. *Brain and Development* 28:521-525.
- Musshoff F, Driever F, Lachenmeier K, Lachenmeier DW, Banger M, Madea B. 2006. Results of hair analyses for drugs of abuse and comparison with self-reports and urine tests. *Forensic Science International* 156:118-123.
- Musshoff F, Madea B. 2007. New trends in hair analysis and scientific demands on validation and technical notes. *Forensic Science International* 165:204-215.
- Nakahara Y. 1999. Hair analysis for abused and therapeutic drugs. *Journal of Chromatography B: Biomedical Sciences and Applications* 733:161-180.
- Nicolis I, Curis E, Deschamps P, Bănzăreanu S. 2009. Arsenite medicinal use, metabolism, pharmacokinetics and monitoring in human hair. *Biochimie* 91:1260-1267.
- Pinheiro MCN, Müller RCS, Sarkis JE, Vieira JLF, Oikawa T, Gomes MSV, Guimarães GA, do Nascimento JLM, Silveira LCL. 2005. Mercury and selenium concentrations in hair samples of women in fertile age from Amazon riverside communities. *Science of The Total Environment* 349:284-288.
- PNHC. 2013. Hair Analysis. Pinewood Natural Health Centre, Toronto.
- Pragst F, Auwaerter V, Sporkert F, Spiegel K. 2001. Analysis of fatty acid ethyl esters in hair as possible markers of chronically elevated alcohol consumption by headspace solid-phase microextraction (HS-SPME) and gas chromatography-mass spectrometry (GC-MS). *Forensic Science International* 121:76-88.
- Pragst F, Balikova MA. 2006. State of the art in hair analysis for detection of drug and alcohol abuse. *Clinica Chimica Acta* 370:17-49.
- Rodrigues JL, Batista BL, Nunes JA, Passos CJS & Barbosa Jr F. 2008. Evaluation of the use of human hair for biomonitoring the deficiency of essential and exposure to toxic elements. *Science of The Total Environment* 405:370-376.
- Sakamoto M, Kubota M, Murata K, Nakai K, Sonoda I, Satoh H. 2008. Changes in mercury concentrations of segmental maternal hair during gestation and their correlations with other biomarkers of fetal exposure to methylmercury in the Japanese population. *Environ Res* 106:270-6.
- Schoeman K, Bend JR, Hill J, Nash K, Koren G. 2009. Defining a lowest observable adverse effect hair concentrations of mercury for neurodevelopmental effects of prenatal methylmercury exposure through maternal fish consumption: a systematic review. *Ther Drug Monit* 31:670-82.
- Seidel S, Kreutzer R, Smith D, McNeel S, Gilliss D. 2001. Assessment of commercial laboratories performing hair mineral analysis. *JAMA* 285:67-72.

- Sela H, Karpas Z, Zoriy M, Pickhardt C, Becker JS. 2007. Biomonitoring of hair samples by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). *International Journal of Mass Spectrometry* 261:199-207.
- Shamberger R.J. 2002. Validity of hair mineral testing. *Biol Trace Elem Res* 87:1-28.
- Shen M, Xiang P, Shen B, Bu J, Wang M. 2009. Physiological concentrations of anabolic steroids in human hair. *Forensic Science International* 184:32-36.
- Silva AMA, Silva Sobrinho AG, Trindade IACM, Resende KT, Bakke OA. 2007. Net and metabolizable protein requirements for body weight gain in hair and wool lambs. *Small Ruminant Research* 67:192-198.
- Souad C, Farida Z, Nadra L, François B, Bougle D, Azeddine S. 2006. Trace element level in infant hair and diet, and in the local environment of the Moroccan city of Marrakech. *Science of The Total Environment* 370:337-342.
- Sukumar A, Subramanian R. 2007. Relative element levels in the paired samples of scalp hair and fingernails of patients from New Delhi. *Science of The Total Environment* 372:474-479.
- Thomson S, Koren G, Van Steen V, Rieder M, Van Uum SH. 2009. Testosterone concentrations in hair of hypogonadal men with and without testosterone replacement therapy. *Ther Drug Monit* 31:779-82.
- Tsanaclis L, Wicks JFC. 2007. Patterns in drug use in the United Kingdom as revealed through analysis of hair in a large population sample. *Forensic Science International* 170:121-128.
- Tsatsakis A, Tutudaki M. 2004. Progress in pesticide and POPs hair analysis for the assessment of exposure. *Forensic Science International* 145:195-199.
- Yasuda H, Yoshida K, Segawa M, Tokuda R, Tsutsui T, Yasuda Y, Magara S. 2009. Metallomics study using hair mineral analysis and multiple logistic regression analysis: relationship between cancer and minerals. *Environ Health Prev Med* 14:261-6.
- Zaida F, Chadrame S, Sedki A, Lekouch N, Bureau Fo, Arhan P, Bougle D. 2007. Lead and aluminium levels in infants' hair, diet, and the local environment in the Moroccan city of Marrakech. *Science of The Total Environment* 377:152-158.