

Announcement by the German Federal Environment Agency (Umweltbundesamt)

Hair Analysis in Environmental Medicine

Opinion of the Human Biomonitoring Commission of the German Federal Environment Agency
(Umweltbundesamt)

1 Introduction

For biological monitoring in environmental medicine readily accessible specimens reflecting average exposure to contaminants over a relatively long period of time are of particular importance. Hence, human scalp hair is used in epidemiological and case studies to assess the internal exposure to metals and metalloids, and other settings. In Germany, and other countries, measurements of minerals and trace elements in hair are offered by private laboratories, and sometimes also by pharmacies and clinical laboratories. This is known as commercial hair analysis and is used to evaluate a person's general state of nutrition and health. These tests are generally realized using inductively coupled plasma-mass spectrometry (ICP-MS), by which specimen analysis of up to 60 elements within the hair is possible. The interpretation of the results is not only restricted to diagnoses of mineral deficiency and heavy metal exposure. They are also used to help in the diagnosis of various diseases and therapeutic recommendations are given.

Although the applicability of hair analysis for measurement of environmental exposure is limited, as has been summarized by various reports [1, 2, 3, 4, 5, 6, 7], hair analysis is widely applied. In view of this fact, the Human Biomonitoring Commission of the Federal Environment Agency has conducted a thorough investigation of this issue and presents its opinion in the present report.

2 Applications of hair analysis

Hair analysis is used for the following purposes:

- detection of medicaments and drugs in forensic medicine
- detection of poisons in clinical and forensic toxicology
- historical investigations
- analysing essential trace element and mineral stores
- evaluating the internal exposure to pollutants

The presence of drugs such as cannabis, cocaine, ecstasy and heroine, as well as nandrolone and other anabolic doping agents can be reliably detected in scalp, auxiliary or pubic hair, long after intake. When new hair is formed, substances in the blood are incorporated into the hair and cannot be completely removed by means of hair washing, colouring and other treatments. Taking into account the growth rate of scalp hair (about 1 cm/month) longer hair strands may give information on drug use over longer periods of time [8].

Nowadays, hair analysis is rarely used in clinical and forensic medicine to detect toxicants such as arsenic and thallium. However, in some cases hair analysis could be a useful adjunct technique. For example, examining longitudinal sections of hair strands can provide insight into the timescale of the poisoning process [9].

Hair analysis can also be used for investigation of historical events: Napoleon's arsenic poisoning, the lead poisoning of Heinrich Heine and of members of the 1845 Arctic expedition (from canned food in tins with lead solder), or the possible drug consumption in ancient Egypt through the analysis of mummy hairs. With hair analysis it could be proven that „Ötzi“ was a vegetarian [10].

Repeated hair trace element analysis realized under well standardized conditions is a suitable method for screening intake changes of various trace elements in food. However, as a single test it is not suitable to evaluate nutritional status, metabolic disturbances, or the causes of nutritional disorders [11, 4]. Hence, under no circumstance should hair analysis be used as a basis for therapeutic or dietary regimens [6].

3 Using hair analysis to evaluate internal exposure to pollutants

3.1 General

A comprehensive summary of hair analysis methods for exposure assessment has been given by Wilhelm and Idel [2]. In 2003 the US Department of Health and Human Services' Agency for Toxic Substances and Disease Registry (ATSDR) released a comment on hair analysis within an expert meeting on hair analysis [6]. According to these sources, hair analysis is not a valid method for determining individual exposure to environmental contaminants except for methylmercury.

At first it has to be clarified for which substances reliable hair analysis methods are available.

As mentioned above, no tested and validated methods are available for organic substances other than methylmercury, pharmaceuticals, and drugs.

Methods for extracting organic toxins from the outer lipid layer of hair to assess external exposure have not been validated satisfactorily. These methods are similar to the application of passive samplers for evaluating individual exposure to air pollutants (personal monitoring). It is noteworthy, in this regard, that both nicotine and cotinine in scalp hair can be analyzed reliably and the results can be used to estimate environmental tobacco smoke exposure [12].

Sensitive methods for detecting metal and metalloid in hair are available. For environmental medicine the following metals are of interest: arsenic, lead and mercury. It is indisputable that metals and metalloids in scalp hair can indicate increased exposure. In many studies exposed groups revealed higher element levels in comparison to controls. This is the case even at low dose exposure, as was shown by a Düsseldorf traffic emission study measuring lead levels in hair and blood of children. This study indicated increased exposure by hair analysis while lead levels in blood did not differ between three study areas [13].

However, the applicability of hair analysis on an individual basis is strongly limited for the following reasons:

- It is difficult to distinguish exogenous and endogenous sources.
- Only little data is available concerning the kinetics of uptake and binding of elements during hair growth whereby various elements are incorporated from the blood into the hair matrix.
- There is a lack of quality assurance methods.
- There is little or no correlation between substance concentration in hair and substance concentration in blood, urine, or target organs.
- The lack of information about confounders such as hair washing, cosmetics, shampoo, perspiration, hair colour and hair type can have a marked effect on the concentrations of substances in hair.

The problem of distinguishing between endogenous and exogenous sources of elements in hair could not be solved despite numerous investigations [14]. This potentially applies for exogenous contamination of hair via air, cosmetics and water (e.g. tap water that is used for hair care, bathwater, rainwater and swimming pool water). The only exception here is the assessment of the exposure to methylmercury, which occurs most exclusively through

ingestion of fish and fish products. A detectable amount of methylmercury is incorporated into hair [15]. Speciation is a promising method that may make it possible to distinguish exogenous from endogenous sources of metals. This method has been used by Mandal et al. [16] to analyze inorganic arsenic compounds and methylated arsenic metabolites separately in hair.

Various studies have attempted to explain the incorporation of elements from the blood into the hair matrix [2] but this mechanism, and the extent to which substances are incorporated into hair, is still not well understood.

At present, no inter-laboratory hair analyses are realized at regularly scheduled intervals for external quality control purposes. However, external quality control is dispensable if hair analysis is to be more widely used than is currently the case because three recent inter-laboratory hair analyses show that the results provided by commercial labs are often unreliable [4, 7, 17]. An additional problem is that no accepted standards have been developed for hair analysis laboratories, in terms of the specimen collection method, as well as variables that must be taken into account (e.g. dwelling location, diet, chemicals in drinking water, lifestyle, age, sex, hair colour, hair treatments, and so on). Rauscher [18] concluded that satisfactorily reproducible hair substance analysis results can be readily obtained if specimens are collected carefully using a suitable method.

Considering basic toxicokinetics, it is not necessary that the substance content in hair correlate strongly with concentration detected in blood, urine, or critical target organs. In fact, it has been found that, in general, for many toxicants – including those whose toxicokinetics have been well described – there is little or no correlation between substance concentrations in hair and other biological matrices [19]. However, this does not apply to methylmercury, for which a close association was found between levels in hair and blood following consumption of contaminated fish from the Rhine river [20]. Also, lead associations between levels in blood and hair have been reported [13, 21].

Occasionally, suspected diagnosis of heavy metal intoxication can be explained by a high exogenous contamination of hair (for examples of case studies see Wilhelm and Idel [22]).

3.2 Significance of selected substances

3.2.1 Methylmercury

Extensive epidemiological data, as well as toxicokinetic data and practical considerations, have shown that hair is the most reliable indicator for individual methylmercury exposure. Usually, measurement of total mercury content in hair will provide sufficient information. However, it can be assumed that clearly more than 50 percent of the mercury content in individuals with elevated alimentary mercury exposure is accounted for by methylmercury and other organic mercury compounds [23].

There are no representative studies of hair mercury content in Germany available. Mercury concentrations in scalp hair from children living in North Rhine-Westphalia range between <0.03 to 1.77 µg/g (geometric mean 0.18 µg/g) [24]. The developing organs of children exhibit a particularly strong response to organic mercury. The onset of developmental disorders and abnormal behaviour in children is associated with a maternal hair mercury content > 5 µg/g [15, 25]. Using a safety factor of five as a basis, a total mercury content in the hair of women of childbearing age should not exceed 1 µg/g [26].

Hair analysis cannot be used to detect exposure from the dental amalgam since this material is inorganic rather than organic mercury.

3.2.2 Lead

In environmental epidemiological studies, the monitoring of lead in scalp hair is a valuable screening method, especially in children. The advantage of this method is that hair specimen collection is inherently non-invasive, and, in children, the risk of hair treatment-induced false positive results is relatively low. However, medical attendance of hair or scalp (e.g. scalp eczema therapy or scalp delousing) must also be taken into account when children's hair is analyzed. Representative data concerning scalp hair lead concentrations in the German population can be found in the Environmental Survey 1990/92 (see section 4).

3.2.3 Arsenic

Since arsenic is rapidly eliminated from blood, arsenic levels in blood are not suitable for environmental medicine issues. Arsenic in urine also reflects only very recent exposure (a few days prior to testing). This holds true for inorganic arsenic compounds and methylated arsenic metabolites in urine, as well. In light of these factors, as well as due to arsenic's pronounced affinity for sulfur-containing substances such as keratin, hair and nails have been used in epidemiological studies as indicator media in measuring exposure to inorganic arsenic compounds. Recent studies have shown that nails (fingernails) reflect internal

incorporation of arsenic more accurately than hair [16, 27]. Arsenic contents in nails reflect exposure to inorganic arsenic at least as accurately [28] and perhaps more accurately [29] than urine. No representative data is available concerning hair arsenic concentration in the German population. The concentration in the literature varies widely, ranging in Europe and the US from 0.01 to 0.2 µg/g.

3.2.4 Selenium

It cannot be determined with certainty whether scalp hair selenium levels correlate with the selenium concentration in blood, tissue, and urine and can thus be used as an indicator of selenium deficiency. The published data is contradictory [30- 40].

Several large epidemiological studies have shown that toenail selenium screening can reliably differentiate the selenium status of different study groups [41- 43]. Hair selenium also appears to be suitable for this purpose [30, 32]. However, hair selenium analysis does not reliably measure selenium status in individuals even if a correlation exists between selenium intake and hair selenium content [44, 45].

The Commission recommends that in order to measure the efficacy of selenium supplements, selenium serum/blood concentration should be measured after 1-3 months. (www.umweltbundesamt.de/uba-info-daten/daten/monitor/pub.htm).

3.2.5 Nicotine and Cotinine

In several environmental epidemiological studies it has been shown that nicotine in scalp hair is a reliable indicator to validate cigarette passive smoker status. With this parameter it is possible to distinguish smokers from non-smokers and also to differentiate between no, low, and high environmental tobacco smoke exposure.

However, cotinine in hair, resulting from passive smoke exposure in non-smokers or light smokers (10 or fewer cigarettes daily), is barely detectable. A reliable differentiation between smokers and low smokers can be done by analysis of levels of nicotine and cotinine in urine along with nicotine in hair. Nicotine content and nicotine/cotinine urine content differ substantially in smokers and non-smokers [12, 46]. Representative data in this regard are available for the FRG (see section 4).

At present, no determination can be made as to the relative proportion of hair nicotine smoke incorporation accounted for by external contamination and internal incorporation [47- 49].

3.2.6 Analysis of trends

Hair analysis may also provide information about heavy metal exposure in specific populations over certain periods of time. For example, the median scalp-hair lead concentration in students in Halle (Eastern Germany) decreased from 0.53 µg/g to 0.15 µg/g between 1995 and 2001 [50].

4 Hair analysis data from the German Environmental Survey (GerES)

The German Environmental Survey (GerES) 1990/92 contains representative hair substance content data that can be used as reference data for epidemiological studies, but that is not valid for the interpretation on an individual basis [21, 46]. Key substances, such as organic arsenic and methylmercury, were not evaluated owing to the relatively small number of specimens available. Since no hair analyses were realized for the environmental survey conducted in 1998 [51] and for the Child Environment Survey 2003/06 [52] it is unlikely that valid reference data for the German population will be available in the foreseeable future.

The methods used in the GerES 1990/92 (sampling, analytical, statistical, etc.) and the results have been described in detail elsewhere by Krause et al. [21, 46]. The present report summarizes the study's key findings. In the environmental survey 1990/92, a series of substances and metabolites in scalp-hair specimens from 4021 adults (age range 25-69) and 736 children (age range 6-10) were analyzed. After being collected using titanium nitride scissors, 4 cm long occipital, proximal and insofar as possible 200 mg specimens were stored in sealed envelopes until the analysis was performed. The analysis first involved washing the specimens in acetone and water in accordance with the International Atomic Energy Agency (IAEA) method. Prior to the trace element analysis, the specimens underwent plasma incineration and were then screened for Al, Ba, Cd, Ca, Cr, Cu, Mg, Pb, P, Sr and Z using inductively coupled plasma-mass spectroscopy (ICP-MS). After undergoing alkaline extraction, specimens weighing more than 200 mg were tested for nicotine and cotinine using high performance liquid chromatography (HPCL). The lead, nicotine and cotinine results can be found in table 1.

Tabelle 1:
Lead, nicotine and cotinine concentration ($\mu\text{g/g}$) in scalp hair of adult (Age of 25 to 69 years old) and children (age of 6 to 14 years old) according German Environmental Survey 1990/92 [46]

Parameter	Population Group	N	n < LOQ	Range	P 50	P 90	P 95
Lead	female	1952	10	< 0.1 - 76.4	0.7	2.3	3.6
	male	1865	4	< 0.1 - 584	1.2	5.4	8.5
	girl	345	0	0.1 - 12.8	0.7	2.1	3.4
	boy	366	2	< 0.1 - 26.9	1.3	4.6	6.2
Nicotine	adult non-smokers	861	120	< 0.1 - 135	1.0	9.4	14.1
	adult smokers	428	9	< 0.1 - 441	10.9	61.8	73.6
	children	255	113	< 0.1 - 21.8	0.2	2.6	5.6
Cotinine	adult non-smokers	872	794	< 0.2 - 11.0	< 0.2	< 0.2	0.3
	adult-smokers	419	218	< 0.2 - 8.2	< 0.2	1.9	2.8
	children	255	242	< 0.2 - 0.5	< 0.2	< 0.2	0.2

Note:

N = sample size; n<LOQ = number below the limit of quantification; P 50, P 90, P 95 = refer to the sample percentile; values below LOQ are set LOQ/2 for calculation purposes

In GerES the following variables were found to influence scalp-hair lead content in the 25-69 age group, in ascending order of importance (overall variance: 22.7%): sex, lead content in household water, amount of time spent outdoors, peak daily temperature, hair growth rate, age of dwelling, and daily cigarette consumption. The variables for the 6-14 age group were as follows (overall variance: 24.9%): sex, age of dwelling, physical activities out of doors, lead content in household drinking water, season, and lead content in outdoor air [53].

The most important factors for hair nicotine and cotinine content are cigarette smoker status and daily cigarette consumption. Three types of non-smoker exposure to passive smoke can be distinguished on the basis of hair nicotine content: none, moderate, and high [12, 46].

A correlation between Pb, Cd, Cu, Cr, cotinine, nicotine, Ca, Mg and P content in scalp hair and blood, serum, or urine was observed for lead in hair and blood and for nicotine and cotinine in hair and urine.

5 Evaluation of reference values

The Commission concludes that hair analysis results are not valid to derive reference values.

Besides the fact that, at present, no standardized analytical method (including sampling, washing procedure, digestion, and measurement) and external quality assurance are available, the Commission concludes that hair analysis is not suitable for the diagnostic purposes of individuals.

6 HBM values?

No toxicological HBM values can be derived for contaminant level in hair, since, except for methylmercury, no dose-relationships between levels in hair and adverse effects are known.

The Commission has verified human biomonitoring values for mercury in blood [15] on the basis of cohort studies conducted on the Faro Islands and in the Seychelles. These studies showed that the lowest neurotoxic effect level on fetuses for total mercury concentration in maternal hair is 5µg/g. However, there are no defined human biomonitoring values for mercury in hair owing to the current lack of external quality control schemes and the fact that no standardized analytic methods are available for specimen collection, washing procedure, digestion, and measurement [15].

7 Conclusion and Perspective

Except for methylmercury, results from hair analysis are not valid either for the evaluation of the individual heavy metal exposure or as a method to detect or exclude individual elevated heavy metal exposure. Any conclusions of such investigations on health relevance of the analyses results are therefore invalid as well.

Hair analysis is inappropriate for determining whether individual subjects are suffering from trace element and mineral imbalances. In the Commission's view, therapeutic or dietary regimens elaborated by pharmacies, natural health practitioners, or commercial hair analysis laboratories (on the basis of hair analysis findings concerning trace elements and minerals) are inapplicable and invalid.

Hair analysis can be used to screen for nicotine, inorganic arsenic compounds, and lead exposure in sufficiently large populations. However, in most cases, hair analysis findings cannot be used to determine the source of toxicant exposure in such groups due to the fact

that elevated concentrations in hair can result from internal incorporation via blood to hair matrix transfer, as well as from external contaminations via air, cosmetics, or water.

In order to determine the validity of hair analysis data from toxicant exposure assessment more proficiently and to use this data to a greater effect, work needs to be done in at least the following areas:

- development of standardized analytic methods for sampling, washing, digestion, and measurement and quality assurance
- investigation of toxicokinetics and of the blood to hair matrix transfer mechanisms
- determination of the correlation between dose and adverse health effects
- identification of metabolites in hair.

Literature

1. Chutsch, M., Krause, C. (1987): Zusammenfassende Bewertung von Haaranalysen. In: Krause, C., Chutsch, M. (Hrsg.): Haaranalyse in Medizin und Umwelt. Gustav Fischer Verlag, Stuttgart/New York
2. Wilhelm, M., Idel, H. (1996): Hair analysis in environmental medicine. *Zbl. Hyg. (jetzt: Int. J. Hyg. Environ. Health)* 198:485-501
3. Wilhelm, M. (2001): Haaranalyse. In: Böse-O'Reilly, Kammerer, Mersch-Sundermann, Wilhelm (Hrsg.): Leitfaden Umweltmedizin. Urban & Fischer, München, 2. Aufl., S. 85-86
4. Drasch, G., Roider, G. (2002): Assessment of hair mineral analysis commercially offered in Germany. *J. Trace Elem. Med. Biol.* 16:27-31
5. Drexler, H., Schaller, K.-H. (2002): Haaranalysen in der klinischen Umweltmedizin. *Dtsch. Ärztebl.* 99: A 3026-3029 [Heft 45]
6. Harkins, D.K., Susten, A.S. (2003): Hair analysis: exploring the state of science. *Environ. Health Perspect.* 111:576-578
7. Seidel, S., Kreutzer, R., Smith, D., McNeel, S., Gilliss, D. (2001) Assessment of commercial laboratories performing hair mineral analysis. *JAMA* 285:67-72
8. Sachs, H. (2003): Haaruntersuchungen auf Arznei- und Suchtstoffe. In: Madea, B., Brinkmann, B. (Hrsg.): Handbuch gerichtliche Medizin. Bd. 2, 177–183, Springer, Berlin, Heidelberg
9. Drasch, G. (2003): Metalle und Verbindungen. In: Madea, B., Brinkmann, B. (Hrsg.): Handbuch gerichtliche Medizin. Bd 2, 198–237, Springer, Berlin, Heidelberg
10. Macko, S.A., Lubec, G., Teschler-Nicola, M., Andrisevich, V., Engel, M.H. (1999): The ice man's diet reflected by the stable nitrogen and carbogen isotopic composition of his hair. *FASEB J.* 13:559-562
11. Kruse-Jarres, J.D. (1997): Interpretation von Haaranalysen. Rückschlüsse auf den Stoffwechsel unmöglich. *Dtsch. Ärztebl.* 94 A-2180 [Heft 34-35]
12. Seiwert, M., Merkel, G., Krause, C., Schwabe, R. (1995): Determination of nicotine and cotinine in urine and hair of the general population of Germany. Annual Conference of the ISEE and the ISEA. *Epidemiology* July 1995. 6: Number 4 Supplement
13. Wilhelm, M., Pesch, A., Rostek, U., Begerow, J., Schmitz, N., Idel, H., Ranft, U. (2002): Concentrations of lead in blood, hair and saliva of German children living in three different areas of traffic density. *Sci. Total Environ.* 297:109-118
14. Kijewski, H. (1993): Die forensische Bedeutung der Mineralstoffgehalte in menschlichen Kopfharen. Verlag Schmidt-Römhild, Lübeck
15. Kommission Human-Biomonitoring (1999): Stoffmonographie Quecksilber – Referenz- und Human-Biomonitoring-(HBM)-Werte. *Bundesgesundheitsbl - Gesundheitsforsch – Gesundheitsschutz.* 42 522-532

16. Mandal BK, Ogra Y, Suzuki KT (2003) Speciation of arsenic in human nail and hair from arsenic-affected area by HPLC-inductively coupled argon plasma mass spectrometry. *Toxicol Appl Pharmacol* 189:73-83
17. Hamilton, T., Schweinsberg, F. (2003): Ergebnisse eines Ringversuches mit Haarproben eines gesunden Probanden – ein Beitrag zur kritischen Bewertung der Haarmineral-Analyse. *Umweltmed. Forsch. Prax.* 8:123-130
18. Rauscher J (2003) Qualitätssicherung in der Haarmineralstoff- und Schwermetallanalyse. *Zeitschrift für Umweltmedizin* 11, 3:142-144
19. Drasch, G., Wanghofer, E., Roider, G. (1997): Are blood, urine, hair and muscle valid biomonitors for the internal burden of men with the heavy metals mercury, lead and cadmium? *J. Trace Elem. Electrolytes* 14: 116–123
20. Schweinsberg, F., Kroiher, A. (1994): Quecksilberbelastung durch Fischkonsum bei Rheinfischern. *Zbl. Hyg.* 195:529-543
21. Krause, C., Babisch, W., Becker, K., Bernigau, W., Helm, D., Hoffmann, K., Nöllke, P., Schulz, C., Schwabe, R., Seiwert, M., Thefeld, W. (1996a): Umwelt-Survey 1990/92 Band Ia: Studienbeschreibung und Human Biomonitoring. Deskription der Spurenelementgehalte in Blut und Urin der Bevölkerung in der Bundesrepublik Deutschland. *WaBoLu Hefte* 1/96, Umweltbundesamt, Berlin
22. Wilhelm, M., H. Idel (1997): Ausgewählte Fallstudien zur "Haaranalyse." *Umweltmed. Forsch. Prax.* 2:247-249
23. Barbosa, A.C., Jardim, W., Dórea, J.G., Fosberg, B., Souza, J. (2001): Hair mercury speciation as a function of gender, age, and body mass index in inhabitants of the Negro River, Amazon, Brazil. *Arch. Environ. Contam. Toxicol.* 40:439-444
24. Pesch, A., Wilhelm, M., Rostek, U., Schmitz, N., Weishoff-Houben, M., Ranft, U., Idel, H. (2002). Mercury concentrations in urine, scalp hair, and saliva in children from Germany. *J. Expo. Anal. Environ. Epidemiol.* 12:252-258
25. Grandjean, P., P. Weihe, R.F. White, F. Debes, S. Araki, K. Yokohama, K. Murata, N. Sorensen, R. Dahl, P.J., Jorgensen, P.J. (1997): Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. *Neurotoxicol. Teratol.* 19:417-428
26. UNEP (United Nations Environment Programme) (2002): Global Mercury Assessment. UNEP Chemicals, Geneva, Switzerland
27. Hinwood, A.L., Sim, M.R., Jolley, D., de Klek, N., Bastone, E.B., Gerostamoulos, J., Drummer, O.H. (2003): Hair and toenail arsenic concentrations of residents living in areas with high environmental arsenic concentrations. *Environ. Health Perspect.* 111:187-193
28. Wilhelm, M., Pesch, B., Wittsiepe, J., Jakubis, P., Miskovic, P., Keegan, T., Nieuwenhuijsen, M. J.; Ranft, U. (2005): Comparison of arsenic levels in fingernails with urinary As species as biomarkers of arsenic exposure in residents living close to a coal-burning power plant in Prievidza District, Slovakia. *J. Expo. Anal. Environ. Epidemiol.*, 15 (1): 89-98
29. Karagas, M.R., Stukel, T.A., Tosteson, T.D. (2002): Assessment of cancer risk and environmental levels of arsenic in New Hampshire. *Int. J. Hyg. Environ. Health* 205:85-94
30. Al-Kunani, A.S., Knight, R., Haswell, S.J., Thompson, J.W., Lindow, S.W. (2001): The selenium status of women with a history of recurrent miscarriage. *Brit. J. Obstet. Gynecol.* 108:1094-1097
31. Steindel, S.J., Howanitz, P.J. (2001): The uncertainty of hair analysis for trace elements. *JAMA* 285:83-85, 2001
32. Zhang, W.H., Neve, J., Xu, J.P., Vanderpas, J., Wang, Z.L. (2001): Selenium, iodine and fungal contamination in Yulin District endemic for Kashin-Beck disease. *Int. Orthopaed.* 25:188-90
33. Frisch, M., Schwartz, B.S. (2002): The pitfalls of hair analysis for toxicants in clinical practice. *Environ. Health Perspect.* 110: 433-36
34. Guvenc, M., Guvenc, H., Karatas, F., Aygun, A.D., Bektas, S. (2002): Low levels of selenium in miscarriage. *J. Trace Elem. Exp. Med.* 15:97-101
35. Hac, E., Krechniak, J., Szyszko, M. (2002): Selenium in human plasma and hair in northern Poland. *Biol. Trace Elem. Res.* 85:277-85
36. Morley, N., Ford, R.P.K. (2002): Hair-element analysis – still on the fringe. *Child: Care, Health & Development* 28: Suppl. 1, 31-34
37. Morton, J., Carolan, V.A., Gardiner, P.H.E. (2002): Removal of exogenously bound elements from human hair by various washing procedures and determination by inductively coupled plasma mass spectrometry. *Anal Chim Acta* 455: 23-34
38. Fang W.X., Wu, P.W., Hu, R.H., Huang, Z.Y. (2003): Environmental Se-Mo-B deficiency and its possible effects on crops and Keshan-Beck disease (KBD) in the Chousang area, Yao county, Shaanxi Province, China. *Environ. Geochem. Health* 25: 267-80

39. Krechniak, J., Szyszko, M., Krzyanowski, M. (2003): Selenium concentrations in human renal cortex, liver and hair in northern Poland. *Biol. Trace Elem. Res.* 92:213-20
40. Kvicala, J., Zamrazil, V., Jiranek, V. (2003): Selenium status of inhabitants of region Jablonec, Czech Republic. *Trace Elem. Electrol.* 20: 83-88
41. Allen NE, Morris JS, Ngwenyama RA, Key TJ (2004): A case-control study of selenium in nails and prostate cancer risk in British men. *Br. J. Cancer* 90:1392-6.
42. Rayman, M.P., Bode, P., Redman, C.W. (2003): Low selenium status is associated with the occurrence of the pregnancy disease preeclampsia in women from the United Kingdom. *Am. J. Obstet. Gynecol.* 189:1343-49.
43. Zhuo, H., Smith, A.H., Steinmaus, C. (2004) Selenium and lung cancer: a quantitative analysis of heterogeneity in the current epidemiological literature. *Cancer Epidemiol. Biomarkers Prev.* 13:771-778
44. Shamberger R.J. (2002): Validity of hair mineral testing. *Biol. Trace Elem. Res.* 87:1-28
45. Klevay, L.M., Christopherson, D.M., Shuler T.R. (2004) Hair as a biopsy material: trace element data on one man over two decades. *Eur. J. Clin. Nutr.* 7
46. Krause, C., Schulz, C., Becker, K., Bernigau, W., Hoffmann, K., Nöllke, P., Schwabe, R., Seiwert, M. (1996b): Umwelt-Survey 1990/92 Band Ib: Human Biomonitoring. Beschreibung der Spurenelementgehalte im Haar der Bevölkerung in der Bundesrepublik Deutschland. *WaBoLu Hefte 2/96*, Umweltbundesamt, Berlin
47. Harley, N. J., Hoffmann, D. (1985) Analysis for nicotine and cotinine in hair to determine cigarette smoker status, *Clin. Chem.* 31:1598-1600
48. Zahlens, K., Nilsen, O.G. (1990): Gas chromatographic analysis of nicotine in hair, *Environ. Technol.* 11:353-364
49. Nilsen, T., Zahlens, K., Nilsen, O.G. (1994) Uptake of nicotine in hair during controlled environmental air exposure to nicotine vapour: evidence for a major contribution of environmental nicotine to the overall nicotine found in hair from smokers and non-smokers, *Pharmacol. Toxicol.* 75:136-142
50. Umweltprobenbank für Human-Organproben Münster. ISUBP 3.30 Analysenauswertung, Gesamt-Kollektive, generiert am 29.5.2002
51. Krause, C., Seifert, B. Schulz, C. (1998): Umwelt-Survey 1997/98. *Gesundheitswesen* 60, Sonderheft 2 577-582
52. Schulz, C., Becker, K., Seiwert, M. (2002): Kinder-Umwelt-Survey. *Gesundheitswesen* 2002; 64 Sonderheft 1; S69-S79, Georg Thieme Verlag Stuttgart
53. Bernigau, W., Becker, K., Hoffmann, K., Krause, C., Friedrich, C., Schulz, C., Seifert, B. (1999): Umwelt-Survey 1990/92. Band X: Blei – Zusammenhangsanalyse. *WaBoLu-Heft 7/99*. Umweltbundesamt, Berlin