Hair as a material for study of medicaments and psychoactive substances

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Abstract
At present it is thought that most xenobiotics, including drugs and medicines introduced into the organisms in different ways, are built in the structure of hair. The analysis of hair found a particular application in confirmation of the fact of addiction to psychoactive substances, identification of the taken drug, clinical diagnostics, and monitoring of treatment of the psychic and neurological diseases. The analysis of children’s hair enables the assessment of exposure to drugs and exposure of a foetus, for instance, to tobacco smoke. Owing to the possibility of detection of anabolic steroids, it is possible to use it for the purpose of controlling the application of doping substances by athletes and racing animals. Such a broad application of the analysis of hair is possible due to the development of modern and sensitive analytical techniques. The techniques available at the moment, such as gas and fluid chromatography combined with mass spectrometry. The article summarizes the current knowledge about application of hair analysis and analytical methods used for these purposes.

Key words: medicaments, psychoactive substances, hair

Introduction
Human hair, owing to unique properties, has become an important source of information on taking xenobiotics or exposure to them.

At present it is thought that most xenobiotics, including drugs and medicines introduced into the organisms in different ways, are built in the structure of hair [1]. Contrary to the study of systemic fluids, from which the xenobiotics are eliminated relatively fast, the determination of xenobiotics in hair provides information on the subject of history of taking substances or exposure to them.

The analysis of hair found a particular application in confirmation of the fact of addiction to psychoactive substances [2], identification of the taken drug [3], clinical diagnostics, [4] and monitoring of treatment of the psychic and neurological diseases. The analysis of children’s hair enables the assessment of exposure to drugs [5] and exposure of a foetus, for instance, to tobacco smoke [6]. Owing to the possibility of detection of anabolic steroids, it is possible to use it for the purpose of controlling the application of doping substances by athletes and racing animals [7, 8].

Such a broad application of the analysis of hair is possible owing to the development of modern and sensitive analytical techniques. The techniques available at the moment, such as gas and fluid chromatography combined with mass spectrometry (GC-MS, LC-MS) enable the determination of organic xenobiotics in concentrations of a few pictograms per gram of hair.

Morphology and physiology of hair
Hair is flexible keratinised fibres which are produced from the epidermal cells. Hair is a heterogeneous structure consisting of about 65-95% of proteins (keratin), 3-5% of melanin, 1-9% of water, 0.25-0.95% of lipids, mineral components and hardly traceable quantities of polysaccharides. Keratin is a protein characterised by a high content of sulphur (~5%) and nitrogen (~20%). Among aminoacids building keratin, cystine which has sulphhydryl groups and disulphite groups that have the ability of chelatation of metals, occurs in the greatest quantities [9-11].

The colour of hair is a hereditary feature. Melanocytes, melanophores are pigment cells producing a pigment in the process of melanogenesis – melanin. Two types of hair melanin are distinguished: eumelanin and feomelanin. Eumelanin is a black-brown pigment, feomelanin is a yellow-red pigment. The hair colour depends on the quantity and type of melanin particles in the hair bulb. The mutual combinations of these pigments give various hues of hair. A strong concentration of the
brown pigment results in black colour. In the case of blondes, the content of this component is low, and its particles have a spiral structure. This component occurs in the external layer of a hair shaft exclusively. With age, the hair becomes darker, and the red hair may become auburn. At the loss of the capability to produce pigment, the hair has ashen colour, and it becomes grey as a consequence of occurrence of air bubbles between the cells [10].

Man is born with full hair covering the whole body; there are no hair only on hands, soles of feet, dorsal surface of distal phalanges, fingers and toes, glans, penis and clitoris and internal surface of prepuce. The hair, called lanugo is composed of delicate, short and light hair, except for hair of the head and eyebrows, where it is thicker, longer and slightly darker. The lanugo falls out just before the delivery or soon after it and is replaced by permanent lanugo which becomes thicker and longer during the period of maturation. In regard to the shape, length, colour, and particularly the typical place of occurrence, we distinguish hair of eyelashes, eyebrows and head. The density, shape and cross-section of hair of the head are subject to significant fluctuations depending on the race. In regard to the shape, we distinguish straight, wavy and curly hair. The number of hairs on the head amounts to about 120000 on average [10].

The hair is produced already in the third month of the foetal life. First the primordiums appear on eyebrow arches and on the upper and lower lip. At the end of the third month and at the beginning of the fourth month, hair appears in greater quantity on the head and starts to cover further skin areas. Already at the end of the seventh month, almost the whole skin is covered with dense and delicate lanugo [12, 13].

Hair is dead keratinized formation and at the moment of complete formation it is not subjected to physiological influences. These factors, however, have an impact on the hair properties in the period preceding its formation. The hair cycle is understood as a period from the beginning of hair growth to its spontaneous falling out and completion of the stationary phase. In each hair follicle, there are three phases occurring one after the other in rhythmic order: growth and full activity phase (anagen), temporary involution phase (catagen) and stationary phase. During anagen lasting 4-8 years on average (according to some sources 2-6 years), the follicle produces the entire hair fibre. During catagen and telogen lasting for a few weeks, which lasts from 4 to 6 months, the follicle is subject to regression, and its cells await the signal to start the next growth phase. In adult people, about 85% of hair is in the growth phase, the remaining 15% is in the stationary phase [14-16].

The hair growth is affected by: age, temperature of environment, type of nutrition and probably some vitamins. The hair on the head is characterised by a particularly long period of growth, which ranges from 5 to 6 years. On average, it reaches the length of about 60-70 cm, although occasionally it may be much longer. The hair grows 1 cm for a month (the standardised hair growth factor amounts to 1 ± 0.3 cm) [17]. The main role in the development of hair is played by mesoderm, whereas the destruction of the hair papilla causes its permanent falling out or even development of a scar. Reversely, the mechanical hair extraction together with its epidermal sheath does not cause this effect, and the removed hair is replaced by a new one, which grows out from the hair bulb.

The hair follicle is surrounded by a rich system of capillaries, which is exceptionally dense at its lower section. The capillaries are most developed in the anagen phase, and they partly disappear in the catagen phase. Apart from this, around the hair follicle, below the sebaceous gland duct, there is a network of circular and longitudinal nervous fibres. They belong to the peripheral autonomous system and reach the skin appendages. The dermal papillae are deprived of nerve ends [10, 14].

**Distribution and incorporation of xenobiotics in hair**

The interest in hair as a biological material for determination of xenobiotics has increased when the long time of deposition of the substance in their structure was proved. However, in order for this material to be used for research purposes, it was necessary to ascertain which basic chemical and pharmacological principles determine the appearance and disappearance of xenobiotics in the matrix. The key issues include the mechanism and place of incorporation of compounds, which is unambiguously combined with the hair structure or its growth. The hair follicles are placed in dermis and surrounded by a network of blood vessels which nourish the hair bulb. Nearby most of the follicles, there are sebaceous glands, apocrine glands and eccrine glands that secrete sweat. In spite of the fact that the sweat ducts reach the dermis, their neighbourhood ensures permanent washing of the hair with this fluid. This exceptional physiology and structure of the hair ensures many possible ways of incorporation and removal of xenobiotics. Blood, sweat, sebum and dermis may constitute a potential source of foreign substances,
which permeate the hair. Also, the infiltration of compounds from the outside environment is possible [18].

The first model of penetration of xenobiotics into hair assumes their diffusion from capillary blood into stem cells at the base of the follicle. In all probability, the foreign substance is bonded with the elements of matrix and pigments. As the cells become longer and older, gradually dying and connecting with each other, they formulate a dead fibre with a built-in xenobiotic. Baumgartner proved that the substances get through the blood stream to a hair proportionally to the concentration [19]. This model, however, was based on purely theoretical assumptions. At present, it is assumed that drugs may penetrate into hair via three ways, in accordance with the proposed “sweat model” [1, 18]. In a way, it is a modification and development of the trap model. The first way in which the xenobiotics build in the hair is their diffusion from blood vessels surrounding the hair bulb [20]. The second way is the so called "sweat way". Eliminating the xenobiotics, the organism subjects them to metabolism and then excretion, mainly with urine. However, part of these substances is removed together with sebum and sweat, which later on moisten and cover the hair during its growth [21]. Substances contained in these secretions settle on the hair surface, from which – as time passes by – they build into it. The third way of penetration of xenobiotics into hair is their external contamination with vapours or physical contact with xenobiotics, which become dissolved in secretions of sweat and sebaceous glands or in water and build into the hair [15, 22].

Not much is known about the environmental pollution of hair. It was ascertained that cocaine may be adsorbed from drug vapours. Therefore, many methods of hair treatment have been developed to protect it from determination of xenobiotics. The methods are different from each other in terms of their effectiveness [24]. Not only in the case of cocaine, but also other compounds is it possible to test numerous biomarkers, and depending on their usefulness, to specify whether the substance was actively taken or whether it penetrated as a result of environmental pollution.

The three main factors affecting the process of building the xenobiotic into the structure of hair include: content of melanin and physicochemical properties – lipophilicity and alkalinity of substance [15]. Apart from this, an important role is played by the structure and colour of the hair itself.

1. The significant affinity of melanin to alkaline compounds was proved and pH of melanocytes ranging from 3 to 5 was indicated [25, 26]. So far not much has been found out about the building elements of hair, responsible for bonding a xenobiotic and its metabolites. The components which most probably may fulfil this function are proteins, melanin and lipids [27], but incorporation into non-melanin fractions is also possible [28]. Larsson and Tjalve, studying the phenomenon of bonding chlorpromazine with melanin, proved that both electrostatic force and van der Waals force play a crucial role in it. The cation form of the medicine is supposedly attracted by anion fragments of melanin. In connection with this, the first stage of bonding is based on the ion exchange. The second one, on the other hand, (van der Waals forces) is based on attraction of aromatic structures to the indole melanin centre [29].

2. The second important factor is the polarity of the xenobiotic. Research proved that polar metabolites penetrate into hair in a smaller quantity than lipophil precursors corresponding to them [30].

3. Acidity and alkalinity are the third important factor. The hair matrix is more acid than blood with pH of 7.4, hence the pH gradient is more favourable to the transfer of alkali than acids and indifferent particles [31].

![Fig. 1. The model of heroine distribution in blood and hair][32]

The complicated mechanism of penetration of drugs into hair causes the hair from various body parts to accumulate different quantities of a given xenobiotic [33]. Hair from the head differs most in respect to the growth speed, but its growth is the fastest (0.2-1.12 mm/day) and one can still collect it easier than other hair. The growth of hair from the beard is the slowest (0.27 mm/
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overdose in reference to varied ways of its application. His work did not lead to a broad popularisation of hair analysis in Germany, but was applied by detectives from the anti-drug departments as court evidence. The later use of the GC-MS method in the hair analysis confirmed its results. However, Arnold was discredited when he wrongly assessed that the positive result of the radio-immunological measurement may be obtained only after taking heroin. It turned out that also codeine gives a good cross reaction. After introduction of GC-MS, the specifically sensitive detection of substances became possible. Since that time, the number of studies and compounds determined in hair has increased significantly. This contributed to the appearance of many disputable issues concerning the applied procedures – the implementation of the process of biological material treatment was suggested, attention was paid to the irregular hair growth, and mechanisms of xenobiotic diffusion for hair were undermined. It was also discovered that the compounds may be partly rinsed out owing to everyday hair hygiene. All the objections constituted a motivation for improvement of hair analysis. The largest area of optimisation was the stage of extraction, as when the substance was once extracted, it was possible to test it just as if it were obtained from a blood or urine sample. The history of extraction from hair covers the use of many agents: methanol [46], enzymes, for instance, pronase [47], glucuronidase [48], extraction in the overcritical state [49].

Cone summed up the use of optional biological materials, including hair, on a global scale [50].

The practical application of analysis of organic xenobiotics in hair

In the recent years, the interest in studies excluding the use of illegal substances or excessive exposure to detrimental substances has increased. Presently, there is no specific law or generally accepted guidelines in Europe for application of the hair analysis as a test for the presence of undesirable substances, even at the workplace or in the road traffic. Many companies have established their own policy of employee control, covering mainly urine tests. Experience and knowledge concerning the hair analysis is developing quite quickly, therefore, its results are of some significance in court cases, among others, in the U.S. [15]. The results of hair analysis in reference to the presence of drugs were presented for the first time in court in 1982 [51, 52], where the positive result of hair analysis in reference to the presence of cocaine by means of the radio-immunological method were used. The tests concerned the alleged victim of rape, who, as the accused claimed, was under the influence of an intoxicant together with him. The accused also testified that they had been meeting with each other and sporadically using cocaine. The victim denied. The tests proved that it was the accused only who occasionally used this intoxicant.

In the U.S., the analysis of xenobiotics in hair is not recommended by the federal law, however, some states allow these tests to be carried out during criminal investigations, in the case of granting the child care and divorces. In the private sector, over ¾ of states permit or do not impose legal limitations in reference to the application of hair as a biological material [50]. In most European countries, the hair tests are in general limited to criminal investigations. In Germany, the determinations of xenobiotics in hair are allowed in the health care services – as a form of monitoring the chronic application of medicines or in the case of prolongation of the driving licence. A similar situation exists in Italy.

Conducting the segmental hair analysis, Uhl described a case in which chronic poisoning of a man by his wife with azeperon – a neuroleptic applied in veterinary medicine – was proved through the hair analysis [53]. Pragst et al. described a case of suicidal poisoning of a woman with amitriptyline. The woman was subjected to permanent therapy with this medicine [54].

The hair analysis is also used to confirm or exclude addiction [55]. At present, one of the most frequent addictions is alcoholism, in which the hair test also finds a broad application. Yegles et al. studied hair in regard to the presence of ethyl glucuronide and ethyl esters of fat acids. Both metabolites turned out to be good quality markers of chronic alcohol consumption. In the positive case, no significant correlation between the concentrations of glucuronide and esters was found, which is probably caused by different mechanisms of formation, incorporation in the hair structure and elimination from organism [56].

The next social problem is addiction to psychoactive substances (narcotics). It is a subject which has been discussed many times in scientific papers, as there is a very broad range of drugs, and the most popular ones include: amphetamine, products of cannabis (marijuana, dope), cocaine and opioids. The analysis of xenobiotics proved that the mean coefficient of positive drug detection was 1.36 higher in hair than in urine [5].

The determination of psychoactive agents in patients participating in the substitutive treatment with the use of the GC-MS method, enabling the detection of 8 amphetamine derivatives [57], confirmed that these agents...
Drugs in hair

were taken by patients from the methadone programme [58]. These studies also proved the correlation between the concentration of opiates and amphetamines in hair and the degree of addiction [59].

Physicians and nurses are one of the professional groups which has an easier access to drugs and controlled medicines [53]. In the case described in literature [60] an anaesthesiologist prescribed over 4000 pills of a painkiller – pentazocine – to various patients within 5 months. He admitted that he himself took a few pills because of a knee bruise. The hair analysis proved a concentration of 200 ng of pentazocine/mg in hair. Although the interdependence between a dose and the concentration is not known, it was clear that the high concentration of this medicine could not be explained by a few days of treatment.

The analysis of xenobiotics in hair is applied not only in the toxicology of addictions, but also in the case of chronic poisonings. It is also more and more often applied as a method of confirmation of exposure of pregnant mothers and their children to harmful xenobiotics. Hair, as a biological material, provides researchers with more information on the history of intoxication than, for instance, a urine or blood sample (mainly due to the possibility of performance of the segmental analysis). The publications describing the long-term exposure to substances other than the addictive ones are not numerous. From time to time, during the occurrence of pathological symptoms (hepatotoxic, neurotoxic) a suspicion of poisoning with an unknown substance arises. The pollution of environment, modifications and pollution of food or even criminal intentions may gradually bring about harmful effects.

The hair analysis is practically used in the post-mortem toxicology. There are many examples of its use in the court medicine, criminology, especially in regard to exposure to a xenobiotic which is distant in time. The time frame of the compound detection in hair is much broader (weeks – months – years) in comparison with the traditional testing material – blood samples, urine samples (hours – days). The long-term application of medicines or poisoning may trigger negative effects in a human organism and gradually lead to occurrence or exacerbation of disease symptoms. For instance, chronic use of metamphetamine causes disorders in the functioning of the cardiovascular system. On the other hand, hair is a useful material in determining deadly intoxication, however, single pieces of information may be found about the attempts to use hair in diagnostics of acute poisonings [61]. The segmental hair analysis (hair divided into 1-2 cm sections) provides information on the history of taking a specific xenobiotic, combining several compounds or changing one compound for another, or occurrence of the abstinence period [62].

The hair analysis is also used in civil cases such as divorces, granting childcare, adoptions, insurances [63].

Taking drugs and medicines by a pregnant mother may have tragic effects for her and for her baby [64-66]. These women most frequently do not admit that they use drugs during pregnancy, being afraid of legal consequences. The tests of baby hair may prove the use of medicines [17] or drugs, or for instance tobacco smoking by the mother [6, 67] and evaluate the impact of such practices on health and life of a baby [6, 68].

The exposure of a foetus to these compounds soon after the birth may cause the occurrence of the so called withdrawal syndrome (abstinence). This syndrome is treated, however the recognition of causes is particularly difficult when the symptoms occurring in newborns are not typical and the addiction of the mother was not discovered. The results of hair tests of newborns may confirm the presence of a given xenobiotic especially when the urine and meconium tests are negative or when these materials are unavailable.

One of the most frequent risks for the foetus (a social problem) apart from alcohol, is tobacco smoke. Its source may be active or passive tobacco smoking (ETS – Environmental Tobacco Smoke) by a mother during pregnancy. The results of conducted studies indicate the possibility of using the measurements of nicotine concentration in hair as a marker of a long-term exposure to tobacco smoke [69, 70]. The main advantage of this approach is a relatively long (lasting even several months) period, during which it is possible to estimate the degree of exposure. It is important especially in conducting epidemiological tests and establishing the etiology of the disease. Therefore, this biomarker and its usefulness for measurement of exposure to ETS in terms of epidemiology must be assessed. The results of the studies conducted so far have indicated that distribution of nicotine in hair is approximately in accordance with the declared average number of cigarettes smoked during a month [55, 71-74]. Kintz proved a correlation (83%) between the nicotine concentrations determined in the hair of newborns and their mothers [75]. Mizuno et al. [72] compared the history of smoking with the nicotine levels in hair, and concluded that it is built in along the hair shaft, whereas Uematsu [74] called hair a unique recorder which collects the data on the individual exposure to nicotine on a current basis. There are also papers where the relation between active smoking
and nicotine level in hair was not proved [71, 76]. In the studies conducted by Koren et al. in regard to a group of mothers and their newborns, no relation was found between the number of cigarettes smoked by mothers and nicotine in their hair or the hair of their newborns [27]. On the other hand, Klein concluded that nicotine concentration in mother’s hair decreased during pregnancy without limiting smoking, whereas the concentration of cotinine did not. This fact may indicate an increased metabolism of nicotine during pregnancy. The quantity of nicotine and cotinine in hair constitutes the total indicator of tobacco exposure [77].

The studies concerning the intrauterine exposure to tobacco smoke (both passive and active) proved a strong correlation between the nicotine levels in the hair of mothers and their newborns. The result of first studies comparing the nicotine level in the hair with cotinine level in urine proves that the level of nicotine determined in the hair of newborns was more correlated with the history of tobacco habit of their parents than the level of cotinine in urine [69]. On top of this, the analysis of cotinine level in the hair of newborns allows the smoking and non-smoking parents to be distinguished. Such a distinction was not possible in the analysis of cotinine in urine. Nafstad et al. conducted the studies on the basis of which it was concluded that the average level of nicotine in the hair of babies exposed to tobacco smoke from more than 10 cigarettes per day was 12.4 times higher than that of the non-exposed newborns, whereas in the case of the newborns exposed to tobacco smoke from less than 10 cigarettes per day it was 3.4 times higher. On the other hand, in the studies conducted by Sęńczuk et al. the correlation between the concentration of nicotine in hair of newborns and concentration of the tobacco smoking marker – cotinine in urine of mothers – was proved [6].

In the general perspective, the hair analysis may constitute a good complementation or clarification of the results obtained in the study of xenobiotics in urine. However in the anti-doping tests in many sport disciplines, the official regulations specify that the unambiguous presence of an illegal agent is possible only after the urine test. The analysis of xenobiotics in hair may be, on the other hand, a method showing the taking of substances retrospectively. The anabolics taken to develop the physical brawn may be detected in the hair after chronic application [78, 79]. Some scientists, however, express their scepticism on the subject of such control, arguing it with the impact of pigmentation and cosmetic treatments on the process of building the xenobiotics in hair.

An interesting application of determination of xenobiotics in hair is the study of archaeological findings. The hair analysis may be used to identify the intoxicants used in the historical times [80]. The tests of hair collected from the 600-year-old Peruvian mummy may constitute an example here. The analysis proved that hair came from a person who used cocaine or stayed in the environment in which it could be found [81].

Sources of errors in the hair analysis

The analysis of xenobiotics in hair has become a routine technique of retrospective study of exposure to xenobiotics. This method is helpful in discovering medicines, doping agents, intoxicants – including drugs - and alcohol metabolites. It enables the use of hair analysis in many areas of life, namely in environmental studies, workplace, ability to drive vehicles, in sport, but also in criminology and court medicine. Apart from this, on the basis of the uniform hair growth, it is possible to determine not only the presence, but also the time of exposure to a given factor in its segmental analysis. However, both the physiological aspects and the analytical performance of the hair study are very complicated, so they are a source of possible errors and incorrect interpretation of results. Already at the first stage of the study, it is possible to commit a mistake by an insufficient collection of information on the subject of purpose of the analysis, or even by taking a decision on selection of hair as a biological material. Then, during description and storage of samples, it is possible to mix them, confuse them, in the next phase – one must avoid using an improper method and conditions of documentation and extraction. The selected technique may be insufficiently sensitive, specific or accurate. Ultimately, there may be a possibility of wrong interpretation of obtained results, related to concentration of the xenobiotic or time of exposure to a given factor [82].

Collection of a hair sample

The careful and accurate collection of the sample is often neglected and the errors are difficult to mend at this stage. In some studies, (especially those connected with criminology, court medicine and related fields) it is indispensable to identify the person from whom the sample comes. Usually, it is a wisp of hair with a diameter of about 3-4 mm, which is tied and cut accurately over the skin surface. In order to obtain a possibly uniform material, hair is collected from the rear part of the head. This area is characterised by the most uniform growth. The permanent growth of hair provides the
most updated information on exposure, and the limited percentage of non-growing hair minimises the deviations of results. It was assumed that each centimetre of hair from the head corresponds to about one month of exposure [74]. It is desirable to mark the proximal end of hair for the segmental study, whereas for the court study – collection of the second sample, which is not tested but stored in case of possible objections [82].

Hydrolysis and extraction of xenobiotics from hair

This is the most sensitive stage of hair analysis. The xenobiotics are strongly built-in the hair structure and partly bonded with proteins, melanin and lipids of the membrane complex of the cell. The level of extraction depends on the structure of the separated compound, state of the hair matrix, material disintegration, polarity of the solvent, length of extraction and application of ultrasounds. There are many described procedures for various substances. The most important ones include hydrolysis by means of sodium hydroxide and extraction to the solid phase or extraction of hair with methanol or a water buffer, supported by ultrasounds. The first one gives good effects in the case of separation of stable compounds in the alkali environment – such as amphetamine. Methanol is applied universally, but extracts are to a great extent contaminated with the hair matrix. The alkali substances are subjected to action of the indiferent or slightly acidic buffer and further extraction to the solid phase. The next quite common error in the hair analysis is too short time of extraction. It is strictly related to the nature of the compound as well as the extraction medium – that is their structure and polarity [82].

Identification and determination of extracted compounds

In order to avoid obtaining a positive result in the case of actual lack of the marked substance, it is necessary to identify it unambiguously. The instrumental methods applied in the hair analysis are developing constantly. The immunological tests may be applied only as preliminary tests and must be confirmed by selective methods as, for instance, chromatographic methods. At present, GC-MS (gas chromatography (GC) combined with mass spectrometer (MS)) with electron ionisation (in the mode of selected ion monitoring (SIM)) is a standard technique. This allows the detection limit of most compounds at the level of about 0.03 ng/mg of hair to be obtained. The negative and positive chemical ionisation allowed the application of the method to be expanded by lowering the limits of detection of certain substances, but at the expense of a decrease in specificity. Apart from this, the long-term technique in the hair analysis is LC-MS (liquid chromatography (LC) combined with mass spectrometer (MS)), due to its advantages in the case of polar analytes [82].

Interpretation of results

The correlation, which is not fully proved for many xenobiotics, between the frequency of exposure to a given factor and the concentration of the substance in hair, is the main problem. Apart from this the degree of incorporation is variable and different in the respective persons (e.g. racial differences). The impact of hair pigmentation is, among other things, characteristic, especially when it comes to building in the alkali compounds. The hair varies in terms of physical condition and their destroyed structure may lead to a faster elimination of the xenobiotic during care and washing. The substances may decompose during bleaching, dyeing and permanent ondulation. A good example of the impact of pigmentation on the incorporation of xenobiotics is grey hair, which – in a simplified perspective – constitutes a mixture of colour hair with white hair. Sometimes it is hardly possible to indicate 5-85% of concentration of a given substance in grey hair in relation to the colour hair. This results from the fact that melanocytes have low pH, which is favorable to accumulation of alkali compounds. Apart from this, adsorption in melanin probably takes place of means of active transport [82].

One of the advantages of segmental analysis of hair is the possibility to establish the time of exposure by determining the place of occurrence of substances in a wisp of hair. However such an interpretation is limited both by experimental and biological inaccuracies. The errors of the first group may include improper hair cutting – not directly and not uniformly over the skin surface. It is necessary to take into account the fact that shortening the strand even by 5 mm corresponds to about a two-week time interval. The wisp of hair is also biologically non-homogenous. Human hair grows in accordance with the growth cycle consisting of three phases – anagen (growth phase), catagen (temporary phase) and telogen (stationary phase). The duration of the respective phases is dependent on the anatomy, age and sex of a given person. In consequence, a wisp of hair contains from 5 to 20% of hair in the telogen phase, which may even be older by half a year than most hair of the anagen phase [82].

To sum up, it must be stated that the application of hair as a material in toxicological and clinical studies, as
well as in the diagnostics plays a greater and greater role. This biological material is used more and more often because of the deep knowledge about mechanism of transporting xenobiotics to hair and improvement of analytical methods. In some cases such as prenatal exposure to psychoactive medicines and substances, or control of abstinence in the treatment of addictions they seem to be already irreplaceable. In spite of unquestionable advantages of hair as the material for detection and determination of xenobiotics, many problems must still be resolved, for instance: durability of xenobiotics in hair, impact of cosmetic treatments, individual differences in binding with hair, and above all, impact of external conditions on hair contamination.

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