

Announcement by the German Federal Environmental Agency

1-hydroxypyrene in urine as an indicator of internal exposure to polycyclic aromatic hydrocarbons (PAH) - reference value for 1-hydroxypyrene in urine

Opinion of the Human Biomonitoring Commission of the German Federal Environmental Agency

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Introduction

Polycyclic aromatic hydrocarbons (PAH) are produced when organic material undergoes incomplete combustion. They are composed of two or more benzene rings and occur, depending on the type of pyrolytic process and of source material, in various compounds, but always in the form of a mixture. Because so many incomplete combustion processes occur, PAH are ubiquitous environmental contaminants.

The significance of this class of substances from the environmental medicine viewpoint is determined by their carcinogenic nature, which is well documented for inhalatory exposure and for certain individual PAH and PAH mixtures. Benzo(a)pyrene (BaP) is the PAH for which we have most information concerning occurrence and transport in environmental media, and also comprehensive toxicological information, so BaP is frequently used as an indicator substance for PAH. According to leading expert bodies, BaP is to be seen “as a cause of cancer for humans” (Kat. 2) [1,2,3].

Test procedures that are sufficiently sensitive to enable determination of certain individual PAH metabolites in urine samples from test persons from the general population are now available to the environmental medicine community, and representative data also exist concerning exposure levels to PAH among the general population in Germany. The Human Biomonitoring Commission has therefore derived a reference value for 1-hydroxypyrene in the urine of the general non-smoking population in Germany in this position paper. In comparison with this value can be used to evaluate individual results recorded in human biomonitoring investigations related to specific incidents. With regard to the excretion of other PAH metabolites in urine, the currently available data are less comprehensive, hence it is not currently possible to derive reference values for these.

Information on the distribution of PAH in the environment and on the toxicology of this class of substances is presented in a number of monographies; sections of these are referred to in the following with special focus on human biomonitoring investigations [4,5,6,7,8,9,10,11,12,13,14].

PAH use, occurrence and distribution in the environment

Polycyclic aromatic hydrocarbons (PAH) form a group of organic compounds which are widespread in the environment as atmospheric, aquatic and soil pollutants [4,11].

Coal-burning, motor vehicle exhausts, coking plants, steel and aluminium producers, and the oil-refining industry are all contributors to the ubiquitous spread of PAH. PAH are contained

in vehicle exhaust fumes, fly ash, used lubricant oil, bitumen, tar, soot, curing smoke, cigarette smoke, etc. Once released, PAH bind to dust particles in the air and spread everywhere in the environment. More than 80 % of PAH are found in the PM_{2.5} fraction. Clean air measures and altered patterns of fuel use have enabled the pollution of the air with PAH to be drastically reduced in the last decades.

Typical BaP concentrations given in the 1990's for rural areas lie between 0.1 and 1 ng/m³ and those given for urban areas lie between 0.5 and 3 ng/m, with the levels at traffic pollution measuring stations likely to be at the upper end of the range given. In the local vicinity of coking plants, concentrations of as much as 30 ng/m³ are measured. The EU estimates that BaP contributes 50 % of the carcinogenic effects of PAH burden [14].

Uptake, metabolism, carcinogenicity of PAH

PAH can be resorbed pulmonarily, dermally or gastrointestinally. Depending on their boiling point, they can be inhaled in free form - as gas - or bound to particles. PAH-bearing particles are often so small that they advance in the lung as far as the alveoli.

Since PAH are highly lipophilic substances which can diffuse well through the lipoprotein layers of the skin, dermal uptake plays an important part in the contact with materials containing PAH [15,16,17].

The human resorption rate of benzo(a)pyrene ingested with food on the other hand is only 10 %. Most of the quantity ingested passes through the alimentary tract without being resorbed. Relatively small proportions of the intake can be found after 1 hour in the lymph, in the bile fluid and in the urine. After resorption, the liver, kidneys, and fatty tissue initially show higher concentrations compared to the blood, central nervous system and muscle tissue. Within 3-4 days redistribution of the PAH causes increased concentrations to occur in the mesenterial lymph nodes, adrenal glands, ovaries and in fatty tissue [18]. Fatty tissue counts as a good repository for PAH [4], where they can be detected even months after being deposited [19].

PAH are metabolised by a whole series of monooxygenases containing cytochrome P 450 during phase I metabolism. These enzymes vary in the efficiency with which they metabolise PAH, classified as carcinogenic, into the corresponding arene oxides, which are considered to be the ultimate carcinogens. A relatively important part in this oxidation process is played by the enzymes CYP1A1 and CYP1B1, among others. It is also significant in this context that the PAH-metabolising enzymes are by no means exclusively found in the liver. CYP1A1, for

example, is also expressed in the lungs, the oesophagus, the stomach and intestines, the placenta and peripheral blood cells. CYP1B1 also exists in the kidneys and in the lungs. Both enzymes can be induced by CYP1A1, by dioxins and in addition, by PAH or by cigarette smoke [11].

Epoxide hydrolases transform the arene oxides produced during the metabolism of PAH into dihydrodiols, or dihydrodiol epoxides into tetrols. In the course of this process, these enzymes may have toxifying or detoxifying properties.

In the so-called phase II metabolism, the epoxides of the PAH are transformed into phenols, diols or tetrols and are conjugated by glutathione transferases, sulfotransferases and UDP-glucuronyltransferases. They are excreted in the form of mercapturic acids, sulphates and glucuronides in the urine [11, 18].

Experiments using mice and rats show that small amounts of resorbed PAH are secreted without being metabolised via the bile fluids into the intestines. The proportions involved increase however with increasing molecular weight of the PAH.

Concerning the behaviour of PAH in human urinary excretion, most is known about pyrene, whose metabolite, 1-hydroxypyrene, has been susceptible since 1987 to routine testing using a procedure based on high efficiency liquid chromatography [20]. The excretion of 1-hydroxypyrene follows elimination kinetics that are biphasic, with one half-life of between several hours and two days duration, and one half-life of approximately 16 days [21]. One part of the incorporated pyrene is thus swiftly excreted, while the other part is stored in deeper locations, such as the fatty tissue, which it only leaves with a time delay to enter the bloodstream and be excreted. Further studies [22,23,24,25,26] have confirmed that the first elimination phase for pyrene has half-life durations of between 4 and 35 hours. Sieber et al.[27] investigated the kinetics of hydroxypyrene excretion in individuals who were occupationally exposed to PAH. Their findings likewise revealed a 2-phase elimination with average half-life durations of approximately 10 hours (6.3 – 15.9 h) and 36 hours (28.8 – 50.1 h).

The “International Agency for Research on Cancer” (IARC) [7] and the German Research Foundation’s “Senate Commission for the Testing of Harmful Work Substances” (*Senatskommission zur Prüfung gesundheitsschädlicher Arbeitsstoffe*) classified eleven PAH as carcinogenic in experiments with animals. Several mixtures containing PAH, including coal tars from brown coal and hard coal, tar pitches, tar oils, and coke oven gases, were

classified as carcinogenic for humans [3].

PAH count as so-called indirect carcinogens, which only develop their mutagenic and carcinogenic properties after metabolic transformation. The ultimate carcinogenic compounds in this process are the dihydrodiol epoxides. These may open the oxirane ring and react with the nitrogen bases of DNA by forming covalent bonds.

PAH which are classified as carcinogenic vary in their potential to cause cancer according to their chemical structure. Those PAH which have a so-called “bay region”, such as benzo[a]pyrene, display an especially high potential to cause cancer [28]. Most recent findings however show that the cancer-causing effect of the so-called “fjord region PAH”, such as dibenzo[a,l]pyrene, is higher by one or two orders of magnitude. Bay region and fjord region PAH are transformed in the human body into dihydrodiol epoxides, whose epoxide ring is largely protected from attack by the epoxide hydrolases [29] (see Figure 1: bay region; fjord region). These dihydrodiol epoxides are so stable that they can reach the cell nucleus and bind to the DNA there. This bond is seen as the initial step in carcinogenesis [30].

The carcinogenic effect of PAH on human skin has been known about for more than 200 years, when first reports were made of an increased incidence of cancer of the scrotum among chimney sweeps [31]. Skin cancer resulting from exposure to PAH has long been a recognised occupational disease subject to compensation rights. More recently, a series of epidemiological studies has established that the inhalative uptake of PAH can trigger lung cancer in humans. Hence lung cancer resulting from inhalative PAH uptake is now also a recognised occupational disease [32]. It is also suspected that PAH may cause cancer in other human organs as a result of uptake via different routes. This applies in particular to cancers of the urinary tract, the gastrointestinal tract and upper sections of the respiratory and digestive tracts [3].

By way of synopsis, the following points are to be noted when considering carcinogenesis triggered by PAH. PAH are taken up in systematic manner and transformed into mutagenic metabolic products by oxidation in many different organs in the human body. These products may make their way into the DNA and in this way potentially give rise to cancer. On the other hand, these intermediate mutagenic products are transformed by a whole range of phase II metabolic enzymes into less toxic substances which are subsequently excreted. Although it is clear that PAH induces toxifying enzymes, i.e. cytochrome P 450 monooxygenases, it is also generally agreed that the majority of enzymes involved in metabolising PAH and their polymorphisms have a levelling effect on the individual's potential cancer risk.

Sources of PAH exposure for humans

The main source of PAH intake is food, on the one hand as a result of airborne PAH precipitating onto cereals, fruit and vegetables, and on the other hand as a result of PAH generated during the preparation of food. For example, smoked food and food grilled on open flames display substantial levels of PAH content [11]. Exposure of humans to PAH from consumption of food containing PAH is discussed in numerous works [33,34,35,36].

A very important source of PAH exposure among the general population is tobacco smoke [4,37,38]. Smokers' intake of pyrene in cigarette smoke is of the same order of magnitude as intake from average food consumption [36].

In individual cases, the therapeutic use of ointments and shampoos containing tar may lead to a high additional exposure to PAH [39].

The use of parquet flooring adhesives containing PAH has been identified as a possible source indoors [40,41,42,43]. Parquet adhesives used in residential construction projects up to the 1950's contained bitumen and tar oils, after which time pure bitumen adhesives were used. Beginning in the middle of the 1970's, adhesives of different composition were used. The PAH contents of tar oils are considerably higher than that of bitumen. In 2002 ARGEBAU produced a document for use in assessment: "Advice for the assessment of PAH exposure from parquet flooring in buildings and measures for its reduction" (*„Hinweise für die Bewertung und Maßnahmen zur Verminderung der PAK-Belastungen durch Parkettböden mit Teerklebstoffen in Gebäuden“, PAK-Hinweise*) [44].

Human biomonitoring parameters for use in estimating internal exposure / demands on health

In the environment or the workplace, PAH always occur in the form of a mixture that can contain as much as several hundred individual substances. For practical as well as for economic reasons, it is not possible for investigations in the environment or at the workplace to cover and quantitatively determine all PAH individually. This is the reason for selecting a single PAH to represent the whole PAH mixture in an environmental sample, which is subjected to quantitative determination. Traditionally, benzo[a]pyrene has been used as the most important PAH indicator in numerous investigations and studies. However, for purposes of quantifying environmental pollution, the United States Environmental Protection Agency (US EPA) recommends using a selection of 16 PAH (see Table 5) [45].

A human biomonitoring investigation (HBM) of PAH is faced with the same difficulties as environmental analyses: it is only possible to investigate a limited number of substances (PAH or their metabolic products) in human body fluids. The selection is made more difficult by the fact that the greater the molecular weight of a PAH, the more it will be excreted in the faeces and thus escape ordinary biological monitoring tests. These are however the PAH with especially high carcinogenic potential.

Urinary excretions on the other hand mainly contain (in measurable quantities) metabolites of PAH with smaller molecular weights, such as naphthalene, phenanthrene and pyrene. These PAH are not, or are substantially less, carcinogenic than PAH with higher molecular weights. Nevertheless, nowadays it is known from numerous HBM studies that these metabolites are suitable to be used as “indicators” of internal PAH exposure. The concentration of unaltered PAH in the bloodstream is so low that it cannot be registered with the instrumental analysis methods currently available.

Hydroxypyrene in urine as a parameter of the internal exposure to PAH

In 1985, Jongeneelen [46] presented for the first time an analytic method (HPLC) which could be used to detect hydroxypyrene, the metabolic product of pyrene, in urine in a relatively simple and reliable manner. Since then, hydroxypyrene has been measured in numerous occupational medicine and environmental medicine investigations across the world. Summaries can be found i.a. in Angerer et al. [47]; Angerer [40]; Brandt and Watson [48]; Strickland et al. [39]; Bouchard et al. [49]; Jacob et al. [50]. The current standard HPLC/FD procedure can be used to detect hydroxypyrene in urine at levels as low as the lower ng/l range. This procedure succeeds - as the results of the 1998 German Environmental Survey show - in demonstrating the dependence of hydroxypyrene excretion for instance on the number of cigarettes smoked [51]. It was also possible to show that shortly after German reunification, inhabitants of the ‘new’ (ex-GDR) German federal states registered urinary hydroxypyrene concentrations that were on average almost three times as high as those of inhabitants of the ‘old’ federal states (i.e. ‘West Germany’).

Urinary hydroxypyrene excretion was also used to assess the PAH exposure situation in connection with the PAH contamination indoors that were discovered several years ago, which had been caused by parquet adhesives containing coal tar. The urinary hydroxypyrene excretion levels of persons who live in apartments with parquet adhesives containing tar did not differ from those of persons living in apartments whose parquet flooring had been laid in some other way or which contained other types of floor covering [40,42,52].

On the basis of the studies available, the Commission concludes that the determination of hydroxypyrene in urine forms a suitable parameter for use in testing the internal PAH exposure of the general population.

Other parameters for estimating internal PAH exposure and its biochemical effects

Following the introduction of hydroxypyrene as a parameter of internal PAH exposure, analytical methods for determining the 5 isomers of hydroxyphenanthrene (1-, 2-, 3-, 4- and 9-hydroxyphenanthrene) were also developed. Since then, investigations of hydroxyphenanthrene excretion have also been carried out among different population groups [50,53,54,55,56,57,58]. These show that the whole population is subject to exposure to phenanthrene, which finds expression in urinary hydroxyphenanthrene excretions that are easily detected by urine analysis. Nevertheless it must be noted that the amount of available data on hydroxyphenanthrene excretion is significantly smaller than that available for hydroxypyrene. Moreover, it has so far failed to be demonstrated that the determination of hydroxyphenanthrene shows advantages over the measurement of hydroxypyrene.

New results concerning the carcinogenic potency of naphthalene [59,60] have meant that this substance has increasingly also been a focus of attention. In the human body, naphthalene gets transformed in significant amounts into 1- and 2-naphthol. These metabolites can be recorded easily using high pressure liquid chromatography methods [61,62,63,64,65]. A particularly suitable method involves multi-dimensional HPLC using a column switching technique [66]. Being slightly volatile and predominantly airborne, naphthalene is a polyaromatic hydrocarbon which occurs everywhere, leading to exposure among the general population that can be reliably tested using the methods described. Preuss et. al. [66] presented a summary of the occupational medicine and environmental medicine issues arising in connection with naphthalene.

The use of high pressure liquid chromatography methods has also made it possible to test for hydroxylated metabolic products of anthracene and chrysene, and even for 3-hydroxybenzo[a]pyrene as a metabolite of BaP [67]. Given the significantly stronger carcinogenic effect of BaP compared with phenanthrene, pyrene, anthracene and chrysene, it would be particularly useful to be able to test for BaP metabolites in a human biomonitoring investigation.

For the detection of 3-hydroxybenzo[a]pyrene (3-HO-BaP), Jongeneelen and colleagues developed a HPLC procedure with a detection limit of 1 µg/l urine [20]. This detection limit is

however at least two orders of magnitude too high to be able to use this procedure to measure the background BaP exposure of the general population. It did prove possible, using a laser induced fluorescence detector (LIF) as the HPLC's detector, to reduce the detection limit to the range 0.5 - 8 ng/l. However, 3-HO-BaP was not found in urine samples from the general population [68].

The BaP tetrols would be even better suited than 3-HO-BaP as parameters for a human biomonitoring investigation. These tetrols are the direct metabolic products of the ultimate carcinogen 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-BaP. To detect BaP tetrols in urine, a highly sensitive gas chromatographic-mass spectrometry procedure was devised. This enabled concentrations ranging from below the detection limit of 1.5 fmol/ml urine to be detected in the urine of smokers [69]. No information is given in this study on the detection of BaP tetrols in the urine of non-smokers. No further investigations into the excretion of BaP tetrols in the general population exist at the present time.

DNA adducts and protein adducts of genotoxic substances in body fluids are parameters for the monitoring of biochemical effects [70,71]. Attempts have been made to detect protein adducts of PAH in human blood. These involved HPLC in conjunction with fluorescence detection, gas chromatography in conjunction with mass spectrometry and also ELISA techniques (enzyme-linked immunosorbent assay) [72,73,74]. It turns out that the procedures used up to now for the detection of PAH protein adducts are neither sufficiently sensitive nor sufficiently specific to be used in HBM investigations. In many cases, it was not even possible to detect a difference in adduct contents between the general population and occupationally exposed persons.

With regard to the detection of PAH DNA adducts in lymphocytes, the situation with respect to specificity appears similar that found with protein adducts. Summaries of these investigations can be found i.a. in Angerer et. al. [75], Brandt and Watson [48], DFG [3]. Evidently, although the methods for monitoring effects that have been available up to now may be highly sensitive (DNA adducts), they are too unspecific to permit reliable and evaluable coverage of DNA adducts in the context of a human biomonitoring study.

These considerations lead the commission to conclude that it is currently not possible to establish reference values for internal PAH exposure among the general population for environmental medicine investigations - neither for the detection of naphthols, phenanthrene, 3-hydroxybenzo[a]pyrene or tetrols in urine, nor for the detection of PAH DNA adducts and PAH protein adducts in blood [76].

Analytical determination of 1-hydroxypyrene in urine

High pressure liquid chromatography has been used from the start for the detection of hydroxypyrene excreted in the urine [77], initially involving acidic splitting of the glucuronides excreted in the urine. Jongeneelen et. al. replaced the acidic hydrolysis with an enzymatic hydrolysis. This method of preparing samples has become the standard [20]. Boos et. al. [78] then developed a HPLC method using a double column switching technique which made it possible to selectively enrich hydroxypyrene and subsequently to separate it from the other accompanying substances on an analytical column. The use of a fluorescence detector made it possible to achieve very low detection limits. This procedure was developed further, so that it became possible to jointly detect the isomeric hydroxyphenanthrenes as well as the hydroxypyrene in one test run. After being tested for reliability and comprehensibility, this form of the method was included in the DFG working materials commission's (*Arbeitsstoffkommission der DFG*) collection of methods "Analyses in biological material" (*Analysen im biologischen Material*) [79]. This procedure has undergone refinements that mean that it is now possible to reliably detect hydroxypyrene concentrations as low as the lower ng/l range. The detection limit is given as values around 10 ng/l. The precision of the method in daily use lies between 5 and 12 % for concentrations of between 100 and 300 ng/l [57].

Gas chromatography was also used for the detection of hydroxypyrene in urine [80,81,82]. However, this requires not only derivatisation of the analytes using diazomethane, but also in addition a liquid-liquid-extraction of the derivate, before it can be analysed using gas chromatography.

Other methods were also developed in which the glucuronides of the hydroxypyrene were detected following immunoaffinity chromatography using synchronous fluorescence spectrometry [83,84].

The methods that have become generally accepted today have been the high pressure liquid chromatography methods in conjunction with fluorescence detection. Compared with other techniques, these methods are distinguished by their use of simple sample preparation methods that are less prone to error. These methods are robust, can be carried out in large series and lead to results that can be compared, even when achieved in different laboratories. Summarising, this procedure for detecting hydroxypyrene in urine can currently be described as the procedure of choice.

The situation regarding data on the internal PAH exposure of the general population

The keen interest of scientific research groups in the search for evidence of PAH exposure in the human body has meant that the database on the excretion of the PAH metabolite 1-hydroxypyrene for the general population has grown continually over the past years (Table 1+2). Taking into consideration the years of investigation and the different air pollution levels in individual countries, the data from different countries for persons not subject to occupational PAH exposure lie in a similar range [85].

The German Environmental Survey 1998 (GerES III) [58,86] presented representative data including data on 1-hydroxypyrene excretion among the adult population in Germany. This included the determination of the PAH metabolite 1-hydroxypyrene in the morning urine of a randomly selected subgroup of 284 women and 289 men aged between 18 and 69 years [58]. The urine samples were subjected to enzymatic hydrolysis, and the metabolites were separated using HPLC and detected using fluorescence spectrometry [87].

The results show that smokers' level of urinary 1-hydroxypyrene content is approximately twice as high as that of non-smokers (Table 3), and that the level increases with the number of cigarettes consumed per day [58]. Figure 2 displays the relative increase in 1-hydroxypyrene content in morning urine depending on the number cigarettes smoked per day [88].

Since smoker/non-smoker status is the dominant factor influencing the excretion of 1-hydroxypyrene and since it obscures the effects of other influencing factors, only the results for non-smokers are presented in what follows. The urinary content of 1-hydroxypyrene among the non-smoking population in Germany lies in the range between undetectable levels (detection limit: 0.012 µg/l) and 1.68 µg/l or 0.90 µg/g creatinine with a geometric mean of 0.10 µg/l or 0.08 µg/g creatinine. This range corresponds closely with data given elsewhere in the literature (see Table 1).

Factors that crucially influence hydroxypyrene content in urine among the non-smoking population are the type of heating system, place of residence (in the 'new' vs. the 'old' federal states), and passive smoking [88]. Significantly higher average concentrations were found for residents using decentrally (as opposed to centrally) operated heating and for residents of the 'new' (as opposed to the 'old') federal states (see Table 3). If there are smokers in the household, passive smokers' urinary 1-hydroxypyrene content levels are predicted to be higher by more than 20 % on the basis of multiple regression analyses. According to these

models, high exposure from passive smoking may even yield urinary 1-hydroxypyrene content levels that are ca. 30 % higher than those of other non-smokers [88].

With the aim of demonstrating a temporal trend in PAH exposure in the population, results of GerES III were accompanied by a parallel investigation of the levels of 1-hydroxypyrene content in the morning urine of a randomly selected subgroup of subjects who had never smoked from the German Environmental Survey 1990/92 (GerES II) comprising 150 adults aged between 25 and 69 years. At the same time, urine samples from 508 children aged between 6 and 12 years who lived in the households of adult subjects of GerES II were tested for 1-hydroxypyrene content. All the analyses were carried out using the same method [87] during the period of analysis of GerES III [58].

In the period between 1990/92 and 1998, no change in internal PAH exposure was detected for adult residents of the 'old' federal states who had never smoked. By contrast, a 60 % decrease was found for adult residents of the 'new' federal states who had never smoked. This means that the average level of PAH exposure among the population of the 'new' federal states has moved closer to the situation obtaining in the 'old' federal states (Table 4). The decrease in the 'new' federal states is accompanied by a decrease in the PAH concentration in atmosphere, which can be explained in terms of reduced emissions from household fuel burning, industry and vehicle use.

It was not possible to prove a correlation of 1-hydroxypyrene excretion in urine among adult non-smokers with the consumption of grilled or smoked foods, nor could such a correlation be shown to obtain with road traffic emissions in GerES III (1998).

The data from GerES II (1990/92) pertaining to non-smoking children and to adults who had never smoked show levels of 1-hydroxypyrene content in urine, both for children and adults, that are substantially higher in the 'new' federal states than the results for child and adult population in the 'old' federal states. The median levels were 0.32 µg/g creatinine in urine of the children and 0.20 µg/g creatinine in urine of adult for the 'new' federal states and 0.14 µg/g creatinine in child urine and 0.08 µg/g creatinine in adult urine for the 'old' federal states. These results further indicate that in 1990/92, higher levels of urinary 1-hydroxypyrene content were found for children than for adults [51]. A possible explanation for the higher urinary concentrations in children might be the fact that children spend more time each day exposed to road traffic (1 h 23 min.) than adults (49 min.) [89], especially since it was only possible to establish a correlation between 1-hydroxypyrene in urine and road traffic emissions in the case of children [51].

According to the regression models, approximately 18 % higher levels are estimated for non-smoking children exposed to intensive passive smoking. For a place of residence close to a road, approximately 17 % higher levels of 1-hydroxypyrene excretion are estimated. For households using coal or wood ovens for cooking, approximately 53 % higher levels are assumed. However, for children as for adults, active smoking of tobacco products was the factor that most strongly influenced 1-hydroxypyrene excretion levels [51].

The content levels established by Angerer [40] for 74 children below 12 years old, who were not subject to additional PAH exposure in the home and who lived in three locations in the 'old' federal states, agree very closely with the results of the 1990/91 GerES (6-12-year-old non-smoking children from the 'old' federal states) despite the differing investigation periods (see Table 2).

An investigation that formed part of the pilot phase to the German Environmental Survey for children (GerES IV) [90] conducted tests that included 1-hydroxypyrene excretion in the morning urine among 389 children and young people aged between 3 and 17 years. The analyses were performed according to Lintelmann and Angerer [87] using a detection limit of 12 ng/l. For the non-smoking children (N=351), the study established median values of 0.14 µg/l or 0.11 µg/g creatinine (median) and a 95th percentile of 0.46 µg/l or 0.38 µg/g creatinine [91].

Reference values for 1-hydroxypyrene in the urine of the general population

The reference value is defined to be the 95th percentile of the values measured for the substance concentration in the relevant body medium of the respective reference population [92]. The reference value is estimated from the 95 % confidence interval of the 95th percentile and given where possible as an absolute value. In accordance with the IUPAC [93] guideline, the respective 95% confidence intervals (CI) of the 95th population percentiles (PP) are calculated and used as the statistical basis for deriving the reference values.

A reference value for adults can be derived using existing data from the representative population data collection of the 1998 GerES. Currently, there are still no representative data that can be used to derive a reference value for children and young people. However, in order to be able also to assess the PAH exposure of children and young people compared to the background exposure, a reference value for non-smoking children aged between 3 and 17 years can be derived using the 1-hydroxypyrene excretion data gathered in the literature

and in the pilot phase of GerES IV. As soon as the data from GerES IV that was begun in 2003 [90] become available, an update will be made.

Based on the data from the environmental survey 1998 (95 % confidence intervals for the 95th population percentiles¹ of 0.42 – 0.57 µg/l urine or 0.27 – 0.35 µg/g creatinine) and the data from the pilot phase of GerES IV (95th percentiles of 0.46 µg/l or 0.38 µg/g creatinine), the Human Biomonitoring Commission derives the following reference value for **1-hydroxypyrene in urine:**

0.5 µg/l or 0.3 µg/g creatinine for the non-smoking general population (aged 3 - 69 years).

For smokers, levels of 1-hydroxypyrene in urine that are approximately twice as high as those of non-smokers should be assumed.

It is emphasized that reference values have no significance for health, but are descriptions of the basic exposure levels among the population investigated obtaining at the time of investigation.

Actions to be taken in connection with the reference value

In cases where the reference value is exceeded and tobacco smoking can be excluded as a possible source, it is advisable that control measurements be carried out. To improve the assessability of the result of the control measurement, it should be ensured that the creatinine concentration of the sample to be tested lies within narrower limits, i.e. in the range from 0.5 to 2.5 g creatinine per litre [94]. Values above the reference values that have been reliably measured (and checked several times) should be taken as grounds for undertaking a search for the causes and sources of this exposure, within reasonable bounds. Possible sources that need to be considered, other than active tobacco smoking, include pollution of the indoors air by small ovens burning fossil fuels, open hearths, building materials, for example defective parquet flooring that is fixed with adhesives containing hard coal tar, and also the consumption of grilled and smoked foods.

Summary

1. 1-Hydroxypyrene is a suitable parameter for use in estimating the actual PAH exposure of the human body resulting from all intake routes.

¹ Excluding urine samples with creatinine contents of <0.3 or >3.0 g/l

2. A heightened level of 1-hydroxypyrene concentration in urine that is maintained over a longer period is to be considered to represent an additional factor contributing to the risk of cancer caused by PAH.
3. Human Biomonitoring values are not derived, on account of the carcinogenic properties of some PAH.
4. The analytic methods for detecting the products of reactions of PAH with DNA and with proteins (adducts) are currently not yet sufficiently sensitive and specific for them to be able to be used for environmental medicine purposes.
5. For the non-smoking section of the general population (aged 3-69 years), a reference value of 0.5 µg 1-hydroxypyrene/l urine or 0.3 µg 1-hydroxypyrene/g creatinine has been given as a description of background PAH exposure.

Brief overview

Test medium	Substance	Sample material	Detection limit	Method
Urine	1-hydroxypyrene (PAH metabolite)	24 h urine / morning urine	0.012 µg/l	HPLC/FD procedure
Reference group for reference values		Test medium	Reference value	
non-smoking general population (aged 3-69 years)		Urine	0.5 µg/l or 0.3 µg/g creatinine	
Sources		Kinetics	Chronic effects	
Tobacco smoke, smoked foods and foods grilled over open fire, adhesives containing hard coal tar, small ovens and open hearths using fossil fuels		Elimination in urine with 2 distinct elimination phases of 10 and 36 hours respectively Storage: fatty tissues	Risk of cancer Organs targeted: skin, lungs, gastrointestinal tract, urinary tract	
Measures to be taken to reduce exposure:				
In cases where values above the reference values are obtained and where tobacco smoking can be excluded as the source: take repeat measurements, ensuring that the creatinine concentration of the sample to be tested lies in the range of 0.5 - 2.5 g creatinine per litre. In case reliable measurements that are checked several times repeatedly yield values above the reference values, a search for the causes and sources should be initiated.				

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Table 1: Levels of 1-hydroxypyrene content in urine in adults in Germany and other countries

Country	Year	Group	Smoker status	N	Statistical data	
Authors [Reference]					50th percentile	95th percentile
Germany						
GerES II Becker et al. [58]	1990/92	General population M,F: aged 25-69; morning urine	NeverS	150	0.14 µg/g creatinine 0.20 µg/l	0.55 µg/g creatinine 0.94 µg/l
GerES III Becker et al. [58]	1998	General population M,F: aged 18-69; morning urine	S	184	0.19 µg/g creatinine 0.25 µg/l	0.73 µg/g creatinine 1.03 µg/l
			NS	389	0.08 µg/g creatinine 0.10 µg/l	0.29 µg/g creatinine 0.53 µg/l
Angerer et al. [95]	?	Schleswig Holstein/ Franken, control group; M,F: aged 18-84; spontaneous urine	S	20	0.24 µg/g creatinine	0.55 µg/g creatinine
			NS	28	0.11 µg/g creatinine	0.28 µg/g creatinine
Angerer et al. [96]	1991/93	Contamination black spot Ruhr region; F: middle-aged; morning urine	S	27	0.48 µg/g creatinine	1.45 µg/g creatinine
			NS	97	0.15 µg/g creatinine	0.46 µg/g creatinine
Goen et al. [97]	1990/95	South Germany, general population	S	21	0.23 µg/g creatinine	0.55 µg/g creatinine
			NS	28	0.12 µg/g creatinine	0.33 µg/g creatinine
		South Germany, general population	S	20	0.28 µg/g creatinine	0.52 µg/g creatinine
		Administrative staff of a waste incineration plant	NS	49	<0.04 µg/g creatinin	0.38 µg/g creatinine
Scherer et al. [98]	1996	Munich and suburbs, M,F: aged 18-70	S	27	AM=0.35 µg/24h	Max=1.2 µg/24h
			NS	42	AM=0.16 µg/24h	Max=0.5 µg/24h
Heudorf [42]	1998	Residents of ex-US housing; M,F aged 20	S	131	0.14 µg/g creatinine	0.47 µg/g creatinine
			NS	289	0.08 µg/g creatinine	0.26 µg/g creatinine
Italy						
Roggi et al. [99]	?	Pavia, general population; M,F: aged 20-79	S	92	0.33 µg/g creatinine	1.1 µg/g creatinine
			NS	327	0.15 µg/g creatinine	0.7 µg/g creatinine
Merlo et al. [100]	1993/94	Genoa, traffic police and controls M,F: aged 35,7 ± 6 years and 38,5 ± 5 years	S	43	AM=0.34-0.44 µg/l*	
			NS	89	AM=0.17 µg/l*	
Canada						
Viau et al. [101]	?	Control group consisting of university and office staff, M,F; spontaneous urine	S	45	GM=0.23 µg/l*	0.63 µg/l* (S,NS)
			NS	95	GM=0.14 µg/l*	

Table 1 (cont.): Levels of 1-hydroxypyrene content in urine in adults in Germany and other countries

Country	Year	Group	Smoker status	N	50th percentile	95th percentile
Authors [Reference]						
Netherlands						
Boogaard und van Sittert [102]	1987/92	Workers without special exposure, spontaneous urine	S/NS	236	0.21 µg/l*	97.5.P.=0.98 µg/l *
Van Rooij et al. [36]	1992	Voluntary subjects not occupationally exposed; M: aged 21-64 years; Morning and spontaneous urine	S NS	37 39	0,48 µg/l * 0,23 µg/l *	95%CI: <0,2-1,5 µg/l * 95%CI: <0,1-0,6 µg/l *
Poland						
Ovebro et al. [103]	1992/93	Non-industrialised region in Silesia	S NS	27 18	GM=0.58 µg/l * GM=0.27 µg/l *	
Sweden						
Levin et al. [85,104]	1988/90	Office employees; Spontaneous urine	S NS	10 14	0.26 µg/l 0.07 µg/l	
Denmark						
Hansen et al. [105]	1989/91	Persons without occupational exposure F/M: aged 21-67 years	S/NS	121	0.02 µg/l*	0.10 µg/l * 95%CI: 0.05-0.30 µg/l *
Czech Republic						
Vyskocil et al. [106]	1995/96	Three different regions, M,F	S,NS	62	0.06-0.23 µg/l *	Max=1.4 µg/l *
Turkey						
Burgaz et al. [107]	?	Control group, university employees; M: aged 21-62 years; spontaneous urine	S NS	14 15	AM=0.64 µg/l * AM=0.46 µg/l *	
USA						
[Buckley et al. [108]	1993	Texas, Rio Grande Valley; M,F: aged 21-73 years; morning urine	NS	12	0.1 µg/l	Max=2.4 µg/l
Santella et al. [109]	?	Volunteers; M,F: aged 45±15 years; 32 % smokers	S,NS	53	AM=0.22 µg/l *	Max=0.8 µg/l *
Chuang et al. [110]	1994/95	North Carolina, persons with low income; M,F; spontaneous urine	S,NS	24	AM=0.09 µg/l	Max=0.36 µg/l
CDC [111]	1999/00	NHANES, persons with low income (?); M,F; aged ≥ 20 years spontaneous urine	S,NS	1309	0.07 µg/l	0.80 µg/l
<p>Notes: N = size of sample; P50, P95 = percentiles; GM = geometric mean; *: Values were calculated according to the formula: 1 µmol/mol Crea = 1.93 µg/g Crea ≈ 3 µg/l given an average excretion of 13 mmol/l [11,85], NeverS = people who have never smoked, NS = non-smokers, S = smokers, M = male, F = female</p>						

Table 2: Levels of urinary 1-hydroxypyrene content in children in Germany and other countries

Country	Authors [References]	Year	Group	N	Statistical data	
					50th percentile	95th percentile
Germany						
	GerES II Seiwert et al. [51]	1990/92	General population, non-smoking children; B/G: aged 6-12 years; morning urine			
			‘Old’ federal states:	299	0.14 µg/g creatinine 0.19 µg/l	0.39 µg/g creatinine 0.63 µg/l
			‘New’ federal states	190	0.32 µg/g creatinine 0.45 µg/l	0.92 µg/g creatinine 1.68 µg/l
Angerer [40]		1997/98	children who were not subject to additional PAH exposure at home			
			≤ 6 years old, Frankfurt:	23	0.15 µg/g creatinine	0.33 µg/g creatinine
			≤ 12 years old, Herzogenaurach:	29	0.20 µg/g creatinine	0.47 µg/g creatinine
			≤ 6 years old, Erlangen:	22	0.16 µg/g creatinine	0.31 µg/g creatinine
			≤ 12 years old Σ	74	0.16 µg/g creatinine	0.31 µg/g creatinine
Heudorf und Angerer [112,113]		1998	Children below 6 years old, residents of ex-US housing in Frankfurt/Main; spontaneous urine	347	0.15 µg/g creatinine	0.47 µg/g creatinine
Pilot study of GerES IV [91]		2001/02	General population from 4 locations in Germany, non-smoking children and youths J/M: aged 3-17 years, morning urine	351	0.11 µg/g creatinine 0.14 µg/l	0.38 µg/g creatinine 0.46 µg/l
USA						
Chuang et al. [110]		1994/95	North Carolina, preschool children from low income households; J/M; aged 2-4 years Spontaneous urine	24	0.13 µg/l	Range 0.009-1.23 µg/l
CDC [111]		1999/00	NHANES			
			B/G: aged 6-11 years	310	0.09 µg/l	0.42 µg/l
			B/G: aged 12-19 years	693	0.11 µg/l	0.64 µg/l
			Spontaneous urine			
Netherlands						
van Wijnen et al. [114]		?	5 locations with varying levels of PAH pollution in outdoor air and soil B/G; aged 1-6 years	644	GM for 1-6 years: 0.3 – 0.5 µg/l*	
Czech Republic						
Fiala et al. [115]		?	Kindergarten in polluted area	42	GM=0.18 µg/l*	Range 0.04-2.8 µg/l*
			Kindergarten in unpolluted area	42	GM=0.15 µg/l*	0.03-0.5 µg/l*
			B/G: aged 3-6 years Morning urine, summer			
Notes: N = size of sample; P50, P95 = percentiles; GM = geometric mean; B=boys; G=girls, *: Values were calculated according to the formula: 1 µmol/mol Crea = 1.93 µg/g Crea ≈ 3 µg/l given an average excretion of 13 mmol/l [11,85]						

Table 3: Levels of 1-hydroxypyrene content in urine ($\mu\text{g/g}$ creatinine) of the general population (aged 18 to 65 years) in Germany in 1998 [58]

	N	P50	P95	GM	CI-GM
Overall	573	0.10	0.48	0.11	0.10 - 0.11
Smoker status*					
Smokers	184	0.19	0.73	0.19	0.17 - 0.21
Non-smokers	389	0.08	0.29	0.08	0.07 - 0.09
Non-smokers					
Heating system*					
Not central	35	0.15	0.61	0.14	0.11 - 0.19
Central	354	0.08	0.24	0.08	0.07 - 0.08
Federal states*					
'New' fed. states	71	0.12	0.45	0.12	0.10 - 0.14
'Old' fed. state	319	0.07	0.24	0.07	0.07 - 0.08

Notes: N = size of sample; P50, P95 = percentiles; GM = geometric mean; CI-GM = approximative 95% confidence interval for GM; values below the limit of quantification (LOQ) are included as LOQ/2; * = significant difference in the GM ($p < 0.01$) by the t-test

Table 4: Levels of 1-hydroxypyrene content in urine ($\mu\text{g/g}$ creatinine) of the never smoking population (aged 25 to 69 years) in Germany in 1990/92 and 1998 [58]

	N	P50	P95	GM	CI-GM
'Old' federal states					
1990/91	75	0.08	0.28	0.08	0.06 - 0.09
1998	182	0.07	0.20	0.07	0.06 - 0.08
'New' federal states*					
1991/92	75	0.20	0.62	0.19	0.16 - 0.24
1998	45	0.10	0.58	0.11	0.08 - 0.14

Notes: N = size of sample; P50, P95 = percentiles; GM = geometric mean; CI-GM = approximative 95% confidence interval for GM; the limit of quantification (LOQ) are included as LOQ/2; * = significant difference in the GM ($p < 0.01$) by the t-test

Table 5: Proposals made by the US-EPA and the DFG on determining PAH relevant to environmental medicine and occupational medicine

EPA list [45]	DFG proposal [3]	Effectively equivalent * Factor
Acenaphthene ^a	Anthanthrene	0.1
Acenaphthylene ^a	Benz[a]anthracene ^b	0.1
Anthracene ^a	Benzo[b]fluoranthene ^b	0.1
Benz[a]anthracene ^b	Benzo[j]fluoranthene ^b	0.1
Benzo[b]fluoranthene ^b	Benzo[k]fluoranthene ^b	0.1
Benzo[k]fluoranthene ^b	Benzo[b]naphtho[2,1-d]thiophene ^d	0.01
Benzo[a]pyrene	Benzo[a]pyrene	1
Benzo[ghi]perylene ^{a,b}	Chrysene ^b	0.01
Chrysene ^b	Cyclopenta[cd]pyrene	0.1
Dibenz[a,h]anthracene	Dibenz[a,h]anthracene	1.0
Fluoranthene ^a	Dibenzo[a,l]pyrene	100.0
Fluorene ^a	Dibenzo[a,e]pyrene	1.0
Indeno[1,2,3-cd]pyrene ^b	Dibenzo[a,h]pyrene	1.0
Naphthalene ^b	Indeno[1,2,3-cd]pyrene ^b	0.1
Phenanthrene ^c	Naphthalene ^b	n.i.
Pyrene ^c	Phenanthrene ^c	0.001
	Pyrene ^c	0.001

^a not carcinogenic

^b weakly carcinogenic, but frequently occurs in relatively high concentrations in the environment

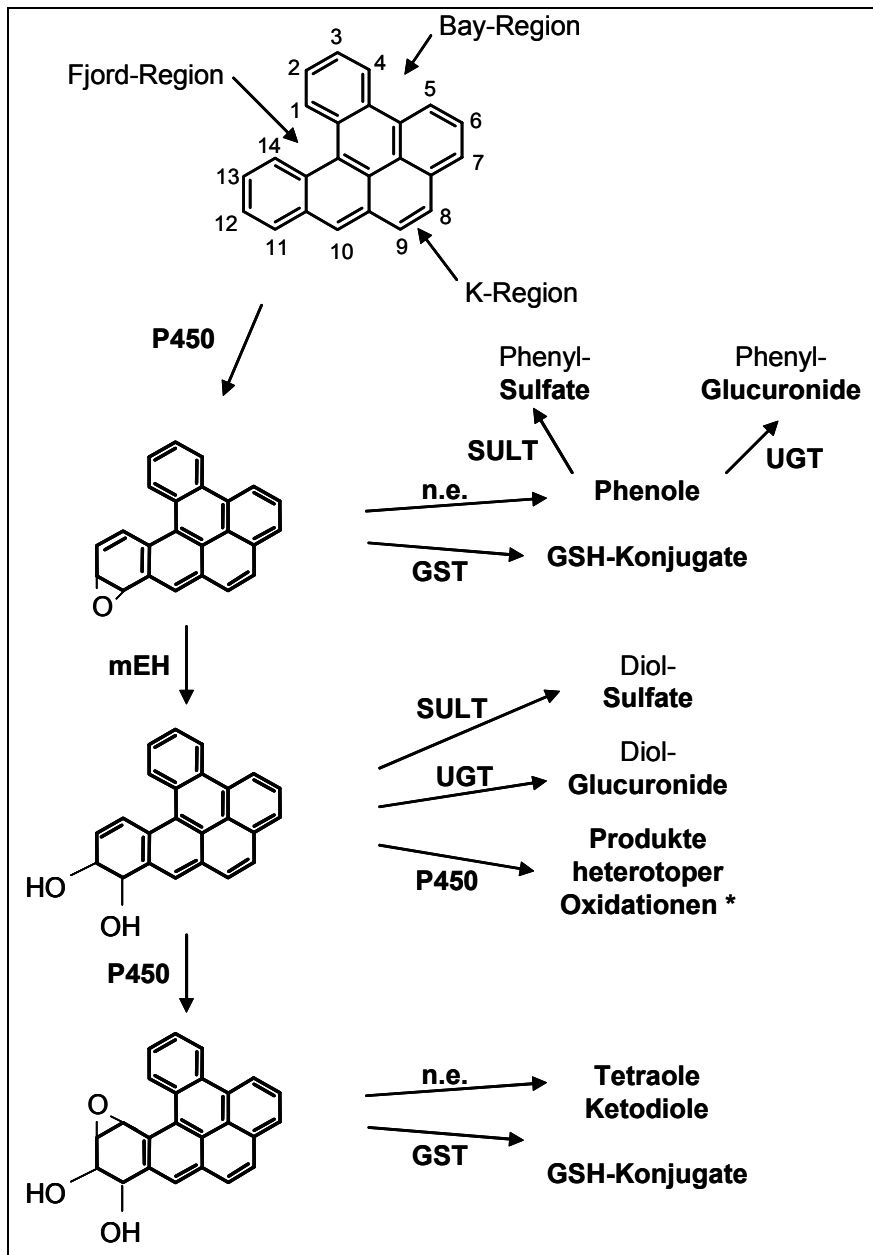
^c not carcinogenic, but is a metabolite normally used in biomonitoring; hence it can be useful to know the exposure data.

^d weakly carcinogenic, emission-specific, occurs in comparatively high concentrations in certain matrices (mineral oil products) and indicative for the class of thioarenes

n.i. no information

* DFG [3]

Figure 1: Phase I and phase II metabolism, illustrated for dibenzo[a,l]pyrene following Luch and Jacob [116]



UGT: glucuronosyltransferase

GST: glutathione S-transferase

mEH: microsomal epoxide hydrolase

n.e.: not enzymatic

P450: cytochrome P450 dependent monooxygenases

SULT: sulfotransferase

*: additional oxidation(s) at one or more positions outside the fjord region (C-atoms 13-14)

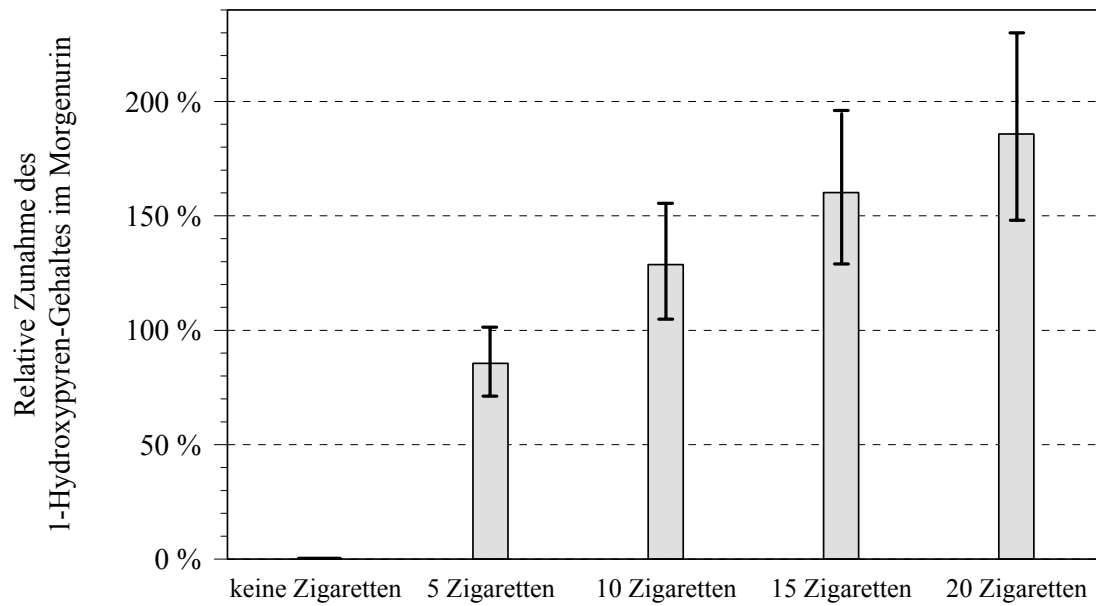


Figure 2: Effect of the number of cigarettes smoked per day on the level of 1-hydroxypyrene content in morning urine (average relative increase of 1-hydroxypyrene content in morning urine with 95 % confidence interval)

Note: This figure shows the relative changes in the content by volume of 1-hydroxypyrene in morning urine as estimated according to the multiple linear regression model, and not levels of absolute 1-hydroxypyrene content.

Source: Federal Environment Agency, GerES III (1998)