

Announcement by the German Federal Environmental Agency

Substance Monograph: Arsenic - Reference Value in Urine

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

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Introduction

Arsenic compounds are ubiquitous in the environmental media. Inorganic arsenic compounds such as arsenic trioxide and arsenic pentoxide originate both from geogenic sources and from industrial emissions. Given their chronic toxicity and carcinogenic effect, they are of high relevance in environmental medicine [1, 2]. Organic arsenic compounds are produced by biologically active systems. Particularly large amounts of such compounds are found in marine animals. Up to the present, they have been rated as being little toxic.

The current arsenic body burden in humans resulting from different routes of exposure can be assessed by determination of the arsenic level in urine. Analysis should be performed only by means of methods validated for human biomonitoring.

Occurrence and use

Occurrence in environmental media

Arsenic is a natural component of the earth's crust, with an average share of 2 – 5 mg/kg. The most significant natural emission is to be attributed to volcanic eruptions. In addition, weathering plays a role. Anthropogenic emissions are mainly due to the use of fossil raw materials and fuels (having a natural arsenic content) in thermal processes. Their emission is promoted by the highly volatile character of arsenic oxides. Important emitters include the non-ferrous metallurgy (in particular, copper smelting), the production of iron and steel, the non-metallic minerals industry with the production of glass and the domestic combustion of fuels. Waste incineration has only a minor share in total arsenic emission today.

In **air**, arsenic is mostly present in particulate form as As_2O_3 [3]. In the vicinity of copper smelters or (coal-burning) power stations, concentrations between 1.4 and $160 \mu\text{g}/\text{m}^3$ were measured some decades ago. In the last decade, arsenic emission has become reduced owing to pollution control measures and decreasing use. In 1990, estimated emissions from motor vehicle traffic and stationary sources in the old and new federal Länder of Germany were 20 t and 100 t, respectively [4]. Ambient air concentrations of arsenic are between 0.5 and $1 \text{ ng As}/\text{m}^3$ in little contaminated regions, and $15 \text{ ng As}/\text{m}^3$ in the vicinity of emitters [5].

Arsenic levels found in natural **soils** are between 0.1 and 20 mg/kg, as a rule. Due to geogenic conditions, such levels may, however, exceed 100 mg/kg. Levels found in contaminated soils, for example in the vicinity of copper smelters and former arsenic production sites, may even exceed 1000 mg/kg [3, 6-8]. Similarly high concentrations may be found in the soil of agricultural land after application of arsenic-containing herbicides [2]. Knowledge is scarce concerning the chemical form

of arsenic in the soil. Part of it is presumably present in organic form as a result of biological activity [9].

Arsenic levels in **ground water** largely depend on geological and local conditions [10, 11]. In ground and surface water, the ratio between As(III) and As(V) depends on the redox potential. In ground waters, up to 50 % of the total arsenic may be found in its trivalent form depending on the prevailing oxygen conditions [12].

In Germany, arsenic is essentially bound to strata of mottled sandstone (Triassic) and Rotliegend (Permian). In regions rich in arsenic, the population may be exposed to above-average arsenic levels in drinking water. As a result of oxidative water treatment, 80-90 % of the total arsenic found in **drinking water** is present in its pentavalent form. As a rule, the levels found are far below the 10 µg/L limit value stipulated by the German Drinking Water Regulations. In 1998, the German Environmental Survey found a mean arsenic level of 0.4 µg/L in domestic drinking water (stagnant-water sample) supplied to the population aged 18-69 years [13]. However, in some regions of the world, very high arsenic levels in the range of 100-1000 µg/L have been detected in drinking water.

In rare cases, high arsenic levels (exceeding 50 µg/L) are present in Germany in water from medicinal springs due to geogenic conditions. The limit values fixed in the German Mineral Water Regulations (1991) are: 50 µg/L for natural **mineral waters**, spring water and table water, and 5 µg/L, for water used for the preparation of infant formula. Arsenic levels found in ready-to-drink beverages are far below 10 µg/L, as a rule.

In the majority of **foods**, arsenic levels are below 2.5 µg per kg. Arsenic levels detected in seafood and some species of marine fish are markedly higher, and the major part of the arsenic is present in the form of organic compounds such as arsenobetaine or arsenocholine, which accumulate in the marine animal [14]. Concentrations detected in seafood are stated to be in the range of 1 - 15 mg/kg fresh weight (normal values) up to 150 mg/kg (contaminated waters), and for marine fish, ca. 3 mg/kg. In contrast, the concentration found in freshwater fish is no more than about 0.3 mg/kg. In other foods, arsenic concentrations are mostly below the detection limit of 0.05 mg/kg fresh weight [15]. Arsenic levels detected in breast milk are mostly low (< 0.3 µg/L) [16]. Since 1942, arsenic has been banned in Germany as a component of pest control products used in viticulture. However, elevated arsenic levels may be found in imported wine.

Use

The naturally occurring arsenic sulfides, auripigment and realgar were used already in ancient Egypt as a yellow **dye**, for make-up purposes and as auxiliary products in leather processing. Auripigment was important in alchemy since it was known to produce a golden gleam when rubbed on silver.

In quantitative terms, the use of arsenic trioxide in zinc electrolysis and glass production is of major importance in industrial **production**. Today, arsenic is also used in the production of special alloys. Another important field of use is that of gallium or indium arsenide in semiconductor technology. Under the Regulations on Dangerous Substances and Materials (Gefahrstoffverordnung, 1986), the use of arsenic in the Federal Republic of Germany has been limited for a large number of fields. Materials containing more than 0.3 % by weight of arsenic are banned from use in many fields.

In **medical science**, the use of arsenic preparations has been described since Hippocrates' times. Fowler's solution (potassium arsenite containing ca. 1 % arsenic trioxide) is used to treat psoriasis. At the beginning of the 20th century, the introduction of organic arsenic compounds such as Salvarsan constituted a breakthrough in the treatment of a great number of bacterial diseases such as syphilis. Research to find new, effective arsenic-containing products resulted in the synthesis of more than 30 000 arsenic compounds. However, these compounds have meanwhile been almost completely replaced by antibiotics. Today, only a few preparations are available worldwide, including for example melarsoprol, which is used to treat trypanosomiasis. Arsenic trioxide (white arsenic/arsenicum album) or other arsenic compounds are used in the preparation of some homoeopathic products. Highly toxic levels of arsenic have been found in some Asian medications. Recently, high doses of arsenic trioxide have been used in the chemotherapy of leukaemia [17].

During World War One, arsenic compounds were used as **chemical warfare agents**. Lewisite is an organochlorine arsenic compound. By crosslinking of sulfhydryl groups occurring naturally in the body it causes destruction of tissue both locally (skin, respiratory tract, eye) and in internal organs after systemic absorption, which takes place at a high rate. BAL (British anti-lewisite) was developed as an antidote. It is a compound blocking arsenic compounds with its sulfhydryl groups.

In the past, **pesticides** containing arsenic (e.g. arsenic trioxide) were commonly used all over the world. In the Federal Republic of Germany, they have been banned since 1974. Nevertheless, the presence of residues in imported goods cannot be excluded. A mixture of arsenic, copper and chromium salts is used for wood preservation in a high-pressure process.

Common exposure situations

Exposure through foods

The average weekly intake of inorganic arsenic compounds through foods and drinking water by adults in Germany is estimated to be 1 µg/kg per kg body weight [18]. This is below the PTWI (Provisional Tolerable Weekly Intake) level fixed by the World Health Organization (15 µg/kg body weight and week), which does not take into account the carcinogenic effects of the substance. However, it exceeds the RfD (Reference Dose) level of 0.3 µg/kg and day fixed by the United States Environmental Protection Agency (EPA). Fish and seafood consumption accounts only for the intake of insignificant quantities of inorganic arsenic [19]. Different results were obtained by studies performed in the past. However, the analytical methods used at that time were not adequate for a specific and reliable detection of the various species of arsenic [20].

Fish and seafood constitute the main sources for the intake of organic arsenic compounds by humans. Arsenobetaine and arsenocholine account for the highest share of arsenic intake through fish and seafood. They are followed by dimethylarsinic acid (DMAA) [19], trimethylarsine oxide (TMAO) and trimethylarsine (TMA) [21].

Other routes of exposure

Inhalational absorption of arsenic accounts for less than 0.1 µg/day, as a rule. Tobacco smoking is of minor importance for arsenic absorption [22]. Arsenic absorption through ingestion of soil and dust is negligible in our regions for persons not subject to any specific exposure. However, it plays a role in the case of children living in regions with soils rich in arsenic, mainly in dry regions with soil drifting [23, 24]. Arsenic-containing medicinal products (see above) may cause very high exposure levels.

Kinetics

Systemic absorption

Organic and inorganic arsenic compounds ingested by the oral route are absorbed readily and effectively (ca. 45-90 %) in the gastrointestinal tract [25, 26]. In general, poorly soluble arsenic compounds are absorbed to a lesser extent than readily soluble ones.

Since in the air, arsenic is present in particulate form, pulmonary absorption is characterized by a two-phase process consisting of particulate deposition and subsequent absorption. The first phase is determined by the particle size distribution and the second, by the solubility of the arsenic compounds inhaled. In spite of the availability of a number of results obtained by animal

experiments [27-29] and studies performed in workplace situations [30-32] it is impossible to state general absorption rates. On an average, a deposition of 40 % and an absorption rate of 30 % can be assumed as realistic.

Percutaneous absorption rates differ depending on the chemical form of the substance. They range between <1 % for several inorganic arsenic compounds and a few percent points for several organic arsenic compounds [33-35].

Distribution and accumulation

Absorbed inorganic As(III) and As(V) will spread rapidly to the organs of the human body. After 24 h, ca. 1 % of the amount absorbed will be detected in the blood. The major part is found in muscles, bones, kidneys and lungs, mainly as As(III). In cases of chronic exposure, arsenic will preferentially accumulate in tissues rich in keratin and/or sulfhydryl such as hair, nails and skin. Monomethylarsonic acid (MMAA) and dimethylarsinic acid (DMAA) will hardly bind to tissue. Arsenic can easily cross the placental barrier. It passes into breast milk to a minor extent [36].

Metabolism, excretion

In humans, organic arsenic compounds such as arsenobetaine, dimethylarsinic acid, trimethylarsine oxide and trimethylarsine ingested by the consumption of fish and seafood are largely eliminated by the renal route within a period of ca. 2-3 days. Arsenic sugars, which are found in ample amounts in marine plants and seaweed, are partly metabolized in mammals and have a somewhat longer half-life [37].

A considerable proportion of inorganic arsenic compounds is methylated in the human liver to form monomethylarsonic acid (MMAA) and dimethylarsinic acid (DMAA) [38]. MMAA and DMAA are rapidly eliminated by the renal route. On balance, humans excrete ca. 10-20 % of inorganic arsenic as As(III) and As(V), ca. 15-25 %, as monomethylarsonic acid and 30-60 %, as dimethylarsinic acid [39, 40]. The quantitative results obtained by different working groups vary to a considerable degree. Part of the arsenic is excreted by the biliary route [26]. On the whole, the situation may vary considerably from one individual to the other.

As a consequence of the great number of compartments and metabolic forms involved, elimination is characterized by several phases. The foremost part is excreted within a half-life period of one to several days, depending on the arsenic compound involved, and a minor part, within a half-life period of up to one month [25, 41, 42]. Results of animal experiments cannot be directly extrapolated to humans because considerable differences exist between different species with regard to the arsenic metabolism [39, 43]. As an example, Figure 1 shows the course of arsenic

concentrations in the urine of a 50-year-old test person after the consumption of marine fish (halibut).

Effects in humans

Mechanisms

The toxic effects of arsenic are caused by a variety of molecular mechanisms such as -

- Inhibition of cellular ATP formation as a result of competition with phosphate (acute);
- Inhibition of sulfhydryl groups in enzymes; and
- Damage to chromosomes.

While inorganic arsenic compounds have a high acute and chronic toxicity based on these mechanisms, the toxicity of organic arsenic compounds has so far been rated as very low. This assumption may have to be revised. Recent findings have revealed that MMAA and DMAA, which are formed in the human body from inorganic and presumably also from organic arsenic [44], are cytotoxic [45], have a potential to damage the DNA [46] and are known to promote skin tumours (DMAA) in animal experiments [47]. These first results do not yet suffice to suggest a revision of assessments concerning the importance for humans.

Acute effects

Acute effects are not important from the environmental health aspect but they are from the aspects of forensic and occupational medicine. In this context, their detection by human biomonitoring plays an important role. Owing to their high acute toxicity, inorganic As(III) compounds have been used for centuries as a poison to commit murder. As(III) has a higher mobility and due to its higher bioavailability, it is two to ten times more toxic than As(V). Oral intake of 0.1 g of arsenic (III) may result in death. Shortly after ingestion, severe diarrhoea and vomiting, associated with a garlic-like body odour and neurological manifestations are observed. Capillaries become permeable and the blood will thicken. This is followed by manifestations of shock and renal failure.

In the course of accidents, inhalational exposure to the highly toxic gas, arsine will lead, after a few hours, to massive haemolysis, with red urine as an early sign. Irritant-type chemical warfare agents containing arsenic cause severe damage to the skin and mucosa and after systemic absorption, general organ failure.

Chronic effects

Non-cancer diseases

Long-term exposure to relatively high levels of inorganic arsenic compounds, e.g. through highly contaminated drinking water, will result in polyneuropathy associated with painful peripheral

paraesthesia and skin changes. The latter will become manifest by changes in pigmentation, hyperkeratosis of the skin and white horizontal lines in the nails. An endemic occurrence of damage to blood vessels associated with disturbed circulation was observed in Taiwan and other countries and has become known as blackfoot disease [48].

In recent years, very high arsenic contamination of drinking water has been identified as a severe environmental health problem in several regions of the world, particularly in Bangladesh and West Bengal [11, 49, 50]. In Bangladesh, concentrations exceeding 50 µg arsenic per litre were found in 37 % of wells examined. 20 % of the population exhibited skin changes typical of arsenic, being associated with a high incidence of general weakness and neurological manifestations. More than 100 million people are affected by exposure to high arsenic levels in that country.

Cancer

Arsenic is a carcinogenic substance. The mechanism of the carcinogenic effect has not yet been elucidated. Arsenic is not a chemical mutagen. i.e. it does not attack the DNA directly. However, it leads to chromosomal mutations under both in vitro and in vivo conditions [15, 51]. In addition, there is an inhibition of DNA repair mechanisms. Because of the DNA damage recently described to have been caused also by DMAA and MMAA it cannot be excluded that also organic arsenic compounds may contribute to the carcinogenic character of arsenic.

Skin cancer (basal-cell and squamous-cell carcinoma) is a characteristic disease of persons exposed to high doses of arsenic by the oral route. An elevated incidence was found among psoriasis patients treated with Fowler's solution, among wine growers exposed to arsenic residues in highly contaminated home-made wine after administration of arsenic-containing pesticides, among workers in copper smelters and among people in East Asia, India and South America whose drinking water was highly contaminated with arsenic [2].

Lung cancer is the critical endpoint after inhalational exposure to arsenic (III) and (V) [52, 53]. An effect threshold as sometimes discussed for the oral route of exposure has not been assumed to exist for the inhalational route. Lung tumours are probably linked to a long-term arsenic release from the particulate matter retained in the lungs. According to an assessment by the Länderausschuss für Immissionsschutz (German Länder commission for protection against ambient pollution), arsenic is one of the most important airborne carcinogens found in outdoor air [54]. Lung cancer [56, 56] as well as bladder, kidney and liver cancer have been reported to be promoted also by consumption of drinking water rich in arsenic [57].

Environmental health relevance

In Central Europe and Germany, contamination of the environmental media with arsenic has become massively reduced by regulatory measures in recent decades. Nevertheless, considerable environmental health importance is still attributed to arsenic. Based on the oral and inhalation unit risk levels stated by the US EPA (see section on limit values, guide levels, recommendations), a noticeable carcinogenic risk has to be inferred by environmental health standards. The situation is most dramatic in regions of the world rich in arsenic where exposure may be 100 times higher than that in Germany. Insofar, arsenic has to be rated among the most important environmental contaminants on a worldwide level.

Arsenic determination in human biomonitoring

Biological material

Today, the arsenic body burden in humans is commonly ascertained by determination of the element and its compounds in the urine. Arsenic determination in the blood is practically of no importance. The half-life of arsenic in the blood is only short. Therefore, concentrations in the blood are low and do not permit a sensitive and reliable assessment of the body burden. Hair and nail analyses are of importance in forensic medicine and epidemiological studies to assess episodes of exposure to arsenic in the past. [58].

Standard determination by means of hydride generation atomic absorption spectrometry

The hydride method

The method used most often today for the determination of arsenic in urine is the so-called hydride generation atomic absorption spectrometry (hydride AAS). This method is suitable for the simultaneous detection of inorganic arsenic compounds and MMAA, DMAA, TMAO and TMA, which, however, must be preceded by adequate reduction. Arsenobetaine and arsenocholine are not detectable by this method [59, 60]. Arsenic concentrations that can be detected by urine analysis depend on

- The quantity of the inorganic arsenic absorbed;
- The quantity of the organic arsenic compounds absorbed;
- The sampling and processing techniques employed; and
- The type of the analytical method used.

When hydride AAS is used, arsenic concentrations excreted in the urine will provide an appropriate picture of the body burden of inorganic arsenic only if it can be ensured that the measuring result is not masked by arsenic intake from fish and seafood. This can be assumed to

be the case if the test person has refrained from fish or seafood consumption for at least three days.

Preanalytical phase

The test person should not eat any fish or seafood such as shellfish, crustaceans, etc. for three days before urine sampling is performed. For urine examination, a 24-hour urine sample should be used if possible. If the collection of complete 24-hour urine samples cannot be reliably ensured, an early morning urine sample should be used for arsenic determination. Containers used for collection and transport must be free of any arsenic contamination. The urine should be submitted to the analytical laboratory immediately after having been collected from the test person. If necessary, samples can be stored in a refrigerator for up to 48 hours. If this period is exceeded, storage in a deep-freeze cabinet is recommended (ca. -18 °C). Under such conditions, samples can be stored for up to 6 months.

Analytical phase

Prior to further processing, the refrigerated urine samples are allowed to assume room temperature. Prior to division into aliquots, the samples should be vigorously shaken to provide for an even distribution of sediment.

For the determination of arsenic in the urine by means of hydride AAS (batch method), a method is available that has been verified with regard to its analytical reliability and reproducibility [60]. During performance of this method, which is used in a similar way all over the world, the urine is allowed to react with sodium borohydride in hydrochloric acid solution. The volatile arsenic hydrides are transferred to a quartz cell placed in the optical path of an atomic absorption spectrometer where the hydrides are thermally decomposed and the arsenic extinction is measured instrumentally. The analytical results are evaluated by standard addition procedures. The detection limit achieved by this method is 1 µg/L.

FIAS systems

To avoid the time-consuming discontinuous batch method, many laboratories increasingly use automated flow injection systems (FIAS) for sample processing when employing the hydride technique. In these systems, reagents are added automatically to the samples to be analysed. Generally, experience has shown that if such systems are used for arsenic determination in urine, the levels detected are quite different from those determined with the batch method and systematically, too low. This is probably due to the fact that arsenic species that are more difficult to hydrogenate may easily escape detection. If FIAS systems are used, this potential error should be counteracted by a thorough validation of the method and possibly also by means of an

appropriate preceding reduction and intensive quality assurance. Therefore, this method appears to be unsuitable at present for routine biomonitoring of arsenic in humans.

Quality assurance

For arsenic determination in urine, the general rules of quality assurance in human biomonitoring will apply [61]. For internal measures taken by the laboratory to ensure quality assurance, appropriate control materials are commercially available. Interlaboratory studies on external reproducibility on an international level are offered by the Deutsche Gesellschaft für Arbeitsmedizin und Umweltmedizin (DGAUM - German society of occupational and environmental medicine) and the Centre de toxicologie du Quebec. The interlaboratory studies performed by DGAUM have shown that laboratories holding a valid certificate have produced results that deviated from the rated value by a maximum of 30 % in the concentration range of 3-4 µg arsenic/L urine [62]. At higher concentrations, the deviation of results produced by certified laboratories was less than 20 % [63]. Highly qualified laboratories (reference laboratories) are known to obtain results deviating from the rated value by no more than 10 and 6 %, respectively, for the concentration ranges stated above.

Analytical determination of different arsenic species in urine (speciation)

Today, a number of analytical methods can be found in the literature which consist of an initial chromatographic separation of the different organic and inorganic arsenic compounds excreted in urine and subsequent determination by AAS (atomic absorption spectrometry) or ICPMS (inductively coupled plasma mass spectrometry) [64-69].

Meanwhile, also the Deutsche Forschungsgemeinschaft (DFG) has published an up-to-date method for the detection of arsenic species [70]. When using this method, the individual arsenic species are first separated by means of high-performance liquid chromatography and subsequently, after having been converted into the respective arsenic hydrides, determined by atomic absorption spectrometry. This method is suitable to determine As(III), As(V), MMAA and DMAA in a single analytical run. The latter are the arsenic species considered as important in a toxicological view. Arsenocholine and arsenobetaine are not detectable by this method. Its detection limits are 0.9 (As(III)), 2.3 (DMAA), 1.4 (MMAA) and 2.0 (As(V)) µg arsenic/L urine. The precision to be achieved by this method in an analytical series is 4 - 8 %.

The ranges of tolerance (triple standard deviation of reference laboratories) recorded in first interlaboratory studies performed by DGAUM for the detection of arsenic species in urine were between 25 and 36 %. The levels concerned (10 - 40 µg/L) rather corresponded to concentration ranges being relevant for occupational medicine. For environmental health purposes, the current

detection limits of such methods are not satisfactory. In addition, only a few laboratories are in the position to perform such determinations at present.

Obviously, the advantage provided by arsenic speciation consists in the possibility to separately record and evaluate the different arsenic compounds which vary in their degree of toxicity. However, it has to be taken into account that inorganic arsenic compounds are methylated in the human body. Consequently, the health risk involved would probably be underestimated if a toxicological assessment was based merely on As(III) and As(V) concentrations. Also, arsenic speciation cannot solve the problem linked to the intake of organic arsenic compounds contained in fish and seafood. For arsenic species analysis as well, the urine must not be sampled earlier than three days after consumption of the last meal containing fish. Otherwise, the results obtained will not allow an assessment of their health relevance.

Determination of the total arsenic excreted in the urine

Using the methods of instrumental analysis available today, the total quantity of arsenic excreted in the urine may be determined. For this purpose, the urine samples are first subjected to mineralization by means of oxidizing acids. The arsenic will then be present in the form of inorganic ions. These can be determined by means of the hydride technique described above as well as by means of ICPMS. The determination of total arsenic is used for special purposes such as the overall assessment of arsenic quantities absorbed and excreted. In environmental medicine, the total arsenic level is attributed a minor importance at present since it also covers the arsenic species considered as less toxic. In cohort testing without observing a waiting period after fish consumption, the determination of total arsenic in urine has produced median levels that were about three times as high as those obtained by the batch method. When observing a waiting period after fish consumption of at least three days, the levels detected largely corresponded to each other.

Indication to perform human biomonitoring

An indication to perform human biomonitoring may be based both on public health and individual health considerations. It applies to population groups suspected of being exposed to arsenic to an abnormal extent. Such exposure may be caused for example by industrial emission or dietary arsenic intake. In such cases, biomonitoring will permit an objective proof of an elevated body burden as compared to the general population. Arsenic determination in urine may also be indicated for individual persons if medical history and examination of environmental samples have suggested relatively high exposure levels that should be quantified.

In addition, human biomonitoring is the diagnostic method of choice to reliably elucidate the situation in cases of suspected arsenic poisoning of both chronic (polyneuropathy, pain in the

hands) and acute character (diarrhoea, vomiting, garlic-like body odour, neurological manifestations).

Interpretation of findings of human biomonitoring

When interpreting results it has to be taken into account that arsenic levels found in urine can only reflect arsenic exposure and absorption in the past few days. Conclusions as to a chronic exposure and risk can be drawn only with reservation. For comparison of results with exposure levels described in literature it has to be examined whether the arsenic levels recorded referred to total arsenic or arsenic species that may undergo hydrogenation.

Reference values for arsenic

Derivation of a reference value

The reference value corresponds to the 95th population percentile of the arsenic concentration found in the general population [71]. Reference values for arsenic in urine were derived on the basis of the representative population data obtained under the 1998 Environmental Survey among adults [72,73] who had refrained from fish consumption for at least 48 hours prior to sampling. Since no such data are available for children, no reference value are derived for that group.

The Environmental Survey was performed among the German population aged 18-69 years during the period between October 1997 and March 1999, based on a cross-section random sample representative in terms of community size, age and sex (N = 4741 persons) [72, 73]. Arsenic levels in early morning urine were determined on the basis of standard methods [70] using the hydride batch technique with a determination limit of 0.6 µg/L [73].

As a statistical basis for the derivation of reference values, the respective 95 % confidence intervals (CI) of the 95th population percentiles (PP) were calculated as described in the corresponding IUPAC guideline [74]. Table 1 shows the corresponding parameters for arsenic in urine (µg/L) for the German population aged 18-69 years.

Table 1:

Arsenic levels in the urine of the population aged 18-69 years with no fish consumption for 48 hours prior to sampling - 95 % confidence intervals for 95th population percentiles [73]

	Age	N	CI 95 th PP
µg As/L urine	18 - 69	3924	14.9 – 16.3

Note: N = number of samples; CI 95th PP = approximate 95%-confidence interval for 95th population percentile

The following reference value has been fixed:

Adults (18 - 69 years) having refrained from fish consumption for 48 hours prior to sampling: 15 µg/L urine.

For adults having consumed fish or seafood within the last 48 hours (contrary to the sampling recommendations), the 95th percentile has been found to be about two to three times higher.

Table 2 shows arsenic concentrations as a function of fish consumption, which is the most powerful predictor of arsenic excretion in the urine of the general population in Germany.

Table 2:

Arsenic levels in the urine of the German population aged 18-69 years as a function of fish consumption - German Environmental Survey 1998 [73]

	N	N<BG	P50	P95	GM	CI-GM
Sample						
Total	4741	208	4.1	35.4	3.92	3.81 - 4.03
Fish consumption within 48 h prior to sampling						
Yes	788	6	7.5	48.1	7.91	7.38 - 8.48
No	3924	199	3.7	13.1	3.40	3.30 - 3.50

Note: N = number of samples; n<LOD= number of values below limit of determination (LOD); P50, P95 = percentiles; GM = geometric mean; CI-GM = approximate 95% confidence interval for GM; levels below LOD recorded as LOD/2

Since arsenic excretion in the urine varies considerably as a function of fish consumption, additional environmental exposure is difficult to identify. For that reason and because organic arsenic compounds are assumed to be of minor toxicological importance, the Commission agreed to fix reference values for arsenic in the urine of persons having refrained from fish/seafood consumption for at least 48 hours before sampling.

Factors influencing arsenic concentrations in urine

In a subsample of participants in the Environmental Survey who had stated to have refrained from fish consumption for 48 hours prior to sampling, a significant correlation ($p \leq 0.001$) was found to exist between the arsenic levels detected in the urine (related both to volume and to creatinine) and sex, age, arsenic levels in domestic drinking water (stagnant-water sample), the frequency of consumption of fish and of the consumption of wine, sparkling wine and fruit wine [73]. The differences in arsenic excretion between the subgroups were less pronounced as compared to the influence of a 48-hour waiting period after fish consumption prior to sampling. In Table 3, the distribution of arsenic levels in urine as stratified by these characteristics is shown.

As part of the statistical evaluation, a great number of other potentially influencing variables was examined which did not prove to show any significant correlation to arsenic excretion in urine. These included, among others, variables describing *the housing environment, the home, food behaviour, use of biocides, smoking habits* and *socioeconomic status, the time of the year when sampling was performed* and *the body mass index*.

Examinations of the influence of arsenic levels in the soil have revealed that persons living in residential areas with relatively high arsenic levels in the topsoil (up to 100 mg/kg) did not exhibit any differences in arsenic concentrations in the urine as compared to control persons [75]. Another study involving mean arsenic levels in the soil between 237 and 371 mg/kg resulted in a significantly higher median value in exposed (3.6 µg/24 h) persons as compared to control persons (2.4 µg/24 h) living on soils containing less than 20 mg arsenic/kg [2, 23]. Persons supplied with drinking water from sources rich in arsenic have shown considerably higher arsenic concentrations in their urine than control persons not exposed to drinking water rich in arsenic [15].

Table 3:

Arsenic levels in urine ($\mu\text{g/L}$) of the German population aged 18-69 years - no fish consumption for 48 h prior to sampling - German Environmental Survey 1998 [73]

	N	n<LOD	P50	P95	GM	CI-GM
Sex						
Female	1960	126	3.4	12.1	3.07	2.95 - 3.21
Male	1963	74	4.0	13.8	3.75	3.61 - 3.90
Age (years)						
18 – 19	161	3	4.5	11.2	3.93	3.51 - 4.39
20 – 29	673	21	3.9	14.3	3.79	3.56 - 4.04
30 – 39	912	36	3.9	12.1	3.52	3.33 - 3.72
40 – 49	774	44	3.6	13.5	3.36	3.13 - 3.60
50 – 59	741	46	3.6	12.9	3.26	3.07 - 3.53
60 – 69	663	50	3.2	13.2	2.93	2.71 - 3.16
Frequency of fish consumption						
Several times per week	379	17	4.2	17.3	3.89	3.54 - 4.27
Ca. once per week	1455	76	3.9	13.9	3.55	3.38 - 3.73
2-3 times per week	984	45	3.6	13.8	3.35	3.16 - 3.54
Max. once per month	644	30	3.6	11.3	3.30	3.09 - 3.53
(Almost) never	454	31	3.2	9.3	2.87	2.64 - 3.12
Arsenic levels in domestic drinking water						
> 2 $\mu\text{g/L}$	275	9	4.7	11.7	4.47	4.07 - 4.92
$\leq 2\mu\text{g/L}$	3637	191	3.6	13.2	3.32	3.22 - 3.42
Frequency of consumption of wine / sparkling wine / fruit wine						
Several times per week	454	16	4.3	13.3	3.88	3.58 - 4.21
Max. once per week	1308	57	3.8	12.7	3.55	3.38 - 3.72
Max. once per month	2146	125	3.5	13.2	3.22	3.09 - 3.35
(Almost) never	476	31	3.3	10.3	2.98	2.74 - 3.24

Note: N = number of samples; n<LOD= number of values below limit of determination (LOD); P50, P95 = percentiles; GM = geometric mean; CI-GM = approximate 95% confidence interval for GM; levels below LOD recorded as LOD/2

Measures guided by the reference value

A repeat measurement should be performed in cases of concentrations exceeding the reference value. It should be carried out not earlier than 3 days or preferably, to be on the safe side, 5 - 6 days after the last consumption of fish or seafood. If arsenic levels continue to be elevated on the repeat measurement and consumption of fish or seafood can reliably be excluded, other sources of arsenic could play a role. Sources to be considered should include, among others: drinking water, mineral or medicinal water, sparkling wine/wine/fruit wine, arsenic-containing medicinal products, high soil contamination and high exposure at the workplace. Efforts should be made to reduce elevated arsenic exposure.

In the Commission's opinion, cases of elevated arsenic levels in urine due to environmental exposure do not constitute an indication for chelate therapy [76]. Only in cases of accidents or homicidal/suicidal attempts involving arsenic that are characterized by unequivocal clinical manifestations, patients should be treated with chelating agents. In such treatment, both the clinical picture and arsenic levels found in the urine should be taken into account.

HBM values

At present, the Commission is not in a position to derive toxicologically founded HBM (human biomonitoring) values for arsenic. Essentially, this is due to the fact that contrary to the situation prevailing in occupational medicine, the arsenic detected in urine originates from different sources and consists of various chemical species differing considerably in their toxicity. For the field of occupational medicine, the MAK-Kommission (commission responsible for the establishment of maximum admissible concentrations at the workplace) has fixed a biological guide value of 50 µg/L for inorganic arsenic and methylated metabolites in urine.

Limit values, guide values, recommendations

Classification by the MAK-Kommission

The MAK-Kommission of the Deutsche Forschungsgemeinschaft (DFG) has assigned to Category 1 arsenic trioxide, arsenic pentoxide, arsenic acid, arsenous acid and their salts, e.g. lead arsenate and calcium arsenate. Experience has shown that these substances have a potential for causing malignant tumours in humans. The following relationship was described between occupational exposure and urine levels at the end of the shift of workers (Expositionsäquivalent für krebserzeugende Arbeitsstoffe - EKA):

Classification by the MAK-Kommission

mg arsenic/m ³ air	µg arsenic/L urine
0.01	50
0.05	90
0.1	130

The biological guide value (Biologischer Leitwert - BLW) has been fixed at 50 µg arsenic/L urine.

TRK level

The technical limit concentration in workplace air (technische Richtkonzentration - TRK) is 0.1 mg total arsenic/m³.

Provisional tolerable weekly intake (PTWI)

In 1988, the World Health Organization has proposed a PTWI level of 15 µg/kg body weight for non-carcinogenic effects of inorganic arsenic.

Reference dose (RfD)

If the reference dose (RfD) of 0.3 µg/kg body weight and day stated by the US EPA is not exceeded, no risk of even the slightest chronic effects (hyperpigmentation, keratosis and possible vascular damage) is to be expected.

Unit risk (oral) by EPA

An average oral intake of inorganic arsenic compounds of 0.14 µg/kg body weight and day is stated to result in an additional risk of skin cancer of 2.5×10^{-4} (2.5 additional cases of cancer per 10 000 population).

Unit risk (inhalation) by LAI

The German Länderausschuss für Immissionschutz (LAI - Länder commission for protection against ambient pollution) has stated an estimated unit risk of 4×10^{-3} . This means that among 1 million persons exposed to 1 ng As/m³ air over their entire lifetime, four additional deaths due to lung cancer have to be expected.

Limit values for drinking water

The WHO guide value for (inorganic) arsenic and likewise, the limit value of the German Drinking Water Regulations valid since 1991 and also that of Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption, is 10 µg/L.

Synoptic view

Synoptic view

Matrix	Substance	Sample material	Limit of determination	Method
Urine	Arsenic	50 mL urine (24-h-urine or early morning urine)	0.2 µg/L	Hydride batch method
Population groups for reference values			Matrix	Reference value
Adults (18-69 years), no fish consumption within 48 h prior to sampling			Urine	15 µg/L
Sources		Kinetics		Chronic effects
<u>Inorganic arsenic</u>		<u>Gastrointestinal absorption</u>		Carcinogenic risk
Drinking water		Low rate for poorly soluble compounds		<u>Target organs</u>
Soil contamination		45-90 % for soluble As(III) and As(V) compounds		
Emission from metallurgy		90 % for organic arsenic compounds		Skin, lungs, nervous system
Medicinal products		<u>Inhalative absorption</u>		
Residues of pesticides		30-40 % for soluble compounds		
Wood preservatives		<u>Accumulation</u>		
<u>Organic arsenic</u>		Muscle, bones, nails		
Foods, mainly fish and seafood		<u>Excretion</u>		
Medicinal products		Half-life for excretion in urine ca. 2-4 days for inorganic arsenic; ca. 1-2 days for organic arsenic		
Chemical warfare agents				

Measures to reduce exposure

Levels exceeding the reference value: Repeat measurement not earlier than after a 3-days waiting period after fish and seafood consumption. Levels repeatedly exceeding the reference value: Follow-up examinations of levels in urine and investigation into possible sources. Sources to be considered should include, among others: Local supply with drinking water contaminated with arsenic, mineral or medicinal water, high soil contamination, severe exposure in the working environment, arsenic-containing medicinal products.

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