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ASHRAM

ARSENIC HEALTH RISK ASSESSMENT AND MOLECULAR EPIDEMIOLOGY

FINAL REPORT

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1. INTRODUCTION

There is widespread exposure of European populations to low concentration of arsenic in drinking water (in range 5-30 µg/l) . Given the carcinogenicity of arsenic, such exposure has potentially great public health relevance. However, currently the only evidence of this is an extrapolation of cancer risk from studies where arsenic exposure was much higher than the mean European exposure. Therefore, direct observation of cancer risk in relation to exposure to low concentration of arsenic in drinking water, has potentially large public health implications. An opportunity to conduct a study on this issue arose as in regions of Central Europe there are large populations exposed in last 20 years at low to moderate exposures (in range 10-100 µg/l).

When this project was proposed and accepted for funding there was very little evidence to guide policy makers on the dose response relationships for arsenic and cancer for populations exposed in the 10-100 µg/litre range of concentrations of arsenic in drinking water; furthermore there was a shortage of information on mechanisms of action the interaction between arsenic as a risk factor and other factors, particularly those providing some insight on susceptibility. Since our study has started, more findings have become available on both cancer risk and susceptibility, but these are in most cases based on studies of rather limited size. Another concern of previous studies was that they had in many cases relied on rather imprecise measures of exposure. We set out to address this lack of data and such methodological shortcomings in the literature, by designing a study of risk of cancers of the bladder, skin and kidney in three target areas of central Europe. The areas were chosen to include three major European populations who either have been or are (for some of them) still exposed to arsenic in drinking water.

1.1 OBJECTIVES

This study had the following objectives:

1) To quantify the cancer risks (bladder, skin, and kidney) in relation to arsenic ingestion via drinking water in Hungary, Romania, and Slovakia.

2) To assess the effect of inter-individual variation in arsenic metabolism and DNA repair on carcinogenic risk.

3) To characterise determinants of inter-individual differences in speciation of arsenic in vivo.

4) To review, in the light of the new data, current risk assessment models for arsenic and cancer, and the adequacy of the current and proposed drinking water standards.

1.2 BACKGROUND

Some more detailed background is provided here, based on published literature. Metabolism of inorganic arsenic (As) involves alternating reduction of pentavalent arsenic to trivalent arsenic and addition of methyl groups. The glutathione (GSH) and probably other thiols serve as electron donor in the reduction reactions (Buchet and Lauwerys 1988; Scott et al. 1993; Delnomdedieu et al. 1994a; Delnomdedieu et al. 1994b; Styblo et al. 1995), and the reaction are re-catalyzed by As(V) and MA(V) reductases and methyltransferases, which are only partly identified. The MA(V)-reductase has been suggested to be glutathione-S-transferase omega (GSTO1), which may also reduce As(V) (Zakharyan, R. A. and Aposhian 1999; Radabaugh and Aposhian 2000; Zakharyan, R. A. et al. 2001). The methylation of As(III) and MA(III) is catalyzed by methyltransferases and requires both S-adenosylmethionine (SAM), as the methyl donor, and a thiol for activity (Marafante and Vahter 1984; Buchet and Lauwerys 1985; Smith et al. 1992; Zakharyan, R. et al. 1995; Styblo et al. 1996).

Following exposure to inorganic arsenic by ingestion and inhalation the urinary excretion mainly consists of arsenite [As(III)], arsenate [As(V)], methylarsonic acid [MA(V)] and dimethylarsonic acid [DMA(V)].

The pentavalent methylated metabolites are less reactive with tissue constituents, less acutely toxic, less cytotoxic and more easily excreted in urine, compared with inorganic arsenic (Buchet et al. 1981; Vahter and Marafante 1983; Vahter et al. 1984; Yamauchi and Yamamura 1984; Marafante et al. 1987; Moore et al. 1997; Rasmussen and Menzel 1997; Concha et al. 1998a; Hughes and Kenyon 1998; Sakurai et al. 1998). On the contrary, MA(III) and DMA(III) are highly reactive and more cytotoxic than both As(III) or As(V) (Petrick et al. 2000; Styblo et al. 2000; Vega et al. 2001). Both MA(III) and DMA(III) are genotoxic and much more potent in damaging DNA than As(III) (Mass et al. 2001; Nesnow et al. 2002; Styblo et al. 2002; Kitchin and Ahmad 2003; Kligerman et al. 2003; Schwerdtle et al. 2003a; Schwerdtle et al. 2003b). A series of reports have shown the trivalent methylarsonous acid [MA(III)] and dimethylaesonous acid [DMA(III)] as a fraction in the urine matrices (Aposhian et al. 2000; Le et al. 2000; Mandal et al. 2001), although it is not likely to find high concentrations because the high reactivity render them to bind in tissue (Vahter 2002). Our studies support the fact that very little MMA(III) or DMA(III) is excreted in the urine.

There are marked differences among population groups and individuals in arsenic methylation, especially in the formation of MA. Studies have shown from a few percent MA in urine of indigenous people in the north of Argentina and Chile to 20-30% in Taiwan, while most other populations groups studied have 10-20% (Vahter 2002). This large difference between population groups might indicate genetic polymorphisms in the regulation of enzymes responsible for arsenic metabolism. In a recent study, two individuals, who had markedly different patterns in urinary arsenic compared to other individuals in the study group (N=75), also had a unique polymorphism in GSTO1 gene (Marnell et al. 2003). Other factors that have been shown to influence the metabolism of arsenic is gender and age (Concha et al. 1998a), pregnancy (Concha et al. 1998b), diseases (Buchet et al. 1984; Geubel et al. 1988; Hsueh et al. 1995), and lifestyle (Hopenhayn-Rich et al. 1996b). There are also several studies indicating that nutritional status might influence both arsenic metabolism and toxicity (Hsueh et al. 1995; Milton et al. 2004; Mitra et al. 2004). Thus, it is important to elucidate

which factors influence the metabolism of inorganic arsenic and if that may influence the arsenic-related cancer risk.

On the other hand it seems likely that the amount of MMAV in urine reflects the formation of the toxic MMAIII in the body. There is increasing evidence that a high percentage of MA in urine is associated with arsenic toxicity. Experimental animals, most of which methylate arsenic efficiently to DMA with essentially no MA excretion, show a faster overall excretion of arsenic than do humans (Vahter 2002). Also people with a small percentage of urinary arsenic as MA show less retention of arsenic than those with higher percentage urinary MA. In addition, recent studies indicate increasing prevalence of arsenic-related toxic effects, e.g. skin lesions, skin cancer, bladder cancer and chromosome aberrations, with increasing percentage of MA in urine (Del Razo et al. 1997; Hsueh et al. 1997; Maki-Paakkanen J 1998; Yu et al. 2000; Chen, Y. C. et al. 2003a; Chen, Y. C. et al. 2003b)

Polymorphisms of genes

For details on genotyping of genes see WP4.

From the ASHRAM study, in the present document we report on results of chemical analyses of water and urine, study of arsenic metabolites distributions, genetic polymorphisms, and preliminary case control study analyses.

2. MATERIALS AND METHODS

2.1 ETHICAL ASPECTS AND SAFETY PROVISIONS

In Hungary, ethical aspects and safety provisions of ASHRAM study were approved by the Ethical Committee of the National Health Research Council and the Regional Ethical Committee of the Szentgyörgyi Albert University of Szeged, the respective Regional Committee of the study area. According to the decisions of the Ethical Committee of the National Health Research Council, the Protocol of the Hungarian part of the Study had to be changed in some respect. The most important change was the abolishment of the skin examination by the interviewers planned according to the proposal of the Dermatologists' Meeting hold in Debrecen in June 2002. The reason of this decision was that the Committee did not think the interviewers' expertise to be appropriate for deciding about the nature of the skin lesions. Some small changes were required in the questionnaires, too, mainly for reasons of personal data protection.

In Romania, ethical aspects and safety provisions of ASHRAM study were approved by local Hospitals and Public Health Departments. The Ethical approvals were obtained based on complete ASHRAM protocol submitted to the local Public Health Department and Hospitals at the early stage of the ASHRAM project.

In Slovakia, ethical aspects and safety provisions of ASHRAM study Slovakia were approved by all Ethical Committees established in hospitals (7) and State Health Institutes (4) included in the study. An Ethical committee is not established in State Health Institute Levice, but ethical aspects and safety provisions of ASHRAM study in district Levice were approved by the Ethical committee of

the Hospital Levice, which is relevant local authority. The Ethical approvals were obtained based on submission of the complete ASHRAM protocol to the ethical committees at an early stage of the ASHRAM project.

2.2 QUALITY OF EPIDEMIOLOGY FIELD WORK

Quality of epidemiology field work was assured by standard operating procedures based on:

a. Standard protocols
b. Training activities
c. Pilot study
d. Protocol for Conduct of the field survey
e. Data management protocols

The study protocols are included as Annexes.

2.3 SELECTION OF STUDY POPULATION

2.3.1 Study area

The study area included four adjacent counties of Hungary, two adjacent counties of Romania, and seven districts within two adjacent counties of Slovakia. The number of inhabitants of this area is 2,799,570 and their geographical distribution is presented in Table 1. Further information about the area and its inhabitants are provided in the country-specific reports, included as Appendices.

Table 1. Population in the counties and districts of Hungary, Romania, and Slovakia included in the ASHRAM study area

Country	County or	Districts within county	Population	Population Total
Hungary	Bács-Kiskun	all	520,000	1,734,000
	Békés	all	388,000	
	Csongrád	all	420,000	
	J-Nk-Szolnok	all	406,000	
Romania	Arad	all	462,427	1,065,570
	Oradea	all	603,143	
Slovakia	Banska Bystrica	Banska Bystrica	111 984	687 807
		Brezno	65 909	
		Ziar nad Hronom	48 125	
		Zarnovica	27 634	
	Nitra	Nitra	163 540	
		Levice	121 021	
		Nove Zamky	149 594	
Total				2,799,570

2.3.2 Approach to cases and controls

This procedure was part of an overall field protocol for the conduct of the study, which was developed with contribution of all investigators and included guidance on interviews, as well as sampling of urine and blood; all are included as Annexes. All hospitals in the study areas agreed to participate in the study. The hospital staff, both management and lead clinicians, notified the ASHRAM Local Coordinator of possible new cases and controls. The entry criteria and diagnostic criteria were confirmed by the participating clinicians. The clinicians invited possible participants into the study, and gave each participant an information letter and consent form. Clinicians recorded information on potential cases and controls who were asked to take part but refused to participate in the study. New cases of skin, bladder, and kidney cancer aged 30-79 (<80 years old) were identified by clinicians at the main hospitals in the study area. Cases should have been resident in the study

region for at least 1 year. The analyses of the results was on the cases of cancers included in the following table:

Table 2. Cancers included in ASHRAM study

	ICD-9 codes	ICD-10 codes
Non-melanoma skin cancer	173.0-173.9	C44
Bladder cancer	188.0-188.9	C67
Other urinary tract cancer	189.2-189.9	C66, C68
Kidney cancer	189.0, 189.1	C64-C65

Any new skin cancer could be included in the study, including a skin cancer that occurred in a person who had previously already been treated for skin cancer. This is particularly relevant in a study of arsenic, which is known to cause multiple skin cancers. All diagnoses of cancer including cutaneous carcinoma were confirmed by histological examination.

Definition of controls

Controls were general surgery in-patients and orthopaedic and traumatology patients aged 30-79 (<80 years old). Specific ICD codes for controls included are listed in Table 3. Controls should have been resident in the study region for at least 1 year (principally to avoid inclusion of people who have come to the area in relation to their surgery).

Table 3. Diagnostic categories for controls

Surgical	ICD-10	ICD-9	Orthopedics and traumatology	ICD-10	ICD-9
Appendicities	K35-K37	540-543	Fractures	SO2,S12,	800-829
Abdominal hernias	K40-K46	550-553		S22,S32,	
Duodenal ulcer	K26	532		S42,S52,	
Cholelithiasis	K80	574		S62,S72,	
				S82,S92,	
				TO2,TO8,	
				T10,T12	

Procedure for selection of controls

We invited one control to participate for each case recruited. Compared to the case, the invited control was:

- of same sex;
- within the same 5 years age band (30-34, 35-39 etc);
- of the same country;
- currently (before admission to hospital) resident in the same county/region of the study area,
- have resided in the study area for at least one year during their lifetime (if this last information is not available at time of selection of controls, this may be skipped).
- To achieve a good distribution of control diagnoses, selection was made from the five diagnostic categories in rotation
- To achieve a good geographical coverage and cooperation of the participating hospitals they were regularly sampled. As a complete roster of all eligible controls attending all hospitals in the region is not available in a complied form, they were sampled in order, the frequency of contact in rough proportion to their respective number of controls

Every refusal was noted. Control selection continued until the target numbers of interviews was completed. This procedure allowed a systematic rotation between hospitals, and control diagnosis, and therefore constructed a series of controls that was similar to that of the cases for age group, sex, and county of residence, while minimising the opportunity for systematic error in control selection.

2.4 PROCEDURE FOR QUESTIONNAIRE SURVEY

All participants were informed of the study procedures and gave their written consent.

A face to face interview was conducted with each participant, addressing topics listed in two separate questionnaires: a main questionnaire and a nutrition questionnaire; the latter is described in the nutrition section. The main questionnaire included items on participants' lifetime history of

exposure to sunlight and characteristics of their skin and its response to such exposure. In addition, interviews included items on demographic, socio-economic, medical history, smoking, occupational exposures, drinking and nutritional habits, as well as detailed residential history focused on identification of drinking water sources. Full text of both questionnaires is included as Annexes.

Arrangements for conduct of interviews

The ASHRAM Local Coordinators instructed their field workers to arrange a suitable date for an interview with the case. The interviewers were public health practitioners, 8 in Hungary, 4 in Romania, and 4 in Slovakia. Joint training of interviewers was provided at a workshop held in Cluj, Romania on 3-6 July 2002. For the questions on water consumption (Main Questionnaire, Section D), parallel residences (i.e. where a person spends part of their time at one address, and the rest of the time at a different address) were recorded on separate sheets. Interviewers interviewed a mixture of cases and controls rather than just cases or just controls.

2.5 NUTRITIONAL STUDIES

To date limited studies have investigated the role of nutritional factors and arsenic intake on the risk factors for bladder, skin and kidney cancer in parts of Hungary, Romania and Slovakia. The intake of some macronutrients, some micronutrients, total energy and arsenic by food and by beverages and the specific intake of some food groups will be evaluated using a Food Frequency Questionnaire (FFQ) developed for this study. Particular attention will be paid to the nutritional status and the intake of selected micronutrients. Some studies have reported a role of zinc, methionine, the B group of vitamins and folate in influencing the toxicity of arsenic (National Research Council, 1999) or increasing arsenic-caused disease risks (Engel and Receveur, 1993; Levender, 1977, National Research Council 1999, Hsueh et al, 1995).

Two methods for assessment of nutrients were adopted in this study:

1. most nutrients' intake would be determined by use of a Food Frequency Questionnaire (FFQ)

2. selenium intake would be estimated by blood measurements

2.5.1 Rationale and development of Food Frequency Questionnaire (FFQ)

A Food Frequency Questionnaire (FFQ) was used to assess fluid and dietary intake of the studied population in parts of Hungary, Romania and Slovakia. A unique tool has been developed to collect food data in a uniform way in countries that went through similar changes in food consumption patterns during the period of transition. The year 1989 appears to have been a watershed in the Eastern European food system, from a centralised to a more marked-oriented economy (Szostak and Sekula, 1991, Petrovici and Ritson, 2000, Babinska and Bederova, 2002). Dietary patterns changed and tended to converge with those in Western Europe, especially as regards to an increased consumption of fats, sugar and alcohol (Petrovici and Ritson, 2000, Szostak and Sekula, 1991, FAO, 1998).

The questionnaire originates from a validated and reproduced instrument (Franceschi et al, 1993, 1995, Decarli A, et, 1996) that was used on a series of multi-centre case-control studies carried out in Italy (Franceschi et al, 2000; Negri et al, 2000; Bidoli et al. 2001, 2003, 2005). Data on local food consumptions (Babinska et al, 2002, Socialna Statistika, 2002, Mihailescu et al, 1981, Biro', 1992) provided useful suggestions to adapt the FFQ to the studied population.
This questionnaire was developed to assess the habitual diet of the participants, including total energy as well as intake of selected macronutrients, micronutrients. Estimation of subject's arsenic exposure from oral uptake will be also evaluated.

2.5.2 Evaluation of the food intake by the FFQ

The questionnaire was interviewed-administered by twelve (to check) trained interviewers. The FFQ is divided in two main parts, one concerning the year period before 1989 (**past diet**) and the second part concerning two year before the cancer diagnosis for case or hospital admission for controls (**current diet**). The first part of the FFQ include 112 foods, food groups or recipes grouped into 7

sub-sections: (1) Tea, coffee, milk; (2) Bread, cereals, first courses; (3) Main courses; (4) Vegetables - side dishes; (5) Fruits; (6) Sweets and various foods; (7) Alcoholic and analcoholic beverages. The second part include 35 foods, food groups or recipes aimed to asses current food intake. The questionnaire looked more like a restaurant menu than a shopping list. For most of the foods or foods groups, a serving size was indicated in "natural units" or in "commons portions" (such as a plate of soup or one orange).

The interviewer asked for average frequency of consumption of various foods or food groups, as well as complex recipes. A predefined frequency point scale of 9 categories of choices was provided from "*never*" to "*more than 3 time per day*". To account for seasonal variation of food consumption, the frequency of use of some foods was asked for the season in which the food is mainly available (e.g. orange in winter, grape in summer).

Nutritional status of participants

Total energy intake evaluated by FFQ and body mass index (BMI = weight kg/ height m^2) collected will be used to evaluate the nutritional status of the participants.

Evaluation of arsenic intake by the FFQ

Using the FFQ will be evaluated the oral intake of arsenic by food and by beverages. Data on Arsenic concentration in food was collated from pre-existing sources determinate by the Monitoring System, operated by the Department for Evaluation of Food Contaminants, Ministry of Agriculture (Krizova et al, 2001, 2002, 2004a 2004b, Salgovicova et al 2002a, 2002b, 2002c, Svetlikova et al, 2002). These data will be integrated with primary data collection of arsenic content in a limited number of food items in Slovakia. Seven food items were analysed and Arsenic was determined by Inductively Coupled Plasma Mass Spectrometry (ICPMS). The food items, included in the FFQ, were: Bread, Rice, Milk, Pork meat, Chicken meat, Potatoes, Cabbage. They are frequently consumed by the studied population, but not particularly rich in arsenic.

Evaluation of fluid intake by the FFQ

The fluid intake will be calculated for each participant taking into account the frequency of consumption, the serving size of some questions of the FFQ for the past and current diet. They will be combined together to give each member a score in milliliters. The items are: (1) *Tea, (2)Herbal tea, camomile, mint, lime (3) Coffee,(4) Decaffeinated coffee, (5) Milk with coffee, cappuccino, (6)* Meat soup, with chicken, goose, or turkey, (7) Soup with meat and vegetables, goulash soup, (8) Vegetable soup, with beans, peas, mushrooms, tomatoes, (9) Fruit soup (apple, gooseberry, cherry), (10) *Syrup drinks, (11) Soda water, home made (12) tap water, (13) Coca-cola, Pepsi-cola, other fizzy drinks,(14) Fizzy drinks without sugar, (15) Commercial fruit juices,(16) mineral water.*

2.5.3 Nutrition FFQ Reproducibility and Validation study

In an attempt to develop a valid method to assess nutritional intake of the studied population, we designed a study to test the reproducibility and validity of our tools.

The study population consisted of 90 volunteers recruited by means of Advisory Rooms, present in Regional Institutes of Public Health: Banska Bystrica, Nove Zamky and Nitra representing the same areas as the ASHRAM study population.

A first FFQ was administered at the beginning of the study (Spring 2004). The same volunteers were re-interviewed 6 months later with the same instrument (Autumn 2004). In the months subsequent to the administration of the first FFQ, participants were also contacted by telephone, once a month (10 times) to provide information about dietary consumption on the previous 24 hours.

A sub-study was set up to estimate the exact volume of the drinking vessels commonly used by volunteers (glasses, cups, bowls) at home to drink water, beverages, coffee, tea, soups, etc.,

measuring the volume of water to the level they usually fill when serving a particular drink/soup.

This information will be used to complete and to personalise the total fluid intake.

Seven nutritionists interviewed participants. All food, beverages, taken during the previous 24 hours has been written on paper forms and entered in computer files, by means of the software *Alimenta* (Food Research Institute, 2004). This enables transformation of food items into nutrients, based on the Slovak Food Composition Database (Food Research Institute, 2002), in order to make nutrient analyses possible.

2.5.4 Biomarker of nutritional status - Selenium in whole blood

Because separation of plasma and RBC was considered to interfere too much with the normal duties of the personnel at the hospitals, we measured selenium in whole blood. The concentration of selenium was measured mainly by ICP-MS and partly by AAS. Se in whole blood was determined by ICPSFMS (Element Thermo Electron, Finnigan MAT, Bremen, Germany) using 78Se isotope in high resolution mode (delta m/m=11 000). Samples were prepared by dilution with ammonia-Triton X-EDTA-ethanol mixture (2%-0.0005%-0.0005%-4%) with addition of As at 10 µg/l for internal standardization.

2.6 MEASUREMENTS OF ARSENIC IN WATER AND URINE

2.6.1 Description of methods for As measurements in water

The method used bt Partner 6 for analysing the total arsenic from the collected water samples was hydride generation-atomic absorption spectrometry (HG-AAS). Hydride generation was preferred, because of the improved speed of the analysis and the lack of background signals. In hydride generation system, As (III) is chemically reduced, by sodium borohydride (0.6%, w/v), in acid solution (HCl, 5M), to the gaseous hydride. The sodium borohydride solution is stabilized by adding first sodium hydroxide 0.5%, w/v. The hydride is transported to the heated quartz cell where is dissociated in the analyte and hydrogen. This allows the atomic absorption of the analyte to be measured. This process separates the analyte element from its chemical matrix, eliminating matrix interference effects in the atomization process.
Determination of total arsenic requires that all inorganic arsenic compounds to be in the As(III) state. The potential As(V) is quantitatively reduced to As(III) by the action of a reductant solution consisting of potassium iodide 5% and ascorbic acid 5%. Reduction step took about 45 minutes at room temperature. If solutions are not pre-reduced with potassium iodide, and the analyte is retained as As(V), the analytical sensitivity is about 20-30% from that obtained for As(III). Apparatus used : Atomic Absorption Spectrometer, Varian 110, PC controlled, equipped with vapor generation system, VGA 77. Working conditions: Wavelength = 193.7 nm, Lamp current = 10 m A, Slit width = 0.5 nm, Air flow = 3.50 L/min, Acetylene flow = 1.50 L/min.

All analyses of water samples were accompanied by a strict QC procedure. Every fifth sample was measured in duplicate (complete sample preparation). After 10 samples the blank value was determined in duplicate, one calibration solution was determined in duplicate and at least one Certified Standard Reference Material (NIST 1640) was analysed. An internal reference material was also used. The internal reference material was analysed in the laboratory of KFUG, and a concentration of 48.1 +/- 0.5µg As/l (n=3) was found. The results obtained for the CRM were included in quality control charts.

Four Round Robin exercises were organized by KFUG, details of methods and results are given in the QA/QC section of the present report.

Comments on the methodologies

Metabolites of inorganic arsenic in urine

For details concerning chemical analysis of arsenic metabolites in urine, see WP3. We have adjusted the concentrations of urinary arsenic by specific gravity to the average 1.017 g/ml to compensate for variation in dilution of the urine due to variation in fluid intake, exercise etc ($adj.conc = conc. \cdot ((avg.sg - 1)/(sample\,sg - 1))$). We have chosen to adjust for specific gravity instead of creatinine, mainly because creatinine excretion is influenced by muscle mass, and thus by gender, nutrition etc. (Suwazono et al. 2005). For example, because men excrete more creatinine than women, they will get lower creatinine adjusted arsenic concentrations than women at the same exposure.

2.6.2 Trivalent organoarsenic compounds

The present section reports on the justifications for addressing the question: "If it can be measured reliably, does MA(III) represent a species of arsenic that is worth analyzing in the context of its putative extreme toxicity?". This same section also reports on the analytical chemistry methods used to answer the above question.

2.6.2.1 Background on the toxicity of MA(III) and the rationale for the inclusion of a substudy on this topic within ASHRAM

It is well known that chronic exposure to inorganic arsenic is connected with numerous adverse health effects like increased risk for cancer of skin, lungs, kidney, and urinary bladder, hyperkeratosis, pigmentation changes, diabetes, effects on the circulatory and neurological system as well as on liver and kidneys (NRC 2001). In the body inorganic arsenic is methylated in an alternating reductive and

oxidative methylation process, mainly to methylarsonate MA, and dimethylarsinate DMA. Since these methylated metabolites show low toxicity compared to the ingested inorganic species, the methylation process has been considered as a detoxification process (Buchet et al 1981). This has been questioned since several studies have shown that the trivalent intermediate metabolites methylarsonous acid MA(III) and dimethylarsinous acid DMA(III) are more geno- (Mass et al. 2001, Ahmad et al. 2002), and cytotoxic (Petrick et al. 2000) and more potent protein inhibitors (Lin et al. 1999, Petrick et al. 2001, Styblo et al. 2000) than arsenite. Nevertheless, to what extent these species are released in the human body and thus contribute to the toxic effects of arsenic, is not known.
Even though MA(III) and especially DMA(III) turned out to be rather instable (Gong et al 2001), (in a urine sample spiked with MA(III) >90% was oxidised within 5 months when stored at –20°C) they have been identified in urine of individuals chronically exposed to inorganic arsenic in their drinking water (Le et al. 2000, Le et al. 2001, Mandal et al. 2001, Aposhian et al. 2000, Mandal et al 2004, Valenzuela et al 2005) and in patients treated with arsenic trioxide against promeoleucytic leukemia (Wang et al 2004). In several studies it was claimed that the MA(III) concentration could have been underestimated due to long storage times (several months).

In the ASHRAM study we:

- synthesised iododimethylarsine and diiodomethylarsine as standard compounds and developed methods for the determination of MA(III) and DMA(III) in urine
- Investigated sample preparation procedures previously used when the trivalent organoarsenicals have been detected
- investigated 1500 urine samples for methylarsonous acid
- investigated urine samples of patients undergoing arsenic trioxide treatment

2.6.2.2 Synthesis of MA(III), DMA(III) and method development

Both substances were prepared as iodines which where then hydrolysed to the corresponding acids. CH_3AsI_2: This compound was prepared from methylarsonic acid and potassium iodide upon reduction in hydrochloric acid solution with SO_2. The crude product was dried over phosphorous pentoxide. For purification the dry solid was extracted with anhydrous diethyl ether. After cooling the saturated solution below -15°C yellow needles were formed. The melting point of the synthesized diiodomethylarsine was 29-30°C. For the determination of the purity ^{1}H-NMR in $CDCl_3$ or D_2O

were performed. The NMR in D_2O showed one signal at d=1.20 (lit. d=1.24) and the NMR in $CDCl_3$ gave two signals, one at d 3.11 (lit. d=3.10) and one at d=1.58 (lit. d=1.54) for H_2O. $(CH_3)_2AsI$: This compound was prepared from dimethylarsinic acid and potassium iodide upon reduction with SO_2. The formed yellow oil was separated and distilled under reduced pressure (bp. 156°C). 1H-NMR was performed in D_2O and $CDCl_3$.

An existing method for the determination of arsenite, dimethylarsinic acid, methylarsonic acid, and arsenate was modified to determine methylarsonous acid within the same chromatographic run. Under optimised conditions (20 mM ammonium phosphate buffer pH 5, 5% MeOH) the five most toxic arsenicals could be separated within 10 minutes. The detection limit was 1 µg As/L without hydride generation. When hydride generation was employed additionally the detection limit was improved by one order of magnitude.

Stability of methylarsonous acid in aqueous solution:

The stability of methylarsonous acid in water at a concentration of 100 µg As/L was investigated over a five weeks period. The results have shown that storage at 4°C in a refrigerator did not results in oxidation of MA(III) to MA(V). Briefly, the developed methods allowed the determination of MA(III) with a detection limit of 0.1 µg As/l. Due to the retention behavior and instability of DMA(III) the achieved detection limit was only 5 µg As/l. This detection limit together with the reported instability (Gong et al 2001) made it impossible to find dimethylarsinous acid in the urine samples that had been frozen at -20ºC for several months.

2.6.2.3 Sample preparation procedures employed when trivalent organoarsenicals have been detected

A careful inspection of the literature revealed that the trivalent organoarsenicals have been found quite often when dimercaptopropane sulfonic acid (DMPS) was given hours before the urine collection. Before the analysis with hydride generation atomic absorption spectrometry (HG-AAS) or hydride generation atomic fluorescence spectrometry (HG-AFS) the urine samples have sometimes been acidified with HCl and even heated. In order to test a possible artifact formation due to this samples preparation we have performed a systematic study on this procedure.

In a first experiment a solution containing 100 µg As/l as MA was heated (80ºC, 90ºC, or 100ºC) in the presence of 1 M HCl. After cooling the samples were immediately chromatographed with special emphasis on MA(III). The results show that HCl at the chosen temperatures does not reduce MA to MA(III). In a second set of experiments HCl and DMPS were added to a 100 µg As/l MA solution and heated again. In the solution heated to 90ºC ~5% MA(III) was detectable. In a further experiment MA was spiked to a urine sample to a concentration of 100 µg/l and heated to (60ºC, 70ºC, or 80ºC). At all three temperatures the formation of MA(III) up to 5% could be observed. It is very interesting that heating of MA in urine already produced MA(III). Although, there is no proof one could speculate that the thiols (or other reducing compounds) in the urine could be responsible for the reduction of the pentavalent MA. When we spiked additionally DMPS to a urine containing MA the formation of MA(III) was even more pronounced (up to 10% methylarsonous acid formed). It is evident from these results that a possible artifact formation due to the sample preparation used in the early papers that have found MA(III) and DMA(III) cannot be excluded. Moreover, it has to be stated clearly that in most of these papers only one set of chromatographic conditions, quite often with questionable chromatographic resolution, has been employed. In light of this one has to think about the results of a very recent paper (Valenzuela et al 2005) where DMA(III) was the dominant arsenic compound in the urine samples from an area in Mexico with high arsenic concentrations in drinking water. The analytical method employed there was pH-dependent hydride formation with subsequent chromatography and AAS detection.

2.6.2.4 Methylarsonous acid and dimethylarsinous acid in urine samples from the study area

In four Hungarian, two Slovakian and two Romanian counties 1511 urines samples have been collected in order to determine the recent arsenic exposure as well as the methylation efficiency.
According to the work from Gong et al. (2001) MA(III) in urine at room temperature is completely oxidized after 3 days, at –20°C or 4°C 30% of MA(III) were oxidized within 1 day. When MA(III) was spiked into urine and stored for 30 days at 4°C 98% were oxidized whereas when stored at –20°C only 60% of the MA(III) were oxidized. These authors also reported that DMA(III) is completely oxidized after 17 hours when stored at –20°C. Similar stabilities were reported by Del

Razo et al. (2001) although this group stated a remarkable difference in the stability of the trivalent arsenicals in urine samples from three different donors.

The protocols developed for the study included freezing of the urine samples to –20°C immediately after collection. The samples were stored and transported frozen until the analysis. Even during the chromatography the samples were cooled to 4°C. It is important to mention that neither preservatives nor any other chemicals were added to the samples until analysis. Although the developed protocol is not capable to preserve complete species integrity one could expect to find MA(III) in some of the samples. Only in 8 (0.5%) out of 1511 samples traces of MA(III) were found the highest of which was 0.4 µg As/l. These eight samples were not noticeable with respect to any other arsenic compound determined. The arsenate concentration in these samples was very low (< 5 µg/l to 0.9 µg/l) indicating that oxidation by air had been successfully avoided.

2.6.2.5 Urine samples from patients undergoing arsenic trioxide treatment

As trivalent arsenic compounds were not detectable in the urine samples collected within the Ashram study we thought that the rather low arsenic concentrations in the study could be a possible explanation for the absence of these highly toxic arsenicals. To overcome this problem we analysed urine samples of patients undergoing arsenic trioxide treatment. We expected to find rather high concentrations of the trivalent arsenicals in these samples.

In a first set of experiments urine samples of a 25 year old woman were collected over 6 weeks during a treatment because of acute promyelocytic leukemia (APL). She received 0.15 mg per kg body mass per day as arsenic trioxide. The urine samples were collected in the morning and immediately after the intravenous admission of As_2O_3. After collection the samples were frozen at –20°C until the analysis. During the investigation the highest arsenic concentrations in the urines detected were, 1.2 mg As/l (median 0.28 n=47) As(III), 2.52 mg As/l (median 0.50 n=46) DMA, 2.65 mg As/l (median 0.54 n=46) MA, and 0.11 mg As/l (median 0.028, n=46) As(V). Although we have designed the whole experiment with special emphasis on the trivalent arsenicals we were not able to detect MA(III). Again improper storage (-20°C) could have been the reason for the absence of the trivalent organoarsenicals.

Therefore, we decided to investigate urine samples of four patients exposed to high levels of inorganic arsenic in form of treatment with arsenic trioxide. After collection the samples were immediately frozen in liquid nitrogen (-180°C), so species conversion could be excluded. The urine samples were taken from four patients suffering from plasmacytma. At the day of sampling, the patients had been on arsenic therapy for 3 (patient D) and for five weeks, respectively (patients A, B, and C). All the patients were more than 50 years old.

The treatment included two intravenous injections of As_2O_3 (20-30 mg As) per week, depending on body weight and physical condition. Additionally, the patients got an injection of 1 g of vitamin C. Spot urine samples were taken before and after the treatment. The samples were immediately frozen in liquid nitrogen until analysis (~ 3 days). Immediately before the analysis the samples were thawed to room temperature, diluted, and filtered through a 0.22 µm Nylon filters and analyzed.

2.7 EXPOSURE TO ARSENIC IN DRINKING WATER

The description of the arsenic exposure assessment method in the present section is divided in three: first, the overall approach is presented; second, arsenic data sources are described; and third, the method for estimating arsenic exposure in the study cases and controls will be described.

2.7.1 Overall approach to exposure assessment

In the ASHRAM study, one of our aims was to quantify the risks for three cancers (bladder, kidney and skin) in relation to cumulative exposure to arsenic. Of all the possible routes and modalities of exposure to arsenic, three were identified as of possible importance in this study population: (i) exposure from water sources; (ii) occupational exposure; and (iii) exposure from food items. It was initially considered that exposure from water sources was likely to be by far the largest component of the overall exposure. Secondly, the task of reconstructing past exposure for each individual was very complex and even daunting, and it has not been successfully achieved by previous epidemiological studies of arsenic and cancer. Thirdly, within the time scale of the project it was not considered feasible to produce analyses of cancer risk based on solid estimates of exposure from all possible sources, or even all the three types of sources identified as of possible importance in this population. For all these reasons, arsenic exposure reconstruction focused on exposure via drinking water, and the methods used to address this are described in greater detail than the methods used to ascertain occupational and food exposure.

One of the main difficulties in epidemiological studies which examine the relationship between drinking water contaminants, and disease outcomes, is the accurate assessment of individuals' consumption of tap water (Shimokura et al. 1998). A recent workshop identified improving methods for measuring water consumption patterns as a primary research need for epidemiological studies (Arbuckle et al. 2002). This challenge is further confounded where a study is retrospective, e.g. a

case-control study such as ASHRAM, and history of tap water consumption (and hence exposure) needs to be determined at an individual level (Fletcher et al. 2004).

There are two main issues associated with estimating past exposure to arsenic: change in the arsenic dose of individuals due to changes in water supply (Colt et al. 2002), and change in arsenic dose due to changes in the volume of water consumed by individuals (Shimokura et al. 1998). In parts of the ASHRAM study area, significant exposures to arsenic have occurred in the recent past, and for some of these populations significant exposures are still occurring. However in some districts, levels of exposure have reduced following mitigation efforts. The ASHRAM study therefore needs to take into account these changes of exposure potential over time.

In the ASHRAM study area, people use a variety if water supplies including public distribution systems and private wells. Changes in water supply are therefore very complex, but one approach is to obtain water samples from all water supplies used by study subjects throughout their lifetimes (Colt et al. 2002). However, this is an arduous task and the extent to which this is successful is likely to be low. For example, a recent study found that only 47 % of past wells could be successfully sampled (Colt at al. 2002). A different approach could be to use hydrogeological information in order to estimate spatial variation in the concentration of arsenic in different aquifers. For example, the United States Geological Survey (USGS) published a series of maps describing the distribution of arsenic in groundwater (Ayotte et al. 1999). Since the publication of these maps, there has been increasing interest in using this information for making cost-benefit estimates for drinking water regulations and/or predicting arsenic-related health risks for different regions of the United States (Ryker 2001). Although a similar approach was explored, it was decided that the characterisation of a large sedimentary basin such as the Great Hungarian Plain was not practical within the timeframe of the ASHRAM project.

For this study; a methodology was devised which combined self-reported (questionnaire) consumption of drinking water with measured concentrations (either as part of the study, or routine historic data) of arsenic in that water supply. This approach was used to reconstruct an individual exposure history for each participant in the ASHRAM study.

Occupational exposure to arsenic is associated with several industrial and agricultural activities. A section of the main questionnaire is addressing occupational exposures in general, as possible causes of bladder cancer and possibly other cancers, and also as possible source of occupational exposure to arsenic. ASHRAM applied well-validated methods for assessment of occupational exposures.

2.7.2 Arsenic data sources

Arsenic in water was estimated using data from two sources: (1) Measurements in water; (2) Historical data, (3) As arsenic exposure via food. To these, we intend to add (4) occupation. These will be dealt with in succession here.

(1) Arsenic water measurements

Five water sources were sampled for their arsenic content: those at the most recent, the longest, the earliest residential dwellings in the lifetime of the participants, and the most recent and the longest sources at the occupational location of the participants. Only residential sources were taken into account for the analyses presented here. Technicians trained in the water sampling procedures agreed by the investigators [see more detailed protocols on request/on the website] identified all the selected residences and conducted the sampling. All water samples were analysed by the same laboratory in Cluj-Napoca using AAS method, and under supervision of chemists at the University of Graz, Austria and the Regional Public Health Institute of Banska Bystrica, Slovakia. Results of interlaboratory comparisons demonstrated good agreement. Ten per cent of the analyses were replicated at the laboratories of two of the authors (WG and MV), demonstrating agreement within 5%

(2) Historic arsenic data

Arsenic concentration in water changed at several points in time in Hungary and Slovakia, following interventions to reduce exposure. Ignoring this would produce error in the estimate of past arsenic

intake in ASHRAM. No interventions have been introduced in Romania, so no reconstruction of historic concentration was required there. A database on historic water concentration in all the settlements where an ASHRAM participant lived was produced by the Hungarian and the Slovak investigators. For each year since 1980 in Hungary, and since 1989 in Slovakia, each settlement was assigned an arsenic concentration based on measurements made by the authorities. The Hungarian and Slovak information from historic arsenic data was incorporated in the main ASHRAM database.

These data were used to assign concentrations to water supplies in communities where we could not obtain measurements, or, for communities with higher arsenic concentrations in the drinking water in the past, to the relevant time period. Table 1 illustrates this with a sample of the estimated concentrations by year for some Hungarian towns in the study areas. This was combined with the measured data under the following principle: If we had taken water samples from the water supply network from this town then the results of ASHRAM sample analyses were used in preference to the recent historical archive estimates, going back in time until there was a step to a higher estimated concentration (for example back to 1995 in Bekes – prior to then the archive estimates were used).

Table 4. Sample historical observations on arsenic concentration in water for a series of Hungarian towns.

Settlement	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002
ABÁDSZALÓK	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13
ÁGASEGYHÁZA	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
AKASZTÓ	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
ALATTYÁN	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
APÁTFALVA	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
APOSTAG	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
ÁRPÁDHALOM	52	52	52	52	52	52	52	52	52	52	52	52	52	52	40	40	40	40	40	40	28	28	28
BÁCSALMÁS	64	64	64	64	64	64	64	64	64	64	64	34	34	34	34	34	34	34	34	34	34	34	34
BÁCSBOKOD	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18
BÁCSBORSÓD	42	42	42	42	42	42	42	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
BÁCSSZOLOS	41	41	41	41	41	41	41	41	41	41	41	41	41	41	41	41	41	41	41	41	41	41	41
BAJA	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
BALLÓSZÖG	17	17	17	17	17	17	17	17	17	17	17	14	14	14	14	14	14	14	14	14	14	14	14
BALOTASZÁLLÁS	64	64	64	64	64	64	64	64	64	64	46	46	46	46	46	46	46	30	30	30	30	30	30
BÁTYA	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
BÉKÉS	220	220	220	220	71	71	71	71	71	25	25	25	25	25	17	17	17	17	17	17	17	17	17
BÉKÉSCSABA	100	100	100	66	66	66	66	89	89	89	30	30	20	20	17	17	17	17	17	17	17	17	17
BÉKÉSSZENTANDRÁS	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
BEREKFÜRDO	60	60	60	60	60	60	60	60	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
BESENYSZÖG	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18
BÓCSA	80	80	80	80	80	80	80	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
BORDÁNY	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14
BOROTA	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24
BUCSA	198	198	198	198	198	198	198	53	53	43	43	43	43	43	43	43	43	43	43	43	43	43	43
BUGAC	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22

Some further details on the process of reconstructing historic As data in Hungary and Slovakia are presented in this section.

In Hungary, the detailed data on which the historical estimates were based on the following data sources summarizing arsenic concentrations measured by different laboratories (mainly by the National Institute of Environmental Health (previously called as National Institute of Public Health, NIPH):

- The data found in the original logbooks of the instruments used in the NIPH in the 1980-ies were entered into the computer
- The data stored on paper records at the Békés County Public Health Institute were also entered into the computer
- A study document prepared some years ago by Dr Csanády on the history of the assessment of arsenic exposure via drinking water, the various types of interventions and their results contained data on the arsenic concentrations before and after remediation in 70 settlements
- The full database of the country-wide survey on arsenic content of piped drinking water carried out between 1997-99
- Data from more recent measurements in various settlements where changes in the source(s) of drinking water had occurred.

A list of settlements mentioned in the questionnaires either as a residence or a workplace of a patient was created, and s spreadsheet of the relevant concentrations assembled by community. All settlements had a separate worksheet in an Excel file where all the available arsenic concentrations (with the relevant dates) were put together and a certain concentration of arsenic was assigned for each year to each settlement based on an expert's judgement using the above-mentioned data. This way a table was created for identifying the arsenic concentration of the piped drinking water in each settlement in each year. This table was then sent to the four County Public Health Institutes for comments. As a result, some changes were still performed and the final archive database was approved. This way the best possible estimation of the archive arsenic concentrations of the piped drinking water was reached.

In Slovakia, a special effort was devoted to the evaluation of the measurements methods used in survey of As in drinking water in the past. Based on the review of As determination methods in drinking water used in State Health Institutes and in Middle and West Slovakia Waterworks and Sewerage Authorities it was established that the results of As concentrations from public water supply systems after 1989 were of good quality and could be used in exposure assessment. A database of historical As concentrations in public water supplies was constructed taking the results of As measurements performed by above mentioned institutions (about 1500 results) into consideration. The database contains a historical review from 1989 to 2004, separately for each resident included into study (330 residencies). The public water supplies where As concentrations in drinking water were reduced significantly due to measures realized with respect to change of As limit value, approved by means of the Decree of Ministry of Health of the Slovak republic on the value of 10 µg/l of water were evaluated separately.

2.7.3 Arsenic exposure indices

Three indices of exposure to arsenic were studied:

A. Current exposure
B. Life time exposure
C. Peak exposure

A. Current exposure

Current exposure is based on measurement of arsenic in water, and it is particularly relevant for analyses of the relationship between water exposure and urinary biomarkers. We defined two variables related to current exposure:

(i) Current arsenic concentration. This is the As concentration in the most recent residence, and is measured in micrograms/liter.

(ii) Current arsenic dose rate. This is the current As concentration multiplied by the daily local residential water intake, and is measured in micrograms/day. Here, the appropriate estimate of total daily fluid intake from the FFQ is that based on recent fluid intake, and this was applied.

B. Life time exposure

Cumulative exposure combines measurements in water with historic data, and applies both to our knowledge of the participants' residential history. At the time of writing this report, only residential history was taken into account. A simple estimate of cumulative arsenic depends heavily on the proportion of missing data, and therefore a lifetime average dose rate was computed instead.
We defined three variables related to cumulative exposure: (i) Life time average arsenic concentration, (ii) Life time average arsenic dose rate, and (iii) Cumulative arsenic dose. The respective definitions, unit and methods of measurement are presented here.

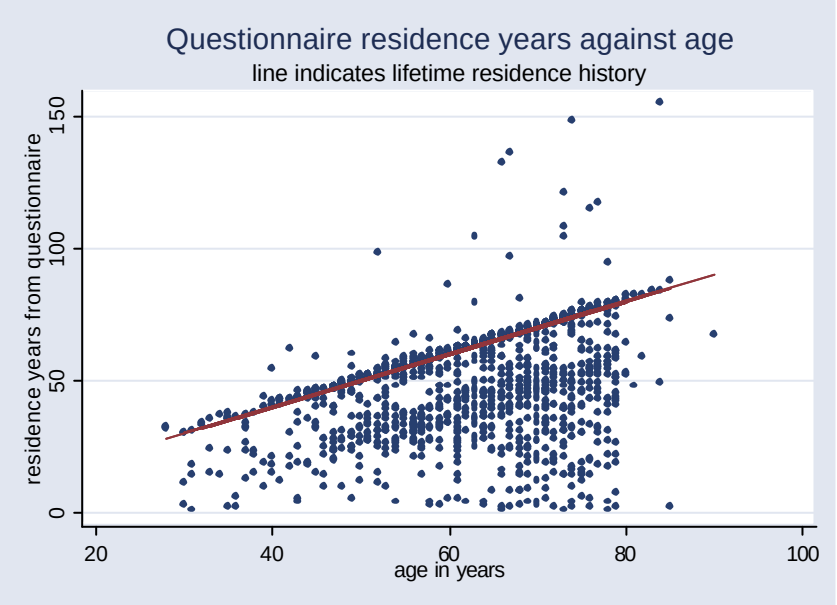
(i) Life time average arsenic concentration. This is the average As concentration over a participant life time, taking into account all the residences, and a combination of historic (from public surveys in the past and current (from ASHRAM) estimates of arsenic concentration in water. Life time average arsenic concentration is measured in micrograms/liter.

Because some of the participants do not have a full residence history (ie, one which covers the whole of their lifetimes) the estimate of cumulative arsenic concentration could be inaccurate. If arsenic concentrations were higher in the past than during the period covered by the historical data this estimate could be an underestimate. In some participants there was overlap between residence times. This was checked during data cleaning and the teams suggested that this could be ascribed to the possession of second homes by some of the population. Overlaps between the first and second residences were addressed during data cleaning in order to provide the most accurate possible description of recent water arsenic concentration. Examination of these data showed that the vast majority of these residences overlapped in the same town so that no adjustment to the concentration of this overlap period was needed. For six people, the first and second residences overlapped in different towns. For these six, an "overlap mean" concentration was generated by taking the mean of

imputed or observed arsenic concentrations in each town and this, weighted by the number of years spent in the overlap period, contributed to the cumulative lifetime exposure estimate. For the remaining residences, some participants (n=358) had a total residential exposure time that exceeded their age. The difference in most cases was very slight; the 90th percentile of the size of the difference was 3 years. The remaining 32 people had between 4 and 74 years' overlap, with a median of 15 years' overlap.

The graph shows the number of residence years covered by the questionnaire against participants' age.

Figure 1. Number of years for which participants provided information on their residential history, in relation to age of participants



The vast majority of observations fall on or below the y=x line; in other words, for most participants the residence years from the questionnaire sum to their age in years or less than their age in years as discussed above.

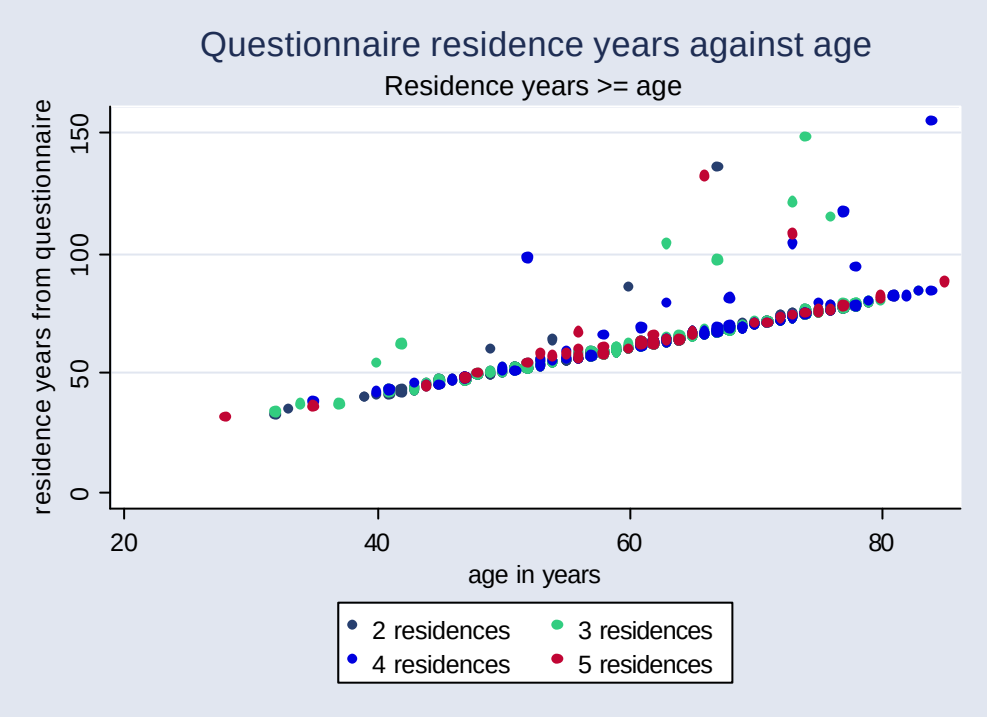
Table 5 shows summary statistics for residence years, by number of residences given on the questionnaire.

Table 5. Number of residences reported by participants, and average duration of residence in years

Number of residences	Number of residence years			
	N	**Min**	**Mean (sd)**	**Max**
1	170	15	58.7 (15.7)	80
2	419	1	51.9 (19.6)	136
3	417	1	49.9 (20.5)	148
4	255	1	49.1 (21.5)	155
5	158	2	48.7 (18.9)	132
6	30	26	50.4 (13.5)	72
7	12	20	41.9 (13.6)	76
8	11	20	45.1 (14.0)	65
9	1	47	47	47
Whole sample	1473	1	51.1	155

High values for residence years are to be found where participants report between two and five residences; the graph shows residence years against age for those people with 2-5 residences whose residence years equalled or exceeded their age at the time of the questionnaire.

Figure 2. **Participants who reported years of residence above their years of age**



The following table shows the distribution of proportion of lifetime covered by the residence years in the questionnaire by county (proportions greater than 1 refer to the overlap time discussed above).

The first part of the table includes overlaps, the second excludes them.

Table 6. Proportion of lifetime for which residential information, leading to possibility of estimation of past exposure to arsenic, is available.

	Summary statistics for proportion of lifetime covered by the questionnaire					
County	**Number of participants**	**Minimum**	**10th percentile**	**50th percentile**	**90th percentile**	**Maximum**
Bacs	261	0	0.6	1	1	1.5
Bekes	55	0.2	0.6	1	1.5	2
Csongrad	102	0	0.6	1	1	2
Jasz-Nagykun-Szolnok	110	0.1	0.5	1	1	1.1
Bihor	164	0	0.3	0.9	1	2
Arad	296	0	0.2	0.7	1	1
Banska Bystrica	224	0	0.5	1	1	1
Nitra	261	0.1	0.5	1	1	1.7
Total	1473	0	0.4	1	1	2

	Summary statistics for proportion of lifetime covered by the questionnaire					
County	**Number of participants**	**Minimum**	**10th percentile**	**50th percentile**	**90th percentile**	**Maximum**
Bacs	164	0	0.4	0.9	1	1
Bekes	30	0.2	0.5	0.8	1	1
Csongrad	72	0	0.5	1	1	1
Jasz-Nagykun-Szolnok	80	0.1	0.4	0.9	1	1
Bihor	135	0	0.2	0.7	1	1
Arad	273	0	0.2	0.6	1	1
Banska Bystrica	177	0	0.5	0.8	1	1
Nitra	184	0.1	0.5	0.9	1	1
Total	1115	0	0.4	0.7	1	1

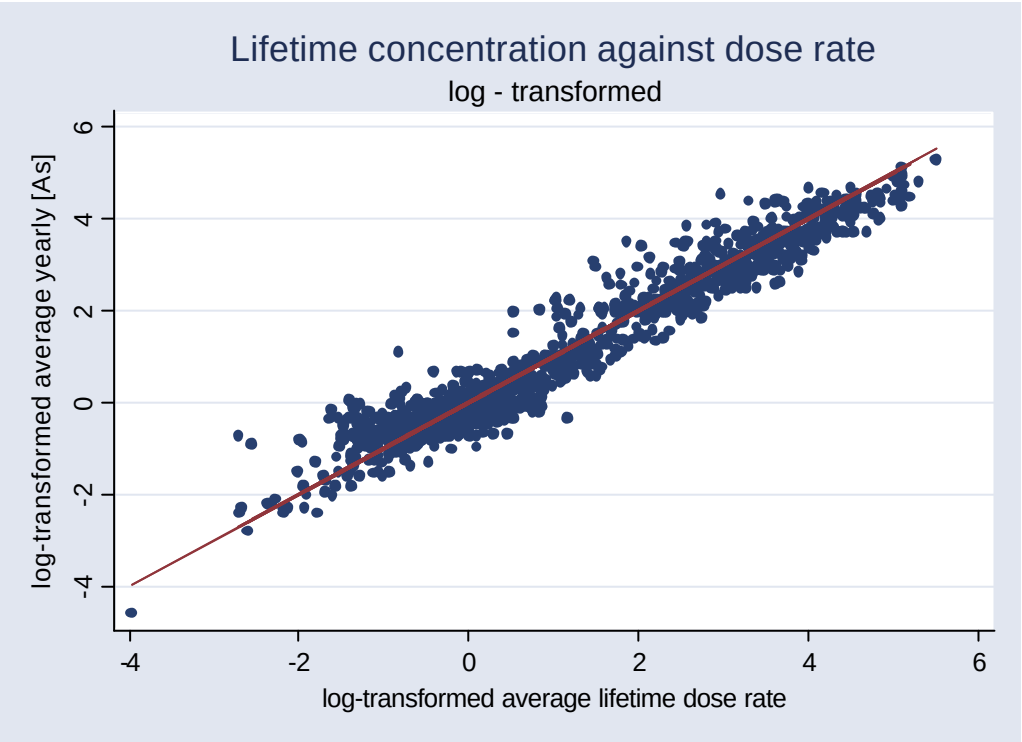
(ii) Life time arsenic dose rate

This was computed as the life time average concentration multiplied by the volume of "local daily water" ingested by the participant. Life time arsenic dose rate is measured in micrograms/day. Here, the appropriate estimate of total daily fluid intake from the FFQ is that based on past (pre-1989) fluid intake, and this was applied.

There is a very strong relationship (correlation 0.97) between life time concentration and life time dose rate, and in initial analyses the life time concentration was used as a proxy of life time exposure

The graph in Figure X shows the relationship between lifetime concentration and lifetime dose rate.

Figure 3. Comparison of arsenic lifetime concentration and life time dose rate.



(iii) Cumulative arsenic dose.

First, the "total volume of past local daily water" was computed, by multiplying the "local daily water" (an estimate relative to an average day) by 365.25 (to obtain one year's volume), and then by age (in years). This provided an estimate of the total volume of water ingested by participants over their entire life. As two separate estimates of "local daily water" were available from the FFQ: before

1989 and after 1989, the former was used to compute "total volume of past local daily water". Then, the cumulative dose was computed, by multiplying the "total volume of past local daily water" by the life time concentration. Cumulative dose is measured in grams.

C. Peak exposure.

Peak exposure is based on the highest arsenic exposure in one year of the life of the participant. Two measures can be identified:

(i) Peak arsenic concentration , defined as the highest arsenic concentration in water in one year of the life of the participant.. It is measured in micrograms/liter.
(ii) Peak arsenic daily dose rate, defined as peak concentration multiplied by the "total volume of past local daily water". It is measured in micrograms/day.

Summary of exposure indices
This is presented in Table 7 on the following page.

Table 7. Summary of indices of exposure to arsenic*.

	Not including water volume		Including water volume	
	Definition	**Period over which calculated**	**Definition**	**Period over which calculated**
CURRENT	Current arsenic concentration. (micrograms/liter) =the arsenic concentration in the most recent residence	n/a – could be historic data if no measurement has been taken or could be our measurements	Current arsenic dose rate (micrograms/day) = current residential As concentration* "recent" local daily water (from recent section of FFQ)	n/a – could be historic data if no measurement has been taken or could be our measurements
LIFE TIME	**Life time average arsenic concentration (micrograms/liter)** =the average arsenic concentration over a participant life time, taking into account all the residences, and a combination of historic (from public surveys in the past) and current (from ASHRAM) estimates of arsenic concentration in water.	Total non-missing residential lifetime	Life time average arsenic dose rate (micrograms/day) = life time average concentration multiplied by the volume of past "local daily water" (per day) ingested by the participant (from pre-1989 section of FFQ). **Cumulative arsenic dose (grams)** = average life time As concentration * total volume of past local daily water (from pre-1989 section of FFQ) Note: total volume of past local daily water=local daily water*365.25*age(years)	Non-missing residential lifetime Lifetime (age in years)
PEAK	Peak arsenic concentration (micrograms/liter) = the highest arsenic concentration in water in one year of the life of the participant.	Highest ever calculated – could be in a year for historical data or our measurement	**Peak arsenic daily dose rate (micrograms/day)** =peak concentration*total past local daily water	Highest ever calculated – could be in a year for historical data or our measurement

*The three exposure indices in bold format are those used to produce the estimates of arsenic cancer risk in the present document.

2.8 SURVEY OF TOTAL ARSENIC CONCENTRATIONS IN FOOD ITEMS FROM SLOVAKIA

Within the Ashram study arsenic in food was assessed by a Food Frequency Questionnaire. In order to check available results from a Slovakian database for nutrients and other metals in food a small survey of several food items in Slovakia was conducted. The major goal of this study was to verify the quality of the data available from the Slovakian database as many of the arsenic concentrations listed were only assigned because the results were below the detection limits.

Only a short list of the food items most likely to be associated with arsenic exposure was feasible within ASHRAM, as food was not initially considered an important source of exposure in this population mainly exposed to water. The criterion for selection of food items for As analysis was that they were likely to lead to As exposure, either because the literature reported relatively high arsenic content, or more importantly because they are common food items leading to a large intake for the average diet in Slovakia, thereby leading to potentially significant intake of As even for comparatively small As concentration in the food. Based on these considerations and on the examination of As food content from previous surveys in Slovakia, the food items sampled were seven: dark bread, white rice, semi-skimmed milk, pork meat, chicken meat, potatoes (skinned and not skinned), and cabbage.

Samples were collected in two regions: Banska Bystrica and Nitra. Since 1989, the type of food retail outlets in these regions has changed considerably, with the introduction of several new supermarkets. In each region, the samples were collected in one main city, in two smaller towns and in some villages, as outlined in the following table, reflecting regional consumption patterns, wholesales, and distribution of the Slovak population. A protocol for sampling was developed describing the procedure for selection and transport. On 25 November 2004, the same seven food items were collected in Banska Bystrica (large and small shop), Nitra (big and small shop), Brezno (small shop), Ziara nad Hronom (small shop), Hlinik nad Hronom (small shop), Valaska (small shop), Levice (big shop), Nove Zamki (small shop), Kozarovce (small shop), and Tvrdosovce (small shop) and delivered for analysis at the laboratories of Partner 3, Graz University.

The wet samples (milk, pork meat, chicken meat, potatoes, and cabbage) were freeze-dried and the loss of water was recorded in order to calculate all the results on a wet mass basis. Thereafter, the samples were ground in a Retsch ZM 1000 ultracentrifugal mill equipped with a titanium rotor and a titanium sieve (0.25 mm). An aliquot of these samples (~0.5 g) was digested with HNO3 in quartz vessels in a microwave assisted autoclave. The temperature was ramped in 45 minutes to 250°C and then this temperature was kept for 45 minutes. This digestion procedure ensured that the residual carbon in the samples in close to zero which is very important for arsenic analysis at trace concentrations. After digestion the samples were transferred quantitatively into 50 ml polypropylene tubes in and filled to 20 ml with Milli-Q water (18.2 MΩ*cm).

The total arsenic concentration in these samples was determined with an Agilent 7500ce inductively coupled plasma mass spectrometer equipped with an octopole reaction system. To remove possible interferences on m/z 75 He was used as reaction gas. For calibration the arsenic standard solutions were acid matched. For QA/QC appropriate SRMS (NIST Wheat Gluten, Tomato leaves) were treated in the same way as the samples.

In a first series of experiments one sample of the different food items was randomly selected and analysed for homogeneity. The results are shown in the following tables. All results are expressed on a wet mass basis.

Table 8. Homogeneity of the arsenic concentration within one food item

Sample Name	[ug As/kg]	Sample Name	[ug As/kg]	Sample Name	[ug As/kg]
Bread 6.1	10.2	Rice 6.2	183	Milk 6.3_1	0.6
Bread 6.1_2	9.5	Rice 6.2_2	188	Milk 6.3_2	0.6
Bread 6.1_3	8.2	Rice 6.2_3	188	Milk 6.3_3	0.6
Bread 6.1_4	8.0	Rice 6.2_4	190	Milk 6.3_4	0.9
Bread 6.1_5	8.9	Rice 6.2_5	188	Milk 6.3_5	0.6
Mean	9	**Mean**	187	**Mean**	0.7
SD	1	**SD**	3	**DEV**	0.1

Sample Name	[ug As/kg]	Sample Name	[ug As/kg]	Sample Name	[ug As/kg]
Pork meat 6.4	2.6	Chicken 6.5	38.4	Cabbage 6.7_1	1.0
Pork meat 6.4_2	1.4	Chicken 6.5_2	41.1	Cabbage 6.7_2	1.5
Pork meat 6.4_3	1.4	Chicken 6.5_3	42.6	Cabbage 6.7_3	1.0
Pork meat 6.4_4	1.3	Chicken 6.5_4	38.2	Cabbage 6.7_4	1.2
Pork meat 6.4_5	2.3	Chicken 6.5_5	38.4	Cabbage 6.7_5	1.0
Mean	**1.8**	Mean	40	**Mean**	**1.1**
SD	**0.6**	SD	2	**DEV**	**0.2**

Sample Name	[ug As/kg]
Potatoes 6.6	97.9
Potatoes 6.6_2	113
Potatoes 6.6_3	100
Potatoes 6.6_4	110
Potatoes 6.6_5	208
Mean	**126**
SD	**46**

The heterogeneity of the arsenic concentration within one food item was remarkable. At higher arsenic concentrations as e.g. in rice it was less than 2% but increased with decreasing total arsenic concentration. The exception was the potato sample which showed a high inhomogeneity (~37%) although the total arsenic concentration was high (~100 µg/kg). The potato sample was not peeled in this experiment. Possible adhering soil particles could be an explanation for these findings. The

rather high arsenic concentrations also indicate contamination from soil adhering to the peel of the potatoes. Therefore, the potatoes were reanalyzed for the total arsenic after peeling. The results are shown in the following table.

As expected the total arsenic concentration was much lower than for potatoes analysed with peel. These findings are significant with respect to the interpretation of urinary arsenic concentrations at low arsenic exposure from drinking water. Many people eat potatoes together with peel and significant amounts of arsenic can increase the urinary arsenic concentration when the arsenic present in the adhering soil is bioavailable.

Table 9. Homogeneity of the total arsenic concentration in a peeled potato sample

Sample Name	[ug As / kg]
Potatoes 6_6	1.9
Potatoes 6_6 replicate 2	2.2
Potatoes 6_6 replicate 3	1.9
Potatoes 6_6 replicate 4	3.0
Potatoes 6_6 replicate 5	7.0
Mean	3.2
SD	2.2

2.9 EXPOSURE TO SUNLIGHT AND OTHER CONFOUNDERS

Information on possible confounding factors of the association of As exposure with cancer was collected by use of interviewer-administered questionnaire.

Three indices of exposure to sunlight were studied, peak exposure (UV-PE) was defined as the highest annual number of hours of exposure of the upper trunk to the sun, cumulative life time exposure (UV-CLTE) as the average life time number of hours of exposure to the sun, skin sensitivity to burns (UV-sens) as an index based on the intensity of the cutaneous reaction to sun

exposure (frequency of burns), and skin complexion (UV-compl) as the self-reported complexion of the skin.

Items on smoking and passive smoking derived from standard questionnaires used by IARC in study of occupational bladder cancer. Other areas covered were medical history including kidney infections and use of medication, as well as number of years of education, self-reported nationality, age and sex. A copy of the full questionnaire is included in the Annexes.

2.10 STATISTICAL METHODS

2.10.1 Data management

Data processing was implemented through Auto Data software which enables to set up a data entry system that uses a fast scanner to enter information from questionnaire form into a database.

2.10.2 Analysis of factors influencing the metabolism

The exposure and metabolite data were not normally distributed. Therefore, Spearman's rank order correlation test was used to test for univariate associations between variables. The metabolic data were continuous variables with a heavy right skew. Log transformations were applied to normalise the distributions and stabilise the variances of regressors for estimation models. The principal metabolic data were a priori defined as %MMA in the urine and %DMA in the urine and univariate test of statistical significance were conducted to look for associations between variables. The relative amount of DMA and MMA in urine was expressed as the percent of total urinary arsenic (%DMA).

Multiple linear regression was conducted, with transformed variables to meet the requirements of normally distributed residuals, to test for associations between variables.

2.10.3 Analyses of nutritional factors

The mean daily intake of nutrients and the corresponding standard deviations will be computed using the corresponding daily frequency of consumption of each food item or recipe (questions) and the nutrient content of each food item of the reference portion size. Careful data inspection will make sure than no implausible level of nutrient intake was present.

It will be evaluate if other characteristics of the study participants influenced the results. Data could be adjusted for total energy intake, centre, sex, age and interval between the two interviews.

FLUID INTAKE

The total fluid intake will be calculate for all participants in ml/day, and subjects can be grouped in categories of fluid intake. Odds ratios for different level of fluid intake or categories of fluid intake will be computed between case and controls.

Reproducibility and Validation of the FFQ

The reproducibility of the FFQ will be evaluated by means of the Pearson correlation-coefficient intake of nutrients calculated from the first and the second questionnaire.

For the purpose of assessing the reproducibility of the 112 questions concerning frequency of consumption of food items and recipes correlation coefficient (r) will be calculated between intakes of food items calculated from the first and the second administration of the questionnaire. Various aggregations of food items, which could be similar to other or turn out to be a source of misinterpretation, could be considered in order to increase the FFQ reproducibility.

2.10.4 Case control analyses

Choice of models

Logistic regression models were developed with inclusion of sun exposure indices, age, sex, number of years of education as proxy for socio-economic status, and alternative arsenic exposure indices. The arsenic exposure indices were log-transformed to stabilise variance in the estimation models. Models for the cancer outcomes were constructed for each outcome in turn. Univariate models were constructed using the log-transformed peak dose rate described above and log-transformed lifetime mean dose rate; these univariate models adjusted for clustering by county using fixed-effects analysis. Multivariable models were then constructed using both of the exposure indices in turn and a range of a priori confounders which varied for each outcome. For the basal cell carcinoma outcome, a priori confounders were sun exposure, age and sex; for the kidney cancer outcome the a priori confounders were a medical history of kidney infection (a binary response variable), age and sex; for the bladder cancer outcome a priori confounders were exposure to cigarette smoke (measured as a continuous variable, total pack-years of smoking over the lifetime), age and sex.

Random effects vs fixed factor models

The intra-class correlation coefficients for all of the log-transformed As exposure variables by country were close to 0.6 (see table). In other words, a good proportion (~60%) of the variance is due to country effect; therefore adjustment for country needed to be made. A fixed-effects model was suitable in this case because the distribution of people across country was fairly even. Additionally, the case sampling distribution varies by county within country so we adjust for clustering by county. No adjustment for country is therefore necessary because county is a proxy for country.

The multivariable models described above all therefore used fixed-effects logistic regression with the county as the level 2 unit of exposure.

Table 10. Intra-class correlation coefficents for the log-transformed As exposure indices by country and county

Clustering variable	Intra-class correlation coefficient for log-transformed peak dose rate (asymptotic SE)	Intra-class correlation coefficient for log-transformed average lifetime dose rate (asymptotic SE)
Country	0.60 (0.24)	0.59 (0.24)
County	0.57 (0.14)	0.56 (0.15)

Role of nutrients

This case-control study can provide insight on the relationship between nutrient intake, arsenic intake and bladder, skin and kidney cancer. It will estimate the subject's arsenic exposure from oral uptake by food and beverages, consequently the odds ratio for oral uptake of arsenic will computed between case and controls, for different level of arsenic exposure.

Odds ratios for different level of intake for selected macronutrients and micronutrients will be computed between case and controls.

The nutrient intake of the studied population will be compared with the Recommended Dietary allowances for the population of Slovak Republic (RDA, 1997).

To analyses the effect of the intake of food items or food groups on the cancer risk, all questions of the FFQ will be grouped into 19 food groups: (1) milk, (2) coffee and tea, (3) bread and cereals dishes, (4) soups (5) eggs, (6) poultry (7) red meat, (8) processed meat (9) fish (10) cheese, (11) raw vegetable (12) cooked vegetables (13) potatoes (14) pulses (15) citrus fruits (16) other fruits, (17) cakes, (18) sugars and candies (19) artificial sweeteners. The sum of the weekly frequency of intake of food items or recipes included in the same group will be distributed into different level of consumption based on the entire population (case and control). Odds ratios will be computed.

3. RESULTS

3.1 STUDY POPULATION.

Recruitement of cases and controls was carried out through 20 months, starting in January 2003 and ending mid-September 2004. Of 1836 patients invited, 1566 or 85.3% consented to taking part in the study (Table 11). Later, 71 of these did not have histological confirmation of the diagnoses, and a further 7 were excluded for other reasons, leaving 1488 observations available for analysis.

Table 11. Recruitment of study population

Number of patients	Country			Total
	Hungary	Romania	Slovakia	
Invited in study	626	480	730	1836
Consenting	571	480	515	1566
Excluded due to absent or negative histology	38	13	20	71
Excluded due to other reasons (death, move from study area)	2	0	5	7
Included in database for analyses	531	467	490	1488

In total the study recruited 1488 valid participants in all of whom 948 were cancer cases (625 skin, 214 bladder, 109 kidney) and 540 were hospital-based controls. The distribution of cancer cases and controls across the three countries is shown is Table 12.

Table 12. Cases and controls participating in ASHRAM

	Hungary		Romania		Slovakia		Total	
	N	%	N	%	N	%	N	%
All skin cancer	177	28.3	218	34.9	230	36.8	625	100.0
BCC	160	30.2	158	29.9	211	39.9	529	100.0
Other skin cancer	17	17.7	60	62.5	19	19.8	96	100.0
Bladder cancer	72	33.6	69	32.2	73	34.1	214	100.0
Kidney cancer	33	30.3	24	22.0	52	47.7	109	100.0
Controls	249	46.1	156	28.9	135	25.0	540	100.0
Total	531		467		490		1488	100.0

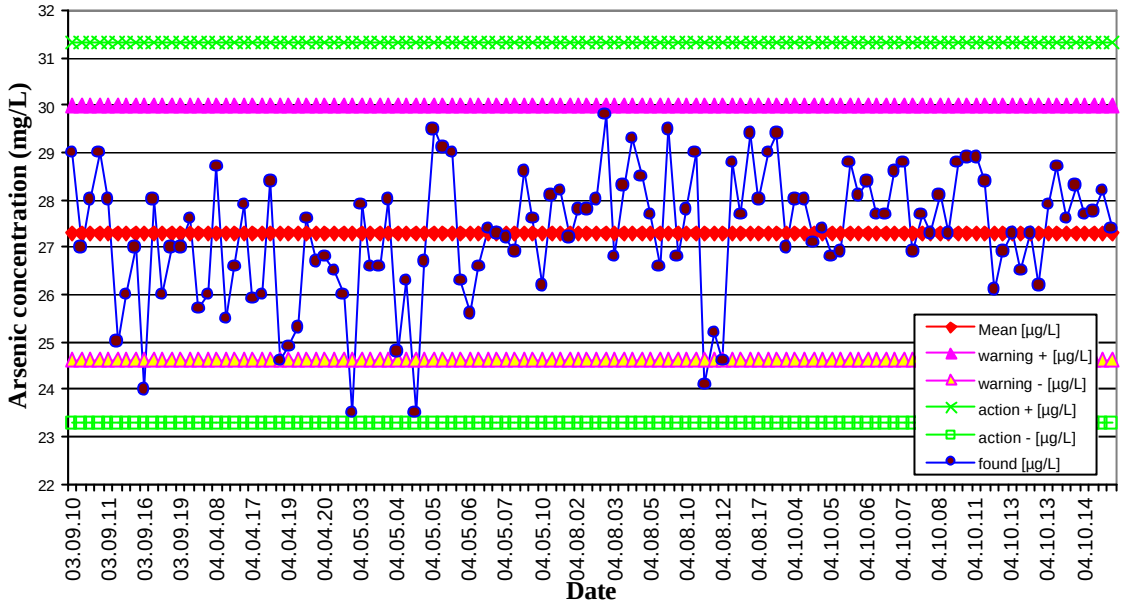
3.2 ARSENIC MEASUREMENTS IN WATER

3.2.1 Concentration of arsenic in water samples

Altogether 1410 water samples were analysed with the following distribution: 524 from Hungary, 690 from Romania and 196 from Slovakia. The results of the analyses were reported to LSTHM and KFUG within 4 weeks after the reception of each batch of samples in form of a Microsoft Excel file. The report file contained the results and the quality control chart for the analysed batch, and also the following points: date and time of analysis, analyst and any changes from the described procedure.

The control chart for the water samples analyses using the NIST 1640 Certified Standard Reference Material is presented below.

Control Chart NIST 1640



In general, the concentrations of arsenic in the currently used water, median 1.0 µg/L, were low. About 20% had concentrations above the recommended guideline set by WHO to 10 µg/L. The highest concentrations were seen in Hungary, the median water arsenic concentration was about 19 fold higher than in Romania and 17 fold higher than in Slovakia (Table 1, 2 and 3).

Table 13. Water concentrations in Hungary and Hungarian regions

	Hungary	Bacs	Békés	Csongrad	Jasz-Nagykun-Szolnok
Mean	14.7	10.9	17.4	23.5	14.2
Median	13.3	7.7	17.0	19.0	15.7
10th perc.	1.0	0.9	7.0	6.4	0.8
90th perc.	30.1	26.2	27.0	39.0	26.0
N	521	257	54	100	110

Table 14. **Water concentrations in Romania and Romanian regions**

	Romania	Bihor	Arad
Mean	**3.8**	2.3	4.7
Median	**0.7**	0.5	0.7
10th perc.	**0.4**	0.4	0.4
90th perc.	**5.8**	4.0	5.9
N	**456**	163	293

Table 15. **Water concentrations in Slovakia and Slovakian regions**

	Slovakia	Banská Bystrica	Nitra
Mean	**1.9**	2.0	1.9
Median	**0.8**	0.7	0.9
10th perc.	**0.5**	0.5	0.6
90th perc.	**2.4**	2.4	3.1
N	**484**	221	263

3.3 ARSENIC MEASUREMENTS IN URINE

3.3.1 Concentration of arsenic in urine samples

In general, the concentrations of arsenic in urine (total urinary arsenic metabolites; Sum As) were low in the Ashram study – in most cases below 30 µg/L (adjusted to specific gravity 1.017 g/ml). The highest exposure, assessed from urinary arsenic, was seen in Hungary, where the median concentration was about 3.5 fold higher than in Slovakia and 5.5 fold higher than in Romania (Table 9, 10 and 11).

Table 16. Urine concentrations of inorganic arsenic and its metabolites in Hungary and Hungarian regions

		Hungary	Bacs	Békés	Csongrad	Jasz-Nagykun-Szolnok
DMA adj. [µg/L]	Mean	**14.9**	13.5	18.2	21.4	10.7
	Median	**10.9**	10.2	12.5	17.4	7.8
	10th perc.	**3.2**	2.8	5.9	5.4	2.8
	90th perc.	**32.3**	29.0	41.5	42.5	19.9
	N	**512**	254	54	98	106
MA(V) adj. [µg/L]	Mean	**3.8**	3.3	4.5	5.6	2.8
	Median	**2.3**	2.0	3.1	3.7	1.9
	10th perc.	**0.7**	0.5	1.3	1.5	0.7
	90th perc.	**8.0**	7.3	9.1	12.7	5.0
	N	**512**	254	54	98	106
iAs adj. [µg/L]	Mean	**2.2**	1.7	2.3	4.2	1.5
	Median	**1.3**	1.1	1.4	2.8	1.1
	10th perc.	**0.2**	0.1	0.5	0.7	0.2
	90th perc.	**4.8**	4.3	5.5	7.3	2.9
	N	**512**	254	54	98	106
Sum As adj. [µg/L]	Mean	**20.9**	18.5	25.1	31.3	14.9
	Median	**15.3**	13.9	17.4	24.9	11.2
	10th perc.	**4.6**	3.9	8.6	8.8	4.0
	90th perc.	**45.6**	38.7	50.4	57.0	28.5
	N	**512**	254	54	98	106

Table 17. Urine concentrations of inorganic arsenic and its metabolites in Romania and Romanian regions

		Romania	Bihor	Arad
DMA adj. [µg/L]	Mean	**4.7**	4.5	4.7
	Median	**2.0**	2.3	1.9
	10th perc.	**0.9**	0.8	1.0
	90th perc.	**8.3**	8.3	7.7
	N	**460**	163	297
MA(V) adj. [µg/L]	Mean	**0.9**	0.8	1.0
	Median	**0.4**	0.4	0.4
	10th perc.	**0.0**	0.0	0.0
	90th perc.	**1.8**	1.8	1.6
	N	**460**	163	297
iAs adj. [µg/L]	Mean	**0.5**	0.4	0.6
	Median	**0.2**	0.2	0.2
	10th perc.	**0.0**	0.0	0.0
	90th perc.	**1.0**	1.1	1.0
	N	**460**	163	297
Sum As adj. [µg/L]	Mean	**6.1**	5.7	6.3
	Median	**2.7**	3.0	2.6
	10th perc.	**1.1**	1.0	1.2
	90th perc.	**10.9**	11.4	10.9
	N	**460**	163	297

Table 18. Urine concentrations of inorganic arsenic and its metabolites in Slovakia and Slovakian regions

		Slovakia	Banská Bystrica	Nitra
DMA adj. [µg/L]	Mean	**5.0**	4.9	5.0
	Median	**3.4**	3.1	3.5
	10th perc.	**1.6**	1.4	1.6
	90th perc.	**9.0**	8.8	9.1
	N	**484**	223	261
MA(V) adj. [µg/L]	Mean	**0.9**	0.9	0.9
	Median	**0.6**	0.6	0.6
	10th perc.	**0.2**	0.1	0.2
	90th perc.	**1.8**	1.9	1.6
	N	**484**	223	261
iAs adj. [µg/L]	Mean	**0.4**	0.4	0.4
	Median	**0.2**	0.2	0.3
	10th perc.	**0.0**	0.0	0.0
	90th perc.	**0.9**	0.9	0.8
	N	**484**	223	261
Sum As adj. [µg/L]	Mean	**6.2**	6.1	6.3
	Median	**4.4**	4.3	4.6
	10th perc.	**1.9**	1.6	2.2
	90th perc.	**11.8**	11.5	11.8
	N	**484**	223	261

3.3.2 Results of trivalent organoarsenic compounds analyses

Table 19. Urinary arsenic metabolites of patients treated with As_2O_3

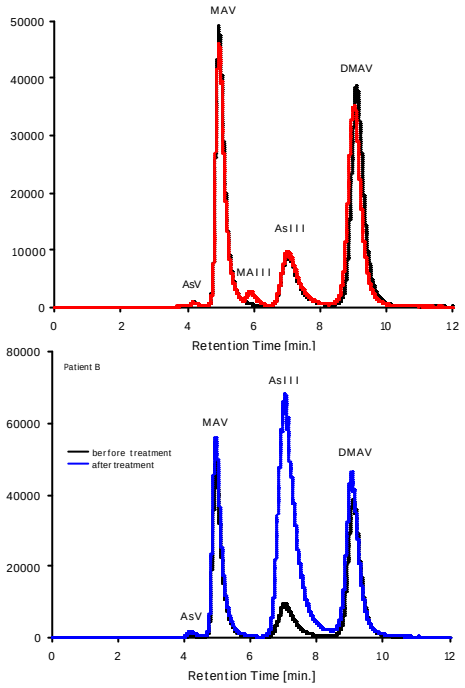
	Concentration mg As / L							
	A1	**A2**	**B1**	**B2**	**C1**	**C2**	**D1**	**D2**
As(III)	2.7	11	1.2	8.9	0.36	2.1	0.073	1.9
DMA	1.6	1.7	3.4	3.8	2.3	4.9	1.1	1.6
MA	0.41	0.35	3.0	3.1	1.6	3.3	0.21	0.33
As(V)	n.d.	0.36	0.034	0.12	0.028	0.048	n.d.	0.063
Sum	4.7	13.2	7.6	15.9	4.3	10.4	1.4	3.9

	Concentration %							
	A1	**A2**	**B1**	**B2**	**C1**	**C2**	**D1**	**D2**
As(III)	57	82	16	56	8	21	5	48
DMA	34	13	45	24	54	47	79	42
MA	9	3	39	20	37	32	15	8
As(V)	0	3	0	1	1	0	0	2

In all the urine sample within this trial a low As(V) concentration was observed indicating that the storage in liquid nitrogen was successful. We did not observe the typical excretion patterns of 60-70% DMA, 10-20% MA and 10-30% inorganic arsenic (Vahter 2002) in the urine of these patients, except maybe for patient D, had been only 3 weeks on therapy. All the other patients showed elevated As(III) or MA concentrations.

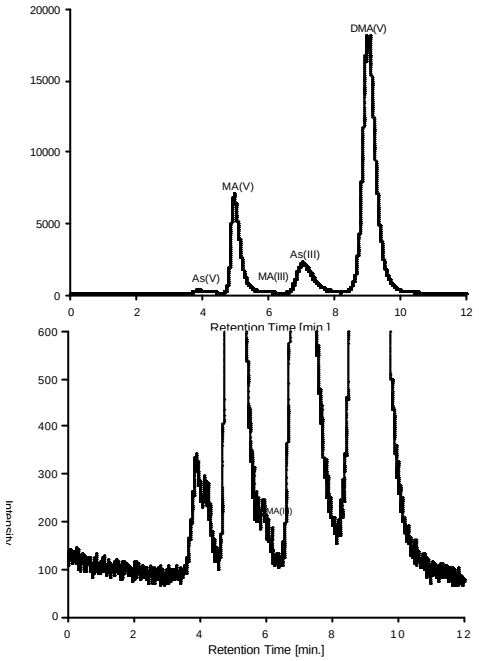
In order to detect possible traces of MA(III) after the huge As(III) concentrations chromatography was optimized for this purpose (Figure 1).

Figure 4. **Urine sample of patient B spiked with 10 µg As / l as MA(III)**



Only in the urine of patient A MA(III) could be detected at trace concentrations (Figure 2). As the concentrations were so low a reliable quantification was not possible. But the concentration of MA(III) can be estimated to be less than 0.5% of the sum of the hydride forming arsenic compounds. In all the other urine samples MA(III) and DMA(III) were below the detection limit.

Figure 5. Urine sample of patient A before (top) and after treatment



The concentrations of MA(III) are much lower as published data (e.g. Valenzuela et al 2005). Arsenic concentrations in the urine samples of the treated patients are comparable or even higher than the arsenic concentrations in urine reported for people exposed to arsenic via drinking water in Romania, Inner Mongolia, Mexico and Bangladesh. Nevertheless, it was not possible for us to detect significant amounts of trivalent organoarsenicals although samples have been stored immediately after collection until analysis in liquid nitrogen. As the methods used in our study are superior with respect to detection limits and chromatographic resolution and we used two independent chromatographic systems (in contrast to the published data) we have to conclude that:

1. trivalent organoarsenicals are not significant metabolites in human urine

2. the instability of MA(III) and especially DMA(III) makes them unsuitable as arsenic exposure markers in human urine
3. excretion of trivalent organoarsenicals is varying significantly among humans
4. published results on the trivalent organoarsenicals have to be questioned

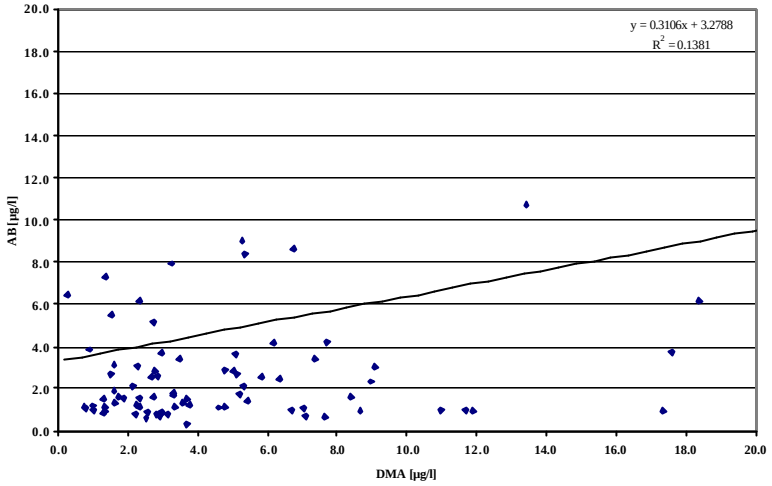
3.3.3 Cationic arsenic compounds and arsenosugars in urine samples

Although the predominant route of arsenic intake is water certain food items such as mushrooms Slejkovec et al. (1997), fish Sörös et al. (2005), mussels, Schmeisser et al. (2004), and seafood Edmonds & Francesconi (2003), can significantly contribute to the hydride forming arsenicals [As(III), DMA, MA, As(V)] excreted in human urine. Therefore, we analysed in a subset of 202 urine samples the cationic arsenic compounds arsenobetaine, trimethylarsine oxide, arsenocholine, the tetramethylarsonium cation, and the glycerol arsenosugar. The compounds were separated on a Zorbax LC-SCX strong cation-exchange column using a pyridinium formate buffer at pH 2.3 (adjusted with formic acid) as mobile phase. The compounds were determined with ICPMS in the effluent of the chromatographic column.

Trimethylarsine oxide, and arsenocholine were below the detection limit of 0.5 µg As/l. The tetramethylarsonium cation was detectable only in one urine sample at a concentration of 1.8 µg As/l. This urine sample was found to be the highest for arsenobetaine as well (59 µg As/l). Arsenobetaine was above the detection limit of 0.5 µg As/l in 89 out of 202 samples (44%). The mean arsenobetaine concentration was 5.5 µg As/l the median was 1.9 µg As/l.

The hydride active arsenic species As(III), DMA, MA, and As(V) did not correlate with the arsenobetaine concentration (Figs. 3, 4).

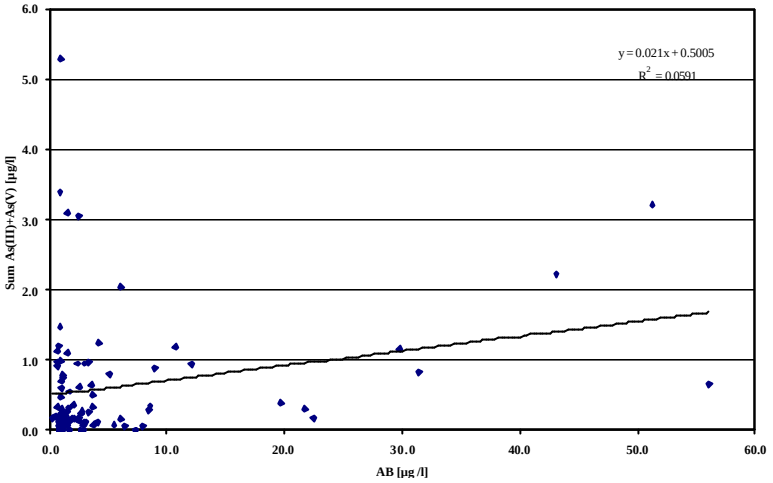
Figure 3 Correlation of DMA versus AB



From these results we can conclude that the high arsenic concentrations in urine (above 10 µg/l) are not coming from (sea)food consumption but are most likely originated from high arsenic concentration in the current water supply.

In WP 3 it is discussed that at the low exposure level a significant portion of the urinary arsenic is coming from food as the ratio arsenic in drinking water versus urinary arsenic is not 1:1 (see WP3)

Figure 4 Correlation of AB versus inorganic arsenic



Arsenosugars:

Arsenosugars were determined in all the urine samples analyzed within the Ashram project using a method that we have developed during the Ashram study (Schmeisser et al. Anal Chem. 2004). Arsenosugars were not detectable in any of the urine samples.

3.4 QA/QC PROGRAMME

1st Round Robin exercise April 2002: Water samples: Tap water was spiked with arsenate at two different concentrations: 8.0 µg As/l (water level 1) and 60.0 µg As/l (water level 2). The samples were analysed in the laboratories in SHI (Slovakia), EHC (Romania), KI-IMM (Sweden), NCPH (Hungary), and KFUG (Austria).

Table 20. 1st Round Robin: reported arsenic concentration in the two different water samples.

Country	Water level 1	Water level 2
EHC Romania	2.53	21.53
KFUG Austria	8.5 ± 0.02	61.5 ± 0.4
SHI Slovakia	7.62	39.6
KI-IMM Sweden	8.0 ± 0.05	56 ± 2.3
NCPH Hungary	6.9	70

The overall mean of the reported concentrations was 6.7 ± 2.4 µg As/l and 49.7 ± 19.3 µg As/l, respectively. According to the Dixon and Grubbs outlier tests only water level 1 are acceptable. For water level 1 the results from EHC Romania and NCPH Hungary are detected as outliers. Recalculation of the mean and standard deviation for water level 1 without the outliers results in 8.0 ± 0.41 µg As/l which is in very good agreement with the spiked arsenic concentration of 8.0 µg As/l. For water level 2 the results reported from EHC, SHI, and NCPH have been identified as outlier. Urine samples: The urine level 1 sample was unspiked urine. Only the lab from KFUG was able to detect 1.3 ± 0.2 µg As/l as DMA. For all the other labs the concentration on DMA was below the detection limit of their methods (0.5 and 0.1 µg/L), and they were not able to detect it. The urine level 2 was spiked with 4.8 µg As/l as As(III), 4.8 µg As/l as As(V), 8.0 µg As/l as MA and 32.0 µg As/l as DMA. The samples were analysed in the laboratories in SHI (Slovakia), KI-IMM (Sweden), and KFUG (Austria).

Table 21. **1st Round Robin: reported arsenic concentration in the two different urine samples.**

Country	Sample	Inorg. As	DMA [µg/L]	MA [µg/L]
KFUG Austria	Urine level 1	N.D.	1.3 ± 0.2	N.D.
	Urine level 2	9.4 ± 0.9	35.2 ± 1.4	8.6 ± 1.1
SHI Slovakia	Urine level 1	N.D.	N.D.	N.D.
	Urine level 2	8.9	30.4	9
KI-IMM Sweden	Urine level 1	N.D.	N.D.	N.D.
	Urine level 2	11 ± 0.4	30 ± 1.8	8.4 ± 0.9

A mentioned above for urine level 1 (unspiked urine) no results from the labs in Slovakia and Sweden were available. The concentrations determined in Slovakia, Sweden and Austria agree within the acceptable range for urine level 2.

2nd Round Robin exercise July 2002: Water samples: Tap water was spiked with arsenate at two different concentrations: 12.0 µg As/l (water level 1) and 44.0 µg As/l (water level 2). The samples were analysed in the laboratories in SHI (Slovakia), EHC (Romania), KI-IMM (Sweden), NCPH (Hungary), and KFUG (Austria).

Table 22. **2nd Round Robin: reported arsenic concentration in the two different water samples.**

Country	Water level 1	Water level 2
EHC Romania	12.3 ± 1.1	43.9 ± 4.3
KFUG Austria	11.6 ± 0.2	43.0 ± 0.4
SHI Slovakia	13.5	50.1
KI-IMM Sweden	12.0 ± 0.6	44.0 ± 1.5
NCPH Hungary	11.1 ± 0.65	42.8 ±0.58

The overall mean of the reported concentrations was 12.1 ± 0.9 µg As/l and 44.8 ± 3.0 µg As/l, respectively. For water level 1 all the labs produced results, that fit well into range of ± 1 standard deviation. No outlier was produced for the sample water level 1. For water level 2 the value from Slovakia was out of the ± 1 SD area, if all data (including outliers) were used for the calculation of

the interlaboratory mean. The interlaboratory mean for water level 2 without outliers was 43.4 µg As/L (true value 44 µg/L).

Urine samples: The urine level 1 sample was spiked to 2.0 µg As/l as As(V), 2.0 µg As/l as As(III), 3.0 µg As/l as MA, and 3.0 µg As/l as DMA. The urine level 2 was spiked spiked to 5.0 µg As/l as As(V), 6.0 µg As/l as As(III), 10.0 µg As/l as MA, and 36.0 µg As/l as DMA. The samples were analysed in the laboratories in SHI (Slovakia), KI-IMM (Sweden), and KFUG (Austria).

Table 23. 2nd Round Robin: reported arsenic concentration in the two different urine samples.

Country	Sample	Inorg. As	DMA [µg/L]	MA [µg/L]
KFUG Austria	Urine level 1	4.6 ± 0.1	5.9 ± 0.1	3.9 ± 0.1
	Urine level 2	11.1 ± 0.3	39.2 ± 0.3	10.9 ± 0.1
SHI Slovakia	Urine level 1	5.4 ± 0.6	5.0 ± 0.7	4.1 ± 0.3
	Urine level 2	8.9 ± 0.5	1.7 ± 0.5	8.4 ± 0.3
KI-IMM Sweden	Urine level 1	N.D.	N.D.	N.D.
	Urine level 2	13 ± 0.5	42.0 ± 1.2	10.0 ± 0.1

For urine level 1 for the species only data from the labs from KFUG and SHI were available. Sweden did not send back results for the arsenic species. The results for DMA were higher, than the added value to the urine. The difference between the added and the founded value for DMA must be the “blank value” from the urine, that were used for the preparation of the round robin samples. Also the reults for DMA in the urine level 2 reported from the labs in Sweden and Austria were higher than the added value. The results for urine level 2, that came back from SHI Slovakia were outliers for all species, that were added to the urine. The results from the other two labs, were in good agreement with the added values to the urine sample (level 2), when the “blank value” is added to the value for DMA.

3rd Round Robin exercise March 2003: Water samples: Tap water was spiked with arsenate at two different concentrations: 16.0 µg As/l (water level 1) and 56.0 µg As/l (water level 2). The samples were analysed in the laboratories in SHI (Slovakia), EHC (Romania), KI-IMM (Sweden), NCPH (Hungary), and KFUG (Austria).

Table 24. **3rd Round Robin: reported arsenic concentration in the two different water samples.**

Country	Water level 1	Water level 2
EHC Romania	11.1 ± 0.42	62.9 ± 2.312
KFUG Austria	15.8 ± 0.4	54.9 ± 0.7
SHI Slovakia	15.6 ± 0.09	55.0 ± 0.56
KI-IMM Sweden	13.0 ± 1.4	53 ± 1.4
NCPH Hungary	15.1 ± 0.42	57.6 ± 1.58

The overall mean of the reported concentrations was 14.1 ± 2.0 µg As/l and 56.7 ± 3.9 µg As/l, respectively. According to the Dixon and Grubbs outlier tests all results for water level 1 are acceptable. The overall mean is not within the agreed value of 10%. For water level 2 the result reported from EHC has been identified as outlier at the 95% level. Recalculation of the mean and standard deviation without this results in 55.1 ± 1.9 µg As/l which is in very good agreement with the spiked arsenic concentration of 56 µg As/l.

Urine samples: The urine level 1 sample was spiked with 5.0 µg As/l as As(III) and 8.0 µg As/l as DMA. The urine level 2 was spiked with 10.0 µg As/l as As(III), 12.0 µg As/l as MA and 30.0 µg As/l as DMA. The samples were analysed in the laboratories in SHI (Slovakia), KI-IMM (Sweden), and KFUG (Austria).

Table 25. **3rd Round Robin: reported arsenic concentration in the two different urine samples.**

Country	Sample	Inorg. As	DMA [µg/L]	MA [µg/L]
KFUG Austria	Urine level 1	5.2 ± 0.2	9.5 ± 0.4	0.11 ± 0.01
	Urine level 2	15.0 ± 0.3	29.9 ± 0.7	12.1 ± 0.2
SHI Slovakia	Urine level 1	6.6 ± 0.14	N.D.	Not added
	Urine level 2	35.7	24.1	10.5
KI-IMM Sweden	Urine level 1	8 ± 1	8 ± 1	Not added
	Urine level 2	16 ± 3	28 ± 1	13 ± 3

Due to some technical problems SHI was not able to report correct concentrations. The concentrations determined in Sweden and Austria agree within the acceptable range although inorganic arsenic in the Urine level 1 was reported quite high. At the PI meeting in Falmouth it was agreed the SHI will change their method to HPLC separation and detection by HG-AAS in order to improve the results.

4th Round Robin exercise October 2003: Water samples: Tap water was spiked with arsenate at two different concentrations: 21.0 µg As/l (water level 1) and 84.0 µg As/l (water level 2). The samples were analysed in the laboratories in SHI (Slovakia), EHC (Romania), KI-IMM (Sweden), NCPH (Hungary), and KFUG (Austria).

Table 26. 4th Round Robin: reported arsenic concentration in the two different water samples.

Country	Water level 1	Water level 2
EHC Romania	20.9 ± 0.731	90.9 ± 2.485
KFUG Austria	21.4 ± 0.8	82.8 ± 2.9
SHI Slovakia	22.1 ± 0.76	84.4 ± 3.59
KI-IMM Sweden	22.0 ± 0.6	90.0 ± 1.3
NCPH Hungary	21.3	85.2

The overall mean of the reported concentrations was 21.5± 0.5 µg As/l and 86.7 ± 3.7 µg As/l, respectively. According to the Dixon and Grubbs outlier tests all results for water level 1 and water level 2 are acceptable. Also the overall mean agree very well with the added concentrations.
Urine samples: After the discussions during the PI meeting in Poiana Brasov it was agreed that all the urine samples will be analysed at KFUG for the arsenic speciation because of the low concentrations found in the first set of samples. The methods used at KI-IMM and SHI are not capable to analyse the arsenic species at these low concentrations. The quality control will be reversed that means after analysing the samples at KFUG for the arsenic species, a subset of samples (~10%) with higher arsenic concentrations will be analysed in KI-IMM and SHI. Therefore, the urine samples were this time spiked with higher concentrations. The urine level 1 sample was spiked to 12.0 µg As/l as As(V), 14.0 µg As/l as As(III), 32.0 µg As/l as MA, and 40.0 µg As/l as DMA. The urine level 2 was

spiked spiked to 18.0 µg As/l as As(V), 20.0 µg As/l as As(III), 40.0 µg As/l as MA, and 70.0 µg As/l as DMA. The samples were analysed in the laboratories in SHI (Slovakia), KI-IMM (Sweden), and KFUG (Austria).

Table 27. 4th Round Robin: reported arsenic concentration in the two different urine samples.

Country	Sample	Inorg. As	DMA [µg/L]	MA [µg/L]
KFUG Austria	Urine level 1	25.6 ± 1.5	39.5 ± 0.5	31.3 ± 0.6
	Urine level 2	35.6 ± 2.0	65.7 ± 0.6	37.2 ± 2.9
SHI Slovakia	Urine level 1	28.8	43.4 ± 5.8	28.0 ± 2.6
	Urine level 2	38	77.5 ± 3.4	38.5 ± 3.8
KI-IMM Sweden	Urine level 1	22	34 ± 2	33 ± 2
	Urine level 2	33	56 ± 3	41 ± 4

The results obtained from KFUG and SHI for inorganic arsenic in urine level 1 are in good agreement with the spiked concentration. The concentration reported by KI-IMM was about 15% lower. The situation for DMA was quite similar. The value reported from KI-IMM was again 15% lower than the spiked concentration. The concentrations reported for MA were within the agreed range. A similar situation was observed for the results obtained for urine level 2.

3.5 RELATIONSHIP BETWEEN WATER AND URINE ARSENIC

Arsenic concentrations in water and urine, and their relevance for exposure assessment

In the evaluation of associations between arsenic exposure and cancer risk, the lifetime exposure to arsenic is based on the concentration in the drinking water. The estimates of arsenic exposure based on water arsenic concentrations were validated by comparing the concentrations of arsenic in the currently used water with the urinary arsenic concentrations. The latter is considered a good indicator of ongoing exposure. Following exposure to inorganic arsenic, the half-time in the body is 3-4 days (NRC 1999).

Urine-water relationship

There was a significant correlation between arsenic in urine and arsenic in the currently used water (Linear regression: $\ln SumAs = 0.9561 + 0.4262 \cdot \sqrt{curr\ waterconc}$; $R^2=0.48$, $p<.001$). The intercept (where the water concentration theoretically is 0 µg/L) was 2.60 µg/L, indicating exposure from other sources, e.g. via food. This is also supported by the high concentrations of arsenic in urine compared to that in the water; the ratio between median urinary arsenic and median water arsenic was 5.3. Usually, this ratio is 1-2.

3.6 INTERINDIVIDUAL VARIATION IN ARSENIC METABOLISM

Urinary metabolites of inorganic arsenic

Overall, about 7% of the urinary arsenic was inorganic arsenic, 16% MA, and 77% DMA. Compared to previous reports on populations exposed to arsenic via drinking water (Vahter 2002), the present

study shows a lower percentage of inorganic arsenic and a higher percentage of DMA. That was mainly due to intake of DMA via food (see below).

Exposure level

The relative distribution of urinary arsenic metabolites was clearly related to the exposure level. The %DMA decreased significantly with dose**.** It is well known that acute arsenic poisoning decreases the methylation rate due to inhibition or saturation of the enzymes involved in the metabolism of arsenic (reductases and methyltransferases; GST?) (Csanaky et al. 2003). Also, a few reports have shown that in people exposed to high concentrations of arsenic in drinking water, the percentage of urinary DMA decreased and that of MA increased slightly with increasing exposure (Hopenhayn-Rich et al. 1996; Del Razo et al. 1997; Goering et al. 1999). However, those levels were much higher than in the present study. In the present study there was a cluster of 100% DMA values at the lowest water arsenic concentrations, and the main decrease in %DMA was around 2 µg/L. This might be due to influence from intake of DMA via food. The intercept between Sum As plotted against current water concentration was 2.6 µg/L, indicating a significant contribution of arsenic from food at the low exposure levels. Previous studies have shown exposure to DMA in food, or arsenosugars in food, (see WP 2), which are metabolised to DMA in the body (Francesconi et al. 2002), which may explain elevated DMA percentages in the low concentration range.

The detection limits for the arsenic compounds using HPLC-HG-ICPMS were 0.1 µg As/l for As(III), DMA, and MA and 0.5 µg As/l for arsenate. Assuming a DMA concentration of 0.5 µg As in a urine sample and a normal distribution of arsenic species in the urine 60-70% DMA, 10-20% MA and 10-30% inorganic arsenic falsified methylation patterns would be obtained. DMA is still above the detection limit whereas the other arsenicals (As(III), MA(V), and As(V) would be below the detection limit of the method. This would always result in 100% DMA which can be seen in quite a few of the samples.

Because of the influence of food and analytical precision on the percent of DMA in the low concentration range, we decided to evaluate other factors influencing the metabolite pattern only at concentrations above 2 µg/L, at which the influence from food was less obvious. Thus we

subtracted all Sum As concentrations with 2 µg/L in the further analysis. The sample size after this exclusion is 1168 individuals. Figure 3 and 4 shows the lowess fit (a local regression model is fit to each point and the points close to it to give a view of the overall relationship between x and y variables) between Sum As and %DMA before subtracting with 2 µg/L and after, respectively.

Figure 6. **Relationship between Sum As and %DMA in all 1456 samples. (Lowess fit)**

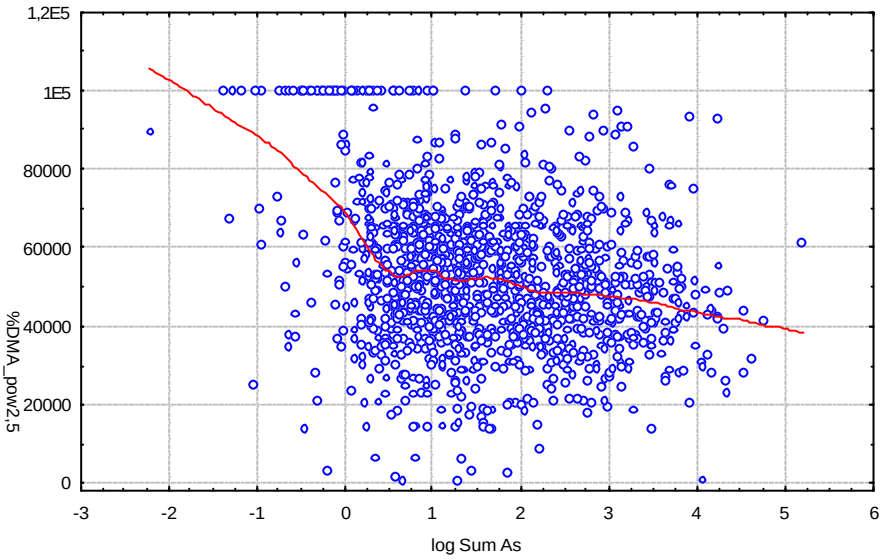
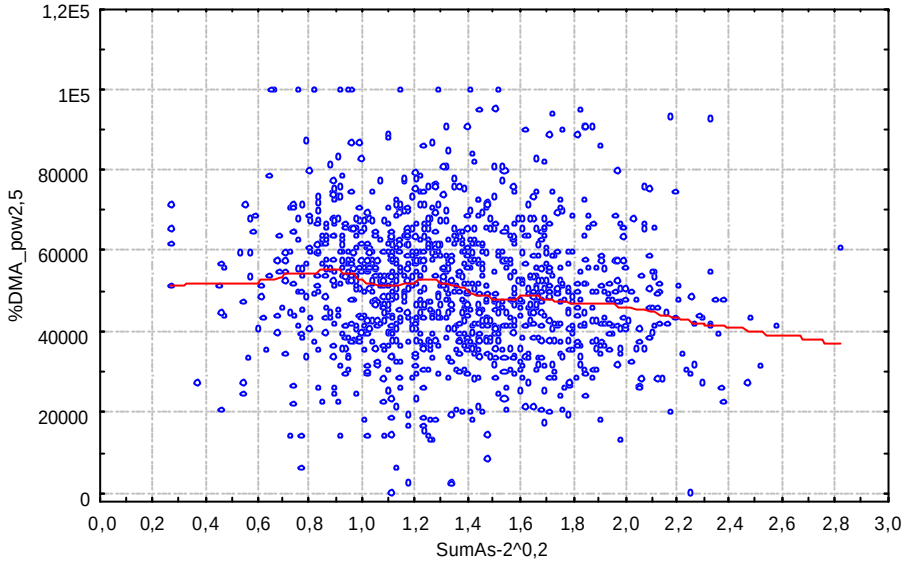


Figure 7. Relationship between Sum As subtracted by 2 µg / L and %DMA in 1168 samples. (Lowess fit)



Factors influencing the metabolism

The exposure and metabolite data were not normally distributed. Therefore, Spearman's rank order correlation test was used to test for univariate associations between variables. The relative amount of DMA in urine, expressed as the percent of total urinary arsenic (%DMA) was correlated to geographical area, skin and bladder cancer, gender, BMI and total urinary arsenic. Borderline correlations were found between %DMA and selenium (p=0.09) and polymorphism on exon 7 of MTHFR gene (p=0.09). The relative amoun of MMA (%MA), was correlated to country, skin cancer, gender, BMI and total urinary arsenic. Inorganic arsenic, %iAs, was correlated to country, bladder and kidney cancer, gender, age, selenium and total urinary arsenic. Borderline correlations were found between %iAs and polymorphisms on exon7 of MTHFR gene (p=0.07) and of GSTO1 gene (p=0.09).

Multiple linear regression was conducted, with transformed variables to meet the requirements of normally distributed residuals, to test for associations between variables. In tables 7, 8 and 9 the results of the multiple linear regression models are presented. Six different factors were identified to influence on the metabolite pattern in urinary arsenic; gender, diagnosis, BMI, age, Sum As and selenium. The results of the present study showed a significant difference between men and women. In accordance to other studies men had higher %iAs and %MA and lower %DMA than women (Hopenhayn-Rich et al. 1996; Concha et al. 1998a). In contrast to other studies the cancer cases in the present study had lower %iAs and %MA and higher %DMA than controls. This might be due to change of water source after being diagnosed since the exposure was clearly influencing the metabolite pattern of urinary arsenic. There are several studies indicating that nutritional status might influence both arsenic metabolism and toxicity. In animal experiments, a reduced methylation capacity, e.g. by a low intake of protein, choline or methionine resulted in a decreased arsenic methylation and a marked increase in tissue retention (Vahter and Marafante 1987). In addition several studies have indicated that people with poor nutrition is more susceptible to arsenic toxicity than others (Chen, C. J. et al. 1988; Hsueh et al. 1995; Guha Mazumder et al. 1998; Milton et al. 2004; Mitra et al. 2004). Although the BMI in the present study (average=26) did not indicate poor nutrition, there was an increase in %DMA and decrease in %MA and %iAs with increasing BMI. There was no major changes in arsenic metabolites with age - there was only a slight decrease in %iAs with increasing age, indicating a small increase of methylation efficiency with increasing age.

Table 28. **Results from the multivariate model of %iAs (adj. R2=0.05). Variables influencing %iAs are diagnosis, gender, age and BMI.**

BMI=26 (average) Age=47 (10th precentile)			BMI=26 (average) Age=65 (average)			BMI=26 (average) Age=76 (90th percentile)		
%iAs	**Man**	**Woman**	**%iAs**	**Man**	**Woman**	**%iAs**	**Man**	**Woman**
Control	11.0	9.1	Control	9.7	8.0	Control	9.0	7.5
Bladder	8.6	7.1	Bladder	7,6	6.3	Bladder	7.0	5.8
Skin	10.2	8.4	Skin	8.9	7.4	Skin	8.3	6.9
Kidney	7.8	6.4	Kidney	6.9	5.7	Kidney	6.4	5.3

Age=65 (average) BMI=22 (10th precentile)			Age=65 (average) BMI=26 (average)			Age=65 (average) BMI=33 (90th percentile)		
%iAs	**Man**	**Woman**	**%iAs**	**Man**	**Woman**	**%iAs**	**Man**	**Woman**
Control	10.1	8.4	Control	9.7	8,0	Control	9.1	7.5
Bladder	7.9	6.5	Bladder	7.6	6,3	Bladder	7.1	5.8
Skin	9.4	7.7	Skin	8.9	7,4	Skin	8.4	6.9
Kidney	7.1	5.9	Kidney	6.9	5.7	Kidney	6.4	5.3

Table 29. **Results form the multivariate model of %MA (adj. R^2=0.05). Variables influencing %MA are diagnosis, gender, BMI and SumAs.**

SumAs-2=12 (average) BMI=22 (10th percentile)			SumAs-2=12 (average) BMI=26 (average)			SumAs-2=12 (average) BMI=33 (90th percentile)		
%MA	**Man**	**Woman**	**%MA**	**Man**	**Woman**	**%MA**	**Man**	**Woman**
Control	18.7	17.1	Control	17.7	16.2	Control	16.1	14.6
Bladder	16.9	15.4	Bladder	15.9	14.4	Bladder	14.3	12.8
Skin	17.5	16.0	Skin	16.6	15.1	Skin	14.9	13.5
Kidney	18.5	17.0	Kidney	17.6	16.1	Kidney	15.9	14.4

BMI=26 (average) SumAs-2=1 (10th percentile)			BMI=26 (average) SumAs-2=12 (average)			BMI=26 (average) SumAs-2=30 (90th percentile)		
%MA	**Man**	**Woman**	**%MA**	**Man**	**Woman**	**%MA**	**Man**	**Woman**
Control	17.4	15.9	Control	17.7	16.2	Control	18.2	16.7
Bladder	15.6	14.1	Bladder	15.9	14.4	Bladder	16.5	15.0
Skin	16.2	14.8	Skin	16.6	15.1	Skin	17.1	15.6
Kidney	17.2	15.7	Kidney	17.6	16.1	Kidney	18.1	16.6

Table 30. **Results form the multivariate model of %DMA (adj. R^2=0.06). Variables influencing %DMA are diagnosis, gender, BMI, SumAs and Selenium.**

SumAs-2=12 (average) BMI=22 (10th percentile) Se=100 (average)			SumAs-2=12 (average) BMI=26 (average) Se=100 (average)			SumAs-2=12 (average) BMI=33 (90th percentile) Se=100 (average)		
%DMA	**Man**	**Woman**	**%DMA**	**Man**	**Woman**	**%DMA**	**Man**	**Woman**
Control	72.0	75.1	Control	73.3	76.4	Control	75.7	78.6
Bladder	75.6	78.5	Bladder	76.9	79.8	Bladder	79.1	81.9
Skin	73.9	76.9	Skin	75.2	78.2	Skin	77.5	80.3
Kidney	74.7	77.6	Kidney	76.0	78.9	Kidney	78.2	80.5

BMI=26 (average) SumAs-2=1 (10th percentile) Se=100 (average)			BMI=26 (average) SumAs-2=12 (average) Se=100 (average)			BMI=26 (average) SumAs-2=30 (90th percentile) Se=100 (average)		
%DMA	**Man**	**Woman**	**%DMA**	**Man**	**Woman**	**%DMA**	**Man**	**Woman**
Control	74.0	77.0	Control	73.3	76.4	Control	72.3	75.4
Bladder	77.5	80.3	Bladder	76.9	79.8	Bladder	75.9	78.9
Skin	75.8	78.7	Skin	75.2	78.2	Skin	74.2	77.2
Kidney	76.6	79.5	Kidney	76.0	78.9	Kidney	75.0	78.0

BMI=26 (average) Se=76 (10th percentile) SumAs-2=12 (average)			BMI=26 (average) Se=100 (average) SumAs-2=12 (average)			BMI=26 (average) Se=129 (90th percentile) SumAs-2=12 (average)		
%DMA	**Man**	**Woman**	**%DMA**	**Man**	**Woman**	**%DMA**	**Man**	**Woman**
Control	74.1	77.1	Control	73.3	76.4	Control	72.5	75.6
Bladder	77.6	80.4	Bladder	76.9	79.8	Bladder	76.1	79.0
Skin	75.9	78.8	Skin	75.2	78.2	Skin	74.4	77.4
Kidney	76.7	79.5	Kidney	76.0	78.9	Kidney	75.2	78.1

Two genes which are thought to be involved in arsenic metabolism were investigated in the present study. Glutathione S-transferase omega 1 (GSTO1) has been suggested to be the MA(V) reductase, and methylenetetrahydrofolate (MTHFR) is involved in the folate dependent remethylation of homocystein to methionine. The results of the linear regression models are shown in table 10. A mutation in the GSTO1 gene seems to decrease the %iAs, although the %MA and %DMA were not significantly affected. Mutations on both exon 4 and 7 of the MTHFR gene was associated with an increase in %MA (almost 20% increase) and a corresponding decrease in %DMA. No difference was seen when only one of the two exons had a mutation.

3.7 INDICES OF EXPOSURE TO ARSENIC

3.7.1 Residential exposure to arsenic

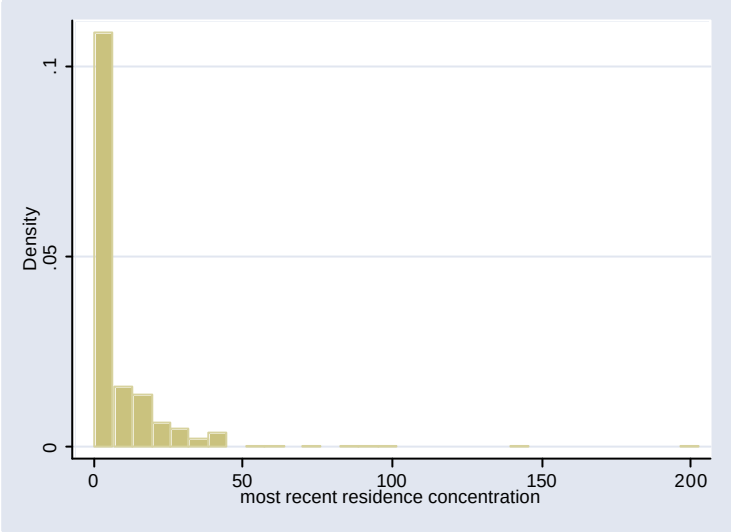
Of the several possible indices of exposure to arsenic in drinking water, four are presented here (Table 31 and Figures 8, 9, 10,and 11). The current As concentration is related to current As intake, and it is the most suitable index to be studied in relation to urinary As concentration, as the latter reflects exposure over the preceding hours and days. Life time average As concentration, cumulative As dose, and peak As daily dose are all representations of exposure plausibly related to cancer, and they were suitable for use in the statistical analyses of cancer risk.

Table 31. Indices of residential exposure to arsenic in drinking water in the ASHRAM participants

Country	Summary statistics				
	Number of observations	**Minimum**	**Maximum**	**Mean (sd)**	**Median**
		Current As concentration (micrograms/liter)			
Hungary	524	0.10	143.0	14.61	13.26
Romania	458	0.06	196.0	3.80	0.70
Slovakia	486	0.01	39.06	1.91	0.84
Overall	1468	0.01	196.0	7.03	1.00
		Life time average As concentration (micrograms/liter)			
Hungary	528	0.11	167.29	26.83	18.03
Romania	460	0.06	196.00	4.02	0.70
Slovakia	485	0.01	143.84	3.54	0.84
Overall	1473	0.01	196.0	12.03	1.16
		Cumulative As dose (grams)			
Hungary	517	0.02	47.22	7.35	4.69
Romania	458	0.01	70.43	1.07	0.20
Slovakia	482	0.00	43.53	0.78	0.24
Overall	1457	0.0004	7.04	0.32	0.036
		Peak As daily dose (micrograms/day)			
Hungary	524	0.20	242.14	43.78	27.35
Romania	460	0.07	250.43	5.10	0.85
Slovakia	487	0.09	270.00	6.34	1.30
Overall	1471	0.07	270.00	19.29	2.14

Figure 8. **Distribution of current As concentration (micrograms/liter). A shows the un-transformed values, and B the log-transformed values.**

A



B

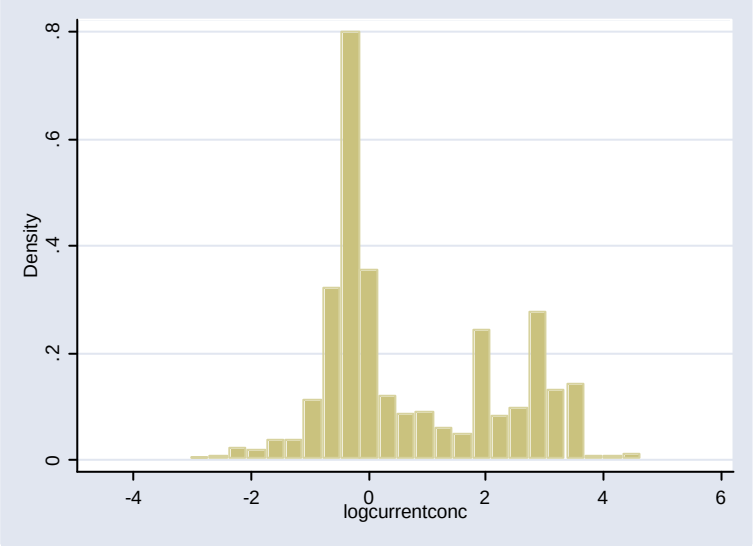
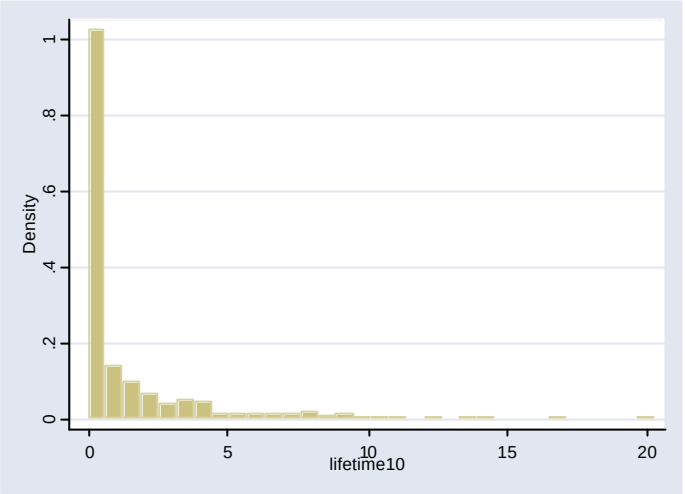


Figure 9. **Distribution of lifetime average As concentration (10 micrograms/liter). A shows the un-transformed values, and B the log-transformed values.**

A



B

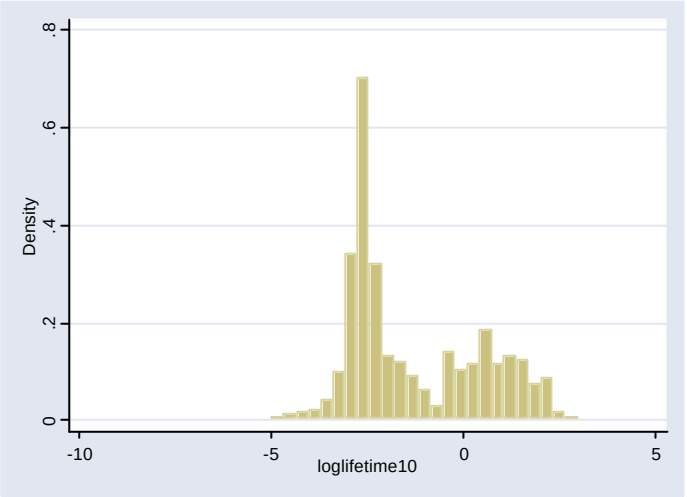
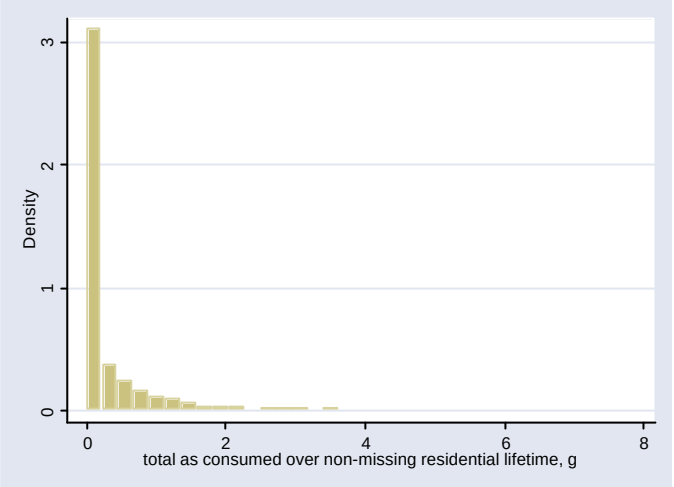


Figure 10. **Distribution of cumulative As dose (grams). A shows the un-transformed values, and B the log-transformed values.**

A



B

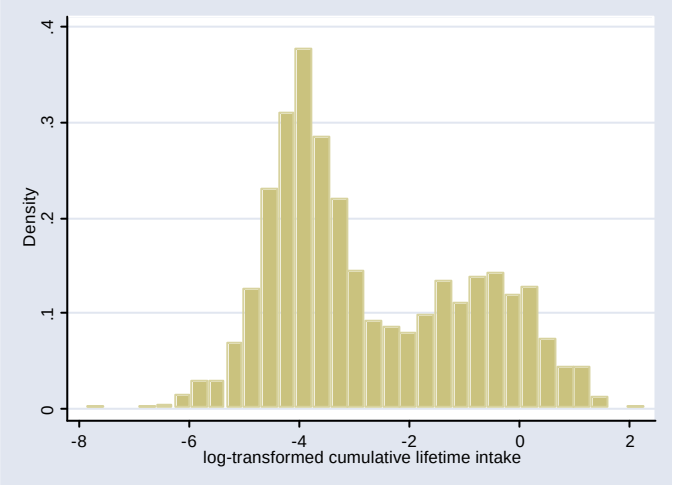
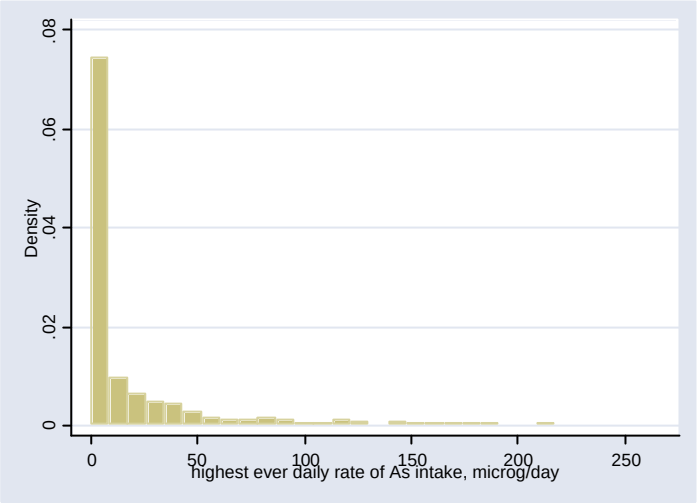
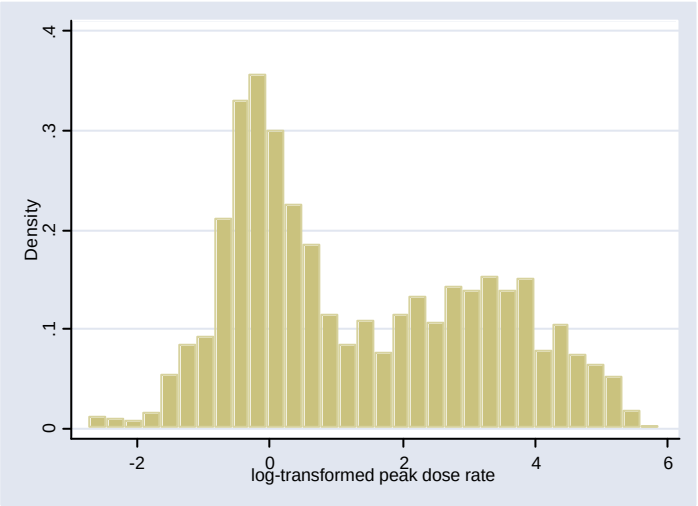


Figure 11. **Distribution of peak As daily dose (micrograms/day). A shows the un-transformed values, and B the log-transformed values.**

A



B



3.7.2 Occupational exposure to arsenic

Expert assessment of occupational exposure to arsenic and other risk factors for skin, bladder, and kidney cancer was conducted on the ASHRAM population, based on information from the main

questionnaire. The agreement of the expert coders assessing exposures of individual jobs was monitored during workshops in the course of the study, and was judged adequate to provide information comparable across the three study countries. Data management of occupational exposure data was postponed due to the large effort in terms of resources required to complete the construction and validation of several exposure indices based on residential exposure. Therefore, initial case-control analyses of arsenic risk have not included occupational exposure information.

3.7.3 Exposure to arsenic via food

The total arsenic concentrations found in the food items from Slovakia are listed in the following tables.

Table 32. Arsenic content in selected food items

Sample Name	[ug As / kg]
Bread 1.1	3.7
Bread 2.1	8.6
Bread 3.1	9.7
Bread 4.1	25.7
Bread 5.1	29.1
Bread 6.1	10.2
Bread 7.1	8.5
Bread 8.1	11.7
Bread 9.1	6.6
Bread 10.1	9.3
Bread 11.1	8.6
Bread 12.1	10.9
Mean	**11.9**
DEV	**7.6**

Sample Name	[ug As / kg]
Rice 1.2	184
Rice 2.2	48
Rice 3.2	256
Rice 4.2	130
Rice 5.2	147
Rice 6.2	183
Rice 7.2	166
Rice 8.2	199
Rice 9.2	129
Rice 10.2	179
Rice 11.2	137
Rice 12.2	141
Mean	**158.3**
DEV	**50.2**

Sample Name	[ug As / kg]
Milk 1.3	0.8
Milk 2.3	0.5
Milk 3.3	0.7
Milk 4.3	0.5
Milk 5.3	0.6
Milk 6.3	0.6
Milk 7.3	0.5
Milk 8.3	0.6
Milk 9.3	0.5
Milk 10.3	0.7
Milk 11.3	0.6
Milk 12.3	0.4
Mean	**0.6**
DEV	**0.1**

(the Table continues on the following page)

(Table 32 continuation)

Sample Name	[ug As / kg]		Sample Name	[ug As / kg]		Sample Name	[ug As / kg]
Pork meat 1.4	1.8		Chicken meat 1.5	24.6		Cabbage 1.7	0.4
Pork meat 2.4	2.2		Chicken meat 2.5	35.4		Cabbage 2.7	0.9
Pork meat 3.4	14.1		Chicken meat 3.5	46.3		Cabbage 3.7	0.7
Pork meat 4.4	49.7		Chicken meat 4.5	3.7		Cabbage 4.7	0.3
Pork meat 5.4	2.3		Chicken meat 5.5	3.7		Cabbage 5.7	1.2
Pork meat 6.4	2.6		Chicken meat 6.5	38.4		Cabbage 6.7	1
Pork meat 7.4	2.7		Chicken meat 7.5	27.5		Cabbage 7.7	0.4
Pork meat 8.4	2.4		Chicken meat 8.5	28.2		Cabbage 8.7	0.3
Pork meat 9.4	4.7		Chicken meat 9.5	9.6		Cabbage 9.7	0.8
Pork meat 10.4	1.8		Chicken meat 10.5	34.3		Cabbage 10.7	2.1
Pork meat 11.4	0.9		Chicken meat 11.5	55.1		Cabbage 11.7	1.2
Pork meat 12.4	3.5		Chicken meat 12.5	32.8		Cabbage 12.7	0.9
Mean	**7.4**	7.39	**Mean**	**28.3**	28.30	**Mean**	**0.9**
DEV	**13.8**	13.77	**DEV**	**16.0**	16.03	**DEV**	**0.5**

Sample Name	[ug As / kg]		Sample Name	[ug As / kg]
Potatoes 1.6	24		Potatoes peeled 1.6	2.4
Potatoes 2.6	16.3		Potatoes peeled 2.6	1.2
Potatoes 3.6	48		Potatoes peeled 3.6	0.8
Potatoes 4.6	27.3		Potatoes peeled 4.6	0.6
Potatoes 5.6	26.5		Potatoes peeled 5.6	11.6
Potatoes 6.6	97.9		Potatoes peeled 6.6	1.9
Potatoes 7.6	21.4		Potatoes peeled 7.6	0.5
Potatoes 8.6	30.8		Potatoes peeled 8.6	1.3
Potatoes 9.6	32.9		Potatoes peeled 9.6	0.4
Potatoes 10.6	27.6			
Potatoes 11.6	8.1			
Potatoes 12.6	35.7			
Mean	**33.0**		**Mean**	2.3
DEV	**22.7**		**DEV**	3.6

As expected the rice samples contained the highest total arsenic concentration. It is well known that rice accumulates arsenic (D'Amato et al. 2004, Heitkemper et al. 2001). The arsenic speciation in rice can vary a lot. Heitkemper et al 2001 found inorganic arsenic to account for 11-91% of the extractable arsenic, while the rest was DMA. In the study of D'Amato et al. 2004 a similar picture was obtained. Inorganic arsenic was dominant followed by DMA and small amounts of MA.

The other investigated food items showed generally low total arsenic concentrations with the exception of the chicken meat. These findings were rather surprising. A possible explanation could be fish meal fed to the chickens. Therefore we analysed the chicken samples for arsenobetaine (AB) a typical marker for fishmeal.

Table 33. Arsenobetaine in chicken

Sample name	AB [ug As / kg]	Total Arsenic [ug As / kg]	% AB of As total
Chicken meat 1.5	25.2	24.6	103
Chicken meat 2.5	35.4	35.4	99.8
Chicken meat 3.5	42.7	46.3	92.1
Chicken meat 4.5	b.d.	3.7	/
Chicken meat 5.5	b.d.	3.7	/
Chicken meat 6.5	33.1	38.4	86.3
Chicken meat 7.5	25.8	27.5	93.8
Chicken meat 8.5	26.0	28.2	92.2
Chicken meat 9.5	4.8	9.6	49.7
Chicken meat 10.5	29.2	34.3	85.2
Chicken meat 11.5	59.9	55.1	109
Chicken meat 12.5	33.3	32.8	102

In all the chicken meat samples with a total arsenic concentration above 20 µg As/kg arsenobetaine accounted for more than 80% of the extractable arsenic. These findings support the idea that fishmeal was used for feeding the animal. As arsenobetaine is not forming a hydride it does not contribute to the arsenic species determined in the urine samples.

Arsenic and its compounds in fish samples from Hungary

Fish is popular in Hungary. As not only marine fish is accumulating arsenic we have investigated arsenic and the arsenic speciation in fish samples from the Ashram study area as well as from outside the area (control samples). The arsenic species were determined by HPLC-ICPMS on methanol

extracts of the muscle tissue from the fish. Catfish (Claries gariepinus) were raised in geothermal water where the average total arsenic concentrations were 161 µg As /l (contaminated sites) and 15.1 µg As /l (control); they were all fed an artificial diet containing 2880 µg As /kg total arsenic, mostly present as arsenobetaine. In the catfish the accumulated total arsenic (2370-5040 µg As/kg) was found mostly in the form of arsenobetaine suggesting that uptake of arsenic was dominated by their diet. Carp (Cyprinus carpio) was cultured in surface lakes with no significant arsenic pollution, and had total arsenic concentrations ranging from 52.3-344 µg As /kg. A big portion of this arsenic, however, was not extractable into methanol, in contrast to the catfish samples where essentially quantitative extraction into methanol was achieved. It is very likely that the arsenic compounds in the carp samples are lipid soluble arsenicals of which not much is known until now (Schmeisser et al. 2005).

The arsenic species in the carp differed markedly from those in the catfish in that no arsenobetaine was detected. Most samples of carp from the investigated sites contained low concentrations of As(III), As(V), MA, and DMA, and no other compounds were detected. The four individuals from the control site, however, all contained appreciable levels of an arsenosugar (phosphate arsenosugar). Indeed, this arsenic species dominated the speciation pattern for these carp representing about 80% of the sum of species. The contrast between these two freshwater aquaculture species regarding total arsenic and arsenic species has relevant toxicological aspects in terms of food safety. The catfish, despite appreciable concentrations of total arsenic, present no human health concern because almost all the arsenic is present as the harmless arsenobetaine. In the case of carp, the total arsenic concentrations are so low that even if the arsenic were all present as potentially toxic forms it would not represent a health risk to human consumers of these fish.

Comparison of arsenic intake from food in ASHRAM and other studies

In Table 34, the arsenic content of the seven items analysed for this study, are shown in comparison with the arsenic content of the same items determinate in other studies: the Slovakian Monitoring System (Krizova et al, 2001, 2002, 2004a 2004b, Salgovicova et al 2002a, 2002b, 2002c, Svetlikova et al, 2002), the PHARE project, a study on the health impact of environmental pollution, carried out in Slovakia in 1994 (Proceedings of PHARE project, 1995) and a market basket study carried on in the United States (Schoof et al, 1999). For the Slovakian Monitoring System and the PHARE project

information about sampling plan and analytical methods are incomplete. The highest total arsenic concentrations occurred in rice (158.3 µg/kg) and chicken, meat (28.3 µg/kg) as reported by other studies (Schoof, 1999, Tao et al, 1999, Dakeba et al, 1993) and they can result to be main contributor to the total arsenic intake. Potatoes with skin result to be rich in arsenic, due to the contact with the soil during growing, but it turns to contain a lower content if peeled, as reported by Schoof and collaborator (Schoof, 1999). Nevertheless, the variability in arsenic concentrations of the data reported can be directly related to the different analytical method, the different arsenic concentration in the soil (geographical area) where food was grown and also the accumulative capacity of food (Delgado-Andrade et at, 2003). Differences in results may be due to both differences in geographical areas and sampling or analytical methods.

Table 34. Arsenic in food items, comparison of ASHRAM and other sources

Food Items	ASHRAM, project mean (µg/kg)	Slovakian MS$ mean (µg/kg)	PHARE, project 1995& mean (µg/kg)	Schoof et al, 1999 # mean (µg/kg)
Bread, dark	11.9	20.31£	31£	-
Cabbage	0.9	7.92	- **	#
Chicken, meat	28.3	10.47	17	86.4
Milk, cow	0.6	10.16	26	2.6~ - 1.8^
Pork, meat	7.4	11.55	- *	13.5
Potatoes, with skin	33.0	11.58	58	-
Potaotes, peeled	2.3	-	-	2.8
Rice	158.3	34.43	35	303

$ Slovakian MS = monitoring System, by Ministry of Agriculture, Slovakia 1993-2003

& PHARE project: Study in the health impact of environmental pollution, Slovakia, 1995

Schoof et al, 1999: Market basket survey of As in food.

£ Unspecified bread

** Fresh vegetables = 42 µg/kg

#: Lettuce = 1.4µg/kg and Spinach = 5.1µg/kg

~ Skimmed milk

^ Whole milk

* Meat, unspecified = 42 µg/kg

3.8 NUTRITION SURVEYS

This section describes the work carried out in order to develop a database on intake of nutrients possible modifying the effect of arsenic on cancer, and the methodological developments underlying the construction of such database. The results concern first, the descriptive results from the FFQ, second, the validation and repeatability studies, third, the work to compile a database of nutrient intake for ASHRAM participants, and fourth, descriptive results of selenium analyses.

3.8.1 Nutritional status of participants

In Table 35 is presented the average body mass index (BMI) of participants of this study that is calculated as weight kg/ height m^2. Weight and height has been collected during interviews and they refer to two year before the cancer diagnosis for case or hospital admission for controls. BMI is 26.79 (median is 26.44 - data not shown in the table) showing that our population is above the normal range of weight (BMI 18.5-24.9). In Table 36 subjects has been grouped in 3 sets of BMI. The number of underweight defined as BMI < 18.5 is very low, only 0.6%, whereas overweight/obese subjects, defined as BMI above 25, is high: 63.1% of the entire population. Male present a higher percentage than the female: 64.2% and 61.9% respectively. Cases present a slightly lower BMI (62.9) than controls (63.5).

In Table 37 subjects have been grouped by BMI, by sex and by country of origin. Romania presents the highest percentages of subjects with normal weight: male 42.8% and female 45.2. BMI is higher in Hungary and Slovakia, in particular between female 62.3% in Hungary and 69.7% in Slovakia, compared with published data, 49.1% and 51% respectively (International Obesity task force, 2005). The percentages of underweight are very low in all three countries.

Table 35. **Mean, SD, Minimum, Maximum of BMI in total subjects, by sex and by cases and controls.**

	BMI #				
	N. (%)	**Mean**	**SD**	**Min**	**Max**
Overall	1462	26.79	4.22	17.30	42.91
Male	798 (54.6)	26.78	3.93	17.75	10.90
Female	664 (45.4)	26.80	4.54	17.30	42.91
Cases	931 (63.7)	26.68	4.06	17.30	42.91
Controls	531 (36.3)	26.98	4.48	17.58	41.59

BMI =weight kg/ height m^2

Table 36. **Participants of the study grouped in three set of BMI, by sex and by cases and controls.**

	Overall		**Male**		**Female**	
BMI	**N.**	**%**	**N.**	**%**	**N.**	**%**
Overall						
< 18.49	9	0.6	4	0.5	5	0.8
18.5 – 24.99	530	36.3	282	35.3	248	37.3
> 25	923	63.1	512	64.2	411	61.9
Tot	1462	100.0	798	100.0	664	100.0
Cases						
< 18.49	4	0.4	2	0.4	2	0.5
18.5 – 24.99	341	36.6	184	35.1	157	38.7
> 25	586	62.9	339	64.6	247	60.8
Tot	931	100.0	525	100.0	406	100.0
Controls						
< 18.49	5	0.9	2	0.7	3	1.1
18.5 – 24.99	189	35.6	98	35.9	91	35.3
> 25	337	63.5	173	63.4	164	63.6
Tot	531	100.0	273	100.0	258	100.0

Table 37. **Participants of the study grouped in three set of BMI, by sex and by country of origin.**

	Hungary		Romania		Slovakia	
	Male	Female	Male	Female	Male	Female
BMI	N. (%)	N. (%)	N. (%)	N. (%)	N. (%)	N. (%)
Overall						
< 18.49	0 (0)	3 (1.3)	2 (0.8)	2 (0.9)	2 (0.7)	0 (0)
18.5 – 24.99	91 (32.3)	86 (36.4)	104 (42.8)	98 (45.2)	87 (31.9)	64 (30.3)
> 25	191 (67.7)	147 (62.3)	137 (56.4)	117 (53.9)	184 (67.4)	147 (69.7)
Tot	282 (100)	236 (100)	243 (100)	217(100)	273 (100)	211(100)

3.8.2 Results from methods development: reproducibility and validation

80 subjects (89%) have completed the two FFQs and ten repeated 24hR. The drop out rate is low: 10 subjects (11%) were not available to complete both FFQs and for interviews. Median interval between the two questionnaires is 6 months.

3.8.3 Development of nutrient database

A food composition database is a key-point in the interpretation of nutritional epidemiology studies, since conflicting results and discrepant findings may be due to the lack or inaccuracy of these data (Willett et al, 1998). A food composition database for the ASHRAM study will be developed in order to analyse the food intake evaluate by FFQ. Data available on the nutrient composition of food will be collected from the three participants countries. At the moment, The Slovak Food Composition Table (Food Research Institute, 2002) is used to calculate the composition of the diet in terms of total energy and macronutrients, micronutrients for the past and current diet. Nutrients available will be checked and missing values will be covered using data coming from other sources (Salvini et al, 1996).

3.8.4 Selenium measurements in blood

Selenium status

Since selenium has been shown to influence the metabolism and toxicity of arsenic (NRC 1999), the selenium status were determined in this population. Blood selenium concentrations in relation to Ashram countries and areas are shown in Figure 1 and 2. The selenium concentrations do not indicate poor selenium status, and they were slightly higher in the Ashram population than in a population from the Czech Republic (Batáriová et al. 2005). The median blood selenium concentration in the Ashram population was 100 µg/L compared to 82 µg/L in the Czech population.

There was a weak but significant relationship between selenium in whole blood and urinary arsenic (linear regression: $Sum\ As^{0.2} = 1.07 + 0.003 \cdot Se;\ R^2 = 0.02;\ p < 0.001$). This might indicate a co-exposure of arsenic and selenium.

3.9 RESULTS OF CASE CONTROL STUDIES

First, the effect of arsenic on cancer risk is presented from models where an As exposure index is considered as a linear factor, and results were computed with a range of adjustments for possible confounders. Second, As effect is presented from models where As metabolites are considered as effect modifiers.

3.9.1 Effect of arsenic as a linear factor on cancer risk

The effect of arsenic on cancer risk was analysed for three exposure indices: lifetime average As concentration, cumulative As dose, and peak As daily dose rate. Each of these were considered as linear factors. Results of logistic regression models all include country as a fixed effect. The effect of arsenic on BCC, bladder and kidney cancer was estimated first from models including age and gender only ("crude" OR), then from models also including a key further potential confounder. For BCC, skin complexion was the strongest predictor of cancer risk when compared to duration of sun exposure or tendency of skin to burn when exposed to sun. Therefore skin complexion was chosen as the factor representing UV exposure in this population, and included in a model to estimate As effect on BCC. For bladder cancer, a quantitative estimate of number of cigarettes ever smoked over the lifetime was the strongest predictor of bladder cancer risk when compared with other smoking-related variables. Therefore, total number of cigarettes (as categorical variable in two levels) was chosen as the factor representing smoking exposure in this population, and included in a model to estimate As effect on bladder cancer. For kidney cancer, history of kidney infection was chosen as a variable of interest as potential confounder for inclusion in a model estimating As effect on kidney cancer risk. Results of crude and adjusted models are presented in Table 38, 39, and 40.

Table 38. **Results of logistic regression analyses on effect of arsenic exposure on risk of basal cell carcinoma (BCC) in ASHRAM population**

	OR crude	95% CI	OR adjusted	95% CI
Lifetime average As concentration (10 micrograms/L)	1.17	1.08 – 1.27	1.16	1.07 – 1.26
Cumulative As dose (grams)	1.44	1.09 – 1.91	1.42	1.07 – 1.88
Peak As daily dose rate (micrograms/day)	1.0052	1.0007 – 1.0097	1.0046	1.0001 – 1.0092

All three exposure indices are associated with risk of BCC, and are all statistically significant in both the crude and adjusted models. Adjustment for skin complexion reduces slightly the observed effect of As. For bladder cancer, no statistically significant association was found with life time average As concentration, but one was observed when the effect of cumulative As dose is examined, and smoking is taken into account as a confounder.

Table 39. **Results of logistic regression analyses on effect of arsenic exposure on risk of bladder cancer in ASHRAM population**

	OR crude	95% CI	OR adjusted	95% CI
Lifetime average As concentration (10 micrograms/L)	1.01	0.90 – 1.12	1.05	0.94 – 1.17
Cumulative As dose (grams)	1.39	0.98 - 1.99	1.49	1.03 - 2.15
Peak As daily dose rate (micrograms/day)	1.0089	0.9989 - 1.0114	1.0052	0.9989 - 1.0114

Kidney cancer risk appears to have a similar pattern to BCC and bladder cancer in relation to As exposure, though the only statistically significant associations are with peak As exposure.

Table 40. Results of logistic regression analyses on effect of arsenic exposure on risk of kidney cancer in ASHRAM population

	OR crude	95% CI	OR adjusted	95% CI
Lifetime average As concentration (10 micrograms/L)	1.07	0.96 – 1.20	1.08	0.96 – 1.21
Cumulative As dose (grams)	1.55	0.98 - 2.45	2.73	0.92 - 8.09
Peak As daily dose rate (micrograms/day)	1.0074	1.0011 - 1.0138	1.0071	1.0008 - 1.0136

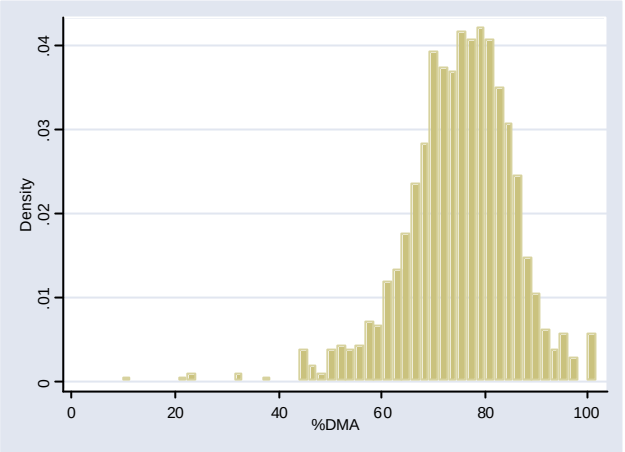
3.9.2 Effect of arsenic on cancer, by two groups of DMA and MMA

To address the objective (2) of the project "to study the effect of inter-individual variation in arsenic metabolism on carcinogenic risk", analyses were conducted looking at effect of arsenic exposure by two groups of DMA% and MMA% : below and above median.

The distribution of DMA % and MMA % is shown in Figure 12 and Table 41.

Figure 12. **Distribution of percentage DMA (A) and percentage MA (B) in ASHRAM participants**

A



B

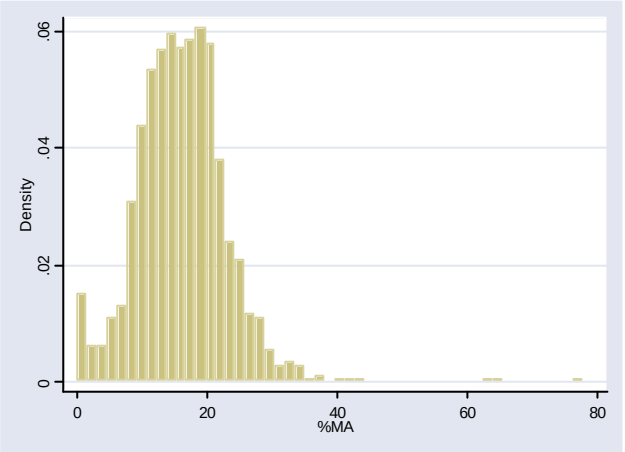


Table 41. **Range of DMA and MA concentration when the distribution of each metabolite concentration is divided in two groups below and above the median value**

	Minimum DMA value	Maximum DMA value	Minimum MA value	Maximum MA value
Metabolite percentage below the median	9.8	75.9	0	15.94
Metabolite percentage above the median	76.0	100	15.95	76.2

Effect of As for each level of metabolite was estimated by logistic regression producing odds ratios and 95% confidence intervals for BCC, bladder and kidney cancer. In addition an odds ratio was computed for the comparison of As effect in highest group vs the lowest group of metabolite, with correspondent 95% CI (this is the OR of the interaction term for the correspondent level). All the analyses control for county, age, gender, and a major possible confounder: skin complexion for BCC, smoking for bladder, and kidney infection for kidney. Analyses were conducted for two indices of As exposure: (i) cumulative dose (grams in lifetime), and (ii) peak dose rate

The pattern is very similar for both As exposure indices. Tables 42 and 43 report the results for cumulative dose and peak dose. There is a pattern in the direction anticipated : effect of As on cancer is stronger in the groups of participants with low DMA% or high MMA%. This pattern is statistically significant for BCC.

In addition, the same models were also estimated having restricted the analyses to those participants for whom the sum of As metabolites is more than 5.5 microgram/L, but the results do not change significantly. Controlling for effect of selenium also does not remove the effect of As. In both cases, the point estimates of arsenic effect as well their confidence intervals can be interpreted in the same way as for the previous analyses.

Table 42. **Effect of As life time exposure by metabolite level. The table reports a comparison of effect of arsenic cumulative dose in grams on cancer between two groups of DMA% and MMA%: below and above the median value. The stratum-specific odds ratios (OR_{ss}) estimate effect of arsenic on cancer as a linear effect, for each of two groups of DMA% and MMA%. The odds ratios comparing As effect in higher metabolite group to As effect in lower metabolite group (OR_{hl}) are shown in the column on the right (interaction between higher and lower group).**

				OR_{ss}	95% CI	OR_{hl} (95% CI)
DMA	**BCC**	DMA%	<76%	1.59	1.13 – 2.23	0.65 (0.41-1.04)
			>=76%	1.04	0.70 – 1.54	p=0.070
	BLADDER	DMA%	<76%	1.66	1.09 – 2.54	0.74 (0.37-1.48)
			>=76%	1.23	0.66 – 2.29	p=0.397
	KIDNEY	DMA%	<76%	1.52	0.82 – 2.84	0.92 (0.46-1.85)
			>=76%	1.41	0.81 – 2.45	p=0.822
MMA	**BCC**	MMA%	<16%	1.14	0.79 – 1.66	1.35 (0.86-2.10)
			>=16%	1.53	1.08 – 2.17	p=0.191
	BLADDER	MMA%	<16%	1.30	0.75 – 2.27	1.21 (0.64 -2.30)
			>=16%	1.58	1.02 – 2.44	p=0.563
	KIDNEY	MMA%	<16%	1.45	0.81 – 2.59	1.06 (0.53-2.14)
			>=16%	1.54	0.85 – 2.80	p=0.587

Table 43. **Effect of As peak exposure by metabolite level. The table reports a comparison of effect of arsenic peak dose rate on cancer between two groups of DMA% and MMA%: below and above the median value. The stratum-specific odds ratios (ORss) estimate effect of arsenic on cancer as a linear effect, for each of two groups of DMA% and MMA%. The odds ratios comparing As effect in higher metabolite group to As effect in lower metabolite group (ORhl) are shown in the column on the right (interaction between higher and lower group).**

				OR_{ss}	95% CI	OR_{hl} (95% CI)
DMA	**BCC**	DMA%	<76%	1.0074	1.0018 – 1.0129	0.9974 (0.9844 -0.9992)
			>=76%	0.9991	0.9929 – 1.0054	p=0.030
	BLADDER	DMA%	<76%	1.0078	1.0002 – 1.0155	0.9936 (0.9825-1.0049)
			>=76%	1.0014	0.9919 – 1.0109	p=0.264
	KIDNEY	DMA%	<76%	1.0075	0.9982-1.0168	0.9985 (0.9882-1.0089)
			>=76%	1.0060	0.9985-1.0135	p=0.776
MMA	**BCC**	MMA%	<16%	1.0001	0.9938 – 1.0064	1.0066 (0.9992-1.0140)
			>=16%	1.0067	1.0011 – 1.0123	p=0.080
	BLADDER	MMA%	<16%	1.0028	0.9940 – 1.0117	1.0038 (0.9931 - 1.0148)
			>=16%	1.0067	0.9988 – 1.0146	p=0.486
	KIDNEY	MMA%	<16%	1.0057	0.9975 – 1.0140	1.0026 (0.9923-1.0129)
			>=16%	1.0083	0.9999 – 1.0166	P=0.624

3.10 RELEVANCE OF RESULTS FOR ARSENIC RISK ASSESSMENT

Arsenic risk assessment is affected by uncertainties due to genetic variations, metabolism variation, and nutrition variation between populations and individuals. Based on the results obtained in ASHRAM, currently available estimates of risk seem warranted (Smith 1992) (see also section 3.12 below for risk assessment in the ASHRAM study area). Based on the data obtained in the ASHRAM study, it will be possible to examine several aspects of arsenic risk assessment in further analyses, in order to further refine risk assessment models.

3.11 RISK MANAGEMENT

This section was prepared by Environmental Health Center, Romania, and reports on work conducted as part of WorkPackage 9

The workpakage (WP) no. 9 was intended to address some of the arsenic exposure related problems in the area investigated under ASHRAM project.

The major objectives of the WP 9 consists of:

1. Problems encountered to what the exposed residents require to change contaminated water intake
2. Developing a central running water system and/or interim mitigation options, such as bottled water
3. Practical solutions based on the integration of the consumer's perspective

During the first phase of the WP 9 were conducted several field surveys in order to identify the no. of localities with arsenic in drinking water related problems (about 100 localities), the total no. of inhabitants in each locality, maps of the area, no. of wells/village, depth of well, age of well, no. of inhabitants drinking water from each well, and other information such as tape water where available and the like.

Some specific local data (example)

SETTLEMENTS (No. of inhabitants)	TYPE OF WATER SUPPLIES (% of people drinking water)	ARSENIC CONC. (mg/L)	DEPTH (m)	AGE (years)
CIUMEGHIU (2,056)	deep well -drilling (75 %)	176	140	> 10
CIUMEGHIU (2,056)	public artesian well (25 %)	166	200	> 30
TAMASEU (2,834)	private well (0.15 %)	102	20	> 30
SEPREUS (2,757)	deep well -drilling (100 %)	99	150	> 50
APATEU (2,600)	deep well -drilling (38.5 %)	84	100	> 50
AVRAM IANCU (2,076)	hydro -power microstation (10 %)	82	180	> 20

Within WP 9 was carried out a field survey in collaboration with Mark Hopson from Imperial College. There were two in country activities (3 days each) and a three weeks collaborating work at the Environmental Health Center (EHC).

The first field visit consisted of:

- meeting with local authorities at the Arad county level
- collecting additional data on drinking water facilities in the investigated villages
- based on EHC and local authorities data there were 3 villages selected to be included in a risk communication/management program

The selected localities were identify based on 3 different problems these locations are facing with in terms of drinking water quality:

- Sepreus –central running water system (CRWS) – relative "good" drinking water quality, with local inhabitants using mostly artesian (high arsenic concentration) water for drinking and cooking purposes
- Zerind – CRWS –"bad" drinking water quality, with local inhabitants using mostly artesian (high arsenic concentration) water for drinking and cooking purposes
- Pilu – lack of CRWS, with local inhabitants using almost only artesian (high arsenic concentration) water for drinking and cooking purposes

Local photos concerning artesian wells, people filling their recipients with artesian water, and other such aspects, were taken during the field work.

The team spent several ours during each visit talking to the people in the area in order to find out about reasons they use the artesian water for drinking and cooking, whether they are aware about arsenic exposure via drinking water and which could be the major related health outcomes, what do they think about measures and decisions on decreasing exposure, cutting the pathway and improve their health status.

During the second visit some time was spent to get information and see the place in order to elaborate a description of the area, the main activities in the area, the major surface water sources in the area, and other such information.

During the work at the EHC was prepared an overview of the methods available for the underground water monitoring, including quotations at the time, and a review of techniques required to remove arsenic from drinking water.

Based on all these activities and reports some mitigation options were elaborated for each of the 3 localities.

1. Sepreus the village with the CRWS, "good" drinking water quality, with local inhabitants using mostly artesian (high arsenic concentration) water for drinking and cooking purposes, was part of several activities undertaken in the area: 3 meetings with local public health

department, family doctors, community representatives, some people from the local community. The discussions were focused on several topics as follow:

- problems on tap water quality
- advantages of using tape water
- quality of deep underground water
- disadvantages of high arsenic concentration in drinking water
- evolution of no. of people using tap water
- costs related to tap water supply
- no. of people bending underground water
- risk communication strategy

2. Zerind the village with the CRWS, "bad" drinking water quality, with local inhabitants using mostly artesian (high arsenic concentration) water for drinking and cooking purposes, was part of several activities undertaken in the area: 3 meetings with local public health department, local water department, local EPA, local government, community representatives. The discussions were focused on several topics as follow:

- improving water quality monitoring program (local Public Health Department)
- discussions about improving water treatment technologies (lack of funds emerged out of the main discussions)

3. Pilu the village without a CRWS, with local inhabitants using almost only artesian (high arsenic concentration) water for drinking and cooking purposes, was part of several activities undertaken in the area: local public health department, local EPA, local family doctors, local community representatives. The discussions were focused on several topics as follow:

- making people aware about arsenic in drinking water health related problems
- use of bottled water (lack of money for some people in the village)
- proposed activities to identify funds for a central drinking water system

After all these activities carried out so far some conclusions were drawn out:

4. local authorities were very much involved in the work done in the area

5. a tremendous need to identify funds for improving water quality and/or implementing a central drinking water system
6. county governor contacted Water Research Center (WRC) in UK – ambiguous answer from WRC
7. it was decided that the results from ASHRAM study will be used by local authorities to seek funds to address major problems with regard to new alternatives for drinking water supply

In collaboration with the Public Health Department in Arad it was elaborated some sort of a training activity for local specialist and public representatives, under a local project.
The project is addressing the following issues:
- a need for further development of a critical mass of specialist within Arad county with relevant training and expertise, to build on develop existing capacity within the villages with high arsenic concentration in drinking water and surroundings
- to develop and apply methods of standardization and quality control for drinking water in health-related arsenic areas, to facilitate harmonization of water quality measurements, control and standard access in the "arsenic villages" and neighborhood communities. This would also improve the internal validity and would provide external validity for comparison of results across the county.
- to develop the capacity for the systematic and scientific appraisal of the problems of drinking water with high arsenic concentration and health status of the population, to allow a realistic appraisal of current problems
- to focus on knowledge based on high arsenic drinking water and health issues of important "client" groups, including public, policy makers, NGO's, etc.
- to assist in communication of factual information on high arsenic drinking water risks to such groups in a way that might have a positive impact on drinking water quality and health policy
- to promote alternatives for "good and safe" drinking water quality

The scope of the project is being to:

- elaborate common methodologies in order to get comparable date bases across the region

- community awareness, communication strategies, social marketing in order to enforce decisions and measures, to improve drinking water quality based on community needs

The major objectives are:

- strengthen the capacity to undertake high quality drinking water monitoring at the county level and in the localities with high arsenic concentration in drinking water and surrounding communities
- establish comparable base-line date on drinking water quality and health in the localities with high concentration of arsenic in drinking water and/or "bad" tap water quality
- prepare a detailed characterization of risk perception in general public, develop, pilot and evaluate local strategies for communication, with a view to encouraging a convergence of views on health impact-based priority setting among key stakeholders and the public

To achieve the above mentioned objectives several activities are needed:

- availability of reliable and comparable data, organizational and methodological guaranties of reliability which consists of: validated procedures, harmonization and standardization, qualified personnel, quality assurance and quality control and auditing
- to integrate QA/QC in drinking water quality monitoring and health related aspects, to optimize the quality of data on drinking water quality measurements, in order to ensure a good infrastructure for risk perception/communication
- to link the risk perception to health in order to contribute to the process of disseminating a trusted health and "good and safe drinking water" message
- to determine goal of communication, target groups, content of the communication, to choose communication methods and means, to make sure the communication is well organized, to evaluate the communication results
- to empower citizens and help them to feel they can have a positive effect and influence on improving drinking water quality
- to accept the public as a valued partner for improving drinking water quality, to create o coalition (citizens, NGO'S, mass media, government, local government, industries, etc.)

which is crucial to the success of a regulatory system. Public has to reinforce the "good and safe drinking water quality" views and ensure that the government does not ignore this major concern

This activities were undertaken during the last year of the ASHRAM project, and will continue to be undertaken using new inputs such as ASHRAM results. The results of the ASHRAM project will contribute to the development of the above mentioned program, and will be very useful to support new applications to access available funds in order to decrease the arsenic exposure via drinking water in the area.

3.12 ESTIMATE OF PUBLIC HEALTH BURDEN

3.12.1 Estimate of public health burden in Hungary

Based on risk assessment for arsenic cancer risk previously published (Smith et al 1992), we computed the fraction of cancer attributable to arsenic in the Hungarian study area. This area includes four Hungarian counties with a population of 1,580,156. We estimated that the overall number of deaths (annually) in this population is 1519, and the arsenic-attributed number of deaths (annually) is 306. This corresponds to a proportion of bladder, kidney, and lung cancer deaths attributable to arsenic in this area of 20.2% (306/1519). The represents a very considerable proportion of all cancer cases in the study region, and it would appear to justify the commitment of the Hungarian Parliament and the efforts of the affected municipalities to implement plans for further reduction of exposure to arsenic in drinking water.

3.12.2 Arsenic exposure in Europe

In 2005 the quantitative reconstruction of ground water arsenic distribution in Europe is difficult due to the great variation in national and regional monitoring systems, and very partial availability of monitoring data in the public domain. Qualitative information about As in ground water across Europe is summarized in this section.

United Kingdom

In the mining areas of Cornwall and Devon, ore processing released As to the atmosphere in particle form. This was then deposited on soil and water. In fields and private gardens As concentration can be above 1000 micrograms/kg. In much of the surface water of Cornwall soluble As ranges between 10 and 50 µg/L. Water treatment with aluminium hydroxide has been used to remove As when present to a concentration of 10 µg/L in the majority of the wells used for abstraction. Processing of arsenopyrite led to arsenic oxides emissions; these form deposits that in time form largely insoluble iron As compounds.

France

Water of several French regions contain As at concentrations above 10 µg/L. In the Auvergne over 100,000 people drink water containing As above 10 µg/L. In the Allier and Puy-de-Dôme districts, As concentrations have a range 10-190 µg/L and 14-27 µg/L, and the exposed population are approximately 32,400 and 87,000, respectively.

Switzerland

There are three areas with elevated As concentration of natural origin: (i) cantons in the North East, with thermal spa waters rich in As; (ii) Jura, with iron deposits in calcar…. and clay; (iii) the Alps, with mineral deposits and crystalline rock strata. The spa thermal springs of Baden, Zurzach, Schinznach, and Bad Saeckingen can contain above 130 µg/L of As. In the Ticino canton, As

concentration in drinking water has been monitored since 1996, and several areas use drinking water with As above 10 µg/L, with small areas reaching an average of 80 µg/L and peaks of 300 µg/L. In Vallese canton, a survey in 1999 found that about 14,000 people are estimated to drink water containing between 12 and 50 µg/L of As.

Germany

In northern Bavaria there is an area of 2,500 km^2 with 160 wells containing As at concentrations between 10 and 150 µg/L. These ground waters are part of Upper Triassic sandstone, and it is thought that As is release via natural processes.

Italy

Several regions in the Po river basin are affected, in the Turin area As concentration ranges to 80 µg/L, in the Ostiglia district it reaches above 50 µg/L, and in several provinces in the central Padana plain As is above 10 µg/L. In the Veneto region, a 1995 survey of high risk Comuni identified 27% of wells with As above 10 µg/L. In Tuscany, ground water surveys since 1971 have identified several water sources containing As at concentrations above 10 µg/L, with peaks of 670 µg/L at springs in the province of Pisa. In Northern Lazio many surface waters and lakes have As concentrations above 10 µg/L.

Finland

Ground water in areas not influenced by human activity were found to have concentration of As ranging from 17 to 980 µg/L in surveys conducted in 1993-1994.

Belgium

Rivers in the Schelde region were assessed for As in 1988 and concentrations of up to 3.8 µg/L were found.

Comment on public health burden in Europe

Given the absence of comparable exposure data across European countries, a quantitative estimate of public health burden attributable to arsenic is difficult. However, based on risk assessment

published in the literature, and qualitative information about population prevalence of exposure, it would seem likely that the proportion of arsenic-related cancer is in the range 5-10% of bladder, kidney and lung cancer in several areas of Europe.

4. DISCUSSION

The ASHRAM study addressed the issue of cancer risk in relation to arsenic exposure via drinking water in a European population. A major effort was devoted to characterization of exposure in a variety of its dimensions: from the analytical-chemistry aspects, the human metabolism considerations, and the complexity of assembling life time experience of drinking water consumption. A selection of exposure indices was employed to assess risk of skin (BCC), bladder , and kidney cancer. BCC risk is elevated in relation to a variety of arsenic exposure indices, and the relationship remains significant after controlling for UV exposure. The pattern of bladder and kidney cancer risk is substantially similar to that of BCC, but statistical significance is achieved only for some of the exposure indices.

Selection of cancer cases was dependent on active collaboration of the clinical teams in the hospitals, and in some cases the active collaboration of the local public health authorities as well. The pattern of the data collection was likely influenced by national and local characteristics of the health service and its relation with the public health service. However much effort was devoted to complete recruitment of cancer cases over the period of data collection, it cannot be excluded that county-specific factors affected recruitment. Controls selection as well might conceivably be affected by these factors. ASHRAM emphasized the requirement to recruit controls from not only the hospitals functioning as cancer centres and diagnostic units, but a whole series of smaller hospitals as well. In this way, it was attempted to identify for selection controls representative of the whole population. Nevertheless, the case-control ratio in each country appears to vary considerably within the study, and for this reason, we controlled for county in all analyses relating As exposure to cancer risk.

Analysis of total arsenic content in drinking water is based on established analytical-chemistry techniques, nevertheless it may be of concern that errors in sampling or laboratory procedures may affect estimate of human exposure in the present study. The QA/QC programme demonstrated that the water analyses were of good quality, and could be used for estimating current exposure for all participants. When historic water measurements were used, expert judgement was involved in attributing a As concentration to water sources used by participants in the past. This could be associated with misclassification of past exposure. The records of past As measurements conducted by public agencies in the study area were available to the study, and were assigned to participants by an expert blind to case-control status. The questionnaire interviews obtained an excellent completeness of information on past residences. This, combined with measurements of samples collected as part of the study, and with past measurements, both of which provide values for exposure to arsenic independently of responder or interviewer, makes it unlikely that misclassification of past exposure is systematic. The reconstruction of historic exposure to arsenic in drinking water can therefore be considered adequate for production of exposure assessment estimates that would be a better characterization of overall exposure than possible from current sources alone. In addition, assessment of individual level exposure to arsenic in drinking water has not been often attempted. Most of the studies conducted in Taiwan used an estimate of well water concentration as a proxy of current and past exposure. Though ASHRAM exposure assessment does not include all the possible routes and modalities of exposure, it represent a significant improvement on what has been available before, both in view of the inclusion of information on past arsenic concentrations, and in view of the exposure assessment at individual level.

Population exposure to arsenic, measured as the life time average concentration of residential drinking water, had a mean value of 12 microgram/L, and 95% of the population lay in the range 0.4 to 61.6 microgram/l. This indicates that the majority of the ASHRAM population is exposed to arsenic at concentrations below those in many studies upon which the current arsenic standard is based.

Overall, the ASHRAM study cannot confirm the null hypothesis of no cancer risk in relation to low levels of arsenic exposure via drinking water.

5. CONCLUSIONS

1. A large multi-country hospital based case-control study of arsenic and cancer risk was completed, based on multi-disciplinary work in the fields of epidemiology, toxicology, analytical chemistry, and genetics.

2. Exposure to life time average arsenic concentration in residential drinking water ranges from 0.01 to nearly 200 micrograms/l, with a mean of 12. Ninetythree percent of the population were below 50 micrograms/l.

3. We have found a positive association between exposure to arsenic and all three cancers sites, BCC, bladder cancer, and kidney cancer.

4. The relationship is stronger for skin cancer, which may just reflect larger numbers and larger statistical power to detect an effect.

5. The exposure metric which shows the most significant fit varies between cancer sites, with BCC most strongly associated with life time average arsenic concentration, bladder cancer with cumulative arsenic dose, and kidney cancer with peak arsenic daily dose rate. It is not known if this is a true difference or just a chance finding.

6. Arsenic species in urine produced by human metabolism of ingested inorganic arsenic were identified in the ASHRAM population. This is a substantially larger population on which

arsenic speciation has been characterized for arsenic species at such low levels than previously reported.

7. Overall, about 7% of the urinary arsenic was inorganic arsenic, 16% MA, and 77% DMA. Compared to previous reports on populations exposed to arsenic via drinking water (Vahter 2002), the present study shows a lower percentage of inorganic arsenic and a higher percentage of DMA. That was mainly due to intake of DMA via food.

8. DMA and MMA modify the cancer risk attributed to arsenic, and cancer risk is increased for the subgroups with low percentage of DMA, as well as the subgroup with high percentage of MMA. This effect is significant for BCC. The effect modification is in the direction expected based on toxicology.

9. Advances were made in the field of arsenic chemistry, with clarification of the probable artefactual nature of MA(III) measurements reported in the recent literature, and the identification of two previously unknown arsenic compounds.

10. Advances were made in the field of cancer genetics, with identification of genetic polymorphisms associations with risk of BCC.

RECOMMENDATION

Given the confirmed carcinogenicity of As when ingested at low concentrations over several years, and the lack of As data on population burden of arsenic in Europe, it is recommended to invest in sampling for water and soil As concentration using a standardized protocol across several European countries. The information produced will help to estimate more precisely the burden of arsenic-caused cancers, and will guide interventions by health and environment protection agencies.

6. EXPLOITATION AND DISSEMINATION OF RESULTS

6.1 PRESENTATIONS TO POLICY MAKERS

Meetings with national and regional representatives of public health agencies and authorities responsible for management of drinking water were requested, and at the time of writing this report had been held in Hungary and planned in Romania and Slovakia:

- Budapest, Hungary, June 2005
- Banska Bystrica, Slovakia, October 2005
- Cluj, Romania, November 2005

6.2 CONFERENCES

Results of the ASHRAM study have been presented at national and international conferences in the fields of epidemiology, toxicology, genetics, and analytical chemistry. These included:

- the Sixteenth Conference of the International Society for Environmental Epidemiology in New York in 2004, and the Seventeenth Conference of the International Society for Environmental Epidemiology in Johannesburg, South Africa - September 13-16, 2005
- the meeting of the Central European Chapter of the International Society for Environmental Epidemiology, Hungary , 2005

- the meeting on Occupational and Environmental Epidemiology held at Guys Hospital, London, on 28 April 2005

6.2.1 Abstracts of papers presented at international conferences

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6.3 PAPERS

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7. POLICY RELATED BENEFITS

The principal policy related benefits from ASHRAM are related to the direct observation of human carcinogenic risk of arsenic ingestion for concentrations below the old drinking water standard of 50 microgram/l. The implications of this are several. First, there is a direct support of the recently amended guidance for arsenic in drinking water by the World Health Organization and the European Union, set at 10 microgram/l. Clarity on this guidance has been requested by European governments, local agencies, and others in view of the economic implications of strict compliance. This is due to the cost of engineering and other interventions required to provide drinking water with concentration of arsenic below the guidance value. The present study cannot support a relaxed approach to the implementation of the guidance, and this is its second policy benefit. Thirdly, policies referring to health hazards in contaminated land and soil may be affected by these results, as arsenic accounts for the largest proportion of cancer risk attributable to soil contaminants, and the guidance values for arsenic in soil are based directly on the water standard for arsenic. Therefore, results of a study confirming the need of a specific water standard will indirectly affect also soil and other guidance. Fourthly, the study conclusions would support caution in the use of ground water for drinking as a viable alternative to surface water, given the unknown distribution of arsenic in ground water. ASHRAM study recommendation, reported in section 5, highlights the benefits that would be accrued by a systematic approach to estimation of population exposure to arsenic in Europe. Widespread communication of results on population exposure to arsenic in drinking water, to regional and municipal authorities, should be encouraged.

In addition to the consequences of the study for water management, ASHRAM is likely to make a substantial contribution to the literature on arsenic risk and mechanisms of arsenic toxicity. New findings on the chemistry of arsenic, demonstrated the need for careful evaluation of environmental media and biological samples when studying the possible health-related aspects of exposure to arsenic at low concentrations. Exposure assessment methods used in ASHRAM to estimate arsenic exposure in individuals were of traditional design, but have rarely been implemented before in the literature of arsenic epidemiology, as they are very laborious. Estimating lifetime exposure to low concentration of arsenic in drinking water at individual level allowed to estimate cancer risk in a large multi-country study population. This supports the notion that direct epidemiological observation is a feasible approach for addressing vexed questions in environmental health, but only at condition of obtaining collaboration with natural scientists with specialist skills and knowledge in the specific area. In other words, the integration of specialist chemistry and toxicology within an overall epidemiological design and framework was a necessary condition for the production of meaningful results on a topic related to possible health hazards of low doses of a widespread environmental contaminant. Metabolic and genetic factors at individual level were shown to affect cancer risk in ASHRAM, independently of arsenic exposure, and in some cases jointly with it. The advances provided within the framework of ASHRAM in understanding of arsenic chemistry and mechanisms of action are likely to have implications not only for medical science, but also for the design of environmental monitoring systems.

In summary, the relevance of ASHRAM results is twofold: by confirming a cancer risk from ingestion of arsenic they are likely to affect policies on the management of water for drinking, by contributions to knowledge on determination and evaluation of arsenic and its effects they contribute to environmental health sciences and medical sciences, and attempts towards their integration.

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9. ANNEXES

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REPORT ON GENETIC WORK

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REPORT ON ROMANIAN FIELD WORK

REPORT ON SLOVAKIAN FIELD WORK

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