

# Assessing human exposure to inorganic arsenic in high-arsenic areas of Latium: a biomonitoring study integrated with indicators of dietary intake

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*Key words: Inorganic arsenic, exposure assessment, dietary exposure, biomarkers of exposure, arsenic speciation*

*Parole chiave: Arsenico inorganico, valutazione dell'esposizione, esposizione alimentare, biomarcatori di esposizione, speciazione dell'arsenico*

## Abstract

**Background:** In Latium (central Italy), arsenic concentrations exceeding the regulatory limit of 10 µg/L for drinking water are present in groundwater from a large area of volcanic origin. At least in part of the area, high arsenic concentrations have been detected also in soil and phytoavailable geogenic arsenic enters the food chain. As a result, local population may be exposed to inorganic arsenic via water and also through consumption of food with higher than background arsenic concentrations.

**Methods:** A cross sectional study was conducted to assess inorganic arsenic exposure and metabolism in 269 residents of 27 municipalities in the provinces of Viterbo, Rome and Latina. Total arsenic in toenails and the sum of inorganic arsenic and methylated metabolites in urine, the latter determined by HPLC-ICP-MS, were used as biomarkers of inorganic arsenic exposure. All the subjects involved in the study provided samples of the water(s) used for drinking and cooking as well as detailed information on water use. To get an insight into dietary intake from locally-processed food, inorganic arsenic in bread samples collected in affected municipalities of the three provinces was determined and compared to background levels of samples from reference areas.

**Results:** 30% of the sample used bottled water or resorted to water treatment in order to lower the arsenic content <10 µg/L (Group 1), 51% of the sample drank bottled water and used tap water with an arsenic content exceeding 10 µg/L for cooking only (Group 2), 19% of the sample used tap water with an arsenic content exceeding 10 µg/L for both drinking and cooking (Group 3). Nail arsenic was higher for Group 2 and 3 compared to Group 1, whereas all groups had higher nail arsenic than the reference group. The sum of inorganic arsenic and related metabolites in urine was higher in Group 3 than in the other two groups, and higher in Group 2 compared to Group 1. White bread from the study area showed significantly higher inorganic arsenic levels compared to samples from reference areas.

**Conclusions:** Use of toenail arsenic as biomarker of long-term exposure allowed to retrospectively reconstruct exposure irrespective of recent modifications due to changes in water use. In Group 3, urinary concentration of inorganic arsenic and metabolites exceeded the upper limit of the reference concentration range for the Italian population. Inter-individual variability of the efficiency of arsenic metabolism in the study population was substantial indicating that a subgroup of the population is more susceptible to the toxic effects of inorganic arsenic owing to a lower methylation capability.

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## Introduction

Arsenic is widely distributed in the earth's crust as elemental arsenic and as the inorganic ions arsenite (As(III)), the dominant form under reducing conditions, and arsenate (As(V)), the most stable form in oxygenated environments (1). Biogenesis from inorganic forms leads to the occurrence of a variety of organoarsenic species in living organisms. Organic arsenic has a lower toxicity than inorganic arsenic (iAs) - i.e. arsenite and arsenate - which is the critical species in terms of effects on human health and related risk assessment. Human exposure to iAs is a major public health problem worldwide. The International Agency for Research on Cancer (IARC) has established a causal role for oral exposure to iAs on skin, lung, and bladder cancers, and has shown suggestive evidence for liver, kidney, and prostate cancers (2). Key epidemiologic evidence came from populations chronically exposed to high arsenic levels in drinking water ( $>100 \mu\text{g/L}$ ) in southwestern Taiwan (3), Bangladesh (4), northern Chile (5), and Argentina (6). However, there is increasing evidence of adverse effects at moderate to low exposure levels such as those resulting from water arsenic concentrations below  $50 \mu\text{g/L}$  (7-10). Apart from cancer, a wide range of other adverse health effects such as skin lesions, cardiovascular diseases, developmental toxicity, abnormal glucose metabolism, type II diabetes and (at relatively high exposures) neurotoxicity, are likely related to chronic ingestion of iAs (11). Susceptibility to the toxic effects may vary considerably between individuals mainly depending on inter-individual variations in iAs metabolism related to such factors as age, life stage, gender, nutritional status, and genetic polymorphism in the regulation of enzymes responsible for As biotransformation (12).

The general population is exposed to iAs via the diet, with food being the major

contributor to the intake when arsenic concentrations in water are at background levels and drinking water becoming the major source of exposure to iAs when contaminated groundwater is used (11-12). In the European population, the EFSA estimated that cereal-based processed products are the main contributor to dietary exposure to iAs, with wheat bread in the forefront (13). Arsenic enters the food chain through plant crops, which absorb it through their roots according to its phytoavailable levels in soils. The nature of the soil parent material is a major factor determining the iAs concentration in soils even though soils are enriched in iAs compared with their parent rocks (14). Apart from arsenic levels, soil physicochemical properties are important in determining the fraction of the element that is available to plants, especially Fe/Al oxides and clay that adsorb iAs preventing its migration in the soil solution.

In Italy, because of the complexity of geological history and variety of substratum rock types, the natural geochemical background is highly variable with several areas of geogenic arsenic enrichment (15). High arsenic concentrations have been detected in agricultural soil between Milano and Aosta, in the Brenta and Adige Plains (Veneto), in Tuscany (south-west of Firenze), in the Roman and Neapolitan magmatic provinces (Latium and Campania), all along Apulia region, in some sites in Sardinia and in central Calabria (16). Mapping of phytoavailable arsenic in agricultural soils identified high levels in the province of Novara (Piedmont, northwestern Italy), in Veneto (Verona, Padova, Treviso, and Venezia), in the province of Viterbo (Latium) and in the Apulia region (southeastern Italy), especially in the provinces of Foggia and Brindisi (17). Groundwater arsenic contamination is a problem in several areas of Italy due to different conditions, such as mineral deposits (Tuscany and Sardinia), highly reducing environments in alluvial

plains (Veneto, Emilia Romagna and Lombardy) and quiescent or active volcanic systems (Latium, Campania, Basilicata and Sicily) (18-26). Over the last decade, in more or less extended areas of Piedmont, Lombardy, Trentino-Alto Adige, Emilia Romagna, Tuscany, Latium and other Italian regions, alternative water sources or water treatment have been resorted to in order to supply drinking water with arsenic concentrations within the regulatory limit of 10 µg/L. In Latium, until very recently, almost one hundred municipalities were supplied by groundwater from the volcanic aquifers of Cimino-Vico (Viterbo province) and of Monti Sabatini and Alban Hills (Rome and Latina) exceeding the arsenic limit, owing to three consecutive 3-year derogations from the thresholds established by the European Drinking Water Directive 98/83/EC. Notwithstanding such derogations, the problem is still largely open especially in the Viterbo province and in July 2014 the European Commission sent a formal notice to Italy, the first stage in infringement proceedings (27).

The aim of the present study was to assess iAs exposure and metabolism in residents of the above mentioned municipalities, which altogether represent the largest arsenic-affected area in Italy. Apart from iAs ingestion via water used for drinking, cooking and other household practices, the population living in this area is potentially exposed to iAs levels above those expected for the Italian general population also through consumption of locally grown and locally processed food. Our earlier study on Italian agricultural soils identified the Viterbo province as the one with the highest level of phytoavailable arsenic, with a mean value equal to six times the national average (17). In addition to endogenous arsenic in soil, use of contaminated groundwater may enhance iAs entry in the food chain through crop irrigation and we found significant enrichment in iAs levels in locally grown

green vegetables compared with samples from locations with background levels of arsenic (28).

A combination of biomarkers was used in the present study to assess exposure to iAs and susceptibility to its toxic effects. Toenail arsenic was used as long-term biomarker, reflecting chronic exposure to iAs over several months. The sum of urinary iAs and methylated metabolites was used as biomarker of short-term exposure (last few days) whereas the primary methylation index (PMI) – i.e. the ratio between methylarsonate and iAs level – and the secondary methylation index (SMI) – i.e. the ratio between dimethylarsinate and methylarsonate – were used to assess the efficiency of the first and second methylation step respectively, and thus the individuals' capacity to metabolize and detoxify the ingested iAs. The biomonitoring study was integrated with indicators of dietary intake, i.e., iAs content of drinking water, cooking water, and of the bread produced locally. This study documents for the first time iAs exposure of the general population living in an area with high environmental levels of arsenic of natural origin in Italy and characterizes the relevant risk.

## Materials and methods

### *Study population*

On the whole, the population residing in the arsenic affected area of Latium (central Italy) consists of ca. 814,000 persons. The study population comprised 269 participants aged 1-88 years (20% <15 years, 15% >65 years), with a female to male ratio of 1.31. They were recruited in 27 municipalities of three different provinces in Latium, namely Viterbo (17 municipalities, 139 participants), Rome and Latina (10 municipalities, 130 participants), between November 2010 and March 2011. A control group for the evaluation of baseline toenail arsenic

was recruited in the city of Rome ( $n=34$ ), supplied with water having extremely low arsenic concentrations, mainly piped from the mountains around Rieti (some 80 Km away) by the Peschiera-Capore aqueduct.

The study areas within each province were selected based on their levels of arsenic in drinking water identified through chemical analyses carried out before and during sample collection. Each prospective participant was first interviewed by a general practitioner or paediatrician (for children's parents) to ascertain age, duration of residence, drinking water source, and interest in participation. They were asked to participate if they had lived in the study area at least for the last 2 months, used local tap or well water for drinking and/or cooking, and if they were in good health. Recruited volunteers filled in a questionnaire detailing water use, food habits, and other relevant information, provided samples of water(s) (see 'Sample collection') and participated in the biomonitoring study. The study was carried out in adherence with the guidelines of the Ethical Committee of the Istituto Superiore di Sanità and volunteers gave informed consent to participate.

#### *Sample collection*

Volunteers provided samples of all types of water used in everyday life both for drinking and for cooking, including tap water, water from home treatment devices, bottled water and water from local springs. Water samples were collected in pre-cleaned polypropylene tubes and transported to the laboratory where they were stored at ambient temperature until analysis.

Excretion of iAs was assessed by determining individual urinary concentrations of the sum of iAs and related metabolites, as detailed in Cubadda et al. (29). First morning voided urines were collected in polypropylene tubes and transported at ambient temperature to the laboratory where they were stored at  $-80^{\circ}\text{C}$  until analysis. All

subjects were asked to refrain from seafood and mushroom consumption in the four days before urine sampling in order to limit excretion of organoarsenic compounds that might interfere with the assessment of iAs metabolism.

Because of the inter-individual and intra-individual variations in dilution of urine the urinary arsenic concentrations were normalized using specific gravity (*SG*), which was measured by a hand refractometer (ATAGO, Japan). Urinary arsenic species (*U-As*) were adjusted to the overall mean *SG* value of  $1.020\text{ g mL}^{-1}$  of the study group according to the following equation:  $U\text{-As } SG = U\text{-As} \times (1.020 - 1) / (\text{measured } SG - 1)$ . Analytical results were expressed accordingly throughout.

To get an insight into iAs dietary intake from locally-processed food, bread was selected as the single most significant item owing to both the sizeable average daily consumption and the inclusion of substantial amounts of water as ingredient in the preparation of dough. Twenty-six samples of white bread were bought at retail in the arsenic-affected area and 7 in reference areas with background levels of arsenic in water. The samples were homogenized with a blender in the laboratory and submitted to arsenic speciation analysis for the selective determination of iAs.

#### *Analytical determinations*

Ultrapure  $\text{HNO}_3$ ,  $\text{H}_2\text{O}_2$ , and ultrapure deionized water, obtained by a Milli-Q Element System (Millipore, Molsheim, France), were used throughout. Preparation of analytical standards for the determination of total arsenic and arsenic species as well as their purity check are described in Cubadda et al. (2014). Sample handling was carried out in clean room conditions under a laminar flow box (Spetec GmbH, Erding, Germany).

Total arsenic determination in water and urine was carried out by directly injecting the

samples acidified with 1% v/v HNO<sub>3</sub> (and filtered through 0.22 µm PVDF membranes, for urines) in the sample introduction system of the ICP-MS, an Elan DRC II (PerkinElmer, Norwalk, CT). For arsenic speciation analysis of urines and bread extracts a PerkinElmer Series 200 metal-free HPLC system coupled on line to the ICP-MS was used to accomplish separation of arsenic compounds by anion exchange chromatography as detailed elsewhere (28-29). HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> were used to extract arsenic species from bread samples by means of a Milestone Ethos E microwave labstation (FKV, Bergamo, Italy) (28). Nails were manually scraped to remove visible exogenous material and then submitted to an acetone-water ultrasound-aided cleaning procedure. After drying for 24 h, samples were subjected to microwave-assisted digestion with concentrated HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub>.

Trueness assessment was carried out using the control material Seronorm Urine blank (Sero, Billingstad, Norway), the certified reference materials SRM 2669 I and II (Arsenic Species in Frozen Human Urine, NIST, Gaithersburg, MD, USA), and the reference material IMEP-112 (Wheat). In all cases, found values agreed with certified or reference values.

#### *Statistical analysis*

The study population was stratified by water arsenic concentration and water use into three groups. Group 1 used bottled water for both drinking and cooking or resorted to home water treatment devices and consumed water with an arsenic content below 10 µg/L. Group 2 found alternative water sources for drinking (typically bottled water) and used tap water with an arsenic content exceeding 10 µg/L for cooking only. Group 3 used tap water with an arsenic content exceeding 10 µg/L for both drinking and cooking. Descriptive statistics were computed to analyze the characteristics of the three subsamples.

The iAs concentration measured in water, bread and toenails and the sum of iAs and related metabolites in urine were compared among the three water use groups, the two study areas (province of Viterbo vs. Rome and Latina) and between the study area and the reference areas using the Kruskal-Wallis e Wilcoxon-Mann-Whitney tests.

Statistical analysis was performed with STATA 11.2 (StataCorp, 4905 Lakeway Drive, College Station, Texas, USA) setting significance at P<0.05.

## **Results**

### *Water use and arsenic content in water and bread*

In the study population, 30% of the subjects used bottled water for both drinking and cooking, or resorted to water treatment in order to lower the arsenic content of tap water below 10 µg/L (Group 1). The majority of the sample (51%) drank bottled water and used tap water with an arsenic content exceeding 10 µg/L for cooking only (Group 2). The remaining 19% of the sample used tap water with an arsenic content exceeding 10 µg/L for both drinking and cooking (Group 3).

As far as the origin of tap water is concerned, the public water network was the main supplier whereas only 12% of volunteers used groundwater from private wells. Tap water in the Viterbo province had higher arsenic levels compared to those in Rome and Latina provinces (Table 1), but on the whole in both areas about 75% of the households showed arsenic levels >10 µg/L, and 90% exceeded 5 µg/L. Water arsenic levels exceeded 10 µg/L in 64% of the private wells, but 52% of samples exceeded 20 µg/L and some very high values were found (8 samples in the range 35-149 µg/L).

The arsenic content of drinking and cooking water for the three groups of the study population is shown in Figure 1. Group 1 had similar arsenic concentrations

**Table 1.** Arsenic concentration in the water supplied to households.

Area	Arsenic in tap water ( $\mu\text{g/L}$ )			
	Median (min-max)	Mean (S.D)	% samples $>5 \mu\text{g/L}$	% samples $>10 \mu\text{g/L}$
Viterbo province	26.6 (0.4 - 56.5)	21.2 (14.0)	87.5%	77.7%
Latina and Rome provinces	14.1 (0.3 - 148.9)	16.0 (16.6)	86.2%	73.4%

in drinking and cooking water; Group 2 resembled Group 1 as to arsenic concentration in drinking water and Group 3 as to arsenic concentration in cooking water; Group 3 used a single water source and thus the same arsenic concentration appears. It is interesting to note that in the households with sizeable arsenic levels the use of tap water was discontinued for drinking but not for cooking, as shown by some very high values in Figure 1 (Group 2, cooking water). As far as drinking water is concerned, for Group 1 and 2 in most cases several different water types and sources were used by subjects on a daily basis and thus an average arsenic concentration was calculated based on chemical analysis of all the water

samples provided. In terms of water types, the most frequent alternative to tap water was bottled mineral water but in some cases mineral water from local spring was used. Arsenic concentrations in samples of bottled and spring water provided by the participants are shown in Table 2. Some samples of water from local springs did show substantial arsenic levels.

White bread from the different provinces of the study area showed similar average levels of iAs, which were two times higher compared to samples from reference areas ( $p$ -value  $<0.0001$ ) (Figure 2). In agreement with our earlier studies (16, 29), speciation analysis showed that arsenic in bread was present as iAs, due to the fact that both wheat

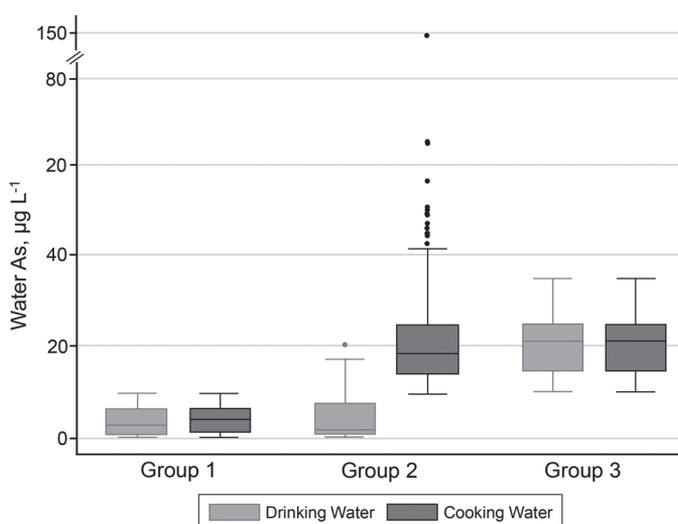


Figure 1. Median, interquartile range, minimum, maximum and extreme values of the concentration of arsenic in drinking and cooking water for the three water use groups.

Table 2. Arsenic concentration in bottled and spring water used for drinking purposes by the study volunteers ( $\mu\text{g/L}$ ).

Sample code <sup>a</sup>	Type <sup>b</sup>	n	mean	SD	Sample code <sup>a</sup>	Type <sup>b</sup>	n	mean	SD
01ANe	B	7	5.6	0.7	18Let	B	9	0.8	0.0
02Ang	B	1	0.3	0.0	19Lev	B	12	7.7	0.4
03Mad	S	1	23.7	0.1	20Ner	B	2	0.2	0.0
04Blu	B	1	0.4	0.0	21Pan	B	1	0.4	0.0
05Cla	B	2	0.2	0.0	22Par	B	3	0.3	0.0
06Coo	B	1	0.2	0.0	23Roc	B	1	0.2	0.0
07Cut	B	2	5.4	0.1	24SBe	B	4	0.5	0.0
08Ege	B	1	9.1	0.1	25SGi	S	1	7.4	0.1
09Fab	B	3	0.5	0.1	25SCh	B	3	0.4	0.0
10Fer	B	5	7.1	0.6	26SCr	B	7	0.2	0.0
12Ard	S	3	15.3	1.5	27SFj	B	1	0.9	0.0
13SVi	S	4	13.7	0.5	28SAg	B	1	4.3	0.0
14Tav	B	13	0.2	0.0	29SAn	B	7	1.6	0.2
15Orv	B	1	6.1	0.1	30Uli	B	1	0.3	0.0
16Fra	B	2	0.3	0.0	31Ver	B	1	1.4	0.0
17Gui	B	5	0.4	0.0	32Viv	B	5	0.2	0.0

<sup>a</sup> Samples were analysed as received from study participants. For  $n \geq 2$ , sample were grouped according to the names provided by study participants and descriptive statistics was calculated accordingly.

<sup>b</sup> B=bottled mineral water; S=mineral water from local springs.

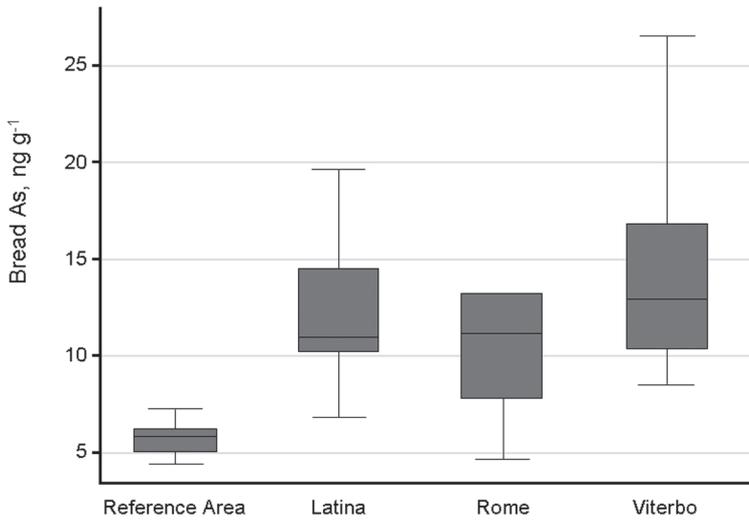


Figure 2. Median, interquartile range, minimum and maximum of the concentration of arsenic in bread from arsenic-affected municipalities of the three Latium provinces and from a reference area.

grain and water contain almost exclusively iAs.

*Overall intake of iAs and biomarkers of exposure*

Arsenic concentrations in toenails of subjects of Group 2 and 3 were higher

compared to those of Group 1 ( $p < 0.001$ ) (Figure 3). Furthermore, all the three groups had higher arsenic concentrations than the reference group ( $p < 0.001$ ), which demonstrates that residents of the affected area had an higher dietary intake of iAs compared to subjects living in areas with

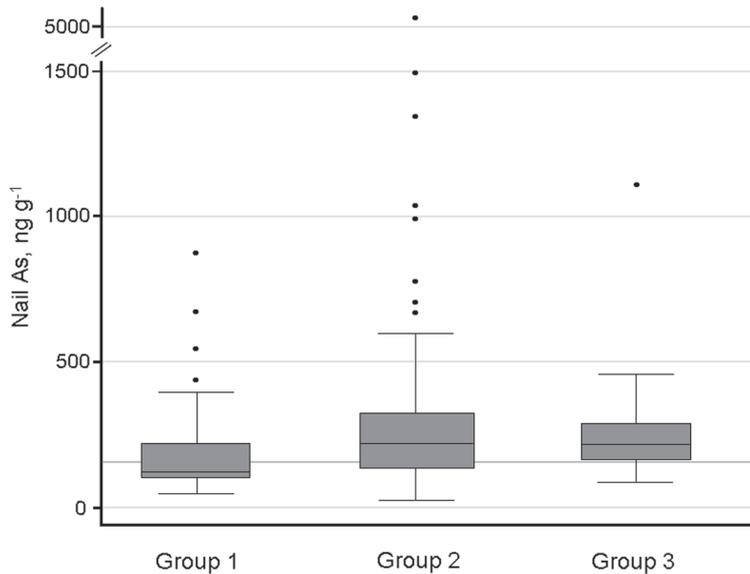


Figure 3. Median, interquartile range, minimum, maximum and extreme values of the concentration of arsenic in nails as a function of the type of water used.

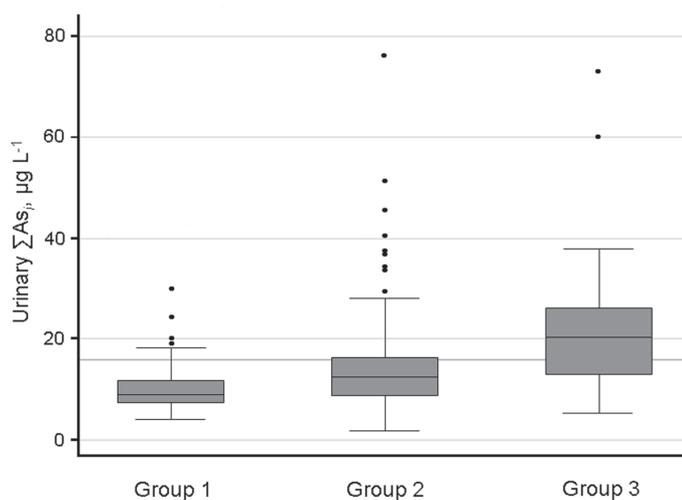


Figure 4. Median, interquartile range, minimum, maximum and extreme values of the of the sum of iAs and related metabolites in urine as a function of the type of water used.

background iAs exposure even though they used drinking and cooking water with arsenic levels  $<10 \mu\text{g/L}$ .

The sum of iAs and related metabolites in urine differed among the three groups and was higher in Group 3 than in the other two groups, and higher in Group 2 compared to Group 1 ( $p < 0.001$ ) (Figure 4).

Independent of water use, residents of the Viterbo province had higher levels of both biomarkers (nail As 292 ng/g, urinary iAs and metabolites  $15.5 \mu\text{g/L}$ ) compared to those of the provinces of Rome and Latina (nail As 211 ng/g, urinary iAs and metabolites  $12.8 \mu\text{g/L}$ ), with a significant difference in the case of nails ( $p < 0.05$ ).

#### *Inter-individual variability in the efficiency of arsenic metabolism*

In the study population, the average PMI was 1.4, with a SD of 1.4. Values ranged from 0.2 to 8.1 highlighting a substantial inter-individual variability. Also the SMI, which had an average value of 7.2, showed a wide variability with a SD of 5.5 and a range of 2.2-39.5.

The percentage of urinary iAs and metabolites was affected by the iAs exposure

level. Subjects of Group 3, which had the highest current exposure to iAs, had higher percentages of iAs and methylarsonate and a lower secondary methylation index, highlighting that the second methylation to dimethylarsinate was less efficient in this group.

## **Discussion and conclusions**

In the study area, both the public water supply and a multitude of wells in rural areas depend on arsenic-rich groundwater of a large volcanic aquifer. The Drinking Water Directive 98/83/EC, transposed into Italian legislation with the Legislative Decree 31/2001, reduced the regulatory limit for arsenic in water intended for human consumption from 50 to  $10 \mu\text{g/L}$ . In December 2003, when the new limit took effect at the national level, derogations were issued allowing use of water with an arsenic content up to  $50 \mu\text{g As/L}$  due to the lack of alternative water sources. The Drinking Water Directive allows for a total of three derogations, each limited to three years. Member States may derogate twice

and, in exceptional cases, they may apply to the European Commission for a third derogation. In October 2010 the EC did not grant a requested third derogation for arsenic at the value of 50  $\mu\text{g/L}$  requested by the national authorities but granted it in March 2011 after a new request was received for a derogated value of 20  $\mu\text{g/L}$ , under the conditions that the derogated water was not used as drinking water by infants and children up to the age of 3.

Public awareness of the problem grew during the second half of 2010 and led to widespread use of bottled water for drinking purposes, whereas the water of the public water supply and private wells was devoted mainly to other household uses including food preparation and cooking. The present study was designed so that both current and past exposure to iAs could be captured. In particular, use of toenail arsenic as biomarker of long-term exposure allowed to retrospectively reconstruct exposure irrespective of recent modifications due to changes in water use. Subjects of Group 2 and 3, which had similar arsenic concentrations in household tap water, showed almost indistinguishable nail arsenic levels highlighting that before recent modifications in water use exposure to iAs was identical. On the other hand, current exposure as reflected by the sum of iAs and metabolites in urine was higher in the subjects of Group 3 since they continued to use tap water for all uses (drinking and cooking). It is interesting that subjects of Group 2 had a current exposure lower than Group 3 but higher than Group 1, highlighting the significant contribution that cooking with high-As water gives to iAs exposure (arsenic levels in drinking water were similar in the two groups).

The most frequent alternative to tap water was bottled water, but in some cases residents resorted to local springs without being aware that arsenic levels in spring water were substantial and in some

cases higher than in tap water supplied to households. Insufficient information to the public on how to reduce risks related to water consumption appeared to be widespread at least at the time the sampling campaign of this study was carried out.

Another issue of public health significance is the widespread use of private wells in rural areas. A lower percentage of wells showed water arsenic exceeding 10  $\mu\text{g/L}$  compared to households connected to the public water supply, however some very high values were found and in a few cases water was used for both drinking and cooking by residents.

The greater iAs level in the bread from study area was certainly due to higher than background arsenic concentrations in the water used in bread processing. In line with this, a trend towards higher iAs concentrations in bread is seen in the Viterbo province coherently with the greater water arsenic levels in this province compared to the other two. Wheat flour, the other major ingredient of bread, likely played a minor role given that the wheat used in bread processing is generally not of local origin in Italy. Apart from bread, we did find that locally-grown green vegetables from the study area had significantly higher iAs levels compared to samples from reference areas (28) suggesting that the dietary intake of the local population was increased independently from water consumption owing to the high environmental levels of arsenic in the study area. A duplicate diet study on a subsample of the study population confirmed that this was the case (28).

The urinary concentration of the sum of iAs and related metabolites in subjects of Group 3 can be taken as an estimate of the historical exposure of the population of the study area to iAs. The average concentration exceeded the upper limit of the reference concentration range of 2–15  $\mu\text{g/L}$  proposed for the Italian population (31) and most individual levels were also largely above. One fifth of the sample had

urinary concentrations twice the upper limit or even higher. Overall, these results highlight dietary arsenic exposures well above the average of the general population, especially in the Viterbo province. On the other hand, EFSA's assessment of iAs exposure in European countries revealed that average dietary intake of iAs is high compared to health reference points and should be reduced (11). Another critical point is the high inter-individual variability of the efficiency of arsenic metabolism in the study population, which shows that a subgroup of the population is more susceptible to the toxic effects of iAs owing to a lower methylation capability. Moreover, the second methylation step (the critical one for iAs detoxification) was impaired with increasing exposure levels and the greater susceptibility to iAs toxicity at higher exposures is another key point to be considered in risk assessment.

The exposure assessment approach presented in this paper supports the ongoing epidemiological studies aiming at identifying health effects from past exposure to iAs of the inhabitants of the high-arsenic areas of Latium.

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#### Riassunto

##### *Valutazione dell'esposizione umana ad arsenico inorganico in aree con abbondanza di arsenico: uno studio di biomonitoraggio integrato con indicatori dell'assunzione alimentare*

**Background:** Nel Lazio, concentrazioni di arsenico eccedenti il limite normativo di 10 µg/L per l'acqua potabile sono presenti in acque sotterranee di un'ampia area di origine vulcanica. In almeno una parte di quest'area sono state rilevate elevate concentrazioni di arsenico anche nel suolo e l'arsenico fitodisponibile di origine geogenica fa ingresso nella catena alimentare.

**Metodi:** È stato condotto uno studio trasversale per valutare l'esposizione all'arsenico inorganico e il suo metabolismo in 269 residenti di 27 comuni nelle province di Viterbo, Roma e Latina. L'arsenico totale nelle unghie dei piedi e la somma di arsenico inorganico e metaboliti metilati nelle urine, la seconda determinata mediante HPLC-ICP-MS, sono stati usati come biomarcatori dell'esposizione all'arsenico inorganico. Tutti i soggetti inclusi nello studio hanno reso disponibili campioni della(e) acqua(e) utilizzate per bere e cucinare e informazioni dettagliate sull'uso dell'acqua. Per acquisire elementi di conoscenza sull'assunzione alimentare da prodotti trasformati in loco, è stato determinato l'arsenico inorganico in campioni di pane raccolti in comuni interessati delle tre province e i livelli misurati sono stati confrontati con quelli di fondo di campioni da aree di riferimento.

**Risultati:** Il 30% del campione usava acqua imbottigliata o ricorrevva al trattamento dell'acqua al fine di diminuire il contenuto di arsenico al di sotto dei 10 µg/L (Gruppo 1), il 51% del campione beveva acqua imbottigliata e usava acqua di rubinetto con un contenuto di arsenico eccedente i 10 µg/L solo per cucinare (Gruppo 2), il 19% del campione utilizzava acqua di rubinetto con un contenuto di arsenico eccedente i 10 µg/L sia per bere che per cucinare (Gruppo 3). L'arsenico nelle unghie è risultato essere maggiore per i Gruppi 2 e 3 rispetto al Gruppo 1, mentre tutti i tre gruppi presentavano un livello di arsenico nelle unghie superiore a quello del gruppo di riferimento. La somma di arsenico inorganico e relativi metaboliti nelle urine è risultata essere maggiore nel Gruppo 3 rispetto agli altri due gruppi e nel Gruppo 2 rispetto al Gruppo 1. Il pane bianco dall'area di studio ha mostrato livelli di arsenico inorganico significativamente più elevati rispetto ai campioni dalle aree di riferimento.

**Conclusioni:** L'uso dell'arsenico nelle unghie dei piedi quale biomarcatore di esposizione a lungo termine ha consentito di ricostruire l'esposizione pregressa indipendentemente delle variazioni recenti dovute a cambiamenti nell'uso dell'acqua. Nel Gruppo 3, la concentrazione urinaria di arsenico inorganico e metaboliti superava il limite superiore dell'intervallo di concentrazioni di

riferimento per la popolazione italiana. La variabilità interindividuale nell'efficienza del metabolismo dell'arsenico nella popolazione studiata è risultata essere marcata e indica che un sottogruppo della popolazione è più suscettibile agli effetti tossici dell'arsenico inorganico a causa di una inferiore capacità di metilazione.

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