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Pregnant women in Timis County, Romania are exposed primarily to low-level (<10 µg/L) arsenic through residential drinking water consumption

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Abstract

Excessive arsenic content in drinking water poses health risks to millions of people worldwide. Inorganic arsenic (iAs) in groundwater exceeding the 10 µg/l maximum contaminant level (MCL) set by the World Health Organization (WHO) is characteristic for intermediate-depth aquifers over large areas of the Pannonian Basin in Central Europe. In western Romania, near the border with Hungary, Arad, Bihor, and Timis counties use drinking water coming partially or entirely from iAs contaminated aquifers. In nearby Arad and Bihor counties, more than 45,000 people are exposed to iAs over 10 µg/l via public drinking water sources. However, comparable data are unavailable for Timis County. To begin to address this data gap, we determined iAs in 124 public and private Timis County drinking water sources, including wells and taps, used by pregnant women participating in a case-control study of spontaneous loss. Levels in water sources were low overall (median = 3.0; range = < 0.5–175 µg/l), although higher in wells (median = 3.1, range = <

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0.5–175) than in community taps (median = 2.7, range = < 0.5–36.4). In a subsample of 20 control women we measured urine biomarkers of iAs exposure, including iAs (arsenite and arsenate), dimethylarsinic acid (DMA), and methylarsonic acid (MMA). Median values were higher among 10 women using iAs contaminated drinking water sources compared to 10 women using uncontaminated sources for urine total iAs (6.6 vs. 5.0 µg/l, $P = 0.24$) and DMA (5.5 vs. 4.2 µg/l, $P = 0.31$). The results suggested that the origin of urine total iAs ($r = 0.35$, $P = 0.13$) and DMA ($r = 0.31$, $P = 0.18$) must have been not only iAs in drinking-water but also some other source. Exposure of pregnant women to arsenic via drinking water in Timis County appears to be lower than for surrounding counties; however, it deserves a more definitive investigation as to its origin and the regional distribution of its risk potential.

Keywords

inorganic arsenic (iAs); arsenic urinary biomarkers; drinking water; exposure; public and private wells; western Romania

1. Introduction

Inorganic arsenic (iAs) is highly toxic and occurs naturally in soils and rocks, from which it can be released to groundwater under specific geochemical conditions (ATSDR, 2007). Relatively high concentrations of dissolved arsenic have been found in aquifers in different parts of the world (Smedley and Kinniburgh, 2002). At a global level, the consumption of water coming from contaminated sources remains the primary non-occupational route of exposure to iAs (WHO, 2001). More than 500 million people worldwide depend on drinking water sources contaminated with iAs concentrations exceeding 10 µg/l, the maximum contaminant level (MCL) currently set by the WHO, the European Union (EU) including Romania, and the U.S. Environmental Protection Agency (EPA), to prevent increased frequencies of arsenic associated cancers (NRC, 2001; WHO, 2004). The most important iAs-prone drinking water area in Europe is the Pannonian Basin, a large lowland area extending over western Romania, Hungary, northern Serbia, northeastern Croatia, and southern Slovakia. The geochemical background of this region is supportive for natural iAs contamination of intermediate-depth drinking water aquifers (100 to 600 m deep). As a result, approximately one million people are potentially at risk for exposure to iAs contaminated groundwater in this area (Rowland et al., 2011).

Arad, Bihor, and Timis Counties in Romania partly overlap the eastern side of the Pannonian Basin on the border with Hungary. While drinking water provided by most Romanian water companies meets or falls below the 10 µg/l EU MCL, more than 45,000 people residing in Arad and Bihor counties are exposed to concentrations above 10 µg/l, and an even higher number are exposed to concentrations below 10 µg/l (Gurzau and Pop, 2012; Gurzau and Gurzau, 2001; Hough et al., 2010). Despite its location in the Pannonian Basin, the close proximity to Arad and Bihor Counties, and use of groundwater aquifers for drinking, iAs levels in drinking water have not been previously reported for Timis County.

Several groups have reported increased risks for adverse pregnancy outcomes in association with exposure to iAs groundwater contamination by >50 µg/L, in particular among women

residing in arsenic-endemic areas of Bangladesh (Ahmad et al., 2001; Kwok et al., 2006; Rahamatullah et al., 2010), but also elsewhere (Bloom et al., 2010; Bloom et al., 2014b). However, there has been little research to date that focuses on levels between 10 and 50 µg/L, or less than 10 µg/L, which are widespread globally (van Halem et al., 2009). Based on previous environmental monitoring studies in the region, we anticipated our population would be exposed primarily to iAs concentrations <50 µg/L (Rowland et al., 2011). Our main objective was to employ a geographic information system (GIS) to describe the spatial distribution of iAs exposure from drinking water sources used by pregnant women residing in Timis County, Romania. The intent was to identify areas in which pregnant women are likely to use residential drinking water sources contaminated by 10 to 50 µg/L, potentially raising the risk for adverse pregnancy outcomes. To accomplish our objective, we collected residential drinking water samples and measured iAs levels in fulfillment of our recent hospital-based case-control pilot study of iAs exposure and spontaneous pregnancy loss. As a secondary objective, we corroborated drinking water exposure by conducting a speciated iAs analysis in urine collected from a targeted subsample of women.

2. Materials and Methods

2.1. Study population

Participant recruitment and data collection were previously described in detail (Bloom et al., 2014a). In brief, residential drinking water sources were identified for cases of spontaneous pregnancy loss (n=150) and for controls receiving routine prenatal care (n=150) at the Gynecology Department of the Emergency County Hospital in Timisoara, Romania (Bega Hospital). Timisoara is the largest city in Timis County. From December 2011 to January 2013, study nurses recruited all cases of spontaneous pregnancy loss receiving treatment at Bega Hospital (88% participation rate) and women with on-going pregnancies as controls matched to cases within one week of gestation (83% participation rate). In a face-to-face interview with a study physician, participants described their average daily water consumption and 'primary' and 'secondary' sources of residential drinking water. 'Primary' water sources were defined as those used most frequently, principally for cooking, bathing, cleaning, and drinking, and were often tap. 'Secondary' water sources were mainly used for drinking and were mostly wells. We collected drinking water samples from a total of 124 reported sources, including community supplies, shallow dug wells (several meters deep), and intermediate-depth drilled wells (100–200 m).

2.2. Laboratory analysis

2.2.1. Drinking water sampling and analysis—We previously described the details of the drinking water sample collection and iAs analysis (Bloom et al., 2014a; Lindberg et al., 2006). Briefly, following collection of drinking water samples into decontaminated polyethylene containers, concentrated analytical grade nitric acid (HNO₃) (Merck, Germany) was immediately added as a preservative. Samples were stored on ice until transfer to the Environmental Health Center (Cluj-Napoca, Romania) for analysis using a Zeenit 700P atomic absorption spectrometer with hydride generation system (Analytik Jena, Germany). The method detection limit (MDL) obtained was 0.5 µg/l. We used an eTrex global positioning system (GPS) handheld device (Garmin International, Inc., USA) to

record latitude and longitude coordinates at each water source. For each pregnancy we estimated average drinking water iAs exposure by type of source in $\mu\text{g/l}$, and then we also weighted measured iAs concentrations by reported water consumption to estimate total daily iAs intake in $\mu\text{g/day}$.

2.2.2. Urine sampling and analysis—Spot urine samples were collected by study nurses at the time of the interview into 50 ml polyethylene containers previously decontaminated with nitric acid and then rinsed. Urine was frozen within 15 minutes of collection and stored at -20°C until analysis. We selected a subset of samples from 20 pregnant control women; $n=10$ ‘unexposed’ using residential water sources with iAs below the MDL and $n=10$ ‘exposed’ using sources with higher iAs ($8.7\text{--}50.3\text{ }\mu\text{g/l}$). Women had lived at their study residence for 0.6 to 29 years. Samples were selected randomly from among the lowest and highest measured iAs concentrations from women with at least two stored urine aliquots. Arsenite plus arsenate ($\text{As}^{\text{III}} + \text{As}^{\text{V}}$), methylarsonic acid (MMA^{V}), dimethylarsinic acid (DMA^{V}), and cationic arsenic (organic arsenic species) were determined using high performance liquid chromatography (Agilent 1100) with Agilent 7700cx inductively coupled plasma mass spectrometry (Agilent Technologies, Waldbronn, Germany) as previously described in detail (Scheer et al., 2012). MDLs were $0.2\text{ }\mu\text{g/l}$ for all arsenic analytes, with the exception of $0.07\text{ }\mu\text{g/l}$ for overall arsenic. We determined specific gravity using a TS 400 Leica total solids refractometer (Leica Microsystems Inc., Buffalo, USA) and normalized arsenic concentrations to the mean specific gravity for the samples (i.e., 1.016), defined as $\text{As}_{\text{normalized}} = \text{As}_{\text{measured}} \times [(1.016-1)/(\text{specific gravity}-1)]$.

2.3. Data analysis

2.3.1 Spatial analysis—Water sampling data were cleaned and processed prior to importation into Stata v.12 (StataCorp LP, College Station, TX USA) for descriptive statistical analysis. The GIS software did not allow for negatives and so values below the MDL were imputed as $\text{MDL}/2$ (Hornung and Reed, 1990). We used *ArcGIS*® *ArcMap*™ v. 10.1 (ESRI, Redlands CA, USA) to develop our GIS maps. To display sampling points as ‘sheet events’, GPS coordinates were transformed into ‘decimal degrees’ (degrees + minutes/60 + seconds/3600) and imported into the 1984 Geographic Coordinate System, World Geodetic System (GCS_WGS_1984). Sheet events were exported into a shape file incorporating municipality, and geographic region borders for our study area were added and superimposed on a base map. We used a kriging method (Oliver and Webster, 1990) to interpolate iAs concentrations between sampling locations and to accommodate location clustering. Appropriate symbols and legends were generated to provide the final GIS maps.

2.3.2 Biomarker analysis—‘Overall’ arsenic included the total sum of iAs as well as cationic (organic) arsenicals. We calculated the total sum of iAs and its metabolites (‘total’ iAs) as $(\text{iAs}^{\text{III}} + \text{iAs}^{\text{V}}) + \text{DMA} + \text{MMA}$. We determined the primary (%DMA) and secondary methylation (%MMA) ratios as $100 \times (\text{DMA}/\text{total iAs})$ and $100 \times (\text{MMA}/\text{DMA})$, respectively. The total methylation ratio (%methylation) was calculated as $100 \times ((\text{DMA} + \text{MMA})/\text{total iAs})$. We compared median urine arsenic measures for 10 women using iAs contaminated residential drinking water sources (i.e., exposed) to 10 women using uncontaminated residential drinking water (i.e., unexposed) by Mann-Whitney U-test.

Across all 20 women, we assessed linear associations using Spearman rank correlation coefficients between urine arsenic measures and average residential drinking water iAs and daily iAs exposure. To maintain consistency with the spatial analysis values below the MDL were imputed as MDL/ 2. SAS v.9.4 (SAS Institute, Inc. Cary, NC USA) was used for analysis.

3. Results

3.1 Drinking water iAs analysis

Figure 1 shows the spatial distribution of 124 sampling points within Timis county as a whole, and inside the capital city Timisoara as an inset. The distribution of sampled water sources was relatively homogenous across the county and across the city. We sampled 44 of more than 100 artesian and non-artesian street wells present in Timisoara with depths equal to or more than 70 m (most 100 m) according to city records. Wells were frequently employed as 'secondary' water sources by participants living close by.

Distributions for water iAs concentrations were right skewed as summarized by Table 1. Values measured in 124 distinct drinking water sources varied from <0.5 to 175 µg/l, with an average of 8.6 µg/l and a median of 3.0 µg/l. There were 29 sources with iAs measured below the MDL. In 39 community-wide sources providing tap water, iAs varied from <0.5 to 36.4 µg/l, with an average of 5.0 µg/l and a median of 2.7 µg/l. In 85 public and private wells iAs varied from <0.5 to 175 µg/l, with an average of 10.3 µg/l and a median of 3.1 µg/l. Average and median iAs measured in primary water sources were 9.8 µg/l and 3.1 µg/l overall, respectively, while for secondary sources, the average and median values were 4.7 µg/l and 3.0 µg/l overall, respectively.

Table 2 describes the distribution of drinking water iAs exposure for 294 pregnancies (Table 2). We excluded n=6 reporting use only of bottled water for drinking. Women described consumption of 1.5 liters of non-bottled water per day on average. The mean daily exposure from primary sources overall was 4.2 µg/l, although the median was <0.5 µg/l. More specifically, mean and median concentrations for water consumed from primary community (tap water) supplies were equal to 1.7 µg/l and <0.5 µg/l, respectively. However, the mean (13.1 µg/l) and median (3.8 µg/l) exposures from primary well sources, were higher. Overall, daily intake from residential water sources was 6.6 µg/day on average with a median of 1.0 µg/day.

3.2 Spatial analysis

The spatial distribution of iAs in Timis County drinking water sources kriged from measured sources is described by Figure 2. Arsenic concentrations above 10 µg/l, were measured in the western, southwestern, northwestern, and central parts of the county (i.e., light and dark orange areas), as compared to sources with <10 µg/l located in the southern, northern, and eastern parts of the county (i.e., green areas). Within Timisoara (Figure 3), the spatial distribution shows higher iAs concentrations, in sampling locations from the central, south, southwestern, western, eastern, and southeastern parts of the city (i.e., red, orange, and yellow areas) as compared with sampling locations from northern, northeastern, and northwestern parts of the city (i.e., blue and green areas). Daily iAs intake, based on average

daily intake from primary and secondary sources, was higher for participants residing in the western, southwestern, northwestern, and central parts of Timis County, as compared to those living in the eastern and northern parts of the county (Figure 4). Within Timisoara, daily iAs intakes were higher for those living in the central, south, southwestern, and southeastern parts of the city as compared to those living in the northern, western and eastern parts (Figure 5).

3.3 Exposure validation analysis

Table 3 describes differences in urine arsenic measures for 10 unexposed and 10 exposed women. Values for $iAs^{III} + iAs^V$, DMA, MMA, and cationic arsenic were above MDLs for nearly all women (95%–100%). Higher median values were suggested for total urinary iAs among exposed compared to unexposed women (6.6 vs. 5 $\mu\text{g/l}$; $P=0.24$). Median values for overall arsenic (7.4 vs. 5.1 $\mu\text{g/l}$) and DMA (5.5 vs. 4.2 $\mu\text{g/l}$) were also higher in the exposed compared to the unexposed women, respectively. Exposed and unexposed women had similar medians, respectively, for $iAs^{III} + iAs^V$ (0.4 vs. 0.4 $\mu\text{g/l}$), MMA (0.5 vs. 0.5 $\mu\text{g/l}$), %DMA (80.9 vs. 79.1), %MMA (12.0 vs. 14.5) and %methylation (91.5 vs. 92.9%). Cationic (organic) arsenicals were higher among the unexposed women (0.7 vs. 0.4 $\mu\text{g/l}$; $P=0.31$). Table 4 describes correlations between average iAs concentrations in residential sources and urine arsenic. We identified positive correlations for average water concentration with urine total iAs ($r=0.35$, $P=0.13$) and DMA ($r=0.31$, $P=0.18$). Correlations were also positive for overall arsenic, $iAs^{III} + iAs^V$, MMA, and %DMA, but negative for cationic arsenic, %MMA, and %methylation. The pattern was analogous for daily iAs exposure. No associations were suggested for arsenic exposure and week of gestation (data not shown).

4. Discussion

In this preliminary study, we described the distribution of drinking water iAs contamination among sources used by pregnant women residing in Timis County, Romania (Bloom et al., 2014a). We measured a wide range of iAs levels across 39 community tap water delivery systems and 85 public and private wells, although most (75%) were below 7.8 $\mu\text{g/l}$. We used GIS to integrate laboratory and spatial data, indicating ‘hot spots’ with drinking water sources likely contaminated by more than 10 $\mu\text{g/l}$ in the western, southwestern, northwestern, and central parts of Timis County, and in the central, south, southwestern, western, eastern and southeastern parts of Timisoara. We also corroborated residential drinking water as a route of iAs exposure in our study population, but perhaps not the most important source, by analyzing urine in a subsample of participants. Still, given the widespread and highly variable nature of drinking water iAs contamination in Timis County and the likely vulnerability of pregnant women to adverse effects, these data may have important public health implications (Bloom et al., 2010; Bloom et al., 2014b; Vahter, 2009).

The western region of Romania, including Timis County draws water, in part or in total, from iAs contaminated aquifers (Gurzau and Gurzau, 2001). In rural areas in particular, such contaminated sources are used for drinking and irrigation. In contrast, urban centers in this region frequently employ surface water (or a combination of surface and groundwater) for

drinking purposes, which is relative free of geogenic iAs. Timisoara draws community water from both surface (Bega River) and ground sources (deep wells in the southeast and the northwest). Contingent on residence and demand (monitored electronically), community supplies are comprised entirely of surface water (northeast), groundwater (southeast), or a mixture thereof (72%:28% on average), accounting for differences in tap concentrations we measured in the various parts of the city. No arsenic mitigation technologies have been implemented. As noted earlier, residents of Timisoara also access more than 100 artesian and non-artesian street wells of various depths. Interactions between thermal, glacial palaeo-, fossil marine, surface, and rain waters have created a complex aquifer system in the study area (Rowland et al., 2011). Sediments in this area are permeable, facilitating groundwater movement with rapid local-level water circulation through shallow aquifers. Arsenic levels $>10 \mu\text{g/l}$ generally have been found in intermediate-depth aquifers (100 to 600 m depth), where water movement is much slower, and the water older, dating from the last ice-age (late Pliocene to Quaternary) (Rowland et al., 2011). Mobilization and release of iAs in local sediments appears to be related to microbial processes, most likely reductive dissolution of iAs bearing Fe-oxides during sediment burial and subsequent diagenesis (Harvey et al., 2002; Islam et al., 2004; McArthur et al., 2004; Nickson et al., 1998), and to the presence of sulfate (Buschmann and Berg, 2009; Kirk et al., 2004; Quicksall et al., 2008). Varsanyi and Kovacs (Varsanyi and Kovacs, 2006) identified a direct correlation between iAs concentration in groundwater and the amount of extractable organic matter and Fe-minerals in sediments.

The wide spatial variability we measured in drinking water iAs sources is likely due in part to the different depths of the wells, and in part to the complex hydrogeological features of the Pannonian Basin. We interpolated drinking water iAs concentrations using a kriging approach, in which a hypothetically horizontal spatial model of the sampled points was assumed (Oliver and Webster, 1990). Variability in the geochemistry of aquifers at different depths across the study area might have introduced error into our interpolation estimates. Even so, this is unlikely to have impacted our exposure estimates as we measured iAs drinking water sources reported individually by all study participants

Our data suggest that contaminated drinking water contributes to iAs exposure in the study population. Due to limited resources, we were only able to analyze 20 urine samples, but we found that the drinking water iAs concentration was positively correlated to a biomarker of iAs internal dose; the higher the drinking water iAs the higher the urine total iAs, as well as the urine DMA, the primary iAs metabolite (Vahter, 2002), and with an opposite pattern for cationic (organic) arsenic. Methylation ratios were similar across groups and unrelated to average drinking water iAs concentrations, indicating little variability in iAs metabolism. Exposure to the organic arsenicals is dietary, unrelated to drinking water, and is not hazardous (ATSDR, 2007). Yet, the modest correlations we measured between iAs exposure assessed using questionnaire data weighted by water sampling, and iAs exposure measured using urine-based biomarkers, indicate non-trivial misclassification or additional uncaptured sources of iAs exposure such as drinking water consumption at the workplace, or dietary sources of iAs; no women reported employment that might have led to occupational exposure. Gestation also impacts arsenic metabolism (Concha et al., 1998); however,

women exposed and unexposed to drinking water iAs had similar gestation lengths and so the impact on our study results was likely to have been modest.

Populations are exposed to iAs primarily through drinking water (WHO, 2001), although food products may also be important sources of exposure in areas with $<10 \mu\text{g/L}$ drinking water contamination. Rice in particular has been identified as an important source of iAs exposure in some populations (Williams et al., 2005). We did not capture dietary data in our study and so we were unable to assess the impact. Yet, rice consumption is infrequent in the study region, as indicated by the results of the Central and Eastern European Arsenic Health Risk Assessment and Molecular Epidemiology (ASHRAM) study (unpublished data). Still, other dietary sources of iAs may explain in part the balance of urine total iAs not associated with drinking water concentrations, and also higher levels of cationic (organic) species among the unexposed women. Daily dietary intake in western countries has been estimated at 8.3 to $14 \mu\text{g/day}$ and from 4.8 to $12.7 \mu\text{g/day}$ for the U.S. and Canada, respectively, with 21% to 40% (1.66 – $5.6 \mu\text{g/day}$ and 1.01 – $5.08 \mu\text{g/day}$) as iAs (Yost et al., 1998), suggesting that in addition to residential drinking water exposure, other sources may raise daily exposures above thresholds for biologic effects in some women given that daily iAs intake ranged from $<\text{MDL}$ – $350 \mu\text{g/day}$ on average, from residential drinking water alone.

Previous studies of the Pannonian basin and western Romania in particular also reported drinking water iAs contamination. A 1995 survey reported 0 to $176 \mu\text{g/l}$ iAs for 134 sources sampled from six towns and 80 small settlements in Arad and Bihor counties in Romania (Surdu et al., 1997). Most (58%) iAs concentrations were less than $10 \mu\text{g/l}$; however, 41.7% of sources exceeded this threshold, including 10.1% above $50 \mu\text{g/l}$. The ASHRAM Study measured iAs in 112 surface and groundwater drinking sources between 2002 and 2004 (Lindberg et al., 2006). The median iAs value for 48 Arad County drinking water sources was $0.48 \mu\text{g/l}$ (range 0.0 – 24.0), and was $0.70 \mu\text{g/l}$ (range 0.0 – 95.0) for 54 Bihor county sources. Another study in the area succeeded in measuring MMA^{III} in human urine for the first time (Aposhian et al., 2000). In our Timis County study, 82.3% of sources had iAs below the WHO and Romanian MCL ($10 \mu\text{g/l}$), while 14.5% exceeded $10 \mu\text{g/l}$, but were below $50 \mu\text{g/l}$, and 3.2% surpassed $50 \mu\text{g/l}$. However, iAs exceeded $10 \mu\text{g/l}$ in over 500 drinking water sources, with 358 greater than $50 \mu\text{g/l}$, for nearby areas in Hungary (Csalagovits, 1999). The highest concentrations ($>800 \mu\text{g/l}$) were encountered in deep-seated geothermal waters in that study ($>300 \text{ m}$). Arsenic concentrations exceeding $400 \mu\text{g/l}$ were reported for drinking water sources in Vojvodina province, located in the Serbian sector of the Pannonian Basin also proximate to our study site; although most were lower (Jovanovic et al., 2011). Overall, iAs levels appear to be lower in the Pannonian Basin than reported for other iAs endemic areas, such as in Bangladesh, India, and Chile (Smedley and Kinniburgh, 2002). Still, Pannonian basin populations, including the nearly 700,000 residents of Timis County, Romania (Romanian National Institute of Statistics, 2014), and pregnant women in particular, are at an increased and ongoing risk for exposure to drinking water contaminated by $<10 \mu\text{g/L}$ iAs; however, there is little data available to determine the impact on adverse pregnancy outcomes at these levels (Mead, 2005).

5. Conclusions

In conclusion, our study results showed that most drinking water sources in Timis County used by our study participants had iAs concentrations below the WHO and Romanian MCL of 10 µg/l, but 34% of sources were greater than 5 µg/l. Higher levels of iAs were measured in drinking water collected from wells as compared to tap water sources, and our spatial analysis suggested a heterogeneous pattern of iAs contamination, with limited areas of drinking water iAs above 10 µg/l. Furthermore, drinking water iAs was associated with urinary iAs biomarkers, supporting increased exposure among pregnant women, although indicative of other non-residential drinking water sources of iAs exposure. Given the widespread and heterogeneous exposure suggested by our data, it is likely that a larger population group in Timis County, including pregnant women, is exposed to drinking water contaminated by <10 µg/L. To reduce the potential public health impact of exposure, a larger and more definitive study is needed to collect sufficient data for implementation of efficient drinking water iAs reduction measures.

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Figure 1.
Water sampling locations in Timis County and Timisoara, Romania (n=124).

Figure 2.
Kriged levels of iAs measured in 124 drinking water sources in Timis County, Romania.

Figure 3.
Kriged levels of iAs in 44 drinking water sources in Timisoara, Romania.

Figure 4.
Daily drinking water iAs intake for women residing in Timis County, Romania.

Figure 5.
Daily drinking water iAs intake for women residing in Timisoara, Romania.

Table 1

iAs concentrations measured in drinking water from sources in Timis County (µg/l)

Water sources	n	Mean	SD	25 th %tile	Median	75 th %tile	Min.	Max.
All measured Timis County sources	124	8.6	21.1	0.59	3.0	7.8	<0.5	175
Community supply (tap water)	39	5.0	7.5	0.55	2.7	6.1	<0.5	36.4
Well and spring water	85	10.3	24.9	0.65	3.1	8.9	<0.5	175
'Primary' water sources ^a	96	9.8	23.7	0.59	3.1	8.2	<0.5	175
'Secondary' water sources ^b	28	4.7	5.6	0.73	3.0	4.8	<0.5	23.1

iAs, inorganic arsenic; Max, maximum value; Min., minimum value; SD, standard deviation.

^a, 'Primary' water sources were defined as those used most frequently, principally for cooking, bathing, cleaning, and drinking, and were often tap;

^b, 'Secondary' water sources mainly used for drinking and almost exclusively wells.

Table 2
iAs concentrations in water consumed by study participants residing in Timis County and arsenic daily intake from drinking water

Water sources	n	Mean	SD	25 th %tile	Median	75 th %tile	Min.	Max.
'Primary' water sources (µg/l) ^a	294	4.2	14.5	<0.5	<0.5	3.3	<0.5	175.1
Community supply (tap water)	230	1.7	3.8	<0.5	<0.5	1.3	<0.5	36.4
Well water	64	13.1	28.6	<0.5	3.8	128	<0.5	175.1
'Secondary' water sources ^b (µg/l)	67	5.1	6.2	0.7	3.1	4.4	<0.5	23.1
Daily intake (µg/day) ^c	294	6.6	23.2	0.4	1.0	5.4	<0.5	350.2

NOTE: n=6 women reporting use only of bottled water for drinking excluded.

iAs, inorganic arsenic; Max, maximum value; Min., minimum value; SD, standard deviation.

^a'Primary' water sources were defined as those used most frequently, principally for cooking, bathing, cleaning, and drinking, and were often tap;

^b'Secondary' water sources were mainly used for drinking and almost exclusively wells;

^cDaily exposure calculated as the average iAs concentration across primary and secondary water sources multiplied by the reported daily water consumption.

Table 3

Median (95% CI) urine arsenic measures between pregnant women with (n=10) and without (n=10) residential drinking water arsenic exposure

Analyte	Avg. iAs water concentration > MDL ^a		Avg. iAs water concentration		P-value ^c
	Median	(95% CI)	Median	(95% CI)	
Overall arsenic (µg/l)	7.4	(4.5, 18.2)	5.1	(4.5, 14.2)	0.52
Total iAs ^d	6.6	(3.9, 10)	5	(3.7, 8.9)	0.24
iAs ^{III} + iAs ^V	0.4	(0.3, 1.1)	0.4	(0.4, 1.1)	0.85
DMA	5.5	(3.1, 8.8)	4.2	(2, 7.2)	0.31
MMA	0.5	(0.4, 1.7)	0.5	(0.2, 1.1)	0.52
Cationic (organic) arsenic	0.4	(0.3, 2.2)	0.7	(0.3, 4)	0.31
%DMA ^e	80.9%	(77.2, 87.6)	79.1%	(52.7, 88.3)	0.52
%MMA ^f	12.0%	(9.1, 18.5)	14.5%	(5.9, 28.3)	0.62
%Methylation ^g	91.5%	(87.7, 95.6)	92.9%	(88.8, 94.2)	0.62

NOTE: Urine values corrected for specific gravity.

Avg., average of up to two residential drinking water sources; CI, confidence interval; DMA, dimethylarsinic acid; iAs, inorganic arsenic; MMA, monomethylarsonic acid; MDL, method detection limit (0.5 µg/l).

^a Average iAs water concentration = 10.24 µg/l;

^b Average iAs water concentration = <0.5 µg/l;

^c Mann-Whitney U-test between exposed & unexposed groups;

^d Total iAs = iAs^{III} + iAs^V + DMA + MMA;

^e %DMA = 100 x (DMA/Total iAs);

^f %MMA=100 x (MMA/DMA);

^g %Methylation = 100 x ((DMA + MMA)/Total iAs).

Table 4

Spearman rank correlation coefficients (95% CI) between arsenic concentrations measured in residential drinking water sources (µg/l) and in urine specimens (µg/l) collected from 20 pregnant women

Analyte	Avg. iAs water concentration		Daily iAs intake via water	
	r (95% CI)	P-value	r (95% CI)	P-value
Overall arsenic	0.24 (-0.23, 0.61)	0.31	0.13 (-0.33, 0.54)	0.58
Total iAs ^a	0.35 (-0.12, 0.68)	0.13	0.25 (-0.23, 0.62)	0.30
iAs ^{III} + iAs ^V	0.07 (-0.39, 0.50)	0.77	0.09 (-0.37, 0.51)	0.69
DMA	0.31 (-0.16, 0.66)	0.18	0.20 (-0.27, 0.59)	0.40
MMA	0.15 (-0.31, 0.56)	0.52	0.25 (-0.23, 0.62)	0.30
Cationic (organic) arsenic	-0.17 (-0.57, 0.30)	0.47	-0.17 (-0.57, 0.30)	0.47
%DMA ^b	0.20 (-0.27, 0.58)	0.41	0.08 (-0.38, 0.50)	0.75
%MMA ^c	-0.17 (-0.57, 0.30)	0.48	-0.06 (-0.49, 0.39)	0.80
%Methylation ^d	-0.06 (-0.49, 0.39)	0.80	-0.06 (-0.49, 0.39)	0.80

NOTE: Urine values corrected for specific gravity.

Avg., average of up to two residential drinking water sources; CI, confidence interval; DMA, dimethylarsinic acid; iAs, inorganic arsenic; MMA, monomethylarsonic acid.

^aTotal iAs = iAs^{III} + iAs^V + DMA + MMA;

^b%DMA = 100 x (DMA/Total iAs);

^c%MMA = 100 x (MMA/DMA);

^d%Methylation = 100 x ((DMA + MMA)/Total iAs).