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Prolonged exposure to arsenic in UK private water supplies: toenail, hair and drinking water concentrations†

D. R. S. Middleton,[abc] M. J. Watts,*[b] E. M. Hamilton,[b] T. Fletcher,[c] G. S. Leonardi,[c] R. M. Close,[c] K. S. Exley,[c] H. Crabbe[c] and D. A. Polya[a]

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Chronic exposure to arsenic (As) in drinking water is an established cause of cancer and other adverse health effects. Arsenic concentrations >10 μg L^{-1} were previously measured in 5% of private water supplies (PWS) in Cornwall, UK. The present study investigated prolongued exposure to As by measuring biomarkers in hair and toenail samples from 212 volunteers and repeated measurements of As in drinking water from 127 households served by PWS. Strong positive Pearson correlations ($r_p = 0.95$) indicated stability of water As concentrations over the time period investigated (up to 31 months). Drinking water As concentrations were positively correlated with toenail ($r_p = 0.53$) and hair ($r_p = 0.38$) As concentrations – indicative of prolonged exposure. Analysis of washing procedure solutions provided strong evidence of the effective removal of exogenous As from toenail samples. Significantly higher As concentrations were measured in hair samples from males and smokers and As concentrations in toenails were negatively associated with age. A positive association between seafood consumption and toenail As and a negative association between home-grown vegetable consumption and hair As was observed for volunteers exposed to <1 As μg L^{-1} in drinking water. These findings have important implications regarding the interpretation of toenail and hair biomarkers. Substantial variation in biomarker As concentrations remained unaccounted for, with soil and dust exposure as possible explanations.

Environmental impact

Arsenic is an established carcinogen, chronic exposure to which has been linked to several cancers (lung, bladder, skin) as well as non-cancerous (cardiovascular disease, diabetes mellitus) health effects. This work consists of a human biomonitoring study (a collaboration between the University of Manchester, British Geological Survey and Public Health England) of 212 volunteers from 127 households with private water supplies from across Cornwall, UK. It is the largest scale exposure biomonitoring study conducted for As and drinking water in the UK to-date and investigates an exposure source for As that, until recently, had not been investigated in depth in the region. The sampling protocol consists of an initial and follow-up water collection spanning a period of either 8 or 31 months which, together with long-term biomarkers such as toenails and hair, allows for the assessment of prolonged arsenic exposure. Furthermore, the methods employed in this paper allow for an assessment of the efficacy of toenail washing procedures given the recognition of the susceptibility of this biomonitoring matrix to external contamination. The demonstration of effective contamination removal from samples in this study will be of great benefit to the wider field.

1. Introduction

Chronic exposure to arsenic (As) in contaminated drinking water is an established cause of lung, skin, bladder and kidney cancer[1] as well as other adverse health effects, posing a global health concern. Five major As endemic regions of the world provide the strongest evidence of this association: north-west and south-east Taiwan;[2] northern Chile;[3] Argentina;[4,5] Bangladesh[6] and West Bengal.[7] Although the aforementioned areas are more severely affected, As contaminated municipal and private water supplies (PWS) have been reported in countries across all inhabited continents.[8] Notable European examples include Hungary,[9] Romania,[9] Slovakia[9] and Serbia.[10]

A survey[11] of PWS in Cornwall, south-west England, reported concentrations exceeding the 10 As μg L^{-1} UK prescribed concentration or value[12] (PCV) and WHO guidance value[13] in 5% of drinking water samples collected ($n = 497$). In a follow-up biomonitoring study,[14] a subset of the same cohort, drinking water As concentrations were positively correlated with urinary As concentrations after the exclusion of arsenobetaine (AB) and

[a]*School of Earth, Atmospheric and Environmental Sciences & William Research Centre for Molecular Environmental Science, University of Manchester, Oxford Rd, Manchester, M13 9PL, UK*

[b]*Inorganic Geochemistry, Centre for Environmental Geochemistry, British Geological Survey, Nicker Hill, Keyworth, Nottinghamshire, NG12 5GG, UK. E-mail: mwatts@bgs.ac.uk*

[c]*Centre for Radiation, Chemicals and Environmental Hazards (CRCE), Public Health England, Chilton, Didcot, Oxfordshire, OX11 0RQ, UK*

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adjustment for hydration (osmolality adjustment). These urinary As concentrations reflected exposure in the preceding 2–4 days.[15] Information on the longevity and temporal variation of exposure in this study group was still outstanding. Two methods that can assess exposure over extended timescales are repeat monitoring of drinking water As concentrations and monitoring of biological matrices, such as toenails and hair, that reflect a longer exposure window than urine. Both approaches were employed in the present study.

There are currently 2460 registered single domestic dwellings served by PWS in Cornwall,[16] with the true number likely to be much greater. No published data on the temporal variation of As concentrations in UK PWS were previously available, but studies elsewhere reported mixed findings. In Nevada, USA, although concentration changes (mean = −3 As μg L^{-1}) were measured in some supplies,[17] with greater changes associated with higher As concentrations, no clear temporal trends were observed between wet and dry seasons. In a related study,[18] strong Spearman correlations (r_s) ($r_s = 0.95$) were reported between As concentrations in the same wells over a period of 11–20 years, with both studies concluding that, for the region, limited measurements are sufficient for predicting exposures over such timescales. Similarly, in Michigan, USA, strong Pearson correlations (r_p) ($r_p = 0.88$) were reported[19] between As measurements taken an average of 14 months apart. Concentrations were affected by point-of-use (POU) treatment systems, highlighting the necessity of collecting treatment usage data. Conversely, a study conducted in Washington, USA[20] reported changes as high as 19-fold in As concentrations measured in the same supply 12 months apart, suggesting that temporal stability of As concentrations varied by region due to geological and geochemical variables, if not inconsistencies in sampling methodologies.[19]

The use of toenail and hair biomonitoring for As exposure offers the assessment of a longer exposure window than that reflected by urine sampling. The affinity of As for sulfhydryl groups in the keratin of nails and hair, the isolation of these matrices from other metabolic processes following their formation and the time taken for them to 'grow out,' makes them attractive for measuring biomarkers of past As exposure.[21] Nails and hair have the added value of a non-invasive collection protocol and few sample transport/storage requirements. Positive correlations between drinking water and biomarker As concentrations have been reported in numerous studies for both toenails[22–25] and hair.[21,26] Increased risk of various cancers, including cutaneous melanoma[27] and small and squamous-cell carcinoma of the lung,[28] have also been positively associated with toenail As concentrations.

Despite the advantages of toenail and hair biomonitoring, caveats apply when using these matrices to assess exposure. Factors unrelated to exposure have been reported to influence As concentrations in hair and nails: namely, the inter-individual variability of growth rates of the biomonitoring matrices, demographic and behavioural factors such as age, gender and smoking,[23] their susceptibility to external contamination[29,30] and the consumption of dietary items such as fruit juices,[31] beer,[32] wine[32] and dark-meat fish.[33]

Average growth rates for fingernails are 0.1 mm per day whereas toenails are estimated to grow by 0.03–0.5 mm per day, meaning that fingernails and toenails reflect exposure windows dating back approximately 6 and 12–18 months, respectively.[34] Hair reflects a period of just a few months, with reported scalp hair growth rates ranging from 0.2 to 1.12 mm per day.[35] Growth rates for both matrices have been demonstrated to vary with demographic factors *e.g.* age and gender,[29,34–36] with obvious implications for interpreting exposure assessments conducted on diverse populations.

The susceptibility of nails and hair to external contamination is well documented, with a range of washing procedures having been implemented.[29,37] The degree of sample contamination likely depends on personal hygiene, hobbies, other behavioural variables and the relative ubiquity of the chemical element of interest. Fingernails are reportedly more prone to contamination than toenails[38] but this does not likely apply to communities who are often barefoot or wear open toed footwear. Contamination of hair and nails from cosmetic products such as shampoos, hair colourings and nail polish is another important consideration. A study[39] of the trace element composition of nail polish estimated that the As contribution from polish, if present, can range from 16 to 633%.

Whilst studies now routinely report the washing of nail and hair samples prior to analysis, few have quantified the degree of exogenous As *versus* As in toenails, or confirmed the removal of exogenous As from samples. One investigation[40] of exposure to As in soils, also conducted in Cornwall, retained toenail washing solutions for As determination. Both the final rinse fractions and a pooled solution of all preceding fractions were retained to quantify exogenous As contamination and confirm its removal from samples. The As content of final rinse fractions accounted for 0.2 to 1.6% of the total As measured in toenails.[40] This provided strong evidence of the efficacy of the washing procedure but, with a sample of 17 volunteers, the performance of this method remained to be validated on a greater scale.

The present study aimed to assess exposure to inorganic As *via* drinking water consumption in a population served by PWS in Cornwall, UK, using hair and toenail biomarkers in addition to initial and follow-up drinking water samples collected up to 31 months apart. Specific objectives were to (i) compare repeat PWS drinking water As concentrations measured either 8 or 31 months apart; (ii) investigate the effects of As concentration, duration between measurements, source type and treatment usage on changes in drinking water As concentrations; (iii) measure the total As concentrations in toenail and hair samples collected from volunteers and assess their relationship with drinking water As concentrations adjusted for other covariables (demographic, behavioural and dietary) and (iv) quantify the potential for external sample contamination to affect As concentrations in toenail and hair samples, including the use of nail polish and hair dye.

2. Experimental

Ethical approval and volunteer communication

Ethical approval was granted by the University of Manchester Research Ethics Committee (Ref 13068) and the NHS Health

Research Authority National Research Ethics Service (NRES) (Ref 13/EE/0234). All volunteers provided written informed consent prior to participating. Individual data feedback to participants was provided through a letter containing specific guidance developed by PHE along with BGS and Cornwall County Council. Participants were given advice on any potential health risks and suggested corrective actions if they had one or more exceedances of the water quality standards. All participants were provided with appropriate contact details for any follow-up enquiries.

Recruitment and sample collection

Environmental monitoring. The sampling frame consisted of 476 households using a PWS that had provided drinking water samples during a previous survey[11] – henceforth referred to as initial sampling (drinking water only). The initial survey was conducted in two parts, with households in east and west Cornwall surveyed in March–April 2011 and March–April 2013, respectively. Information letters were sent to households that participated in initial sampling and, after being contacted by telephone, 127 households were recruited to provide a follow-up drinking water sample. Follow-up sampling took place in November 2013. This resulted in 127 drinking water samples collected either 31 ($n = 51$) or 8 ($n = 76$) months apart depending on whether households were in east (2011 initial collection) or west (2013 initial collection) Cornwall, respectively. Point-of-use drinking water samples were collected using a previously reported protocol[11]

Biomonitoring. Biomonitoring was conducted on one occasion only – at the time of the follow-up drinking water collection in November 2013. Sample collection packs were mailed to volunteers prior to household visits. Volunteers were asked to allow a minimum of 4 weeks for toenail growth (to ensure sufficient mass for analysis) before self-collecting from all 10 toes and storing in polyethylene bags. Hair samples were collected by researchers during visits using an amended version of the COPHES project protocol.[41] Hair >3 cm in length was removed from the nape by twisting into a pencil-width strand before tagging with masking tape. The tape was labelled with an arrow pointing towards the root. Strands were removed with ethanol-rinsed stainless steel scissors as close to the scalp as possible. Hair <3 cm in length was collected in smaller amounts from several locations on the back of the head. The portion of hair >5 cm was discarded with the portion closest to the scalp being retained for analysis.

Additional variables. An exposure/food frequency questionnaire was administered to volunteers using Microsoft Access on a laptop/tablet device. For drinking water related analysis, data on PWS source type, treatment usage, system storage and borehole depth were collected at the time of initial water sampling. For biomonitoring analysis, demographic and behavioural variables – age, gender, current smoking status, nail polish and hair product usage – were collected and, additionally, information on the consumption of select dietary items that have been reported[31–33] to contain As in relatively high concentrations. These were: PWS water consumption (L per day); home-grown vegetable consumption (all year, seasonally, only in pots or never); rice (servings/week); seafood (servings/week); most often consumed seafood type (if reported): white fish (*e.g.* cod, plaice, haddock *etc.*), shellfish (*e.g.* mussels, prawns, cockles *etc.*) and dark-meat fish (*e.g.* salmon, tuna, mackerel, sardines *etc.*); beer (L per day); wine (L per day); cider (L per day) and fruit juice (L per day).

Chemical analyses

Reagents and standards. All aqueous solutions were prepared using 18.2 MΩ deionised water (DIW) (Millipore, UK). Nitric acid (HNO_3), hydrochloric acid (HCl) and 30% hydrogen peroxide (H_2O_2) were Romil-SpA™ super purity grade (Romil, UK). The acetone used for sample cleaning was HPLC grade (Fisher Scientific, UK). Arsenic calibration standards were made using an in-house multi-element stock in which the As contribution was from a 1000 mg L^{-1} PrimAg® grade mono-elemental solution (Romil, UK). Independent 25 μg L^{-1} As QC standards were prepared from a multi-element stock solution of various concentrations with As at 20 mg L^{-1} (Ultra Scientific, USA). A germanium (Ge) ICP-MS internal standard was prepared from a Fluka Analytical 1000 mg L^{-1} stock solution (Sigma-Aldrich, USA).

Sample pre-treatment and dissolution. Toenail samples were cleaned and digested by adapting a previously reported protocol.[40] Visible exogenous debris was removed using a PTFE policeman/stirring rod (Chemware, USA) in a HEPA filtered clean room. Samples with visible nail polish residue (regardless of whether reported in the questionnaire) were further cleaned with acetone and cotton wool. Samples were transferred into clean 25 mL Duran® borosilicate beakers (Schott, Germany), placed in an ultrasonic bath (Fisher Scientific, UK), sonicated at 37 MHz at room temperature for 5 minutes (15 minutes for those with visible varnish) in 3 mL of acetone, rinsed with 2 mL of DIW and then 2 mL of acetone, sonicated for 10 minutes in 3 mL of DIW and twice rinsed with 3 mL of DIW. All rinse aliquots prior to the final, which remained separate, were pooled in PFA vials (Savillex, USA) and evaporated to dryness overnight on a graphite hot block before reconstitution in 5 mL of 1% v/v HNO_3 + 0.5% v/v HCl. Both initial and final rinse fractions were analysed by ICP-MS for total As. The final fraction was analysed separately to assess the effectiveness of the washing procedure and confirm the elimination of exogenous contamination. A schematic of the abovementioned procedure can be viewed in ESI (Fig. S1†).

Toenail samples were dried to constant weight (12 h approx.) in a clean laminar flow hood (Envair, UK) and stored in microcentrifuge tubes in a silica gel desiccator before being weighed (0.1 g or as much as available) into PTFA MARS Xpress vessels (CEM Corporation, UK). Four millilitres of concentrated HNO_3 + 1 mL of H_2O_2 were added and samples were left to rest for 30 minutes until effervescence subsided. Vessels were capped and digested in a microwave assisted reaction system (MARS Xpress, CEM Corporation, UK) on the following heating program: ramped to 100 °C and held for 5 minutes; ramped to 200 °C and held for 30 minutes (100% power: 1200 W). Vessels

were left to cool overnight before their contents were transferred into PFA vials with DIW and reduced to a gel at 80 °C on a graphite hot block. One millilitre of 10% v/v HNO_3 was added to the vessels, which were then heated for 20 minutes at 50 °C followed by the addition of 4 mL of DIW. Digests were stored in polystyrene ICP-MS tubes.

Hair samples underwent the same cleaning and digestion procedure as toenail samples. Whatman Grade B-2 weighting papers (GE Healthcare Life Sciences, UK) and a Milty Zerostat 3 anti-static gun were used to aid the transfer of hair samples between vessels.

Total As determination by ICP-MS. Analysis was performed using an Agilent 7500cx series ICP-MS (Agilent Technologies, USA) fitted with a MicroMist low-flow nebulizer (Glass Expansion, Australia) and an ASXpress rapid sample introduction system (Teledyne CETAC Technologies, USA) using previously reported[42] operating conditions. Drinking water samples were analysed using a previously reported method.[11] Rinse solutions were diluted ×2 and analysed by a method used previously[11] for water samples. Those with visible suspended particulate were passed through a 0.45 μm Acrodisc® syringe filter (PALL Life Sciences, USA). Toenail and hair digests were diluted ×4 with 1% v/v HNO_3 + 0.5% v/v HCl. Helium (He) collision cell mode was used to remove potential polyatomic interferences with the same mass/charge ratio as As (*m*/*z* 75). Signal drift was corrected using a Ge internal standard introduced *via* a T-piece. Analytical limits of detection (LOD) were calculated as 3× the standard deviation of run blanks for drinking water analysis and 3× the standard deviation of reagent blanks for toenail and hair analysis. The LODs for As in drinking water and toenails/hair were 0.02 μg L^{-1} and 10 μg kg^{-1}, respectively.

Quality control. Toenail and hair samples of sufficient mass were chosen for duplicate analysis. Samples were milled to a fine powder using a 6850 Freezer Mill (SPEX Sample Prep, USA) – a cryogenic impact grinder cooled with liquid nitrogen. One pair of duplicates was digested per batch, in addition to 3 reagent blanks. Method accuracy was assessed using Certified Reference Materials (CRMs). Two samples (0.1 g) of NCS DC 73347 Hair (China National analysis Centre for iron and steel, Beijing, China) were digested per batch of hair and toenail samples. Two additional samples (0.1 g) of in-house human toenail reference material (BAPS2014 Human Toenail) were digested per batch of toenail samples. BAPS2014 was produced by pooling the toenail clippings, saved over a period of 2 years, of 2 male volunteers (aged 23 and 38) not knowingly exposed to substantial environmental or occupational As. A homogeneous powder was prepared using the cleaning and milling procedure already described prior to mixing end-over-end for several hours. The accuracy of drinking water and toenail washing solution measurements was assessed using NIST SRM 1643e Trace Elements in Water (National Institute of Standards and Technology, USA).

Statistical analyses

Statistical tests and plot production were performed using R version 3.0.0 (base package).[43] Pearson correlation coefficients with significance tests (*p*-values) and 95% confidence intervals (C.I) were used to assess the strength in relationship between: initial *versus* follow-up drinking water As; well depth *versus* As concentration difference; rinse *versus* digest As concentrations and drinking water *versus* toenail/hair As concentrations. Welch's independent unequal variance tests were used to test for differences in toenail, hair and rinse solution As concentrations between different subsets to account for unequal sample sizes. One-way analysis of variance (ANOVA) was used to test for differences in toenail and hair As concentrations between different age groups. Multiple linear regression models were constructed to assess significant predictors of toenail and hair As in addition to drinking water As. Exploratory analyses revealed positively skewed distributions for drinking water, toenail and hair As concentrations and As concentrations in rinse solutions. To address this, natural log(ln) transformations were applied to these variables prior to Pearson correlations, Welch's tests, ANOVA and multiple regression modelling. For the same reason, geometric means (GM) were calculated instead of arithmetic means. Left censoring was applied to hair As concentrations ($n = 8$) below the analytical limit of detection (LOD) by replacing values with half of the LOD.

3. Results and discussion

Study group

The spatial extent of the study is presented in Fig. 1 and characteristics of households and volunteers are shown in Table 1. Two hundred and twelve volunteers from 129 households reported using their PWS for human consumption and provided either a toenail sample, hair sample or both. This made the present study the largest investigation of long-term exposure to As in drinking water in the UK to-date. Repeated water samples were available for comparison from 127 households, the majority of which were supplied by a borehole. The age distribution of the study group was not representative of the corresponding local rural population, with 63% of volunteers aged over 60. It is noted here that population-based exposure estimates were not the focus of the present paper. Nail polish usage was reported by 17 of the 206 (8%) volunteers who provided toenails, whereas polish was observed on the toenail samples of almost double that number (30, 15%). This underlines the importance of checking nails for visible polish prior to analysis and not relying on questionnaire data alone.

Repeated drinking water measurements

At the initial sampling phase, 125 out of 127 households (98%) had detectable (>0.02 μg L^{-1}) concentrations of total As measured in their drinking water. At follow-up sampling, 126 out of 127 (99%) households had As concentrations >0.02 μg L^{-1}. Only fourteen of the 127 households (11%) exceeded the 10 As μg L^{-1} UK PCV and WHO guidance value at initial sampling with a maximum As concentration of 231 μg L^{-1}. Two households had borderline results (>9 As μg L^{-1}), one of which exceeded the PCV (17 As μg L^{-1}) at follow-up sampling. One further household, below PCV at initial sampling, exceeded at

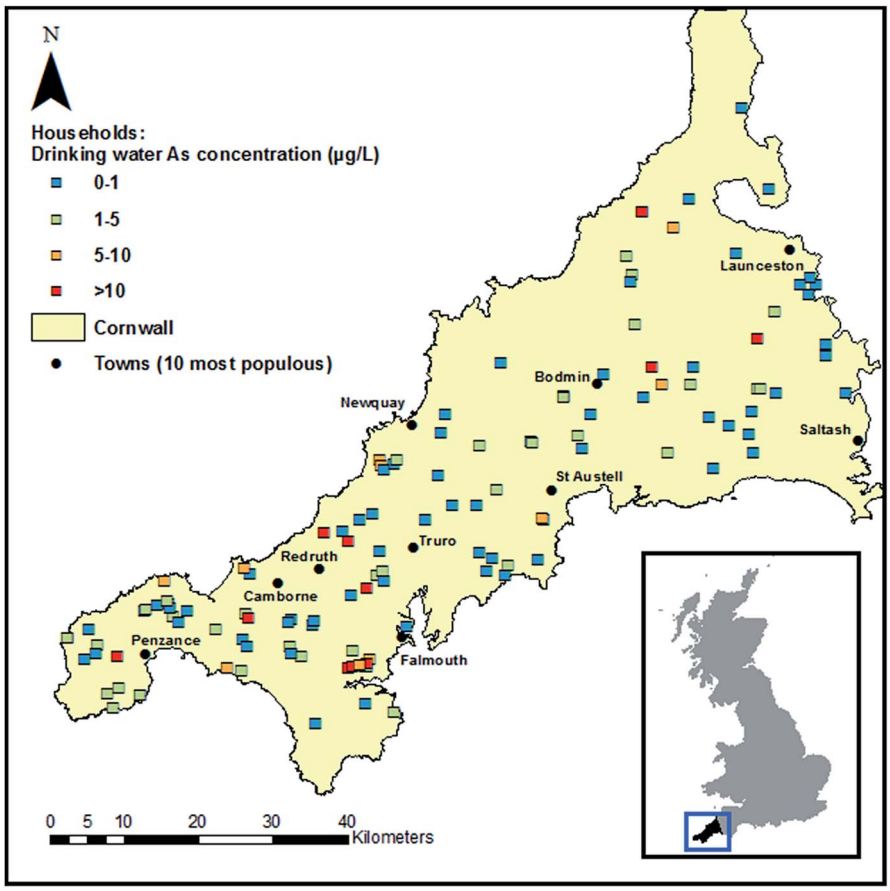

Fig. 1 Map of the Cornwall study area, shown in the context of the UK (excluding Northern Ireland), and the spatial distribution of sampled households. Total As concentrations measured in drinking water samples collected during the initial survey are plotted for reference. Note: no assessment of the spatial controls on As distribution was made in this study. Compiled using ESRI ArcMap 10.1.

follow-up (from 6 to 17 As µg L^{-1}). Only one exceedance dropped below PCV at follow-up sampling, from 14 to <1 As µg L^{-1}. Households who had high As concentrations in their PWS were advised to install appropriate remediation. Changes were not attributed to installation of treatment systems. Of three households that reported installation of any kind of treatment system between initial and follow-up measurements, none were among those exceeding the PCV and the impacts on As concentrations were minimal. Of the 14 households above PCV at initial sampling, 11 reported not installing any additional treatment and data were missing for the remaining three. This has important implications regarding risk awareness and the advice given to households above PCV.

Overall, As concentrations in PWS were stable over both 8 and 31 month periods. Mean differences in As concentrations, initial and follow-up GM As concentrations and Pearson correlation coefficients between initial and follow-up As concentrations are shown in Table 2. Follow-up As concentrations are plotted against their initial counterparts in Fig. 2. In agreement with previous studies,[17,18] strong Pearson correlations were observed between initial and follow-up samples collected both 8 ($r_p = 0.95$) and 31 ($r_p = 0.95$) months apart. A greater mean difference was observed for PWS with >10 As µg L^{-1} due to the higher concentrations reported in this group. The strongest correlation observed was for the subset of households with both iron (Fe) and manganese (Mn) removal systems and pH buffering systems ($r_p = 0.998$) in addition to a lower mean difference to supplies with neither treatment system. This is not unexpected given that supplies with treatment systems installed are not subject to underlying geochemical variations. Although no household in this study group reported using As-specific treatment systems, Fe/Mn removal units have been reported to reduce As concentrations.[11] Of the 62 households where borehole depth information was available, no significant correlation was observed between depth and the difference in As concentration between initial and follow-up sampling. This is consistent with previous studies.[17] Source type influence was only assessed between well and borehole sources due to a limited number of other source types. There was no apparent difference in As concentration changes between well or borehole source types or system storage. An observation was made regarding the correct categorisation of source type. One household in the present study reported using a borehole at initial sampling but on receiving initial results (80.5 As µg L^{-1}) it was discovered to be a disused mine adit (categorised as 'other' in Table 1). This highlights the importance of homeowners seeking the correct characterisation of their PWS when acquiring a new property.

Table 1 Household and study group characteristics

Households	129
Initial and follow-up water sample, *n* (%)[a]	127 (98.4)
Initial sample year, *n* (%)	
2011	51 (40.2)
2013	76 (59.8)
Source type, *n* (%)	
Borehole	111 (87.4)
Well	11 (8.7)
Spring capture	2 (1.6)
Other	3 (2.4)
Borehole depth reported, *n* (%)	62 (48.8)
Mean borehole depth (m)	48
Treatment system, *n* (%)	
Fe/Mn removal	18 (14.2)
pH buffering	60 (47.2)
Storage (*e.g.* water tank) in system, *n* (%)	
Yes	62 (48.8)
No	65 (51.2)
Volunteers	212
Gender, *n* (%)	
Male	109 (51.4)
Female	103 (48.6)
Mean age, years (range)	62 (18–90)
Age group, *n* (%)	
18–29	6 (2.8)
30–39	3 (1.4)
40–49	28 (13.2)
50–59	42 (19.8)
60–69	75 (35.4)
70–79	44 (20.8)
80–90	14 (6.6)
Smoking status, *n* (%)	
Currently smoking	13 (6.1)
Not currently smoking	191 (90.1)
Not reported	8 (3.8)
Provided toenails, *n* (%)	206 (97.2)
Provided hair, *n* (%)	186 (87.7)
Provided both, *n* (%)	180 (84.9)
Cosmetic usage, *n* (%)	
Polish usage reported (if toenails provided)	17 (8.3)
Polish observed on toenails	30 (14.6)
Dye usage reported (if hair provided)	31 (16.7)

[a] Subsequent characteristics and percentages in households section refer to this subset.

Toenail and hair total As

Due to difficulties with sample collection and handling, many hair samples were of low mass at the point of digestion. This prompted the determination of a minimum mass requirement for toenail and hair samples by digesting triplicate samples of NCS DC 73347 CRM in decreasing mass increments (0.1, 0.08, 0.07, 0.06, 0.05, 0.04, 0.03, 0.02, 0.01, 0.005, 0.002 and 0.001 g). Measured As concentrations were plotted against mass of CRM (Fig. S2a†), in the context of the certified value and upper/lower limits. So too were mean recovery and relative standard deviation (RSD) (Fig. S2b†). On the basis of these results, 0.02 g was chosen as the minimum mass requirement, being the lowest mass at which As concentrations were found to be consistently within upper/lower certified limits of the CRM. This value is not universal and may not apply to other studies but was selected to try and maximise the usage of a compromised sample set. Depending on the amount of As in samples, requirements may be lower or higher. The RSD calculated for triplicates at lower masses may also reflect reduced homogeneity of the CRM.

Following the exclusion of samples below the minimum mass, As data were available for the toenails and hair of 200 and 104 volunteers, respectively. All toenail and 96 (92%) hair samples were above the 10 μg kg^{-1} LOD. Arsenic measured in CRM NCS DC 73347 was 273 ± 10 As μg kg^{-1} ($n = 40$), within the certified range of 280 ± 50 As μg kg^{-1}, yielding a mean recovery of 98% with 5% precision. The mean As measured in BAPS 2014 Human Toenail was 93 ± 5 As μg kg^{-1} ($n = 20$). The accuracy of BAPS 2014 measurements could not be assessed, but good precision (5% RSD) was maintained. The mean difference between duplicate digests was 1.1% (7 pairs) and 3.4% (6 pairs) for toenail and hair, respectively.

Summary statistics for toenail and hair As concentrations are shown in Table 3 for different demographic and behavioural subsets. The GM toenail As concentration of all 200 volunteers was 151 As μg kg^{-1} and ranged from 27 to 3354 As μg kg^{-1}. This falls within previously published ranges, with a higher GM and maximum concentration than a study[23] conducted in New Hampshire, USA (GM: 90 As μg kg^{-1}; range: 10–810 As μg kg^{-1}), with comparable levels of drinking water exposure (<0.02–66 As μg L^{-1}). A previous study,[40] conducted in south west England, reported a range of 858 to 25 981 As μg kg^{-1} for individuals exposed to high As in soil, with no exposure to As in drinking water. Although conducted in the same geographic region as the present study, Button *et al.* (2009)[40] investigated individuals living in the direct vicinity of a former As mine, possibly explaining the much higher reported concentrations than the present study. Hinwood *et al.* (2003)[26] investigated the toenail As concentrations of volunteers in different exposure categories in rural Australia: high soil (>30 As mg kg^{-1}); high water (>10 As μg L^{-1}) and low exposure (<10 As μg L^{-1} in drinking water and <30 As mg kg^{-1} in soil). Overall, much higher toenail As concentrations were reported by Hinwood *et al.* (2003), across all categories, than those in the present study. For example, the minimum toenail As concentration in the low exposure group was 1350 μg kg^{-1}, of which only eight volunteers exceeded in the present study. Quantification/removal of exogenous As from toenail samples was cited as a limitation by Hinwood *et al.* (2003) and, therefore, few meaningful conclusions can be drawn from this comparison. Slotnick *et al.* (2007)[44] reported a lower drinking water As GM to the present study (0.59 *versus* 0.88 As μg L^{-1}) and a lower toenail As GM (70 *versus* 151 As μg kg^{-1}). Maximum drinking water and toenail as concentrations were also higher in the present study than those reported by Slotnick *et al.* (2007): 233 *versus* 99 As μg L^{-1} and 3353 *versus* 1260 As μg kg^{-1}, respectively. Other comparable studies include Rivera-Núñez

Table 2 Drinking water As arithmetic mean differences, initial and follow-up As concentration geometric means (GM) and results from Pearson correlations between initial and follow-up As concentrations (ln transformed variables) for different PWS subsets

Subsets	*n*	Mean difference (As μg L^{-1})	Initial total As GM (As μg L^{-1})	Follow-up total As GM (As μg L^{-1})	Pearson correlation (r_p)
All households	127	−0.7	1.0	1.0	0.95
Initial sample year					
2011	51	−1.1	0.8	0.9	0.95
2013	76	−0.5	1.2	1.2	0.95
Initial total As concentration					
<1 μg L^{-1}	67	0.1	0.2	0.3	0.87
1–10 μg L^{-1}	46	−0.1	3.2	2.7	0.68
>10 μg L^{-1}	14	−6.6	36.5	27.9	0.79
Source type					
Borehole	111	−0.8	1.2	1.1	0.95
Well	11	0.4	0.3	0.4	0.97
Treatment system					
Fe/Mn removal only	12	−0.2	1.7	1.6	0.95
pH buffering only	54	−0.2	0.8	0.8	0.94
Both of above	6	−0.3	0.5	0.5	1 (0.998)
Neither of above	55	−1.8	1.2	1.3	0.94
Storage (*e.g.* water tank) in system					
Yes	62	−1.7	1.1	1.1	0.94
No	65	0.3	0.9	1.0	0.95

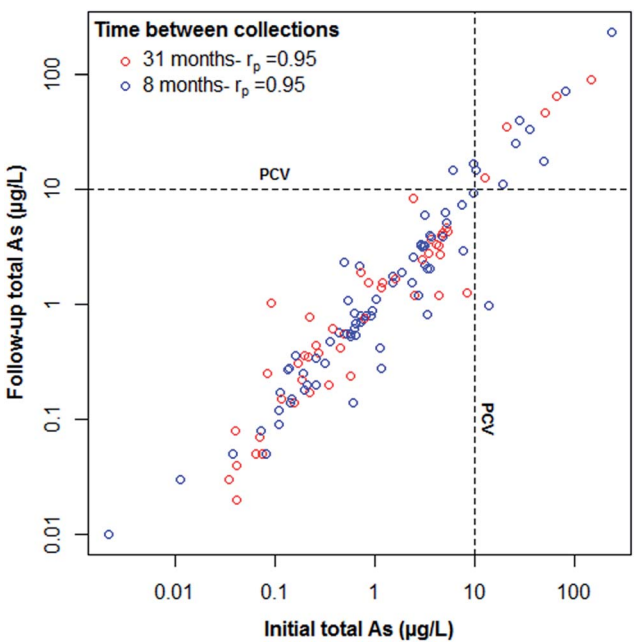

Fig. 2 Follow-up drinking water As concentrations plotted against initial counterparts. Pearson correlation coefficients (r_p) are shown for measurements taken 31 (2011 initial collection) and 8 (2013 initial collection) months apart.

et al. (2011)[45] and Yu *et al.* (2014)[24], with drinking water As GMs of 0.74 and 0.28 μg L^{-1} and toenail As GMs of 90 and 57 μg kg^{-1}, respectively. Widespread As exposure, on the basis of both drinking water and toenail As concentrations, was low in the present study compared to those reported in severely affected areas. Nevertheless, 10 volunteers in the present study exhibited toenail As concentrations above the GM (1010 As μg kg^{-1}) reported by Kile *et al.* (2005)[46] across three villages in Bangladesh – the world's worst affected region – with drinking water As concentrations between 1 and 752 As μg L^{-1} (GM: 6.2 As μg L^{-1}).

The GM hair concentration measured in the present study was 82 As μg kg^{-1} (range: <LOD–2906 μg kg^{-1}). The range reported in the only previous study[47] of hair As concentrations in Cornwall was 890–14 560 μg kg^{-1}. Although Peach and Lane (1998)[47] identified elevated hair concentrations in local residents, they could only speculate as to the likely exposure routes and, with a small study group of five volunteers and no established washing protocols at the time, few comparisons can be made with their study. It is reported that hair As concentrations between 100 and 500 μg kg^{-1} are indicative of chronic exposure and concentrations between 1000 and 3000 μg kg^{-1} are indicative of acute poisoning.[48] The As concentrations of 28 volunteers (15%) in the present study were between 100 and 500 μg kg^{-1} and the concentrations of a further 12 volunteers (6%) were >500 μg kg^{-1}. Of these 40 individuals, 10 were exposed to >10 μg L^{-1} of As in their drinking water. While it is not possible to conclude that these volunteers are either chronically or acutely exposed, where elevations correspond with drinking water As concentrations above PCV, attention is warranted.

Welch's tests (Table 3) detected no significant differences in toenail As between any subsets. Significantly lower hair As concentrations were detected for females (p < 0.001) and

Table 3 Summary statistics for total As in toenail and hair samples for different demographic and behavioural characteristic subsets of the study group. Statistically significant As concentrations between subsets are in bold type with *p*-values calculated by Welch's independent *t*-test on natural log transformed data in adjacent columns. Age group differences were assessed using one-way analysis of variance (ANOVA)

	n (toenails, hair)	Toenail total As (μg kg^{-1}), GM (range)	*p*-Value, Welch test (ANOVA for age groups)	Hair total As (μg kg^{-1}), GM (range)	*p*-Value for Welch's test (ANOVA for age groups)
All	200, 104	151 (26.9–3354)	—	82.6 (<LOD–2908)	—
Gender					
Male	102, 45	155 (26.9–1896)	0.63	**150 (28.8–2908)**	**<0.001**
Female	98, 59	146 (39.1–3354)		**52.5 (<LOD–756)**	
Age group					
18–39	6, 3	214 (8.1–1497)	0.28	89.9 (56.8–128)	0.76 (ANOVA)
40–49	27, 17	204 (57.9–3354)		121 (10.9–2396)	
50–59	41, 20	154 (43–2578)		79.2 (<LOD–756)	
60–69	74, 32	144 (39.1–1896)		67.4 (18.7–2908)	
70–79	40, 24	135 (26.9–1982)		79.7 (11–742)	
80–90	12, 8	111 (39.7–320)		100 (36.5–670)	
Smoking status					
Currently smoking	11,7	209 (100–2578)	0.25	**324 (28.8–2908)**	**0.04**
Not currently smoking	181,93	146 (26.9–1982)		**74.6 (<LOD–2396)**	
Nail polish usage					
Reported/observed	34	131 (44.6–1497)	0.34	—	—
Not reported/observed	166	155 (26.9–3354)		—	
Hair dye usage					
Reported	20	—	—	**41.4 (10.8–756)**	**0.003**
Not reported	84	—		**97.4 (<LOD–2908)**	

volunteers who reported using hair dye ($p = 0.003$). Significantly higher hair As concentrations were detected for smokers ($p = 0.04$). These findings were compared with a previous study[49] investigating demographic and behavioural controls on the composition of hair: Chojnacka *et al.* (2006) reported 150% more As in the hair of smokers, 210% more As in the hair of males and artificially coloured hair was reported to contain 200% more As than naturally coloured hair.[49]

Exogenous As quantification

Analysis of rinse solutions from the toenail washing procedure provided a useful insight into exogenous As contamination. The bar plot in Fig. 3 shows the hypothetical contribution of exogenous As to that measured in toenails if they had not been washed. Rinse concentrations were normalised to the mass of toenail washed to allow comparison with digest concentrations. For toenails without polish, the GM As measured in initial rinse fractions was 9% of that measured in digested toenails, whereas the GM final rinse fraction As concentration only accounted for 0.4%. Firstly, this confirmed the necessity of washing toenails, with a maximum percentage contribution of 716% in the case of one volunteer. Secondly, the low contribution from final rinse fractions indicated the effective removal of exogenous As (maximum contribution: 5%). Furthermore, in agreement with previous findings,[40,50] the washing procedure appeared to have begun to leach endogenous As from toenails by the final rinsing stage. This is indicated in Fig. 4, where no significant

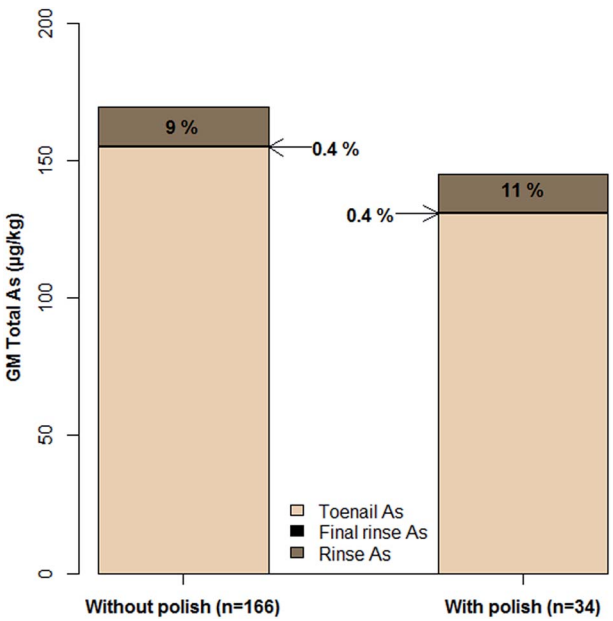

Fig. 3 Geometric mean (GM) As concentrations in toenail samples, initial and final rinse fractions for volunteers with and without observed/reported nail polish. Initial and final rinse fraction As concentrations as a percentage of the As measured in toenail digests are printed on plots.

correlation was observed ($r_p = -0.05$; $p = 0.43$) between initial rinse As concentrations and toenail digest As concentrations (Fig. 4a). Conversely, a significant positive correlation ($r_p = 0.71$; $p < 0.001$) was observed between final rinse As concentrations and toenail digest As concentrations (Fig. 4b). The relatively small hypothetical contributions (5% maximum) of final rinse As concentrations to those in toenail digests suggests that a small degree of leaching is of no great concern in the present study. It is noted that future efforts could be made to determine an optimum degree of washing for toenail samples and maximise the removal of exogenous As whilst minimising endogenous As leaching. It is likely that the optimum number of rinses would depend on the level of contamination on the nail surface – a difficult metric to quantify.

Welch's independent *t*-tests detected no significant differences in digest As concentrations ($p = 0.34$), initial rinse As concentrations ($p = 0.85$), final rinse As concentrations ($p = 0.74$) or percentage contributions from either initial ($p = 0.52$) or final ($p = 0.35$) rinse fractions between samples with and without nail polish. This finding does not dismiss the effects of polish on sample concentrations, as substantial contributions have been demonstrated elsewhere.[39] Several factors may have limited findings on this occasion: misreporting of polish usage/failure to identify polish on samples; ineffective polish removal during washing; low sample size of volunteers with polish and a lack of digestion procedure for rinse solutions/the inability to solubilise As present from polish. Contribution from polish has also been demonstrated[39] as brand dependent and further work is needed to quantify/mitigate the effects of polish usage on biomonitoring studies using human nails as part of a wider review of the effects of surface contamination.

Drinking water and biomarker relationships

Due to the difference in duration between initial and follow-up drinking water samples, follow-up water samples (all of which were collected during the same sampling campaign as the hair and toenail collections) were used as explanatory variables of

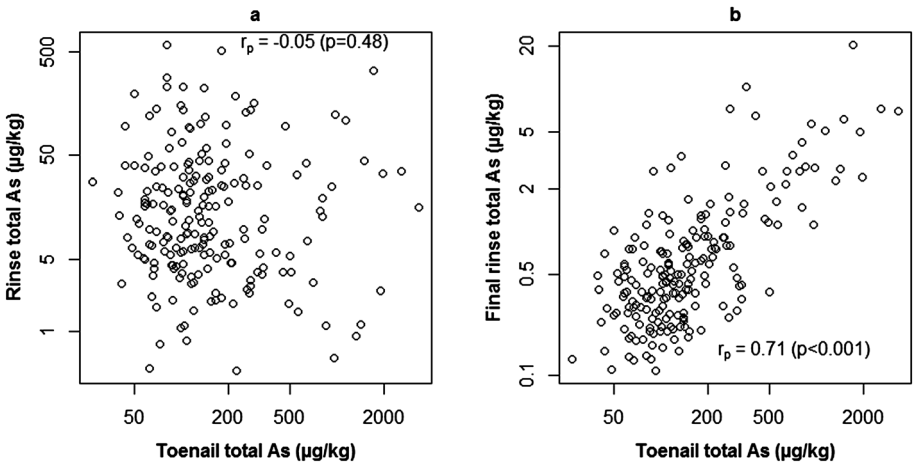

Fig. 4 Initial rinse fraction As concentrations (a) and final rinse fraction As concentrations (b) plotted against toenail digest As concentrations. No significant relationship (r_p) was observed for initial rinse fractions, but a strong significant correlation was evident for final rinse fractions. This suggests (i) effective exogenous As contamination removal and (ii) subsequent leaching of As from toenails.

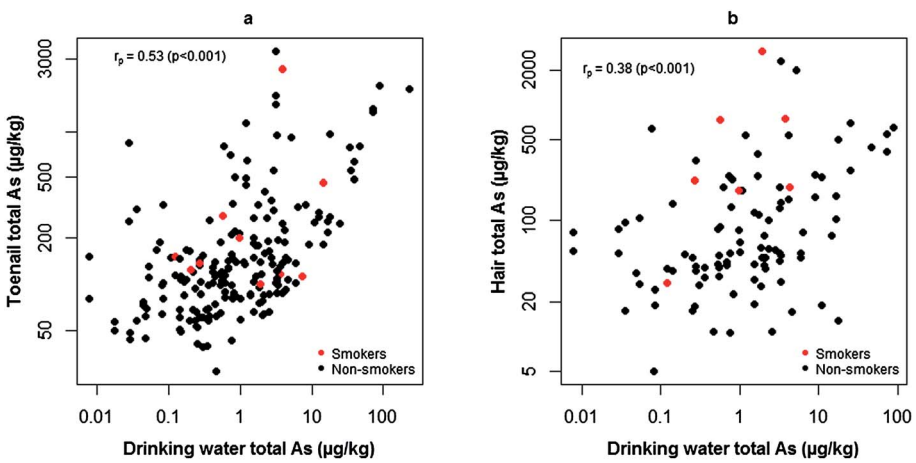

Fig. 5 Significantly positive Pearson correlations (r_p) between toenail (a) and hair (b) biomarker As concentrations and those measured in drinking water.

Table 4 Pearson correlations (r_p) for drinking water As and toenail and hair As for different drinking water As concentration ranges. Moderate/strong correlations (bold type) were only observed where drinking water As exceeded 10 μg L^{-1}

	Pearson's r_p (p-value, [95% C.I])			
	Drinking water As <1 μg L^{-1}	Drinking water As 1–10 μg L^{-1}	Drinking water As >10 μg L^{-1}	Full range
Toenail total As	0.15 ($p = 0.13$, [−0.04, 0.33]) ($n = 107$)	0.12 ($p = 0.32$, [−0.12, 0.34]) ($n = 73$)	**0.86 ($p < 0.001$, [0.66, 0.94]) ($n = 19$)**	0.53 ($p < 0.001$, [0.43, 0.63]) ($n = 199$)
Hair total As	0.11 ($p = 0.45$, [−0.18, 0.38]) ($n = 48$)	0.15 ($p = 0.34$, [−0.16, 0.43]) ($n = 43$)	**0.62 ($p = 0.02$, [0.10, 0.87]) ($n = 13$)**	0.38 ($p < 0.001$, [0.20, 0.53]) ($n = 104$)

biomarker As concentrations for consistency. In agreement with previous findings,[21,23,24,26] significant positive correlations were observed between drinking water and toenail (Fig. 5a, $r_p = 0.53$; $p < 0.001$; 95% C.I: 0.43, 0.63) and drinking water and hair (Fig. 5b, $r_p = 0.38$; $p < 0.001$; 95% C.I: 0.20, 0.53) As concentrations. This confirmed previous findings[14] of human exposure to As from PWS but over a longer timescale. When grouped by drinking water As concentration (Table 4), strong significant correlations were only observed where drinking water As was >10 μg L^{-1} for both toenails and hair. Fig. 5a shows that, for volunteers exposed to drinking water with <10 As μg L^{-1}, a considerable number toenail samples contained notable As concentrations. Given the encouraging results from the assessment of the washing procedure, sample contamination was unlikely to account for these results. Cornwall is a region of elevated environmental As[51] and, as noted previously by Button *et al.* (2009), alternative exposure routes, such as the ingestion of As-bearing soil and dust, are possible explanations for elevated toenail As where drinking water As is low.[40] The investigation of additional exposure routes in the present study population will form the basis of further research.

Fig. 5b depicts similar results for hair to those observed for toenails, albeit with a weaker correlation. Due to problems encountered with sample handling and the difficulty determining the mass of hair washed, assessing the performance of washing was not possible for hair samples. Sample contamination cannot be ruled out as a possible explanation for this weaker correlation. Based on the results from Welch's *t*-tests, cigarette smoking might have accounted for elevated As in the hair of some individuals. Tobacco smoke has been demonstrated[52] to cause elevated As in hair samples from non-occupationally exposed smokers and passive smokers. This pattern was not evident for toenail As concentrations, suggesting external contamination of hair from tobacco smoke among smokers as a possible explanation. Although statistically significant, caution is advised when interpreting these results due to the small number of smokers in the present study group.

Demographic, behavioural and dietary covariables

Multiple linear regression was used to determine significant predictors of toenail and hair As concentrations in addition to drinking water As. These included demographic, behavioural and dietary covariables. Data were stratified into two groups: volunteers with drinking water containing <1 As μg L^{-1} (low) and ≥1 As μg L^{-1} (high). This was to maintain consistency with previous studies[45] that reported a greater predominance of additional, notably dietary, sources of As intake when drinking water concentrations were <1 μg L^{-1}. This stratification resulted in four initial models for toenail (Model 1a, 1b) and hair (Model 2a, 2b) As concentrations as a function of demographic and behavioural variables only.

Coefficients for each model are shown in Table 5. There were no significant demographic/behavioural predictors of toenail As in the low drinking water As group (Model 1a) but both increasing drinking water As and age resulted in a significant increase in toenail As when As in drinking water was >1 μg L^{-1}. The effect of age on toenail As concentration has been reported by previous studies[23] but in the opposite direction to the effect found in the present study. The mechanism of this relationship has not been elucidated. For example, Kile *et al.* (2005) note that toenail growth decreases with age. This may result in a higher concentration of As relative to a lower mass of nail. The high proportion of volunteers in older age groups in the present study may have limited the detection of a positive relationship on this occasion.

Male gender had a significant positive effect on hair As in the low drinking water group. Drinking water As, age, gender (male), dye usage and smoking were all significantly positively associated hair As in the high drinking water group. Findings of the model for hair As in the high drinking water group complimented those of Welch's tests, namely the significantly lower As concentrations in hair collected from females and those who reported using dye. The association with dye usage strengthened with the omission of the gender term. Furthermore, with all but one volunteer reporting dye usage being female and 29% of hair providing volunteers being females that did not report dye usage, the apparent effect of dye implied by Welch's test was an indirect effect of gender. This would be consistent with previous findings[49,53] already discussed regarding lower As in the hair of females. Wolfsperger *et al.* (1994) attributed the higher As in male hair samples to smoking and a higher intake of seafood and wine than females.[53]

To test the influence of food and drink items known to contain As, dietary terms were added to the abovementioned models. None of the dietary variables tested had a significant effect on either toenail or hair As concentrations in the high drinking water group. In the low drinking water group, more servings of seafood per week resulted in a significant increase in

Table 5 Predictors of toenail and hair As concentrations on the basis of multiple linear regression models. Significant coefficients are labelled with (***), (**), (*), and (.) denoting significance to <0.001, <0.01, <0.05 and <0.1, respectively

Model	Terms	β coefficient (significance)
1a. ln(toenail As), drinking water <1 As μg L^{-1}	*Intercept*	*5.309* (***)
	ln(drinking water As)	0.072
	Age (continuous)	−0.01 (.)
	Gender (male)	0.137
	Nail polish usage (true)	−0.268
Adjusted $R^2 = 0.07$	Smoking status (smoker)	0.31
1b. ln(toenail As), drinking water ≥1 As μg L^{-1}	*Intercept*	*5.916* (***)
	ln(drinking water As)	0.469 (***)
	Age (continuous)	−0.018 (**)
	Gender (male)	−0.101
	Nail polish usage (true)	−0.157
Adjusted $R^2 = 0.29$	Smoking status (smoker)	0.005
2a. ln(hair As), drinking water <1 As μg L^{-1}	*Intercept*	*2.646* (**)
	ln(drinking water As)	0.08
	Age (continuous)	0.017
	Gender (male)	0.826 (**)
	Dye usage (true)	−0.159
Adjusted $R^2 = 0.24$	Smoking status (smoker)	0.77
2b. ln(hair As), drinking water ≥1 As μg L^{-1}	*Intercept*	*5.349* (***)
	ln(drinking water As)	0.433 (***)
	Age (continuous)	−0.025 (*)
	Gender (male)	0.810 (**)
	Dye usage (true)	−0.76 (.)
Adjusted $R^2 = 0.42$	Smoking status (smoker)	2.08 (**)
3. ln(toenail As), drinking water < 1 As μg L^{-1}	*Intercept*	*4.662* (***)
	ln(drinking water As)	0.089 (.)
Adjusted $R^2 = 0.04$	Seafood (continuous)	0.081 (*)
4. ln(hair As), drinking water < 1 As μg L^{-1}	*Intercept*	*4.392* (***)
	ln(drinking water As)	0.213 (*)
	Gender (male)	0.905 (***)
	Home-grown vegetables (never)	−0.975 (**)
	Home-grown vegetables (potted only)	0.546
Adjusted $R^2 = 0.33$	Home-grown vegetables (seasonally)	−0.343

toenail As concentration. Specific varieties of seafood were not significant. The model (Model 3) was re-performed with the omission of non-significant covariables and the results are presented in Table 5. A negative association was observed between hair As concentrations and never eating home-grown vegetables. The results of this model (Model 4), with non-significant covariables omitted, are presented in Table 5.

The positive association between seafood consumption and toenail As concentrations and the negative association between home-grown veg consumption and hair As concentrations are of plausible validity. Although seafood derived arsenic species such as arsenobetaine are primarily excreted *via* urine,[54] seafood also contains arsenosugars and arsenolipids which are metabolised into methylarsonate and dimethylarsinate, both of which have been measured in small quantities in human toenails.[40] In the present study, drinking water exposure was the primary focus of the investigation, hence, speciation analysis was not performed. On the basis of these findings, future studies considering dietary sources in low drinking water exposure groups should consider speciation analysis to ensure meaningful results. The negative effect of not eating home-grown vegetables on hair As concentration is consistent with reported high soil As concentrations in the study region[51] and, although values in local vegetables themselves have been found at relatively low concentrations,[55] the ingestion of soil particles adhered to vegetables is a possible exposure pathway.

4. Conclusions

This study is the largest investigation of long-term exposure to As in drinking water in the UK to-date and confirms the presence of prolonged exposure to inorganic As from drinking water of householders with PWS in Cornwall, UK. The temporal stability of As concentrations in PWS suggests that, for this particular region, measurements of As taken in the present are strong predictors of past levels of exposure dating back at least 31 months. Arsenic concentrations measured in toenails and hair were useful in assessing prolonged exposure to As from PWS, in agreement with numerous previous studies. Analysis of washing solutions built on the findings of Button *et al.* (2009)[40] in that the washing procedure employed here was effective in removing exogenous contamination from a large sample set. Both toenail and hair biomarkers were susceptible to the influence of covariables on As concentrations. Although useful

in assessing prolonged exposures to As from drinking water, other factors, such as diet, predominate where As concentrations in drinking water are low *e.g.* <1 μg L^{-1}. A large degree of variation in toenail and hair biomarkers was still unaccounted for in this study, with exposure to soil and dust highly possible explanations in a region of well-documented elevated environmental As. Investigation into the significance of other exposure routes will be the focus of future research.

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