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Seasonal Variation in Dietary and Body Organ Arsenic Concentrations in Wood Mice *Apodemus sylvaticus* and Bank Voles *Clethrionomys glareolus*

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Some of the most arsenic polluted sites in the world are located in south-west England, and in particular in the areas adjacent to the Dartmoor granitic extrusion (Colbourn et al. 1975). In general, these are abandoned mine sites, most of which ceased working around the end of the last century. The most contaminated areas are in the Tamar Valley and have soil arsenic concentrations that can exceed 50,000 µg g^{-1} d.wt. (Kavanagh et al. 1997).

Previous studies have demonstrated that environmental arsenic is ingested and absorbed into the body by wood mice *(Apodemus sylvaticus)* and bank voles *(Clethrionomys glareolus)* on arsenic-polluted sites, including the abandoned mines in south-west England (Erry et al. in press; Ismail and Roberts 1992). The dietary preferences of these small mammal species are well documented and differ between seasons (Corbet and Harris 1991; Hansson 1985). The food items available to mice and voles on the abandoned mine sites vary in their arsenic content (for example, see Porter and Peterson 1975; Benson et al. 1981) and so seasonal variation in diet may result in variation in arsenic intake by small mammals during the year. Furthermore, diet composition can influence gut absorption of arsenic as a result of physical and chemical interactions with trace elements, proteins and fatty materials (Vahter and Norin 1980; Ruby 1996). Thus, seasonal variations in both arsenic intake and diet composition may affect the amount of arsenic absorbed into the body and taken up by the body tissues. Knowledge of any such seasonal variation is important when assessing both the potential toxicological impacts of arsenic on small mammals and the rate of transfer of arsenic to their predators. The aim of the present study was to investigate whether dietary arsenic concentration and tissue residues in wood mice and bank voles differed significantly between late autumn and spring.

MATERIALS AND METHODS

Wood mice and bank voles were trapped on six sites in south-west England in November/December 1995 and May/June 1996. Two sites, Devon Great Consols (DGC) and Gawton United Mine (GUM) were abandoned copper/arsenic mines heavily contaminated with arsenic. Two others, Wheal Exmouth (WEx) and Morwellham Woods (MW) were moderately contaminated with arsenic and the

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remaining sites, University of Exeter Campus (C) and Kolora Park (KP), were uncontaminated control areas. Geometric mean soil arsenic concentrations were 4,320 and 4,520 μg g^{-1} d.wt. on the heavily contaminated sites, 167 and 259 μg g^{-1} d.wt. on the moderately polluted sites and 8-11 μg g^{-1} d.wt. on the control sites (Erry et al. in press). The sites ranged in area from 68 ha (DGC) to 6 ha (C).

Six lines of 10 traps were used on every site. Traps were spaced at 10 m intervals. Each trap-line was placed in a different habitat type, where possible, but in the same location in autumn and spring. The sites varied in size and shape and so no attempt was made to set trap-lines in a similar spatial arrangement at different sites. Traps were baited with carrot smeared sparingly with peanut butter. Carrot was used because it was easily identified in the stomach and indicated when an animal had consumed bait before it died. This was important because analysis of stomach contents was carried out to assess whether dietary arsenic concentrations varied seasonally. During both field seasons, traps were set for five days at each site and were checked daily. Captured rodents were identified to species and sex. They were transported on ice to the laboratory where they were weighed and classed as juveniles (<15 g body weight) or adults (≥15 g body weight). All carcasses were stored at -20°C until analysed.

The numbers of wood mice and bank voles captured in autumn and spring are given in Table 1. Each animal was defrosted and dissected. A sub-sample (c. 200 mg wet weight) of the stomach contents that did not contain any carrot bait, a liver sub-sample *(c.* 200 mg wet weight) and both kidneys were weighed accurately and dried at 70°C to constant mass. Dried samples were re-weighed and cold digested in Aristar grade nitric acid (BDH, Poole, Dorset, UK) for 24 h. They were then hot digested at 120°C for 2-4 h so that all the organic matter was oxidised and no further brown fumes evolved. Stratified random sub-samples of 49 (autumn) and 43 (sspring) dissected adult rodents, balanced for species, sex and site, were analysed to determine arsenic total body burdens. Each carcass was dried to constant mass at 70°C dipped in liquid nitrogen and homogenised using a pestle and mortar. A sub-sample *(c.* 500 mg wet weight) from each homogenate was digested in the same way as the stomach contents except that heating of the digest was ramped (100°C for 2 h, 110°C for 2 h) before the final temperature of 120°C was reached.

Digests of all samples were allowed to cool and were diluted to 30 ml with distilled water. Each digest was shaken and a 10 ml sub-sample evaporated at 110°C to zero volume. Dried digests were re-suspended in 10 ml of 5% hydrochloric acid (BDH, Poole, Dorset, UK: Analar grade) with 0.02 M potassium iodide (Sigma, St Louis, Montana, USA). The samples were covered and left to reduce for a minimum of 24 h. Analysis for arsenic was undertaken using a Philips PU9360 vapour system (for generation of AsH_3) in conjunction with a Pye Unicam SP9 Atomic Absorption Spectrophotometer with the detector set at 193.7 nm and a slit width of 5 nm. The total arsenic detected in the carcass homogenate, the guts, stomach contents, liver and kidney was combined to calculate a concentration for each intact animal.

Table 1. Numbers of small mammals captured. C = Campus; KP = Kolora Park; WEx = Wheal Exmouth; MW = Morwellham Woods; GUM = Gawton United Mine; DGC = Devon Great Consols

		C	KP	WEx	MW	GUM	DGC
Wood Mouse	*Autumn*						
Adult Male		19	8	11	19	9	9
Juvenile Male		1	2	5	5	1	2
Adult Female		6	7	6	11	14	5
Juvenile Female		2	2	3	8	7	2
Total		28	19	25	43	31	18
Adult Male	*Spring*	12	8	7	7	15	6
Juvenile Male		1	2	0	4	0	0
Adult Female		4	9	7	16	13	4
Juvenile Female		0	0	0	0	3	0
Total		17	19	14	27	31	10
Bank Vole	*Autumn*						
Adult Male		5	8	2	17	3	1
Juvenile Male		3	1	2	2	0	0
Adult Female		3	4	0	11	4	0
Juvenile Female		2	2	1	8	1	1
Total		13	15	5	38	8	2
Adult Male	*Spring*	2	1	2	5	2	1
Juvenile Male		0	0	0	0	0	0
Adult Female		3	5	3	8	2	1
Juvenile Female		0	0	0	0	0	0
Total		5	6	5	13	4	2

A blank and an in-house reference material (dry powdered *Agrostis capillaris* from Devon Great Consols) was run with each batch of 40 samples to detect background contamination and monitor the consistency of analysis between batches. A certified reference material (Oyster Tissue, Ref. No 1566a, National Bureau of Standards, US Department of Commerce) was also analysed with every seventh batch of 40 samples to determine the analytical recovery. Mean ± SE recoveries from the certified reference material were 64 ± 2% (n=6) for the winter 1995 analyses and at 71 ± 6% (n=14) for the summer 1996 analyses. Values were blank corrected but were not corrected for recoveries as these were not statistically significantly different (t_{18} = -0.71, P > 0.05). The analytical detection limit was 1 ng ml^{-1} in the acid digest. The limit of detection (LoD), expressed as a dry weight arsenic concentration, was based on average sample weights for each tissue/sample type. The LoDs were 0.18 µg g^{-1} d.wt. for stomach contents; 0.17 µg g^{-1} d.wt. for liver; 0.34 µg g^{-1} d.wt. for kidney and 0.08 µg g^{-1} d.wt. for carcass homogenate. Where values were below 1 ng ml^{-1} in the digest, a nominal

value of 50% of the dry weight concentration LoD was used for statistical analyses.

Data were analysed statistically by Analysis of Variance (ANOVA) using a General Linear Model (GLM) (Ryan et al. 1985). Species, age, sex, site, season and their two-way interactions were used as terms in the models and separate GLMs were run for each tissue type. The data were $\log_{10}$ transformed to meet the underlying assumptions of homogeneity of variance and normality of distribution of the residuals of the ANOVA. Average data are, therefore, given as geometric means (Table 2).

RESULTS AND DISCUSSION

Stomach content arsenic concentrations varied markedly between individuals and differed significantly between sites, being highest in animals from the most contaminated areas (Tables 2 and 3). There was no significant variation with species, sex or age, suggesting that the level of contamination was broadly similar in forage taken by all wood mice and bank voles on any particular site.

There was also no overall significant difference in stomach content arsenic concentrations between autumn and spring (Tables 2 and 3). However, the geometric mean stomach content arsenic concentration was always lower in spring than autumn in rodents from the uncontaminated sites. No such consistent difference was apparent in animals from either the moderately or the heavily contaminated mine sites. The data were reanalysed after being separated into paired sites of similar contamination. This revealed that there was a significant seasonal difference in stomach content arsenic concentration in rodents from the uncontaminated sites ($F_{(1,87)} = 5.13$, $P < 0.05$), but not in animals from the moderately or highly contaminated sites.

Chemical analysis of stomach contents provides a snapshot of the chemical composition of the diet. The findings of this study suggest there was a seasonal difference in the amount of arsenic in the diet of rodents on uncontaminated areas. There is no clear evidence of seasonal variation in the arsenic content of vegetation (Sharma and Shupe 1977 but see Merry et al. 1986). However, the autumn diet of wood mice and bank voles includes a relatively high proportion of mosses, fungi and dead leaves (Hansson 1985), all of which assimilate high concentrations of arsenic (Vetter 1994; Wyttenbach et al. 1997). Higher intake of arsenic by small mammals in autumn on uncontaminated sites might, therefore, be related to greater intake of these foods. Elevated intake of caesium in autumn by wood mice has likewise been attributed to increased intake of fungi (Toal 1999).

Given the observations on uncontaminated sites, the apparent absence of any seasonal difference in stomach content arsenic concentration on contaminated sites was unexpected. The diversity of plant species is limited on arsenic-polluted sites (Benson et al. 1981) and, if also true for other biota, the range of foods

Table 2. Geometric means and minimum-maximum values for arsenic concentrations (µg g-1 d. wt.) in stomach contents (SC), liver (LI), kidney (KI) and whole animal (WA) of wood mice and bank voles captured in autumn and spring. Data for males and females have been pooled. Where no minimum-maximum values are given, n=l. Codes for sites are-given in Table 1.

	C	KP	WEx	MW	GUM	DGC
Wood Mouse						
SC autumn	0.69	0.56	0.66	1.8	6.3	14
	0.08-77	0.08-7.0	0.08-16	0.08-5300	0.08-990	0.55-1000
Spring	0.13	0.27	0.77	2.2	3.7	31
	0.08-0.82	0.08-7.0	0.08-16	0.08-76	0.08-940	7.6-210
LI autumn	0.73	0.75	1.1	1.2	3.4	5.4
	0.08-12	0.27-6.1	0.27-1500	0.08-18	0.27-30	0.79-26
Spring	0.10	0.13	0.13	0.27	1.3	3.2
	0.05-0.64	0.08-1.4	0.08-0.4	0.08-3.9	0.08-20	0.79-26
KI autumn	0.62	0.64	0.97	1.7	2.7	4.9
	0.17-12	0.17-54	0.17-11	0.17-7500	0.17-22	1.0-35
Spring	0.21	0.26	0.37	0.31	1.6	3.8
	0.17-0.68	0.17-3.9	0.17-3.2	0.17-2.9	0.17-21	0.75-23
WA autumn	0.19	0.76	1.9	9.4	11	16
	0.04-0.98	0.37-3.59	0.31-21	1.5-320	4.7-56	3.1-71
Spring	0.19	0.15	1.0	1.6	13	11
	0.08-0.93	0.04-1.07	0.04-6.4	1.6-1.8	2.1-31	3.0-26
Bank Vole						
SC autumn	0.69	0.53	0.72	1.3	7.7	92
	0.08-4.3	0.08-97	0.08-2.3	0.08-79	0.08-430	15-566
Spring	0.12	0.28	0.38	0.68	11	27
	0.08-0.55	0.08-1.3	0.08-7.1	0.08-16	5.8-25	25-30
LI autumn	0.43	0.25	0.21	0.85	1.2	4.3
	0.08-11	0.09-6.1	0.09-2.5	0.09-62	0.09-22	4.2-4.4
Spring	0.29	0.10	0.21	0.16	0.94	0.97
	0.08-33	0.09.0.14	0.07-2.4	0.07-1.4	0.5-2.2	0.44-2.2
KI autumn	0.49	0.39	0.27	0.98	1.3	4.3
	0.17-11	0.17-6.1	0.17-2.5	0.17-62	0.17-22	4.2-4.4
Spring	0.17	0.45	0.42	0.41	1.6	7.9
	-	0.17-6.4	0.17-1.2	0.17-1.5	0.17-7.7	1.4-45
WA autumn	1.56	2.0	0.42	2.75	41	7.8
	0.66-2.4	0.88-6.2	0.25-0.66	0.79-11	4.4-380	0.55-58
Spring	0.37	0.10	0.22	0.54	2.9	2.8
	0.04-4.4	0.04-1.0	0.08-1.4	0.42-0.66	1.1-12	-

available to small mammals may be restricted. This would be likely to reduce any seasonal variation in dietary arsenic content in small mammals. It is also possible that a significant proportion of ingested arsenic on contaminated sites is derived from sources, such as soil, that do not vary seasonally in arsenic content.

Irrespective of site, there were significant seasonal differences in arsenic concentrations in the liver, kidney and whole bodies of rodents; overall means were 70, 47 and 76% lower respectively in spring than autumn (Tables 2 and 3). On uncontaminated sites, this reflected the lower arsenic stomach content

Table 3. Results of the General Linear Model analyses of arsenic concentration using species, age, sex, site, and season as factors in the model.

	Stomach contents	Liver	Kidney	Whole animal
Species	$F_{1,307}=0.03$	$F_{1,357}=6.58^{*}$	$F_{1,350}=2.05$	$F_{1,65}=0.96$
Age	$F_{1,307}=0.73$	$F_{1,357}=0.67$	$F_{1,350}=2.84$	-
Sex	$F_{1,307}=0.04$	$F_{1,357}=0.61$	$F_{1,350}=0.12$	$F_{1,65}=1.52$
Site	$F_{5,307}=12.6^{***}$	$F_{5,307}=9.73^{***}$	$F_{5,350}=8.88^{***}$	$F_{5,65}=12.2^{***}$
Season	$F_{1,307}=2.45$	$F_{1,357}=16.9^{***}$	$F_{1,350}=8.32^{***}$	$F_{1,65}=17.0^{***}$
Species x Age	$F_{1,307}=1.03$	$F_{1,357}=0.73$	$F_{1,350}=1.68$	-
Species x Sex	$F_{1,307}=1.99$	$F_{1,357}=7.34^{**}$	$F_{1,350}=7.59^{**}$	$F_{1,65}=1.11$
Species x Site	$F_{5,307}=0.64$	$F_{5,357}=1.37$	$F_{5,350}=1.04$	$F_{5,65}=1.56$
Species x Season	$F_{1,307}=2.98$	$F_{1,357}=2.08$	$F_{1,350}=1.44$	$F_{1,65}=2.09$
Age x Sex	$F_{1,307}=0.04$	$F_{1,357}=0.01$	$F_{1,350}=0.26$	-
Age x Site	$F_{5,307}=1.69$	$F_{5,357}=0.91$	$F_{5,350}=0.78$	-
Age x Season	$F_{1,307}=0.00$	$F_{1,357}=0.02$	$F_{1,350}=1.61$	-
Sex x Site	$F_{5,307}=1.06$	$F_{5,357}=2.47^{*}$	$F_{5,350}=1.83$	$F_{5,65}=0.60$
Sex x Season	$F_{1,307}=0.39$	$F_{1,357}=1.24$	$F_{1,350}=0.89$	$F_{1,65}=3.23$
Site x Season	$F_{5,307}=2.04$	$F_{5,357}=1.22$	$F_{5,350}=0.95$	$F_{5,65}=0.62$

(*= P<0.05, ** = P<0.01, *** = P<0.001. Whole animal arsenic concentrations were only measured in adult animals and age was not a factor in the GLM for this analysis.)

concentration (and hence probably intake) in wood mice and bank voles in spring. In contrast, the lower organ and body arsenic concentrations of rodents on contaminated sites occurred despite there being no detectable reduction in dietary arsenic concentration in spring. Presumably, either net gut absorption of arsenic from the diet was lower and/or rates of arsenic metabolism and elimination were greater in spring than autumn in animals on contaminated sites.

All other factors except age were significant either as single terms or as interactions in the GLMs for arsenic concentration in the body tissues (Table 3). Wood mice had liver arsenic concentrations that were generally two times greater than those in bank voles (Table 2). Previous work, in which only animals captured in autumn were analysed (Erry et al. in press), had also suggested that liver arsenic concentrations were higher in wood mice than bank voles but the difference between the species was not statistically significant. The detection of a significant difference in the present study appeared to be simply a result of increased sample size as there was no significant interaction between species and season in the GLM (Table 3). The occurrence of a species difference in liver residues, but not in diet, kidney and whole body arsenic concentrations, suggests that wood mice and bank voles differ physiologically in the way they absorb, metabolise or eliminate ingested arsenic.

There were three significant interactions in the GLM models (Table 3). There were significant species/sex interaction terms for both liver and kidney arsenic residues that indicated male wood mice had higher concentrations of arsenic in these tissues than females but the reverse was true in bank voles (Table 2). However, sex had no significant effect on tissue arsenic concentrations when data

for the two species were analysed separately, suggesting that the significant interaction terms were probably Type I statistical errors. There was also a significant sex/site interaction for liver arsenic that indicated males had greater arsenic concentrations in their livers than females at three sites (DGC, KP, MW). These were one each of the control, moderately and highly contaminated sites. That the findings were not consistent between sites of similar contamination status suggests that this interaction term may also have been a Type I statistical error.

In conclusion, seasonal variation in tissue arsenic concentrations was most marked on uncontaminated sites where dietary arsenic concentrations also differed between seasons. However, the amounts of arsenic in the diet and the body tissues were always low. Thus, the significance of seasonal variation in intake and accumulation is likely to be minor in terms of potential adverse effects on small mammals and transfer of arsenic to predators. On contaminated sites, arsenic body burdens in small mammals were generally higher and, as on uncontaminated sites, were greater in autumn than spring. Predators that take similar numbers of rodents from the contaminated sites in the two seasons are, therefore, likely to ingest greater amounts of arsenic in the autumn. However, there is no evidence that such exposure adversely affects avian predators (Erry et al. 1999). Finally, the lower tissue concentrations of arsenic in rodents in spring than autumn suggest that there is no progressive accumulation of arsenic by over-wintering adults on contaminated sites.

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