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Arsenic deposition in tissues of the European hare (*Lepus europaeus*)

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Abstract

The work deals with arsenic deposition in individual biological matrices of the European hare (*Lepus europaeus* Pall.). The aim of this work was to evaluate the arsenic deposition in biological matrices of adult hares distributed by sex, and to highlight the need for monitoring this element in the natural environment. Determination of arsenic concentration was carried out on 11 biomarkers in 105 adult hares from variously loaded areas of the Czech Republic. Individual matrices include the liver, kidneys, brain, adipose tissue, reproductive organs, bone, fur, faeces, lungs, skeletal muscle and the heart. Inductively coupled plasma mass spectrometry was employed as a method to detect arsenic concentrations in the tissues. Arsenic deposition in the monitored biological matrices of adult animals showed no significant differences between sexes. The ratio of arsenic concentration in the skeletal muscle as compared with concentration in other tissues was 1:2.96 in the liver, followed by 1:4.35 in kidneys, 1:1.07 in the heart, 1:2.73 in lungs, 1:3.12 in ovaries, 1:3.30 in testicles, 1:5.90 in bones, 1:114.68 in fur, and 1:60.05 in faeces. Deposition of this element in matrices has a similar character and only differs in concentrations.

Wild game, biomarkers, xenobiotics, contamination

Arsenic is considered a high-risk chemical element which is not only built up uncontrollably over a long period in the natural environment, in wildlife, farm and domestic animals but also in the human population. It is a pollutant with strong carcinogenic, mutagenic and cytotoxic effects to higher organisms (Germolec et al. 1997; Yanamaka et al. 1997; Goering et al. 1999; Mass et al. 2001; Hughes 2002; Dopp et al. 2004).

The European hare (*Lepus europaeus* Pall.) is widespread throughout practically the whole of Europe except northern Scandinavia, Iceland, and Ireland. Hare populations are important components of cultural landscape ecosystems. The population dynamics depends mainly on the interrelations with the environment. Long-term monitoring of these issues point to a certain periodicity in the multitudinousness of individual populations.

Due to its way of life, relatively small territory, short generation interval and processed reference values of haematological and biochemical indicators, this species can be used in environmental monitoring of the anthropogenic loads by foreign substances in the environment, as well as in studying chromosomal aberrations and cancer incidence in these populations.

The aim of this work was to evaluate the arsenic deposition in biological matrices of adult hares distributed by sex, and to highlight the need for monitoring this element in the natural environment.

The monitoring of arsenic in wildlife in the Czech Republic has been somewhat neglected compared to other foreign substances. This element has practically remained unmonitored in game in recent years despite the fact that game is commonly consumed by people.

The biological screening and monitoring of these xenobiotics in the body of game is of high importance from the hygienic point of view. It is primarily used to protect the food chain of the human population. Based on this screening, similar physiological and

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pathological reactions in humans, livestock and wildlife allow determination of health standards for assessing food and contamination of individual components of the natural environment (Páv et al. 1986; Bukovjan and Wittlingerová 1997).

Massanyi et al. (2003) also dealt with the monitoring of chemical element loads in the European hare. The results of arsenic contamination in hares are known in Croatia (Skrivanko et al. 2008). The results show a different level of contamination in game within managed ecosystems.

Chemical elements are monitored not only in the game meat and organs used for human consumption, but in recent years they were also detected in various animal neoplasms. We have encountered this phenomenon in benign and malignant neoplasms in wildlife. Some of these observations were already reported by others and also by our research group (Flux 1965; Karpenko and Bukovjan 1996; Hoffmann and Morl 1985; Hofe et al. 2004; Bukovjan et al. 2011; Kutlvašr et al. 2014).

As proven before, tumours diagnosed in both human and veterinary pathology are usually identical (Bukovjan and Karpenko 1990; 1995) and the international ICD-O classification of neoplasms may be applied to find a common platform and terminology. Selected chemical elements including arsenic were also monitored in the tumours along with the study of these elements in various non-neoplastic tissues (Bukovjan and Karpenko 1996; Bukovjan et al. 2014).

Materials and Methods

The subject of this work was to monitor the presence of arsenic in 105 adult hares older than one year, of which 51 were males (48.57%) and 54 were females (51.43%). The animals came from differently loaded ecosystems of the Hodonín region (42), Litoměřice – Mělník region (32) and Pardubice – Hradec Králové region (31). These groups are well balanced and therefore well statistically interpretable. The tissues were sampled randomly from the brain, subcutaneous adipose tissue, skeletal muscle, myocardium, lungs, liver, kidneys, ovaries, testicles, and faeces. For sampling bones, the femur was used from all tested animals. Fur was also investigated, and it was taken and analysed as a mix from three sampling areas – from the second half of the back and from the left and right shoulder blade region. The samples investigated in this study were collected between the years 2009 and 2013.

The samples were kept frozen at -21 °C until analysis. The examination itself was performed after homogenisation and microwave pressure digestion (EN 13805:2014) of the sample with the aid of optical emission spectrometry (EN 15763:2009) with inductively coupled plasma mass spectrometry ICP/MS (Agilent, model: 7500 ce). Calibration was performed using a standard ICP-MS multi-element calibration standard solution VI (Merck). The method parameters were as follows – the limit of detection of arsenic is 0.003 $mg \cdot kg^{-1}$, the limit of quantification was 0.005 $mg \cdot kg^{-1}$.

The data obtained from animals were tested for normality and homogeneity of distribution. Subsequently, one-factorial ANOVA test was performed to detect the difference in concentration in different tissues ($P = 0.05$, $F = 51.42$). *Post hoc* Fisher LSD test was then performed to detect differences among individual types of matrices.

Results

Determination of arsenic concentrations in different biological matrices was performed in both sexes of the adult European hare. The initial results of the measured values were statistically processed and summarised in Table 1 (female hares) and Table 2 (male hares). The overall results of the hares regardless of the sex distribution are summarised in Table 3. The ratio between the values of arsenic in various matrices, where the concentration of the element in the mass of muscle tissue is equal to 1.00, is indicated in Table 4, and it refers to the entire cohort of examined hares.

Determination of arsenic in the liver of the European hare did not reveal any significant differences in relation to sex. In male hares, the mean concentration of this element was 0.0192 ± 0.021 $mg \cdot kg^{-1}$, and in female hares it was 0.0187 ± 0.020 $mg \cdot kg^{-1}$. Maximum concentrations were also comparable (0.097 $mg \cdot kg^{-1}$ in males and 0.096 $mg \cdot kg^{-1}$ in females) (Tables 1 and 2).

Table 1. Evaluation of arsenic in matrices of adult female hares ($mg \cdot kg^{-1}$).

	Liver	Kidney	Muscle	Heart	Lungs	Brain	Adipose tissue	Ovaries	Bone	Fur	Faeces
Mean	0.01872	0.02929	0.00783	0.00842	0.01729	0.38637	0.07980	0.02224	0.05783	0.72163	0.37860
Min.	0.00460	0.00198	0.00240	0.00190	0.00190	0.00140	0.00150	0.00200	0.00198	0.00730	0.06150
First quartile	0.00688	0.01360	0.00460	0.00390	0.01193	0.00428	0.00285	0.00840	0.01353	0.47588	0.29423
Third quartile	0.02170	0.02570	0.00870	0.00695	0.02100	0.96710	0.00475	0.01120	0.02760	1.00700	0.46245
Max.	0.09660	0.17960	0.02440	0.05600	0.09650	1.39810	0.69180	0.59810	1.29300	1.39110	0.66010
Median	0.00950	0.01895	0.00690	0.00490	0.01510	0.00955	0.00360	0.00910	0.01900	0.62480	0.38565
Mode	0.00770	0.01360	0.00510	0.00490	0.01510	0.00610	0.00360	0.00910	0.01460	0.00000	0.39650
Variance	0.00041	0.00146	0.00002	0.00012	0.00021	0.26664	0.04313	0.00653	0.03116	0.12564	0.01568
Standard deviation	0.02000	0.03783	0.00450	0.01081	0.01419	0.51157	0.20575	0.08008	0.17488	0.35116	0.12406

Table 2. Evaluation of arsenic in matrices of adult male hares ($mg \cdot kg^{-1}$).

	Liver	Kidney	Muscle	Heart	Lungs	Brain	Adipose tissue	Ovaries	Bone	Fur	Faeces
Mean	0.0192	0.0232	0.0086	0.0048	0.0147	0.3990	0.0565	0.0241	0.0349	0.7309	0.3645
Min.	0.0050	0.0024	0.0036	0.0006	0.0012	0.0012	0.0020	0.0063	0.0068	0.2040	0.1010
First quartile	0.0070	0.0130	0.0048	0.0030	0.0108	0.0051	0.0027	0.0084	0.0124	0.4346	0.2800
Third quartile	0.0208	0.0230	0.0089	0.0049	0.0190	0.9671	0.0040	0.0100	0.0210	1.0066	0.4180
Max.	0.0970	0.1840	0.0600	0.0390	0.0510	2.3610	0.6910	0.6113	0.1793	1.4610	0.6110
Median	0.0085	0.0146	0.0070	0.0041	0.0141	0.0069	0.0030	0.0091	0.0185	0.6150	0.3760
Mode	0.0058	0.0141	0.0044	0.0045	0.004	0.0051	0.003	0.0091	0.0141	0.615	0.365
Variance	0.0005	0.0008	0.0001	0.0000	0.0001	11.0575	0.0314	0.0071	0.0022	0.1425	0.0138
Standard deviation	0.0217	0.0284	0.0081	0.0051	0.0091	0.6078	0.1772	0.0845	0.0466	0.3775	0.1174

The values of arsenic in the kidneys are fully comparable in both sexes, and they show no significant differences. In the kidneys of both sexes, higher arsenic concentrations compared to the liver were observed. Detailed statistics are shown in Tables 1, 2, and 3.

The amount of arsenic in the skeletal muscle of hares was minimally different between the sexes. In males, the highest recorded concentration of arsenic was approximately

Table 3. Evaluation of arsenic in matrices of hares regardless of the sex distribution (mg·kg^{-1}).

	Liver	Kidney	Muscle	Heart	Lungs	Brain	Adipose tissue	Bone	Fur	Faeces
Mean	0.01893	0.02636	0.00820	0.00668	0.01605	0.39250	0.06850	0.04670	0.72612	0.37175
Min.	0.00460	0.00198	0.00240	0.00060	0.00120	0.00120	0.00150	0.00198	0.00730	0.06150
First quartile	0.00700	0.01300	0.00460	0.00360	0.01120	0.00470	0.00270	0.01310	0.46100	0.29400
Third quartile	0.02110	0.02510	0.00860	0.00570	0.01910	0.96610	0.00410	0.02360	0.98910	0.43660
Max.	0.09700	0.18400	0.06000	0.05600	0.09650	2.36100	0.69180	1.29300	1.46100	0.66010
Median	0.00910	0.01800	0.00690	0.00460	0.01450	0.00910	0.00310	0.01850	0.61500	0.37990
Mode	0.00580	0.01410	0.00400	0.00490	0.01510	0.00550	0.00300	0.02100	0.61500	0.36500
Variance	0.00044	0.00115	0.00004	0.00008	0.00015	0.31707	0.03751	0.01707	0.13394	0.01480
Standard deviation	0.02084	0.03372	0.00648	0.00872	0.01204	0.56040	0.19274	0.13005	0.36424	0.12106

Table 4. Arsenic concentration ratio in individual matrices (As concentration in muscles = 1.00).

	Muscle	Liver	Kidneys	Heart	Lungs	Brain	Adipose tissue	Repro-organs	Bone	Fur	Faeces
Mean	1.00	2.96	4.35	1.07	2.73	72.50	15.48	3.21	5.90	114.68	60.05
Min.	1.00	0.12	0.17	0.05	0.07	0.09	0.03	0.13	0.15	0.49	4.10
First quartile	1.00	0.75	1.74	0.44	0.79	0.63	0.33	0.97	1.13	56.68	33.65
Third quartile	1.00	4.68	4.75	0.95	3.68	112.98	0.90	2.20	3.89	164.05	79.25
Max.	1.00	14.88	41.82	12.19	12.22	364.47	176.58	75.47	143.67	370.67	145.85
Median	1.00	1.39	2.19	0.81	1.87	1.26	0.47	1.51	1.93	98.75	57.68
Variance	0.00	8.54	44.06	3.38	5.80	13311.41	2003.24	93.89	269.64	5867.69	1034.14
Standard deviation	0.00	2.91	6.61	1.83	2.40	114.82	44.54	9.64	16.34	76.24	32.00

× 3 higher than in female animals (0.0600 mg·kg^{-1} in males and 0.0244 mg·kg^{-1} in females). The mean value of the element in the muscle tissue was 0.0086 ± 0.008 mg·kg^{-1} in male hares and 0.0078 ± 0.0045 mg·kg^{-1} in female hares.

The mean concentration of arsenic in the myocardium differs between the two sexes. It was twice as high in females (0.0084 ± 0.0108 mg·kg^{-1}) as compared to males

(0.0048 ± 0.0051 mg·kg^{-1}). The results between the monitored groups at a specified level of significance were not conclusive ($P = 0.05$). The comparison of arsenic deposition between the skeletal muscle and the myocardium did not reveal any significant differences. Compared to other monitored biomarkers, both skeletal muscle and myocardium may be regarded as biological matrices least loaded by the monitored element.

When evaluating the amount of arsenic in the lungs, virtually identical concentrations were found for both sexes (0.0147 ± 0.0091 mg·kg^{-1} in males and 0.0173 ± 0.014 mg·kg^{-1} in females), with a higher maximum concentration of the element found in females compared to males (0.0965 mg·kg^{-1} vs. 0.051 mg·kg^{-1}).

Compared to other tissues (except for fur and faeces), higher concentrations of the element were detected in the central nervous system. The mean concentration of arsenic in females was 0.386 ± 0.511 mg·kg^{-1} and it was comparable to the findings in males (0.399 ± 0.607 mg·kg^{-1}). Relatively high values, above 1.00 mg·kg^{-1}, were recorded in 11 male individuals compared to 9 female individuals exceeding this level. The majority of individuals with higher levels of arsenic in the brain came from the Pardubice – Hradec Králové agglomeration. Positive correlation ($r = 0.240$) was recorded between the kidneys and the brain.

Arsenic concentrations in the adipose tissue were relatively high in comparison with, for example, parenchymatous organs. In females, the concentration was lower compared to males, but the results were not significantly different between the sexes. Mutual correlations were noted between muscles and fur ($r = 0.300$), and also between adipose tissue and muscles ($r = 0.160$).

Arsenic concentration in the monitored European hare was 0.0241 ± 0.084 mg·kg^{-1} in the testes and 0.0222 ± 0.080 mg·kg^{-1} in the ovaries. These were minimally differing concentrations with a comparable variance within the groups.

In the bone tissue of the individual hares, the concentrations of arsenic were relatively higher compared to the liver, kidneys, myocardium, and skeletal muscle. A higher mean concentration was observed in females than in males (Tables 1, 2). However, in mutual comparison of values, no significant differences were identified.

The highest arsenic concentrations were detected in fur. Comparable mean concentration of the element in fur was detected in both males and females (Table 1 and 2). There was no significant difference between the groups.

The concentration of arsenic in the faeces of the European hare showed minimal variance of the measured values in the groups. The mean arsenic concentration in faeces of the males was approximately half of the values detected in fur. Practically the same was observed in the females (Table 1 and 2). The arsenic content in the faeces shows that this element is excreted from the body in a natural way and the quantity is continuously decreasing in conditions where animals are not exposed to constant contamination.

Discussion

Arsenic is considered a significant environmental pollutant that in general has serious toxic effects on animals. In human toxicology, the absorption, metabolism and excretion of arsenic are well documented. The effect of acute or chronic intoxication depends on sex, age, dose and exposure time (Fowler 1977). In areas with a higher arsenic exposure, consistently elevated concentrations of arsenic in the fur of hares and children's hair were found (Nováková 1973), similarly to the results of the monitoring of this element in the fur of the common vole and wood mouse (Obrusník and Paukert 1973). These results are consistent with our findings of arsenic deposition in the fur of the examined hares.

Monitoring of arsenic concentrations in the organs of hares is not common compared to other elements, especially heavy metals. There are only few studies on the arsenic in animals, such as that by Skrivanko et al. (2008) in Croatia. Their findings are partially comparable with our results. Possible differences in the concentrations can be explained by different levels of contamination of ecosystems by this toxic element.

In the tissues of animals that were diagnosed with malignant neoplastic diseases, arsenic concentrations ranged from 0.010 to 0.040 $mg \cdot kg^{-1}$ in the liver and from 0.020 to 0.050 $mg \cdot kg^{-1}$ in the kidneys, which is comparable with the concentration of the element in the tumour tissue itself (0.02–0.04 $mg \cdot kg^{-1}$). In a benign tumour, concentrations in the liver were higher than in the tumour tissue; concentrations in the kidneys and the neoplastic tissue were comparable (Bukovjan and Karpenko 1996).

Similar results were found in the examination of organs and other tissues, and in an ovarian malignant tumour of a European badger. The concentration of arsenic was 0.07 $mg \cdot kg^{-1}$ in the liver, 0.02 $mg \cdot kg^{-1}$ in the muscle and in the kidney, and 0.03 $mg \cdot kg^{-1}$ in the tumour (Bukovjan et al. 2014).

Arsenic is a significant toxic element that contaminates the environment including wildlife. Deposition of this element in matrices has a similar character and it only differs in concentrations.

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