Inorganic elements in the livers of Eurasian otters, *Lutra lutra*, from England and Wales in 2007 & 2008: a Predatory Bird Monitoring Scheme (PBMS) report

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1.Executive Summary

This is a report on the initial findings of a collaborative study between the Predatory Bird Monitoring Scheme (PBMS) and the Cardiff University Otter Project (CUOP). The study analysed the concentrations of 16 metals and semi-metals in the livers of 107 Eurasian otters (*Lutra lutra*) that had been found dead in 2007 and 2008 and collected by the CUOP. This aim of this work was to determine the current concentrations of inorganic elements accumulated by otters and whether exposure to heavy metals (lead, mercury, cadmium) in particular is likely to be associated with adverse effects. This is the first study of inorganic elements in otter livers from Europe for nearly 10 years.

The otters that were analysed were from England and Wales and included adult and sub-adult males and females. Liver tissue was analysed using Inductively Coupled Plasma mass spectrometry (ICP-MS) techniques.

The concentrations of inorganic elements measured in the present study were within the range previously reported for Eurasian otters in Britain and elsewhere in Europe. Concentrations varied with age and/or sex for some elements. For the heavy metals mercury and cadmium, liver concentrations generally increased with age whereas for lead, juveniles generally had higher liver lead concentrations than adults although for lead these difference were not statistically significant.

Aluminium and chromium were the only elements that varied significantly in concentrations between years. It is unclear whether the inter-year variation in aluminium and chromium represent significant inter-year changes in exposure and/or accumulation or may simply reflect local-scale variation in the provenance of otters and their associated exposure.

The liver concentrations of heavy metals (mercury, cadmium and lead) in all the otters analysed were below those associated with toxic effects in mammals, although liver lead concentrations in a small number of otters were close to the level of concern.

2.Introduction

The Predatory Bird Monitoring Scheme (PBMS; http://pbms.ceh.ac.uk/) is the umbrella project that encompasses the long-term contaminant monitoring and surveillance work in avian predators carried out by the Centre for Ecology & Hydrology (CEH).

By monitoring sentinel vertebrate species, the PBMS aims to detect and quantify current and emerging chemical threats to the environment and in particular to vertebrate wildlife. Our monitoring provides the scientific evidence needed to determine how chemical risk varies over time and space. Such variation may occur due to market-led or regulatory changes in chemical use and may also be associated with larger-scale phenomena, such as global environmental change.

Our monitoring also allows us to assess whether detected contaminants are likely to be associated with adverse effects on individual wild animals and their populations. This is needed to inform regulatory decisions about whether mitigation of exposure is required and what measures might be effective. Monitoring also provides information by which the success of mitigation measures can be evaluated.

Previously the PBMS has used the grey heron, *Ardea cinerea*, as a sentinel to assess levels of contamination in the freshwater environment and to determine whether contamination may pose a risk to wildlife. However, the number of herons received each year by the PBMS is now relatively low (approximately 5/year) which limits our ability to detect temporal and spatial variation. Consequently, we have developed a collaboration with the Cardiff University Otter Project (CUOP) to utilize Eurasian otters, *Lutra lutra*, in place of grey herons as a freshwater monitor. Otters and grey heron both have a high proportion of fish in their diet (Jedrzejewska et al., 2001, Miranda et al., 2008, Cook, 1978, Marquiss and Leitch, 1990, Clavero et al., 2003) and so both are likely reflect contamination in fresh water and near shore fish.

The CUOP analyses the livers of the otters it collects for a selection of persistent organic pollutants (POPs) but not for inorganic contaminants. Linkage of the PBMS and CUOP (both of which are co-funded by the Environment Agency (EA)) provides cost-effective monitoring on the extent and variation in the contamination of the freshwater environment for both POPs and inorganic contaminants.

The PBMS analysed livers from 48 and 57 otters found dead in each of 2007 and 2008, respectively. Livers were analysed for 16 essential and non-essential trace elements, including heavy metals such as cadmium, lead & mercury, which can cause toxic effects in wildlife, including otters (Shore and Rattner, 2001). The aim of this work was to determine the current concentrations of inorganic elements accumulated by otters and whether exposure to heavy metals in particular is likely to be associated with adverse effects.

3.Methods

As part of the Cardiff University Otter Project (CUOP) otters found dead in England and Wales are examined to determine sex, weight and length. Age-class (adult, sub-adult or juvenile) is estimated from a combination of morphometric data and indicators of reproductive activity(Chadwick, 2006). Nutritional and reproductive status, lesions, growths and concretions are also noted. Tissue samples are taken as part of the post-mortem examination, including the liver. A sub-sample of the liver is analyzed for persistent organic pollutants by the Environment Agency's National Laboratory Service, and the results of that analysis are published in reports produced for the Environment Agency; the latest can be downloaded at:
http://publications.environment-agency.gov.uk/pdf/SCHO0307BMKP-e-e.pdf.

Approximately 50 liver samples per year were selected for analysis and the sample stratified over the sex, age-class and location of the otters received by CUOP (Table 1 and Figure 1). The liver samples were analysed for inorganic elements at the Centre for Ecology and Hydrology, Lancaster. The elements quantified are given in table 2 in the results section of this report. The method used, the limits of detection applied and the % recovery data derived from certified reference materials can be downloaded as an addendum to this report at:
http://pbms.ceh.ac.uk/docs/AnnualReports/PBMS_Metals_Otters_2007-8_Addendum.pdf

Concentrations have not been recovery corrected and are presented on a dry weight basis. A common limit of detection was applied across the analysis batches.

Table 1. Age and sex of otters found dead in 2006 to 2008 that are included in this report.

		Age Class			
Year	Sex	Juvenile	Sub-adult	Adult	Total
2006	Male	-	-	2	2
	Female	-	-	-	-
2007	Male	1	11	15	27
	Female	1	10	10	21
2008	Male	6	12	10	28
	Female	5	12	12	29
	Total	13	45	49	107

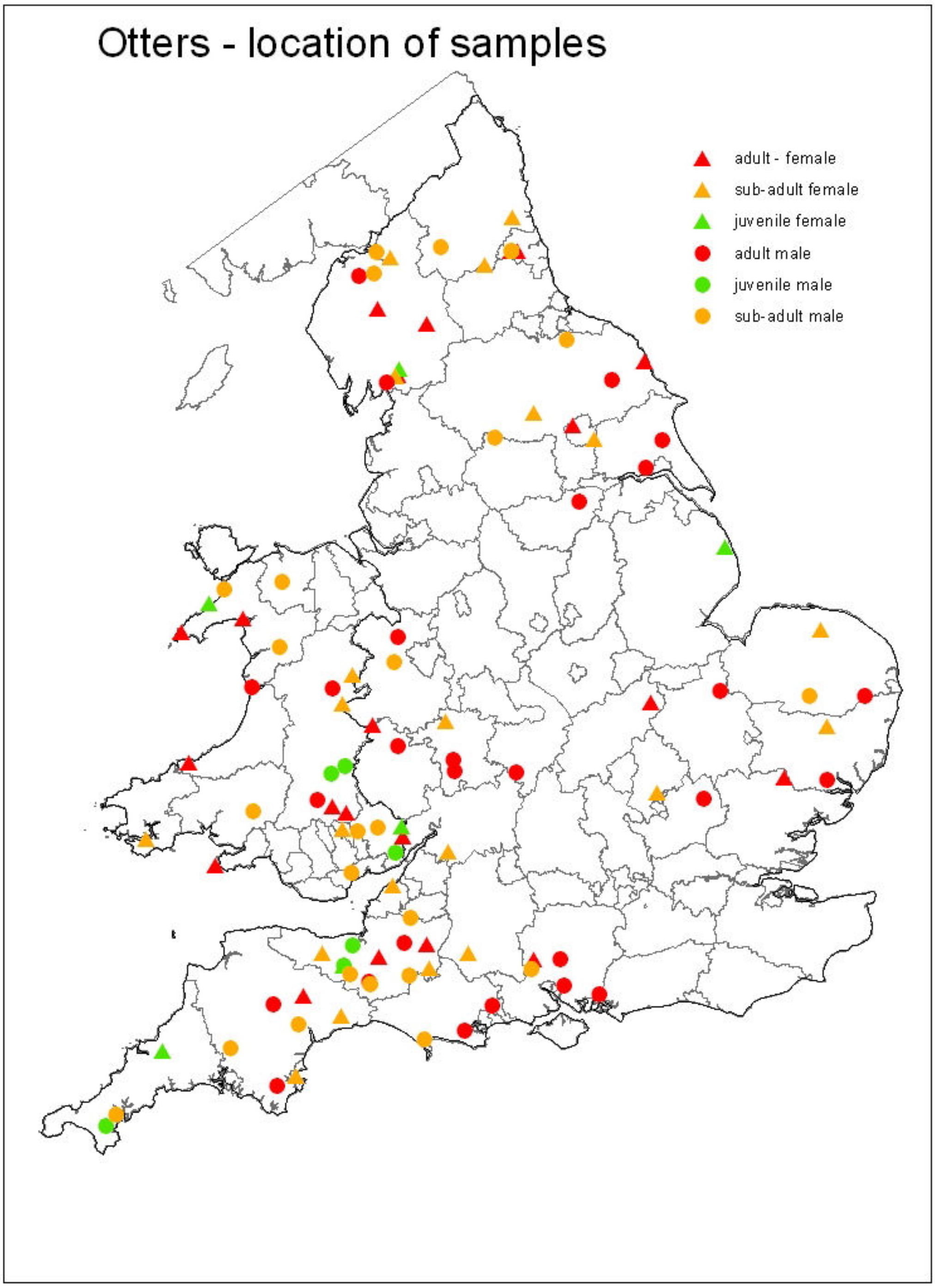

Figure 1. Location of otters, *Lutra lutra*, for which livers were analysed for inorganic elements (total of 107 otters).

4.Results & Discussion

The results for individual otter livers can be downloaded from the PBMS website (http://pbms.ceh.ac.uk/docs/AnnualReports/PBMS_Metals_Otters_2007-8_Addendum.pdf). Frequency distributions for the two years combined indicate that the majority of elements had a left-skewed distribution (preponderance of lower concentrations), e.g. mercury, Figure 2. The exceptions to this were molybdenum (Mo) (Figure 2) and chromium (Cr) which were normally distributed. For antimony (Sb) and aluminium (Al) a large proportion of samples (61% and 97% respectively) were below the limit of detection. As the distributions for most elements were skewed, summary data are presented as medians and Inter-Quartile Ranges (Table 2).

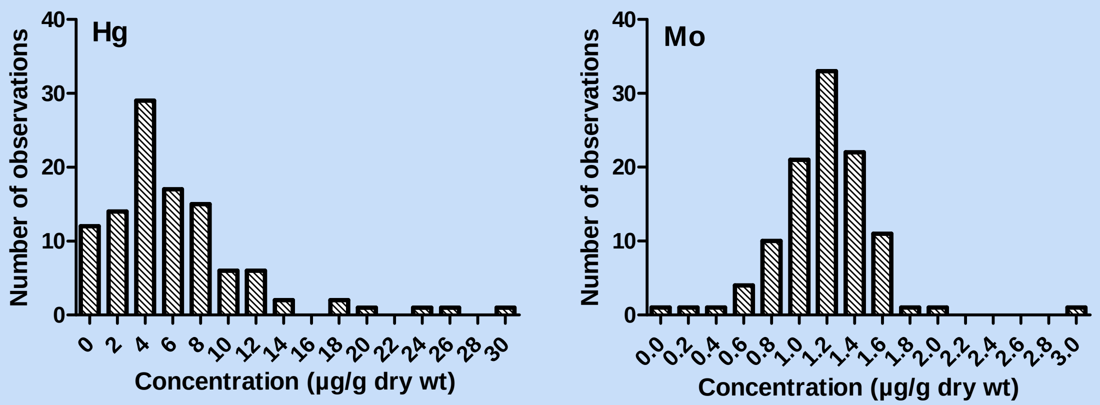

Figure 2. Frequency distribution for mercury (Hg) and molybdenum (Mo) concentrations in livers from otters, *Lutra lutra*, found dead in 2007 & 2008. The Hg histogram shows a typical skewed distribution while Mo residues are normally distributed.

There were no significant differences between years in the liver concentrations of any of the elements studied except for aluminium and chromium which were lower and higher, respectively, in 2008 than in 2007 (Table 2). It is unclear whether the inter-year variation in aluminium and chromium represent significant inter-year changes in exposure and/or accumulation or may simply reflect local-scale variation between years in the provenance of otters and their associated exposure. Further monitoring would be necessary to determine whether there is any progressive change over time in liver concentrations of aluminium, chromium, or any other elements.

Table 2. Median concentrations (µg/g dry weight) of selected inorganic elements in the livers of otters found dead in 2007 & 2008 in England and Wales.

	2007 (N=48)		2008 (N=57)		
Analyte	Median	Inter-quartile Range	Median	Inter-quartile Range	Significant Difference[1]
Aluminum (Al)	1.605	1.163-2.958	ND	ND-ND	***
Arsenic (As)	0.121	0.093-0.233	0.053	0.007-0.350	ns
Cadmium (Cd)	0.238	0.092-0.494	0.171	0.053-0.376	ns
Chromium (Cr)	0.164	0.097-0.266	0.302	0.214-0.370	***
Cobalt (Co)	0.058	0.043-0.087	0.056	0.040-0.079	ns
Copper (Cu)	29.80	22.75-44.30	29.5	22.50-40.70	ns
Iron (Fe)	687.5	446.5-930.5	541	412.5-801.0	ns
Lead (Pb)	0.131	ND-0.330	ND	ND-0.412	ns
Manganese (Mn)	8.305	6.785-10.1	8.98	7.155-10.80	ns
Mercury (Hg)	5.095	3.438-8.298	4.01	1.750-7.345	ns
Molybdenum (Mo)	1.240	1.043-1.433	1.2	0.952-1.335	ns
Nickel (Ni)	0.1045	0.058-0.143	0.074	0.051-0.153	ns
Selenium (Se)	6.145	4.875-7.585	5.88	4.935-7.750	ns
Strontium (Sr)	0.1455	0.074-0.269	0.186	0.128-0.260	ns
Antimony (Sb)	ND	ND-ND	ND	ND-ND	-
Zinc (Zn)	106.5	83.93-129.3	93.5	83.95-112.5	ns

ND indicates non-detected; for Al<1.12, Pb<0.120 & Sb<0.019 µg/g dry weight.
[1] Level of significance tested by Mann-Whitney U test: ns=not significant, *=P<0.05, **=P<0.01 and ***=P<0.001

Data for the two years were combined and then assigned to one of six groups (male adult, male sub-adult, male juvenile, female adult, female sub-adult, female juvenile) to determine whether liver element concentrations varied with sex and age class. Concentrations varied with sex and/or age for some elements and trends for the toxic heavy metals (mercury, cadmium, and lead) are shown in Figure 3.

There were no consistent differences in liver concentrations of these metals between males and females. Mercury and cadmium liver concentrations generally increased with age, although statistically significant differences between age classes were only evident in females (Figure 3). The pattern of accumulation for lead was opposite to that of mercury and cadmium; juveniles generally had higher liver lead concentrations than adults, although these differences were not statistically significant in either females or males (Figure 3). Accumulation of cadmium with age in the liver has been observed in a variety of mammals, although not always in otters, but age-related differences in liver mercury and lead concentrations in mammals are not commonly reported (Shore and Douben, 1994b, Shore and Rattner, 2001). It is possible that such differences may result from age-related differences in diet (and associated contaminant levels).

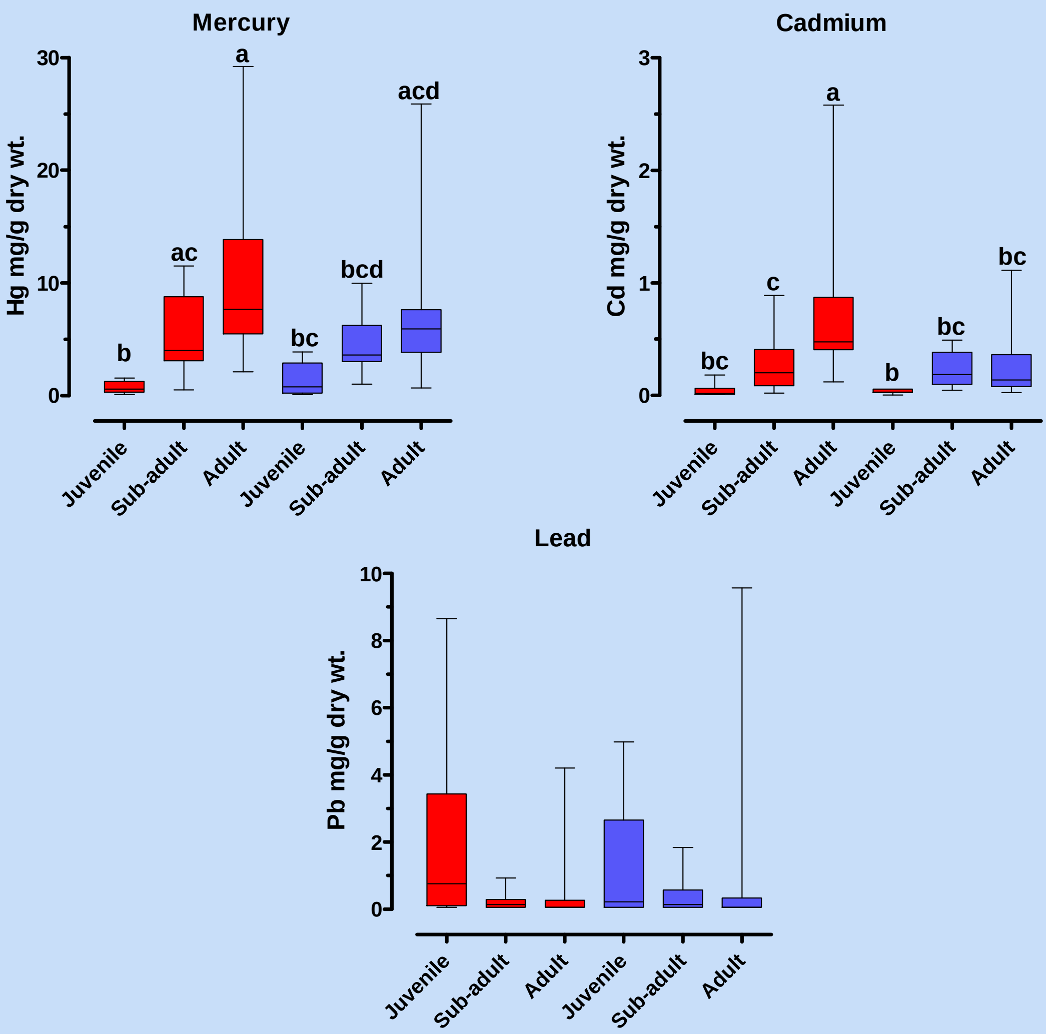

Figure 3. Box and whislers plot of liver concentrations of Hg, Cd, and Pb in otters of different age class and sex. Red and blue boxes indicate female and male otters, respectively. Data were analysed by Kruskall-Wallis test and Dunn's *post-hoc* comparisons across the six sex/age classes. For each element, columns with same superscripts indicate that medians are not significantly different from each other.

Overall, there have been few studies on metal concentrations in the European otter. However, the residues of cadmium, lead and mercury measured in the current study are similar to those reported in otters from Scotland (Kruuk and Conroy, 1991), Great Britain (Mason et al., 1986), and eastern Europe (Gutleb et al., 1998) (Figure 4). Similarly, concentrations of colbalt, copper, chromium, manganese, nickel and zinc are in the same range as those previously reported for Great Britain (Mason and Stephenson, 2001).

Mercury liver concentrations of >25-30 µg/g wet weight have been proposed as indicative of likely adverse effects in mammals (Shore et al., in press). When the dry weight liver mercury concentrations for otters in the present study were converted to wet weight concentrations, based on % moisture content of individual samples (Figure 4), the median concentration was 1.53 µg/g wet weight and the maximum was 9.40 µg/g wet weight.

These concentrations are an order of magnitude and three-fold lower, respectively, than the proposed threshold liver concentrations associated with adverse effects.

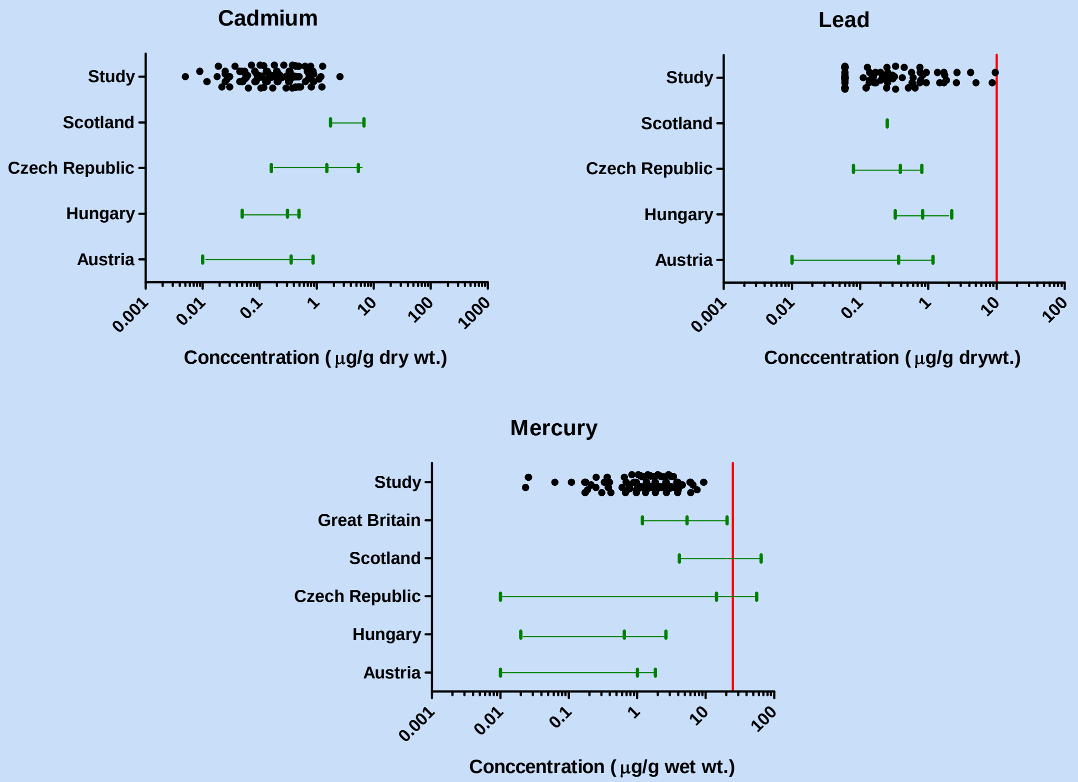

Figure 4. Comparison of data from current study (data for mercury converted to a wet weight concentration) to data from other European studies. Each set of points for previous studies represents minimum, mean and maximum concentrations. Red line indicates concentrations associated with toxic effects. Data are for Great Britain (Mason et al., 1986), Scotland (Kruuk & Conroy, 1991), Czech Republic, Hungary and Austria (Gutleb *et al.*, 1998).

Liver lead concentrations in the otters in the present study were likewise all below the proposed critical level of 10 µg/g dry weight (Ma, 1996). However, the highest concentration measured was 9.57 µg/g. This suggests that liver lead concentrations in some individual otters may reach concentrations associated with toxic effects (Figure 4). Juveniles accumulated the highest median liver lead concentrations and so may be at most risk from lead although it is notable that the otter in our sample that had the highest liver lead concentration was an adult.

Toxic concentrations for cadmium have typically been defined for the kidney rather than the liver; concentration above 105 µg/g dry weight may be associated with cellular damage (Shore and Douben, 1994a). In previous studies of cadmium concentrations in Eurasian otters (Gutleb et al., 1998, Mason et al., 1986), cadmium concentrations in the kidney have been between 1.6 and 2.2 times higher than in the liver. On that basis, median kidney cadmium concentrations in otters in our study would be predicted to be below 1 µg/g dry weight, some two orders of magnitude below concentrations that may be associated with cellular damage.

In summary, the concentrations of inorganic elements measured in the present study were within the range previously reported for Eurasian otters and below those generally associated with toxic effects in mammals. Of the three heavy metals that we measured, lead is the contaminant for which liver concentrations are nearest to the level of concern.

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