Inorganic elements in the livers of Eurasian otters, *Lutra lutra*, from England and Wales in 2009 - a Predatory Bird Monitoring Scheme (PBMS) report

L.A Walker, A.J. Lawlor, E.A. Chadwick[1], E. Potter, M.G. Pereira, & R.F. Shore

Centre for Ecology & Hydrology, Lancaster Environment Centres, Library Avenue, Bailrigg, Lancaster, LA1 4AP
[1]Cardiff School of Biological Sciences, Biomedical Sciences Building, Museum Avenue, Cardiff, CF10 3AX

This report should be cited as:

Walker, L.A., Lawlor, A.J., Chadwick, E.A., Potter, E., Pereira, M.G. & Shore, R.F. [1] (2011). *Inorganic elements in the livers of Eurasian otters, Lutra lutra, from England and Wales in 2009 - a Predatory Bird Monitoring Scheme (PBMS) report***.** Centre for Ecology & Hydrology, Lancaster, UK.

Suggested keywords: Otter; Metals; Liver; Environmental contamination; Monitoring; Pollution; England; Wales

Centre for Ecology and Hydrology Project Number: NEC04288

E-copies of this report:
This report can be requested through the Natural Environment Research Council's Open Research Archive http://nora.nerc.ac.uk/ or can be downloaded directly from the PBMS website

[1]Corresponding author: RF Shore, Centre for Ecology and Hydrology, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster, LA1 4AP, UK. E-mail: rfs@ceh.ac.uk Website: www.ceh.ac.uk

Contents

1. Executive Summary

This is the second report on the 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 15 metals and semi-metals in the livers of 50 Eurasian otters (*Lutra lutra*) that had been found dead between 2007 and 2009 and collected by the CUOP. This report specifically presents data for otters found dead in 2009. The 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.

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.

Five elements, aluminium, antimony, arsenic, chromium, and strontium, varied significantly in concentrations between years. It is unclear whether the inter-year variations in these elements 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 the heavy metals mercury and cadmium in all the otters analysed were below those associated with toxic effects in mammals.

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 50 otters found dead in 2009. Livers were analysed for 15 essential and non-essential trace elements, including heavy metals such as cadmium and 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. The results of analysis of otters that died in 2007 and 2008 are summarised by (Walker et al., 2010).

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. Results of more recent analysis are expected to be available in late 2011.

Since 2007 approximately 50 liver samples per year have been selected for analysis of inorganic elements. The sample was stratified by 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. Lead concentrations were not determined for samples from 2009 because method blanks were contaminated with this element. 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_2009_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 2009 that are included in this report.

		Age Class			
Year	Sex	Juvenile	Sub-adult	Adult	Total
2009	Male	1	12	12	25
	Female	1	12	12	25
	Total	2	24	24	50

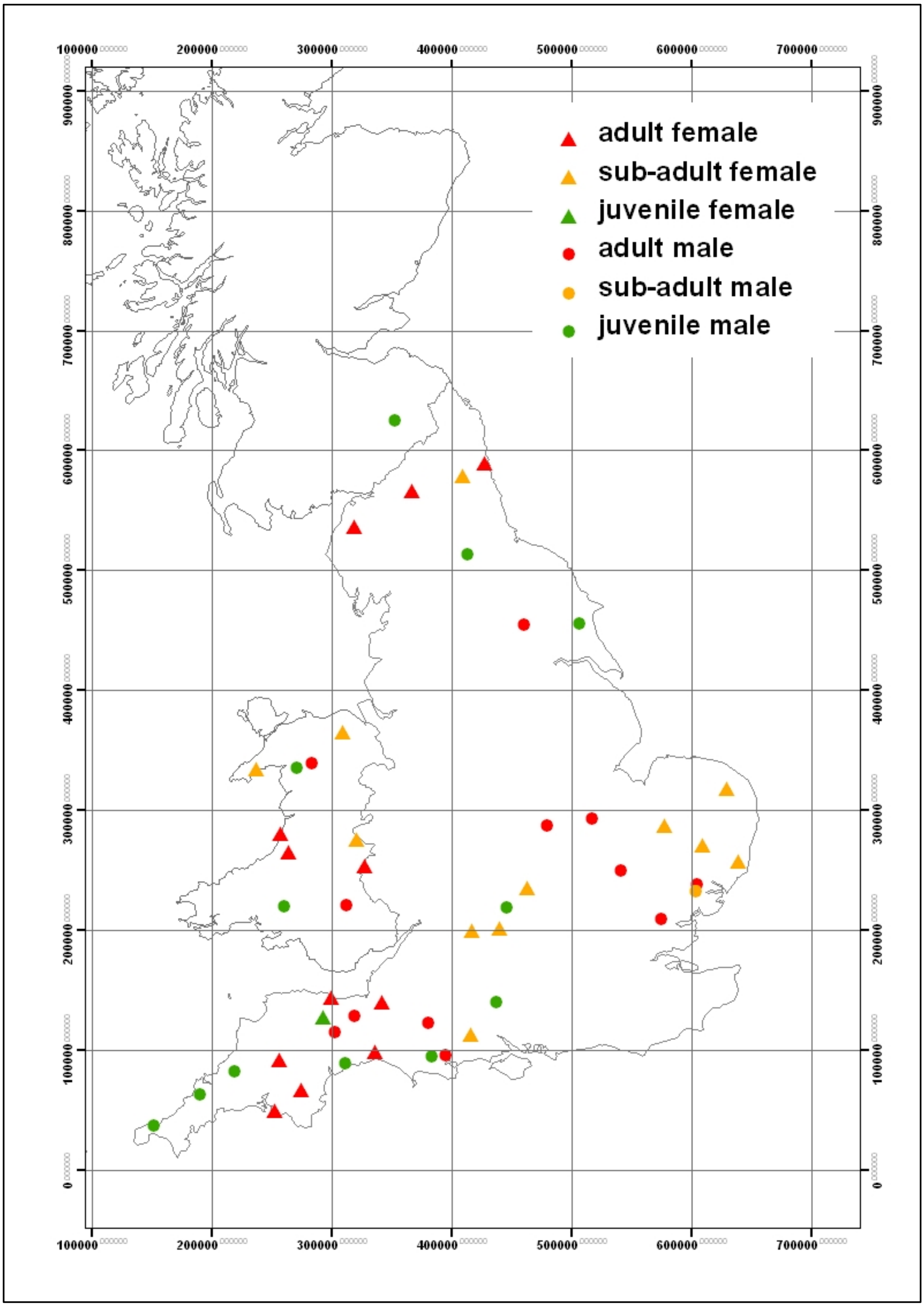

Figure 1. Location of otters, *Lutra lutra*, found dead in 2009 for which livers were analysed for inorganic elements (total of 50 otters).

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_2009_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. 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).

Differences in metal concentrations among years 2007 to 2009 were tested using the Kruskal-Wallis analysis. There were significant differences among years in the liver concentrations of five of the elements studied. However, for two of these elements, antimony and aluminium, the majority of samples (66% and 98% respectively for all years combined) were below the limit of detection and so inter-year comparisons of residue levels may not be representative of the exposure levels in otters for these elements. Median liver concentrations of strontium have increased over the three years studied with samples from 2009 being significantly higher than those measured in 2007 ($P<0.05$). For chromium, samples from 2008 were significantly higher concentrations than in the other two years ($P\leq0.01$) while concentrations of arsenic were significantly lower in 2008 compared to 2007 ($P<0.01$). It is unclear whether the inter-year variation in these elements represents 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 these, or any other elements.

Data for the three years were combined and then assigned to one of six demographic 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. In this report demographic differences are described for the toxic metal/metalloids arsenic, cadmium, copper, and lead, and for the essential elements copper and zinc.

Table 2. Median concentrations (µg/g dry weight) of selected inorganic elements in the livers of otters found dead in 2009 in England and Wales.

Analyte	Median	Inter-quartile Range
Aluminum (Al)	ND	ND-ND
Antimony (Sb)	ND	ND-ND
Arsenic (As)	0.094	0.067-0.182
Cadmium (Cd)	0.167	0.068-0.683
Chromium (Cr)	0.219	0.155-0.306
Cobalt (Co)	0.07	0.052-0.091
Copper (Cu)	31.55	23.95-40.43
Iron (Fe)	731.5	477.3-1013
Manganese (Mn)	7.675	6.855-10.33
Mercury (Hg)	4.955	2.840-9.640
Molybdenum (Mo)	1.24	1.068-1.433
Nickel (Ni)	0.101	0.045-0.165
Selenium (Se)	6.92	5.360-8.805
Strontium (Sr)	0.224	0.141-0.364
Zinc (Zn)	102	85.20-125.0

ND indicates non-detected; for Al<1.12, & 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

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 2). 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 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). Lead concentrations were not determined for samples from 2009, however data from 2007 & 2008 indicated that for juveniles generally had higher liver lead concentrations than adults although these difference were not statistically significant (Walker et al., 2010).

Liver concentrations of the toxic metalloid arsenic and the essential metal copper did not significantly vary among demographic group as also described in a study by Lemarchand *et al.* (2010). For both males and females, zinc concentrations were significantly higher in juvenile animals compared to adults (P<0.05), with sub-adult females also having lower zinc concentration than juvenile females (P<0.05).

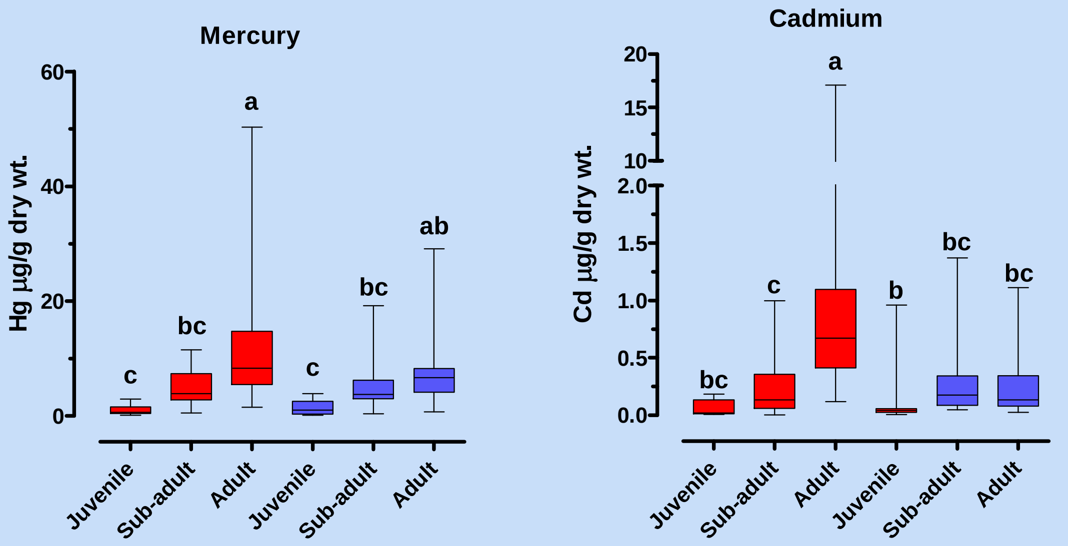

Figure 2. Box and whiskers plot of liver concentrations of Hg and Cd in otters of different age class and sex. Red and blue boxes indicate female and male otters, respectively. Data were analysed by Kruskall-Wallis test and Dunn's *post-hoc* comparisons across the six sex/age classes. For each element, columns with same superscripts indicate that those classes 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 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 3). Similarly, concentrations of cobalt, 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., 2011). 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 3), the median concentration was 1.55 µg/g wet weight and the maximum reported over the 3 years 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.

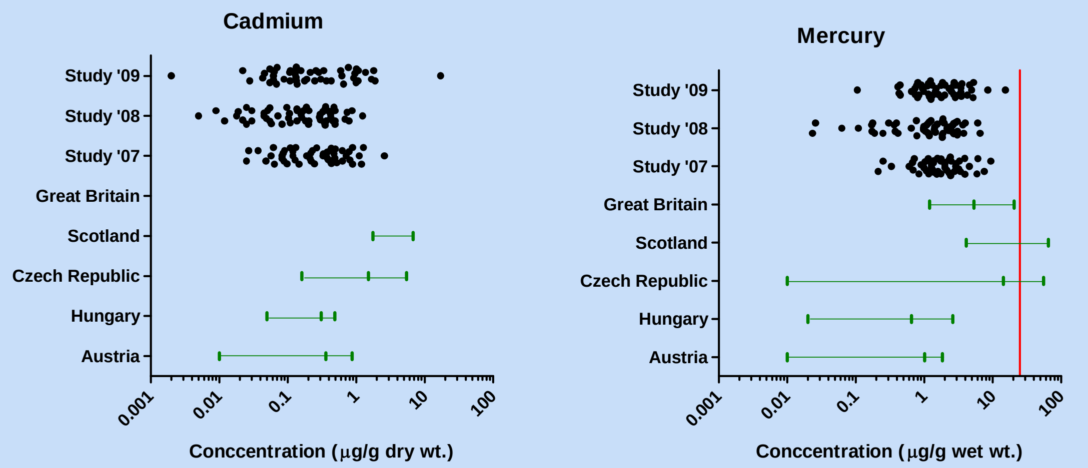

Figure 3. Comparison of data from current study (data for mercury converted to a wet weight concentration) to data from other European studies. Each set of points for previous studies represents minimum, mean and maximum concentrations. Red line indicates concentrations associated with toxic effects. Data are for Great Britain (Hg only; Mason et al., 1986), Scotland (Kruuk & Conroy, 1991), Czech Republic, Hungary and Austria (Gutleb *et al.*, 1998).

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.

5. References

Chadwick, E. A. (2006) A post mortem study of otters found dead in England and Wales between 1992 and 2003. Environment Agency R & D Technical Report W1-084.

Clavero, M., Prenda, J. & Delibes, M. (2003) Trophic diversity of the otter (Lutra lutra L.) in temperate and Mediterranean freshwater habitats. *Journal of Biogeography,* **30,** 761-769.

Cook, D. C. (1978) Foraging Behaviour and Food of Grey Herons Ardea cinerea on the Ythan Estuary. *Bird Study,* **25,** 17-22.

Gutleb, A. C., Kranz, A., Nechay, G. & Toman, A. (1998) Heavy metal concentrations in livers and kidneys of the otter (Lutra lutra) from central Europe. *Bulletin of Environmental Contamination and Toxicology,* **60,** 273-279.

Jedrzejewska, B., Sidorovich, V. E., Pikulik, M. M. & Jedrzejewski, W. (2001) Feeding habits of the otter and the American mink in Bialowieza Primeval Forest (Poland) compared to other Eurasian populations. *Ecography,* **24,** 165-180.

Kruuk, H. & Conroy, J. W. H. (1991) Mortality of Otter (Lutra lutra) in Shetland. *Journal of Applied Ecology,* **28,** 83-94.

Lemarchand, C., Rosoux, R. & Berny, P. (2010) Organochlorine pesticides, PCBs, heavy metals and anticoagulant rodenticides in tissues of Eurasian otters (Lutra lutra) from upper Loire River catchment (France). *Chemosphere,* **80,** 1120-1124.

Marquiss, M. & Leitch, A. F. (1990) The diet of Grey Herons Ardea cinerea breeding at Loch Leven, Scotland, and the importance of their predation on ducklings. *Ibis,* **132,** 535-549.

Mason, C. F., Last, N. I. & Macdonald, S. M. (1986) Mercury, cadmium, and lead in British otters. *Bulletin of Environmental Contamination and Toxicology,* **37,** 844-849.

Mason, C. F. & Stephenson, A. (2001) Metals in tissues of European otters (Lutra lutra) from Denmark, Great Britain and Ireland. *Chemosphere,* **44,** 351-353.

Miranda, R., Copp, G. H., Williams, J., Beyer, K. & Gozlan, R. E. (2008) Do Eurasian otters Lutra lutra (L.) in the Somerset Levels prey preferentially on non-native fish species? *Fundamental and Applied Limnology,* **172,** 339-347.

Shore, R. F. & Douben, P. E. T. (1994a) The ecotoxicological significance of cadmium intake and residues in terrestrial small mammals. *Ecotoxicology and Environmental Safety,* **29,** 101-112.

Shore, R. F. & Douben, P. E. T. (1994b) Predicting ecotoxicological impacts of environmental contaminants on terrestrial small mammals. *Reviews of Environmental Contamination and Toxicology,* **134,** 49-89.

Shore, R. F., Pereira, M. G., Walker, L. A. & Thompson, D. R. (2011) Mercury in Non-marine Birds and Mammals. *Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations, 2nd Edn* (eds W. N. Beyer & J. P. Meador), pp. 609-626. Taylor & Francis, Boca Raton.

Shore, R. F. & Rattner, B. A. (2001) *Ecotoxicology of Wild Mammals,* John Wiley & Sons, Chichester, UK.

Walker, L. A., Lawlor, A. J., Chadwick, E. A., Potter, E., Pereira, M. G. & Shore, R. F. (2010) Inorganic elements in the livers of Eurasian otters, Lutra lutra, from England and Wales in 2007 & 2008: a Predatory Bird Monitoring Scheme (PBMS) report. pp. 13. Centre for Ecology and Hydrology.