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Short Communication

Mercury levels in tissues of Giant otters (*Pteronura brasiliensis*) from the Rio Negro, Pantanal, Brazil ☆

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Abstract

This research reports the first data on mercury levels found in Giant otters (*Pteronura brasiliensis*) from South America. Mercury concentrations were analyzed from different organs/tissues of two animals found dead floating on the water of the Rio Negro in the Pantanal, Brazil. The mean mercury concentration ranged from 2.94 to 3.68 μg/g in hair, from 1.52 to 4.3 μg/g in liver, and from 1.11 to 4.59 μg/g in kidney and was 0.17 μg/g in muscle samples. In comparison with other research, there is no evidence of contamination in these animals and mercury concentrations in tissues appeared to be at levels below those associated with toxicity.

Keywords: Mercury; Giant otter; *Pteronura brasiliensis*; Pantanal (Brazil)

1. Introduction

The Giant otter, *Pteronura brasiliensis* (Zimmermann, 1780), is the largest of all otters species with a total body length typically ranging from 1.5 to 1.8 m and a weight varying from 26 to 32 kg (Duplaix, 1980). They are endemic to South America and distributed throughout the Orinoco, Amazon, and La Plata River basins and numerous localities in the Guyanas (Carter and Rosas, 1997). Giant otters also inhabit several rivers in the upper Paraguay River basin, known as the Pantanal, the Paraguay River itself, the São Lourenço, Itiquira, and Piquiri tributaries in the northern Pantanal, and the Rio Negro and Miranda and Aquidauana Rivers in the southern region (Schweizer, 1992).

The Giant otter is categorized as almost extinct in two countries of its former distribution, seriously endangered in seven countries, and classified as a vulnerable species by the World Conservation Union Mammal Red Data Book (IUCN, 2003). By the early 1970s, pelt hunters had decimated many populations in Brazil, and the remaining are now threatened by increasing exploitation of natural resources like timber, minerals, and fossil fuels. The construction of reservoirs for hydroelectric power generation are rapidly destroying and degrading their habitat (Carter and Rosas, 1997).

Heavy metals like mercury may be partially responsible for the observed decline of the European (*Lutra lutra*) and North American (*Lutra canadensis*) otters in

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many countries (Mason et al., 1986; Halbrook et al., 1994; Evans et al., 2000; Hyvärinen et al., 2003). Diet observation and fecal analysis in various regions have revealed that fish constitute most of the Giant otter's diet (Carter and Rosas, 1997); piscivorous mammals like them, at the top of the food chain and feeding largely on fish, are likely to receive a significant daily dose of mercury as a consequence.

Most of the previous studies of mercury in mammals have measured concentrations in internal tissues like liver and kidneys (Kucera, 1983; Wren et al., 1986; Gutleb et al., 1998). Hair has been used as an indicator of mercury levels in otters in previous studies (Cumbie, 1975; Mason et al., 1986; Halbrook et al., 1994; Evans et al., 1998, 2000), and there is usually a high degree of correlation of mercury levels between different animal tissues (Evans et al., 1998; Mierli et al., 2000).

Although they are an environmental indicator species because of their position in the food chain and therefore a possible early warning system for human health impacts, particularly in fish-eating societies (Evans et al., 1998), only one study on mercury concentration in Giant otters has been published. In this study, Gutleb et al. (1997) used otter spraints and fishes to assess the risk of mercury intoxication in Giant otters in Manu National Park (Peru). The analysis of spraints may help to assess the potential threats to an existing otter population, or it may provide clues to the disappearance of otters from a river, but the interpretation of such results is very difficult because the metal may be associated with those components of the diet, like fish-scales, e.g., that pass unassimilated through the gut (Mason and Macdonald, 1986).

The present paper reports preliminary data on mercury levels occurring in tissues of Giant otters found dead in the Rio Negro in the Pantanal, Brazil.

2. Materials and methods

Tissues (hair, liver, kidney, and/or muscle) were obtained from two Giant otters (both male) between November 2002 and November 2003. They were found dead, though causes unknown on the water of the Rio Negro in the Pantanal, Mato Grosso do Sul State, Brazil during the undertaking of the project Ecology and Conservation of Pantanal Otters, which was supported by Earthwatch Institute and Conservational International. The carcasses were kept frozen until analysis.

The hair samples were excised with clean stainless-steel implements and washed with EDTA 0.01% for 2 h to remove superficial grease and dust to avoid external metal contamination. They were then rinsed twice in double-distilled and deionized water and dried overnight at 40 °C. All samples of internal tissues (30–80 mg fresh weight), and hair (about 10–20 mg dry weight) after weighing, were mineralized for the determination of mercury. Hydrogen peroxide was added to samples of liver, kidney, and muscle tissues, and after 10 min, 5 ml of a solution of concentrated H_2SO_4 and HNO_3 (1:1) was added and placed in a water bath at 60 °C for 15 min and allowed to cool down. After that, 5 ml of 5% $KMnO_4$ solution was added and the extracts were left overnight at room temperature in a dust-free atmosphere. Extracts were neutralized with some drops of a 12% $HONH_3Cl$ solution and a final volume of 25 ml was made up using double-distilled and deionized water just before analyses. The hair procedure was the same as for internal tissues, except that hydrogen peroxide was not added.

Mercury analyses were performed by atomic absorption spectrometry with an AA 1475 Varian instrument equipped with a cold-vapor generator accessory (Varian VGA-76), with sodium borohydride as a reducing agent at Laboratório de Radioisótopos Eduardo Penna Franca from the Federal University of Rio de Janeiro according to Malm et al. (1991).

Accuracy of the mercury concentration was checked by comparison with certified reference samples IAEA-086 and IAEA-085 for hair and lyophilized fish internal laboratory reference sample AFPX5130 (fillet of *Pseudoplatystoma coruscans* from Alta Floresta, Brazil) produced by Radioisotopes Laboratory at Federal University of Rio de Janeiro for internal use. All samples were performed in duplicate, allowing an estimation of analytical variability, and all reagents were p.a. degree.

3. Results and discussion

The average value obtained for mercury in the IAEA-085 hair was 24.4 ± 3.57 μg/g (certified value of 23.2 ± 3.4 μg/g) and for IAEA-086 was 0.58 ± 0.21 μg/g (certified value of 0.574 ± 0.15 μg/g). The average value obtained for internal laboratory reference sample AFPX5130 was 14.3 ± 0.58 μg/g dry weight, close to the local certified value of 13.83 ± 1.32 μg/g dry weight.

Several factors like location, age, sex, and others reported in the literature have been associated with differences in mercury concentration in wild mammals (Wren, 1986). In this work, due to a sample size insufficient to yield adequate power for the statistical tests, it was difficult to identify any one factor that has some effect on mercury accumulation in Giant otters.

Mean mercury detected in Giant otter hair ranged from 2.94 to 3.68 μg/g. Otter hair has been utilized as an indicator of mercury levels in otters in several studies (Cumbie, 1975; Mason et al., 1986; Halbrook et al., 1994; Evans et al., 1998, 2000), in which there was a greater range in mercury concentration. The levels of mercury in the hair of Giant otters from the Rio Negro

were similar to those of *L. canadensis* from Wisconsin (mean, 6.47 μg/g; max, 63.2 μg/g) reported by Sheffy and St. Amant (1982) and can be considered low compared to the results reported for *L. canadensis* from Ontario, Canada (mean, 9.6; range, 4.0–20.0 μg/g; Evans et al., 1998), for *L. lutra* from Finland (mean, 18.5; range, 0.7–61.3 μg/g; Hyvärinen et al., 2003) and from Britain (mean, 18.7; range, 1.3–85.1 μg/g; Mason et al., 1986), and for *L. canadensis* from Maine (mean, 20.3; range, 1.1–33.7 μg/g; Evers et al., 2002) and from Georgia (mean, 21.2; range, 0.5–54.4 μg/g; Halbrook et al., 1994). Sheffy and St. Amant (1982) assume that concentrations of mercury ranging from 1 to 5 μg/g in fur of otters represent normal background levels in these animals.

Wren (1986) suggested that normal mercury concentrations in otter liver should be less than 4.0 μg/g wet weight. The mean concentration of mercury in the liver of one Giant otter (4.3 μg/g) was slightly higher than this value, but it was lower in the other (1.52 μg/g). The mean mercury concentration in the livers of both Giant otters analyzed in this study was higher than in *L. lutra* from Hungary and Austria (0.65 and 1.01 μg/g, respectively), as reported by Gutleb et al. (1998), and in *L. canadensis* from Canada (1.61 μg/g; Evans et al., 2000). However, our results were lower than those for *L. lutra* from Scotland (4.7 μg/g; Mason and Reynolds, 1988) and *L. canadensis* from Georgia (7.13 μg/g; Halbrook et al., 1994) (Table 1).

A background level of mercury is considered to be less than 3.0 μg/g in kidney of otters (O'Connor and Nielsen, 1980; Kucera, 1983). One Giant otter in the present study showed an elevated mercury concentration in the kidney (4.59 μg/g), while the other was 1.11 μg/g (Table 1), but both values are considered to be lower than toxic concentrations for otters and are within the range found in similar studies undertaken with *L. lutra* from Europe (range, 1.35–6.79 μg/g, Mason et al., 1986; range, 1.5–3.0 μg/g, Mason and Reynolds, 1988; <0.01–2.1 μg/g, Gutleb et al., 1998) and *L. canadensis* from North America (mean, 1.42; max, 3.97 μg/g; Evans et al., 2000).

The values found in livers and kidneys of Giant otters were low compared to the findings of O'Connor and Nielsen (1980) and Wren (1985) (Table 1). Experimentally dosed *L. canadensis* died with symptoms of mercurialism and mean levels of mercury in their livers and kidneys were 33.4 and 39.2 μg/g, respectively (O'Connor and Nielsen, 1980). Wren (1985) reported on an otter (*L. canadensis*) that was found dead near a river in Ontario, Canada that was known to be severely polluted with mercury; had a concentration of 96.0 μg/g in the liver and 58.0 μg/g in the kidneys, which suggests that this animal died of mercury poisoning. Tracks in the snow indicated that the animal behaved strangely prior to death: traveling in circles, falling over, and burrowing into the snow. The unnatural behavior of the otter prior to death is consistent with clinical

Table 1
Mercury concentrations (μg/g wet weight) detected in Giant otter (*P. brasiliensis*) liver, kidney, and muscle from the Rio Negro River, Pantanal, in comparison to mercury concentrations reported in otters in others studies

Species	Location	Tissue	Concentration		Reference
			Mean	Range	
P. brasiliensis	Brazil	Liver	2.91	1.52–4.3	This study
L. lutra	Hungary	Liver	0.65	0.02–2.64	Gutleb et al. (1998)
	Austria	Liver	1.01	<0.01–2.1	
L. lutra	Scotland	Liver	4.7	1.0–20.3	Mason and Reynolds (1988)
L. canadensis	Canada	Liver	1.61	Max = 3.35	Evans et al. (2000)
L.canadensis	Georgia	Liver	7.13	2.8–16.3	Halbrook et al. (1994)
L. canadensis	Canada	Liver	33.4	—	O'Connor and Nielsen (1980)
L. canadensis	Canada	Liver	96.0	—	Wren (1985)
P. brasiliensis	Brazil	Kidney	2.84	1.11–4.59	This study
L. lutra	Britain	Kidney	2.27	1.35–6.79	Mason et al. (1986)
L. lutra	Scotland	Kidney	2.1	1.5–3.0	Mason and Reynolds (1988)
L. lutra	Hungary	Kidney	0.68	<0.01–2.1	Gutleb et al. (1998)
L. canadensis	Canada	Kidney	1.42	Max = 3.97	Evans et al. (2000)
L. canadensis	Canada	Kidney	39.2	—	O'Connor and Nielsen (1980)
L. canadensis	Canada	Kidney	58.0	—	Wren (1985)
P. brasiliensis	Brazil	Muscle	0.17	—	This study
L. lutra	Britain	Muscle	1.66	0.61–2.64	Mason et al. (1986)
L. canadensis	Georgia				Halbrook et al. (1994)
	Piedmont	Muscle	1.48	0.82–3.1	
	Coastal plain	Muscle	4.42	0.72–10.27	
L. canadensis	Canada	Muscle	36.0	—	Wren (1985)
L. canadensis	Canada	Muscle	15.7	—	O'Connor and Nielsen (1980)

observations made on minks (*Mustela vison*) suffering from methylmercury poisoning (Wobeser et al., 1976).

The mean of 0.17 μg/g mercury in muscle of the Giant otters was below the mean values found in *L. canadensis* from the lower coastal plain region and Piedmont area of Georgia (4.42 and 1.48 μg/g, respectively; Halbrook et al., 1994). Muscle mercury levels in a wild *L. canadensis* found dead of supposed mercurialism in Ontario, Canada, by Wren (1985) were substantially higher (36.0 μg/g) than terminal mercury levels in muscle of otters experimentally killed with a methylmercury diet (15.7 μg/g; O'Connor and Nielsen, 1980). In *L. lutra* the mean average of mercury in muscle found by Mason et al. (1986) was 1.66 μg/g (Table 1).

Mercury concentrations of 0.12–1.4 μg/g wet weight in a fish diet of mink and otter were associated with adverse health effects (Halbrook et al., 1994). The concentration of 0.1 μg/g mercury fresh weight in fish was proposed to be the tolerable mercury level in fish for consumption by European otters (Hovens, 1992). Some of the most frequent preys of Giant otters, such as *Pygocentrus nattereri* and *Serrasalmus marginatus* (both Serrasalmidae) and *P. maculatus* (Pimellodidae), collected in the Rio Negro showed a similar concentration (Fonseca et al., unpublished data).

The data reported here indicate that the mercury concentrations in these two Giant otters are lower than those known to cause death in otters experimentally dosed with methylmercury and showing clinical signs of mercury contamination. Additional investigations are needed to determine the relative contribution of natural and man-caused mercury inputs to mercury levels observed in wild Giant otters in the Pantanal region.

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References

Carter, S.K., Rosas, F.C.W., 1997. Biology and conservation of the giant otter *Pteronura brasiliensis*. Mammal Rev. 27, 1–26.

Cumbie, P.M., 1975. Mercury levels in Georgia Otter, mink and freshwater fish. Bull. Environ. Contam. Toxicol. 14, 193–196.

Duplaix, N., 1980. Observations on the ecology and behavior of the giant otter *Pteronura brasiliensis* in Suriname. Rev. Ecol. (Terre Vie) 34, 495–620.

Evans, R.D., Addison, E.M., Villeneuve, J.Y., MacDonald, K.S., Joachim, D.G., 1998. An examination of spatial variation in mercury concentrations in otter (*Lutra canadensis*) in south-central Ontario. Sci. Total Environ. 213, 239–245.

Evans, R.D., Addison, E.M., Villeneuve, J.Y., MacDonald, K.S., Joachim, D.G., 2000. Distribution of inorganic and methylmercury among tissues in mink (*Mustela vison*) and otter (*Lutra canadensis*). Environ. Res. 84, 133–139.

Evers, D.C., Yates, D., Savoy, L., 2002. Developing a Mercury Exposure Profile for Mink and River Otter in Maine. Report BRI 2002-10 submitted to the Maine Department of Environmental Protection and Maine Inland Fisheries and Wildlife. BioDiversity Research Institute, Falmouth, ME, USA.

Gutleb, A.C., Schenck, C., Staib, E., 1997. Giant otter (*Pteronura brasiliensis*) at risk? Total mercury and methylmercury levels in fish and otter scats Peru. Ambio 26, 511–514.

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. Bull. Environ. Contam. Toxicol. 60, 273–279.

Halbrook, R.S., Jenkins, J.H., Bush, P.B., Seabolt, N.D., 1994. Sublethal concentrations of mercury in river otters: monitoring environmental contamination. Arch. Environ. Contam. Toxicol. 27, 306–310.

Hovens, J.P.M., 1992. Microverontreinigingen in waterbodems en visbiota in Nederland; de visotter (*Lutra lutra*) als normsteller. Stichting Otterstation Nederland.

Hyvärinen, H., Tyni, P., Nieminen, P., 2003. Effects of moult, age, and sex on the accumulation of heavy metals in the otter (*Lutra lutra*) in Finland. Bull. Environ. Contam. Toxicol. 70, 278–284.

IUCN—The World Conservation Union, 2003. Red List of Threatened Species. IUCN–The World Conservation Union.

Kucera, E., 1983. Mink and otter as indicators of mercury in Manitoba waters. Can. J. Zool. 61, 2250–2256.

Malm, O., Pffeifer, W.C., Reuther, R.E., Souza, C.M.M., 1991. Mercury pollution due to gold mining in the Madeira River Basin, Amazon/Brazil. Ambio 19, 11–15.

Mason, C.F., Macdonald, S.M., 1986. Otters: Ecology and Conservation. Cambridge University Press, Cambridge, UK.

Mason, C.F., Reynolds, P., 1988. Organochlorine residues and metals in otters from the Orkney Islands. Mar. Pollut. Bull. 19, 80–81.

Mason, C.F., Last, N.I., Macdonald, S.M., 1986. Mercury, cadmium, and lead in British otters. Bull. Environ. Contam. Toxicol. 37, 844–849.

Mierli, G., Addison, E.M., MacDonald, K.S., Joachim, D.G., 2000. Mercury levels in tissue of otters from Ontario, Canada: variation with age, sex and location. Environ. Toxicol. Chem. 19, 3044–3051.

O'Connor, D.J., Nielsen, S.W., 1980. Environmental survey of methylmercury levels in wild mink (Mustela vison) and otter (*Lutra canadensis*) from the northeastern United States and experimental pathology of methylmercurialism in the otter. In: Chapman, J. A., Pursley, D. (Eds.), Proceedings of the World Furbearer Conference Inc., Frostburg, MD, USA, pp. 1728–1745.

Schweizer,, J., 1992. A Ariranha no Pantanal: ecologia e comportamento de *Pteronura brasiliensis*. Editora Brasil Natureza Ltda, Curitiba, Brazil.

Sheffy, T.B., St. Amant, J.R., 1982. Mercury burdens in furbearers in Wisconsisn. J. Wildl. Manage. 46, 1117–1120.

Wobeser, G., Nielsen, N.O., Schiefer, B., 1976. Mercury and mink. 2. Experimental methyl mercury intoxication. Can. J. Comp. Med. 40, 34–45.

Wren, C.D., 1985. Probable case of mercury poisoning in a wild otter, *Lutra canadensis*, in northwestern Ontario. Can. Field Nat. 99, 112–114.

Wren, C.D., 1986. A review of metal accumulation and toxicity in wild mammals. Environ. Res. 40, 210–244.

Wren, C.D., Stokes, P.M., Fischer, K.L., 1986. Mercury levels in Ontario mink and otter relative to food levels and environmental acidification. Can. J. Zool. 64, 2854–2859.