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Concentrations of Selected Elements in Illinois Raccoons

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ABSTRACT

We examined concentrations of cadmium (Cd), copper (Cu), lead (Pb), mercury (Hg), selenium (Se), and zinc (Zn) in tissues of raccoons collected in Illinois during 1983-85. Concentrations of Cd, Pb, and Zn in the tissues of Illinois raccoons were variable with regard to concentrations of these elements reported for raccoons from other areas. Cuand Se concentrations were similar to, and Hg concentrations were lower than, previously reported for raccoons from other areas. Concentrations of Pb in livers were lower than in Illinois raccoons collected during the mid- to late-1950s in a previous study. We also compared the tissue concentrations observed in raccoons with those reported for mink and river otters collected in Illinois during 1984-1989 (Halbrook et al, 1996). Cd, Pb, and Se were more frequently detectable, whereas Hg was detectable at a lower rate, in the tissues of raccoons than in mink or river otters. Concentrations of Cd, Pb, and Zn were lower, and Cu and Hg were higher, in the tissues of raccoons as compared with those of mink and river otters from Illinois.

INTRODUCTION

Based on a synthesis of information gathered from the southeastern United States, Bigler et al. (1975) concluded that the raccoon (*Procyon lotor*) was an effective monitor of environmental contaminants, including organochlorine pesticides and heavy metals. Other investigators have examined contaminant levels in raccoons from various locations in North America (e.g. Illinois, Sanderson and Thomas 1961; Wisconsin, Sheffy and St. Amant 1982; Ontario, Wren, 1984; California, Clark, Jr. et al., 1989; Mississippi, Ford and Hill 1990; Alabama, Khan et al. 1995).

The usefulness of raccoons as biomonitors stems from various aspects of their biology and behavior, including high abundance in many areas, ease of capture, close association with humans, relatively sedentary habits, frequent contact with water and sediments, and a diet that may seasonally include items such as fish, crayfish, mussels, frogs, insects, earthworms, and other animals that accumulate contaminants. Raccoons are also relatively long-lived, and able to thrive despite relatively high parasite loads and exposure to a variety of infectious diseases and environmental contaminants. In addition, specimens are often readily available because of nuisance control activities and regulated hunting and trapping.

We analyzed tissues from 62 raccoons collected in 1983-85 in Illinois for concentrations of cadmium (Cd), copper (Cu), lead (Pb), mercury (Hg), selenium (Se), and zinc (Zn). Our objectives were to 1) provide an historic benchmark for concentrations of selected elements in raccoons from Illinois, 2) compare liver Pb concentrations with those previously reported for this species in Illinois, 3) contrast concentrations of selected contaminants in raccoons from Illinois with those reported for other regions, and 4) compare concentrations of selected metals and Se with those reported in mink (*Mustela vison*) and river otters (*Lontra canadensis*) from Illinois.

METHODS

Specimens were solicited from cooperating fur buyers during the 1983-84 and 1984-85 Illinois trapping seasons (Nov-Jan.). Raccoons were captured in drainages in agricultural areas, e.g. along agricultural drainage ditches and wooded stream/river bottoms, of Dekalb, Kane, Kendall, LaSalle, and Richland counties. We combined years except where Method Detection Limits (MDLs) were different. Locations (counties) were also combined to increase sample sizes and because we were interested in the range of observable contamination for agricultural habitats in Illinois.

Kidney, liver, and muscle samples were placed in aluminum foil and frozen until preparation at the Illinois Natural History Survey Analytical Chemistry Laboratory. Duplicate samples were digested with HNO_3 and HCO_4, diluted as necessary, and analyzed for Cd, Cu, Pb, Se, and Zn by inductively coupled argon-plasma emission spectroscopy (ICP-AES) with a Jarrell-Ash Model 975 Plasma AtomComp spectrometer. Hg concentrations were determined by cold-vapor atomic absorption spectrometry with a Fisher Model HG3 Mercury Analyzer. Twenty ml H_2SO4, 10 ml HNO_3, and 20 ml 5% $KMnO_4$ were added to duplicate 1.0 g. samples for 15 minutes before adding 10 ml 5% $K_2S_4O_8$ and placing in a hot water bath. Crystalline $KMnO_4$ was added until a constant color remained, after which samples were cooled and diluted to 100 ml before analysis. Measures of accuracy and precision, including standards, matrix spikes, and blanks were acceptable.

Differences in mean concentrations between sexes and age classes were compared using the Student's t-test (Zar 1984). Kidney Cd, Cu, Hg, and Pb, and liver Cd and Hg were log-transformed prior to statistical analyses to improve distributions. Sexes were combined, as no significant variation in residue concentrations between genders was detected ($P \geq 0.13$). Previous studies have also indicated a lack of variation in contaminant levels between the sexes in raccoons (Clark, Jr. et al. 1989; Herbert and Peterle 1990; Khan et al. 1995; Sanderson and Thomas 1961). We detected significant age-related variation in liver Cd and Hg levels; differences in mean kidney Cd concentrations between age groups were substantial though not statistically significant. Data from both age classes of raccoons (juvenile < 1 year of age; adult ≥ 1 year of age) were initially combined to allow comparison with published information; subsequently we discuss comparisons of age-related variation in metal concentrations. The relationship between Cd and Zn concentrations was compared using simple linear correlation (Zar 1984). For statistical purposes, concentrations below the Method Detection Limit (MDL) were entered at 2 MDL, if ≥ 60% of the samples had concentrations > MDL. Otherwise, descriptive statistics represent data > MDL. The Method Detection Limit is the "minimum concentration of a substance that can be identified", and is "related to the standard deviation of the measured value at or near zero concentration of the analyte" (Glaser et al., 1981:1426). Data reported for raccoons in other studies are provided in Appendix A for the interested reader.

RESULTS AND DISCUSSION

Concentrations of Selected Elements in Raccoon Tissues

Cadmium (Cd)

Cd was detected in the kidneys of nearly half of the raccoons collected in 1983-84 (Table 1). With the exception of specimens from Ontario, the mean concentration of Cd in renal tissue of raccoons in this study was lower than reported for other areas (Appendix A). The mean and maximum Cd concentrations were lower in liver than in kidney tissue (Table 2). The MDL was higher in 1983-84 than in 1984-85, thus the number of detections was higher during the latter period, although the maximum values were similar. The mean hepatic Cd concentration in our 1984-85 sample was considerably lower than in raccoons from Michigan and New York, but was similar to that reported for raccoons from Ontario (Appendix A).

The mean Cd concentration was higher in the kidneys of adult ($\bar{x}$ = 1.35 ppm, n= 9) than juvenile ($\bar{x}$ = 0.74 ppm, n= 22) raccoons, as was noted by Herbert and Peterle (1990) for raccoons from Michigan. However, renal Cd concentrations in our study were highly variable, thus the difference between age classes was not statistically significant (P= 0.18). The mean concentration of Cd in liver was greater in adult (0.42 ppm, n= 24) than in juvenile (0.25 ppm, n= 38) raccoons (P= 0.03). Herbert and Peterle (1990) also found higher concentrations of Cd in the livers of adult as compared with juvenile raccoons. Cd and Zn are known to co-accumulate in the kidney and liver of birds (Scheuhammer and Templeton 1990). However, in our study only weak relationships existed between renal (r= 0.14, *P*= 0.45) and hepatic (for 1984-85, r= -0.12, P=0. 54) Cd and Zn in raccoons. Cd was not detected in raccoon muscle tissue (Table 3).

Copper (Cu)

Cu was detected in the kidneys of all but two of the raccoons collected in 1983-84 (Table 1). The mean concentration of Cu in liver was 31% higher than in kidneys (Table 2). The mean concentrations of Cu in the kidneys and livers of raccoons in our study were similar to that reported for this species from Ontario (Appendix A). The mean concentration of Cu in muscle tissue was considerably lower than in kidney and liver tissue (Table 3).

Lead (Pb)

Pb was detected in 23% of the raccoon kidneys examined (Table 1). Concentrations of Pb in kidneys of raccoons with concentrations > MDL were lower than reported for raccoons from Alabama and New York, but were higher than in raccoons from Michigan and Florida (Appendix A). Pb was detected in 16% of the livers examined, and the mean hepatic concentration was 46% higher than the renal concentration (Table 2). The hepatic Pb concentrations of raccoons with concentrations > MDL were considerably lower than reported for Illinois raccoons collected nearly three decades prior to our study (Appendix A). It is tempting to speculate that this decline in Pb in Illinois raccoons between 1958-59 and 1983-85 resulted from efforts to reduce lead in the environment, i.e. the use of unleaded gasoline. The raccoons examined by Sanderson and Thomas (1961) were collected from various counties throughout Illinois including Du Page (which borders Kendall and Kane counties) and Richland counties. With the exception of Michigan, liver Pb levels in Illinois raccoons in our study were lower than reported for other areas (Appendix A). Pb was detected in few muscle samples (Table 3).

<u>Mercury (Hg)</u>

Mercury was detected in the kidneys of 70% of the Illinois raccoons examined (Table 1). The mean renal concentration observed in this study was lower than in raccoons from other areas (Appendix A). Hg was detected in a higher proportion of livers than kidneys, and the mean concentration was higher in hepatic tissue (Table 2). The mean liver Hg concentration in our sample was lower than in raccoons from other areas of North America (Appendix A). Mean Hg concentrations were significantly greater in livers of adult (0.20 ppm, n= 24) than juvenile (0.04 ppm, n= 38) raccoons (P= 0.002). The frequency of detection and mean concentration of Hg in muscle was lower than in liver and similar to that of kidney tissue (Table 3).

<u>Selenium (Se)</u>

Se is an essential element, and the MDLs for Se in our study were high. Consequently, Se was detectable in only 48% of the kidneys from Illinois raccoons (Table 1). The mean concentration of Se in kidneys was similar to those of raccoons from Ontario and New York (Appendix A). The maximum hepatic Se concentration we observed was similar to mean values for raccoons from Ontario and Michigan, but was below the range of values observed in raccoons from Kesterson National Wildlife Refuge, California, an area known to be contaminated with high concentrations of Se (Table 2). Few raccoon muscle samples contained detectable concentrations of Se (Table 3).

<u>Zinc (Zn)</u>

Zn was detected in all of the raccoon kidneys examined (Table 1). The mean concentration of Zn in kidneys was higher than reported for raccoons from Michigan, but considerably lower than in this species from Florida (Appendix A). Zn was detected in all of the liver samples analyzed (Table 2), and the mean and maximum concentrations were higher than in kidney tissue. The mean hepatic Zn concentration in our study was essentially identical to that observed in raccoons from Ontario (Appendix A). Zn was were detectable in all of the raccoon muscle tissue samples, and the mean concentration in muscle was higher than in liver and kidneys (Table 3).

Heavy metal concentrations in the tissues of raccoons from Illinois were variable (e.g. higher, lower, or similar) with regard to those in raccoons from other portions of the United States and Canada. Collectively, raccoons in prior studies were sampled from a variety of environments including urban areas, near sewage treatment plants, along highly agriculturalized drainages, downstream of industrial areas, and “natural” habitats, along with collections of a broader nature (e.g. county-wide basis). Hamir et al. (1994) found higher blood lead concentrations in raccoons inhabiting urban areas than in specimens occupying rural areas. Most of the studies we referenced for comparative purposes were conducted within 5 years of ours, and so were temporally comparable. Our specimens were collected from highly-agriculturalized drainages, without access to large urban areas or concentrated industrial or manufacturing activity. Thus, the tissue concentrations we observed may be considered background values for raccoons inhabiting agricultural areas in Illinois.

Although raccoons are considered to be tolerant of exposure to environmental contaminants (Sanderson and Thomas 1961; Herbert and Peterle 1990; Hamir et al. 1999), little information exists with regard to critical tissue concentrations in this species. Hamir et al. (1999) failed to detect clinical signs, changes in hematology, or important histopathology in dosed raccoons with liver and kidney Pb concentrations, respectively, of as high as 77 and 31 ppm. Sanderson and Thomas (1961) failed to detect differences in body condition in raccoons with livers containing from 0 to 32 ppm Pb. However, an inverse relationship between relative adrenal weight and liver Pb concentration was observed in that study. The authors suggested that a combination of Pb exposure and pneumonia or injury may have been responsible for illness or death observed in some animals they examined. Our maximum value (3.96 ppm) was similar to the minimum liver Pb concentration (4 ppm) for animals in their study which were sick or found dead. Liver and kidney Pb concentrations above 10 ppm (4.38 ppm wet weight) and 25 ppm dry weight (8.42 ppm wet weight), respectively are considered diagnostic of acute Pb poisoning in wild mammals (Ma 1996). Abnormal behavior and microscopic anatomical changes in renal and hepatic tissue were documented in a raccoon with a liver Pb concentration of 35 ppm wet weight (Diters and Nielsen 1978). Although some minor histological changes were noted in several animals, Clark, Jr. et al. (1989) concluded that liver Se concentrations of up to 31 ppm dry weight were having no negative impacts on raccoons. The maximum Se concentration in raccoons from Illinois (converted to dry weight) fell below the range of values observed in that study. After summarizing the available literature on Cd in wildlife, Eisler (1985) concluded that renal or hepatic concentrations of > 10.0 mg/kg wet weight were indicative of cadmium exposure, and that tissue concentrations of 13.0 to 15.0 mg/kg "probably" posed a hazard to animals at higher trophic levels. Thompson (1996:351) considered Hg concentrations of ≥ 30 mg/kg wet weight in liver or kidneys to be "lethal or at least harmful" to wild mammals. Based on these studies, it seems unlikely the concentrations of heavy metals and Se we observed were having a direct impact on raccoons inhabiting agricultural drainages in Illinois.

Comparisons with mink and river otters from Illinois

A need exists for comparative information on exposure and accumulation of contaminants among species to identify sentinel species which may be used as environmental indicators or biomonitors. We compared the rate of detection and mean concentrations of selected elements observed in raccoons in our study with published information for mink and river otters collected in Illinois during the 1980's (Halbrook et al., 1996).

Cd was detected more frequently in liver tissue of raccoons (77%) than mink (55%) even though the MDL was higher for the raccoons (0.07 vs. 0.04 ppm). Cd was not detected in livers of river otters at an MDL of 0.10 ppm (Halbrook et al., 1996). Pb was detected more frequently in the livers of raccoons (16%) than mink (10%), even though the MDL was higher for raccoons (0.81 ppm vs. 0.68). Lead was not detectable in the livers of river otters at a lower MDL (0.05 ppm) than in either raccoons or mink. Hg was detectable in a larger proportion of mink (97%) as compared with raccoon (87%) livers at the same MDL (0.005 ppm). Hg was not detected in the livers of river otters; however, the MDL for that species was rather high (2.0 ppm). Detection limits for Se were rather high (1.27 to 4.0 ppm); subsequently this element was detected in few samples. However, the detection rate for Se in kidneys was considerably greater in raccoons (48%) than in mink (21%), even though the MDLs were similar (1.27 and 1.35 ppm).

Mean concentrations of some elements were consistently lower (with the exception of Cd in muscle), while others were higher, in the tissues of mink and river otters than in the raccoons we examined (Table 4). For the most part, the maximum observed concentrations reflected these patterns. The exceptions were the maximum kidney Cu concentration which was higher in raccoons than river otters, and the maximum renal Hg concentration, which was slightly higher in raccoons than in mink. The maximum concentration of Se in the kidneys of mink was also lower than in the kidneys of raccoons.

Differences in tissue Hg concentrations between raccoons and the semi-aquatic mink and river otter may result from differences in food habits. As compared with raccoons, the diets of mink and river otters may contain a higher proportion of fish and other aquatic life (Lotze and Anderon, 1979; Lariviere, and Walton, 1998; Lariviere, 1999) which may accumulate high levels of this metal (Eisler, 1987; Wren et al., 1995). Sheffy and St. Amant (1982) also reported that raccoons had lower concentrations of Hg in their tissues than both mink and river otters. However, river otters from Ontario had lower liver and similar kidney Hg concentrations, when compared with raccoons from the same watershed (Wren, 1984). Such differences could reflect regional variation in diets or Hg bioavailability.

Cu and Zn are essential elements, the levels of which normally are regulated by physiologic processes (Eisler 1993, 1997). Thus, differences in tissue concentrations among raccoons, mink, and river otters may represent normal, inter-specific variation in concentrations of those metals. The fact that the tissue Cu and Zn concentrations we observed in raccoons were similar to those in raccoons from Ontario (Wren, 1984), and that raccoons in that study had higher tissue Zn, and lower liver and muscle Cu, concentrations than river otters, supports this hypothesis.

The reasons for higher concentrations of Cd and Pb in the tissues of raccoons can only be speculated. Seasonally, the diet of raccoons may contain a substantial proportion of earthworms, grubs, ground-dwelling insects, and other invertebrates (Schwartz and Schwartz, 1981; Hoffmeister, 1989) which may accumulate Cd and Pb from the soil; raccoons also have a high rate of soil ingestion (Beyer et al., 1994). These habits, coupled with their greater tolerance for human activity and more-terrestrial foraging habits than the semi-aquatic mink and river otter may increase their exposure to these metals (e.g. by foraging along roadsides, at industrial/waste sites, and fertilized agricultural fields, etc). Additionally, raccoons are considered to be tolerant of contaminant exposure and degraded habitats, whereas mink and river otters are sensitive to exposure to environmental contaminants (Aulerich and Ringer 1977; Tillitt et al. 1996; Wren et al. 1995).

Thus, mink and otters may avoid degraded waters where raccoons continue to forage (e.g. streams degraded by sewage effluent), or may succumb to the effects of certain contaminants (e.g. Cd and Pb) at tissue concentrations tolerated by raccoons.

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Table 1. Frequency of detection and concentration (ppm wet weight) of selected elements in kidneys of raccoons collected during 1983-85 in Illinois.

Element	MDL[a]	Frequency of Detection[b]	Concentration $\bar{x}$ ± SE (min-max)
Cadmium	0.53	14/31	1.70 ± 0.31 (0.67-4.29)
Copper	0.22	29/31	3.31 ± 0.31 (0.11-6.57)
Lead	0.71	7/31	0.98 ± 0.09 (0.72-1.35)
Mercury	0.005	22/31	0.029 ± 0.01 (0.0025-0.254)
Selenium	1.27	15/31	2.14 ± 0.17 (1.31-4.0)
Zinc	0.25	31/31	26.1 ± 1.1 (16.5-49.3)

[a] Method Detection Limit
[b] number of samples having detectable concentrations/total number of samples

Table 2. Frequency of detection and concentration (ppm wet weight) of selected elements in livers of raccoons collected during 1983-85 in Illinois.

Element	MDL[a]	Frequency of Detection[b]	Concentration $\bar{x}$ ± SE (min-max)
Cadmium	0.53[c]	4/31	0.78 ± 0.12 (0.60-1.09)
	0.07[d]	24/31	0.29 ± 0.04 (0.035-1.01)
Copper	0.29	59/62	4.32 ± 0.54 (1.27-26.70)
Lead	0.81	10/62	1.43 ± 0.36 (0.81-3.96)
Mercury	0.005	54/62	0.10 ± 0.04 (0.0025-2.06)
Selenium	1.3	4/62	1.82 ± 0.26 (1.31-2.45)
Zinc	0.25	62/62	34.9 ± 1.3 (19.1-58.3)

[a] Method Detection Limit
[b] number of samples having detectable concentrations/total number of samples
[c] 1983-84
[d] 1984-85

Table 3. Frequency of detection and concentration (ppm wet weight) of selected elements in muscle of raccoons collected during 1983-85 in Illinois.

Element	MDL[a]	Frequency of Detection[b]	Concentration $\bar{x}$ ± SE (min-max)
Cadmium	0.53[c]	0/31	-----
	0.07[d]	0/31	-----
Copper	0.29	54/62	1.21 ± 0.07 (0.32-1.91)
Lead	0.71[c]	1/31	0.79
	0.81[d]	2/31	1.04 ± 0.07 (0.97-1.11)
Mercury	0.005	47/62	0.015 ± 0.001 (0.0025-0.052)
Selenium	1.28	4/62	1.80 ± 0.17 (1.41-2.17)
Zinc	0.11	62/62	51.5 ± 1.36 (31.0-88.8)

[a] Method Detection Limit
[b] number of samples having detectable concentrations/total number of samples
[c] 1983-84
[d] 1984-85

Table 4. Mean concentrations of selected elements in tissues of mink and river otters from Illinois (from Halbrook et al., 1996) relative to mean concentrations observed in raccoons in the current study.

	Mink			River Otter		
Element	Kidney	Liver	Muscle	Kidney	Liver	Muscle
Cadmium	Lower	Lower	Higher	NA	Lower/ND	NC
Copper	Higher	Higher	Higher	NA	Higher	Higher
Lead	Lower/ND	Lower	NC	NA	Lower/ND	NC
Mercury	Higher	Higher	Higher	NA	ND	ND
Zinc	Lower	Lower	Lower	NA	Lower	Lower

NA= not available, tissue not analyzed
Lower/ND= analytes not detectable at detection limit lower than mean concentration in raccoons
NC= no comparison; insufficient samples of both species with detectable concentrations of analyte
ND= not detectable; detection limit considerably higher than for raccoons

Appendix A. Mean concentrations (µg/g or ppm wet weight) of selenium and selected metals in kidney and liver tissue of raccoons as reported in previous studies.

Element	Tissue	Locale	Concentration	Source
Cadmium	Kidneys	ONT	1.22	Wren, 1984
		MI	3.48	Herbert and Peterle, 1990
		FL	2.48	Hoff et al., 1977
		NY	6.40[a]	Valentine et al., 1988
	Liver	ONT	0.23	Wren, 1984
		MI	0.79	Herbert and Peterle, 1990
		NY	0.85[a]	Valentine et al., 1988
Copper	Kidneys	ONT	3.99	Wren, 1984
	Liver	ONT	4.8	Wren, 1984
Lead	Kidneys	MI	0.20	Herbert and Peterle, 1990
		FL	0.47	Hoff et al., 1977
		AL	4.95	Khan et al., 1995
		NY	3.11[a]	Valentine et al., 1988
	Liver	IL	6.1	Sanderson and Thomas, 1961
		MI	0.24	Herbert and Peterle, 1990
		AL	3.24	Khan et al., 1995
		CT	6.2	Diters and Nielson, 1978
		NY	3.57[a]	Valentine et al., 1988
Mercury	Kidneys	ONT	1.1	Wren, 1984
		WI	1.36	Sheffy and St Amant, 1982
		MI	0.27	Herbert and Peterle, 1990
		AL	0.24	Khan et al., 1995
		NY	0.18[a]	Valentine et al., 1988
	Liver	ONT	4.5	Wren, 1984
		WI	2.01	Sheffy and St. Amant, 1982
		GA	1.43, 2.34, 4.53	Bigler et al., 1975
		MI	1.18	Herbert and Peterle, 1990
		AL	0.41	Khan et al., 1995
		NY	5.82[a]	Valentine et al., 1988
Selenium	Kidneys	ONT	1.9	Wren, 1984
		MI	ND[b]	Herbert and Peterle, 1990
		NY	3.08[a]	Valentine et al., 1988
	Liver	ONT	2.8	Wren, 1984
		MI	2.29	Hebert and Peterle, 1990
		CA (Kesterson)	4.40 to 10.5	Clark, Jr., et al., 1989
		CA (reference)	0.30 to 1.99	
		NY	0.96[a]	Valentine et al., 1988
Zinc	Kidneys	ONT	29.5	Wren, 1984
		MI	18.6	Herbert and Peterle, 1990
		FL	75.9	Hoff et al., 1977
	Liver	ONT	34.4	Wren, 1984
		MI	44.4	Herbert and Peterle, 1990

[a] published as dry weight; converted to wet weight, kidneys 2.283, liver 2.967.
[b] Not Detectable