CONTAMINANT CONCENTRATIONS IN SMALL MAMMALS FROM THE SHEBOYGAN RIVER AREA OF CONCERN

Sean M. Strom
WI Department of Natural Resources
Bureau of Wildlife Management

February 22, 2013

INTRODUCTION

In 1993, a small mammal community study (Seeley 1993) discovered measurable levels of polychlorinated biphenyls (PCBs) in small mammals collected from three floodplain areas along the Sheboygan River AOC. This was the first study that showed PCB contamination in the Sheboygan River had found its way into the terrestrial food chain. These floodplain areas represent important foraging areas for wildlife that target riparian invertebrates and emergent insects.

In laboratory settings, mink (*Mustela vison*) have shown an extreme sensitivity to PCB contamination. Even at low levels of exposure, mink reproduction can be impaired. Mink also sit high on the food chain in riparian areas, consuming a wide variety of prey, including fish, amphibians, and small mammals. Much of this prey, such as fish and small mammals, have tested positive in the past for contamination, especially PCB contamination. The wide variety of prey consumed, and the sensitivity to low levels of contamination make mink extremely useful as a bio-indicator of the health of an ecosystem. It has long been suspected that mink populations in the AOC are limited due to the PCB contamination. Previous efforts to locate mink in the area have been unsuccessful, despite the apparent prevalence of suitable habitat (Seeley 1993).

The goal of this study was to collect samples of common small mammals for contaminant analysis (PCBs, polybrominated diphenyl ethers (PBDE), metals, and organochlorine pesticides) that reflect local conditions within the Sheboygan River Area of Concern floodplain. This provided an idea of the current extent of contamination of small mammals within the Upper and Middle River floodplain areas.

Specific objectives of the small mammal trapping and analysis include:

1) Establish the concentrations of the various contaminants (PCBs, PBDEs, metals, organochlorine pesticides, and fluorinated compounds) in small mammals and mink inhabiting the floodplain.
2) Compare contaminant concentrations in small mammals inhabiting the contaminated floodplain with concentrations in small mammals from a control or uncontaminated site.
3) Conduct a qualitative comparison between data collected during the current project with historical data from the same area.
4) Compare contaminant results with known toxicity threshold tissue concentrations.

A second goal of this study was to attempt to trap mink from the AOC and an uncontaminated area upstream in order to compare the number of mink captured in the AOC to the control. The results will help us determine whether there is a difference in mink populations that might be caused by the PCB contamination in the AOC. Tissue samples from the mink carcasses were submitted for contaminants analysis.

METHODS

Small mammal trapping

Small mammals were collected from six sites in floodplain riparian areas of the Upper and Middle River segments (as delineated by the U.S. EPA for the Superfund project) of the Sheboygan River AOC and from one control location upstream of the areas of known contamination (Figure 1). At each individual location, a total of four traps were set. Three of the traps were large Sherman live traps, while the fourth trap was a small-sized Tomahawk live trap. Targeted species at each location included 2 white-footed mice (*Peromyscus leucopus*), 2 meadow voles (*Microtus pennsylvanicus*), and 2 masked shrews (*Sorex cinereus*).

Trapping efforts continued at each location until the targeted number of specimens was collected. For the six test sampling sites, the target was two white-footed mice, two meadow voles, and two masked shrews per station, and for the control location, the target was four individuals of each species. Traps were checked daily and upon capture of an animal, information on each animal was collected including tail length (mm), foot length (mm), ear length (mm), weight (grams), sex of the specimen, species, and whether it was found dead, euthanized, or was released. Non-target and questionable species will be photographed, measured to assist identification, and then released on site.

During the planning of this study, we recognized that our proposed sample size would limit the ability to do statistical comparisons and therefore we expected any comparisons would be qualitative in nature. Our final sampling plan struck a balance between the level of effort required to collect and analyze samples with available resources.

Mink trapping

The WDNR contracted with a local mink trapper to collect mink carcasses from two stretches of the Sheboygan River (Figure 2). The first location was the Upper River and the upper half of the Middle River segments of the Sheboygan River AOC. The second location was an area at least five miles upstream and outside of the AOC. The brain, kidneys, and liver were removed from the mink and placed individually into sterile collection bags. Brains and kidneys will be archived in the freezer for possible future contaminant analysis.

Sample Processing and Analysis

Individuals collected for contaminants analysis were brought back to the WDNR Service Center lab for processing. Entire carcasses for *Sorex, Peromyscus, Microtus* and *Blarina* (short-tailed shrew) species were submitted for analysis. In addition, liver samples from mink and weasels were also submitted for analysis. All samples were placed individually into sterile collection bags with a unique identification label. Whole-body and liver samples were kept frozen until submitted to the Wisconsin State Lab of Hygiene (WSLH) for analysis. All samples were analyzed for the following parameters:

- PCBs (congeners)
- PBDEs
- Organochlorine pesticides
- Perfluorinated compounds

- Cadmium
- Lead
- Mercury
- Percent fat

RESULTS

PCBs

The highest concentrations of PCBs in small mammals from the AOC were observed in the 2 shrew species (Table 1). A total of 6 short-tailed shrews were collected from 2 different sites within the AOC. No short-tailed shrews were collected within the control site. PCBs were detected in all 6 individuals (range 0.045 – 8.93 µg/g). The sample size for masked shrews was smaller than that for short-tailed shrews. Only 1 masked shrew sample was collected in the control area, so statistical comparisons could not be made between shrews collected within the AOC. PCB levels in masked shrews from within the AOC appear to be slightly less than those observed in short-tailed shrews. In both species, considerable variation in PCB concentrations was observed both between sites and among individual shrews.

A total of 24 white-footed mice were collected from 6 different sites within the AOC while 10 individuals were collected from the control area. PCBs were detected in every individual from the AOC (range 0.001 – 2.25 µg/g) (Table 2) but were not detected in any of the individuals from the control area. PCB levels from white-footed mice within the AOC were significantly greater than levels observed from control site mice ($p = 0.000$). Within the AOC, there was considerable variation between sites and among individuals. In regards to PCBs, floodplains "04" and "06" appear to be the most contaminated while floodplain "01" appears to be the least contaminated.

Only 3 voles were collected during the sampling period, none of which were collected from the control area. Levels of PCBs in voles from the AOC were slightly higher than levels observed in white-footed mice (Table 1). The sample size of voles ($n = 3$) is too small to make any meaningful inferences as to whether voles are experiencing adverse impacts.

Only 2 mink and a short-tailed weasel were collected within the AOC and only 3 mink were collected from the control area. Liver PCB levels from mink collected within the AOC were significantly greater than levels from mink collected outside the AOC (Table 1). No mink were collected within the AOC during the initial small mammal assessment conducted in 1993 (Seeley 1993), therefore a historic comparison was not possible.

A total of 4 muskrats were also collected during the sampling period. No muskrats were collected from the control area, so comparisons between levels observed in AOC muskrats cannot be made. PCB levels observed in AOC muskrats were relatively low compared to the other species groups (Table 1).

Metals

Many of the samples analyzed for Pb exceeded their quality control standards and therefore the confidence in the accuracy of the analysis is low. The relative percent difference (RPD) ranged from 30 – 50%. The laboratory repeated the analysis with similar results regarding the RPD and

the small amount of sample available precluded digesting a larger sample weight. As such, we are not able to interpret any of the Pb results for any of the samples.

Concentrations of both Cd and Hg were low in samples of white-footed mice. There was no significant difference either in Cd concentration ($p = 0.40$) or Hg concentration ($p = 0.12$) between samples collected from the AOC and samples collected from the control area. Cadmium was also detected at low levels in all of the other species groups. Mercury was detected at low levels in all species groups, but was only detected in 1 of the muskrats collected and only 1 of the voles collected. The highest levels of both Cd and Hg were observed in mink collected from the AOC.

POLYBROMINATED DIPHENYL ETHERS (PBDEs)

Brominated diphenyl ethers were only detected in 11 samples (Table 2). One sample from white-footed mice, 2 samples from short-tailed shrews, 3 samples from masked shrews, 1 sample from voles and 4 of the mink samples had detectable levels of PBDEs. Of the 17 different PBDE congeners analyzed, only BDE #47, BDE #99, and BDE #153 were detected. The low level of detection of PBDEs prevents us from conducting any statistical analyses on the data.

PERFLUORINATED COMPOUNDS (PFCs)

Perfluorinated compounds were detected in every sample analyzed (Table 2). In samples of white-footed mice, concentrations of PFCs were significantly greater in samples collected from the control area compared to samples from the AOC ($p = 0.000$). The small sample sizes of the other species collected prevents any additional statistical analyses between concentrations within the AOC and concentrations from the control area. The highest levels of PFCs were observed in mink/weasels collected within the AOC.

DISCUSSION/MANAGEMENT IMPLICATIONS

The sample size for the present study is too small to make any inferences regarding population level effects due to exposure to PCBs for any of the species groups. However, the data does indicate that small mammals within the Sheboygan River AOC are accumulating PCBs as well as other contaminants.

For all species except mink and muskrat, the whole carcass had to be analyzed in order to obtain enough sample for the desired suite of contaminants. Criteria levels based on whole carcass concentrations do not exist, so for species where the whole carcass was analyzed, a qualitative comparison was made to critical concentrations in the liver (if available). While comparing liver concentrations with carcass concentrations are not directly compatible, it can provide an indication as to whether toxic responses may be occurring in some individuals.

PCB threshold concentrations in liver tissue do not exist for mice, vole, or muskrat species. A threshold liver concentration of 2.3 µg/g wet weight has been suggested for shrew species (Zwiernik et al 2011). Extending this threshold value to carcass concentration, individuals of white-footed mice may have PCB levels which approach concentrations where adverse impacts occur. Similarly, individuals of both short-tailed and masked shrews had carcass concentrations above this threshold, suggesting individuals within the AOC may be accumulating high enough levels of PCBs for adverse impacts to occur.

We observed a shift in the presence of Peromyscus species throughout both the AOC and the control area. During the Seeley study (1993), the deer mouse (*Peromyscus maniculatus*) was one of the most common species encountered. However, no deer mice were captured in the present study and the white-footed mouse (*Peromyscus leucopus*) was the most common species encountered. Due to the shift in species composition as well as the limited sample size, direct comparisons between the historic samples and current samples are difficult. In addition, the number of PCB congeners analyzed by the SLH has changed over time, further compounding the difficulties of comparing data. However, on a qualitative basis, it appears that total PCB levels in samples of *Peromyscus* species have declined slightly since they were last sampled in 1993. Similarly, PCB levels appear to have decreased in short-tailed shrews compared to levels observed in the 1990s. No mink samples were collected within the AOC in the 1990s, so comparisons with historical samples cannot be made.

Only 1 mink was collected within the AOC boundaries. A road-kill mink was also collected within the AOC and submitted for analysis, but the origin of this particular animal is unknown. A PCB threshold concentration of 2.0 µg/g in the liver has been proposed for mink (Zwiernik et al 2011). One mink collected within the AOC and the short-tailed weasel had liver PCB concentrations that approach this criteria concentration suggesting individuals within the AOC may have PCB levels which could cause adverse health impacts.

The lack of mink collected within the AOC raises suspicion that the population is limited due to the contaminated habitat, especially considering that quality mink habitat is present within the AOC boundaries. We do not have enough data to suggest population level effects are occurring within the AOC, but the lack of mink in suitable habitat at least suggests PCB exposure may be playing a role in the lack of mink within the AOC.

While our sample sizes for nearly all species were relatively small, based on the low concentrations observed, it is unlikely small mammal populations within the AOC are experiencing any adverse impacts based on exposure to metals, PBDEs, or other organochlorine pesticides. However, the absence of mink in the AOC raises concerns about contaminants limiting population growth of this species. Tissue threshold concentrations for PFCs have not been established for small mammals, so it is not possible to determine whether any adverse impacts are occurring from exposure to PFCs. However, the fact that PFCs were detected in every sample submitted suggests they may become a concern in the future.

CONCLUSSIONS

In conclusion, small mammal populations from several different species groups and feeding niches within the AOC are accumulating PCBs. The small sample size of our collection prevents any robust statistical analysis, but we did observe higher PCB concentrations in samples from white-footed mice that were related to specific floodplains, suggesting certain floodplains may be more contaminated than others. Likewise, while our sample size was too small to infer any PCB related population level effects, our data suggests that individuals within several different species groups could be experiencing adverse health impacts as a result of exposure to PCBs within the AOC. We conclude that small mammals can be useful indicators of PCB accumulation in contaminated floodplains.

ACKNOWLEDGEMENTS

Special thanks to Dale Katsma and Dustin Miller for the collection and trapping of the small mammals. Ron Arneson helped coordinate the sample submission to the WI State Laboratory of Hygiene and his help is greatly appreciated.

Table 1: Total PCB concentrations in small mammals collected from the Sheboygan River AOC and a control area

Species	Area	Collection Date Range	Sample Size	Mean Total PCBs (µg/g)	Range
White-footed mouse	AOC	08/03/11 – 09/14/11	24	0.369	0.001 – 2.25
White-footed mouse	Control	08/04/11 – 09/13/11	10	ND	ND
Jumping mouse	AOC	09/16/11	1	0.027	NA
Short-tailed shrew	AOC	08/30/11 – 09/20/11	6	2.91	0.45 – 8.94
Masked shrew	AOC	09/01/11 – 09/29/11	3	2.48	0.39 – 5.90
Masked shrew	Control	09/16/11	1	0.11	NA
Meadow vole	AOC	08/17/11 – 08/30/11	3	1.07	0.94 – 1.26
Mink	AOC	11/07/11 – 03/13/12	3	1.20	ND - 1.86
Mink	Control	11/06/11 – 12/26/11	3	0.03	0.003 – 0.066
Muskrat	AOC	01/21/12 – 02/01/12	4	0.20	0.11 – 0.40

ND = Not detected

NA = Not applicable

Table 2: Total PCB concentrations white-footed mice collected from different floodplains within the Sheboygan River AOC and a control area

Floodplain ID	Location	Collection Date Range	Sample Size	Mean Total PCBs (µg/g)	Range
01	County Highway A	08/10/11 – 09/14/11	7	0.03	0.007 – 0.08
02	River Wildlife	08/10/11 – 09/23/11	2	0.11	0.07 – 0.15
03	Lower Pheasant Field	08/09/11 – 09/09/11	5	0.11	0.001 – 0.18
04	Riverbend Peninsula	08/03/11 – 08/26/11	5	0.73	0.15 – 2.25
05	Riverbend West	08/26/11 – 09/02/11	2	0.14	0.14 – 0.15
06	Kohler Stables	08/03/11 – 08/25/11	3	1.3	0.79 – 2.05
07 (Control)	River Oaks	08/04/11 – 09/13/11	11	ND	

ND = Not detected

Table 3: Environmental contaminants in small mammals collected from the Sheboygan River AOC and a control area.

Species	Area	Sample Size	DDE (µg/g)	Cd (ng/g)	Hg (ng/g)	Pb (µg/g)	Total PBDEs (ng/g)	Total PFCs (ng/g)
White-footed mouse	AOC	24	12.6	9.11	15.5	176.8*	0.11	6.32
White-footed mouse	Control	10	ND	7.27	10.6	263.5*	ND	73.79
Jumping mouse	AOC	1	ND	5.6	ND	0.142*	ND	89.3
Blarina	AOC	6	175.5	154.3	104.0	0.440*	0.4	94.6
Short-tailed shrew	AOC	3	0.05	110.5	35.5	0.21	1.5	6.87
Short-tailed shrew	Control	1	ND	**	**	**	1.0	8.96
Meadow vole	AOC	3	ND	11.5	2.0	0.198	0.8	30.97
Mink	AOC	3	0.009	68.8	767.0	0.40*	1.7	469.5
Mink	Control	3	0.007	33.4	347.3	0.08*	1.3	308.1
Muskrat	AOC	4	ND	26.4	1.5	0.09*	ND	180.9

*Numerous samples failed QC (high relative percent difference), statistical comparisons were not possible.
**Not enough sample for analysis
ND = Not detected

REFERENCES

Seeley, A. L. 1993. Small mammal populations along PCB contaminated sections of the Sheboygan River, Wisconsin. Summer internship project for the Wisconsin Department of Natural Resources through the University of Wisconsin, Stevens Point.

Zwiernik, M., F. Vermeulen, and S. Bursian. 2011. Toxicological implications of PCBs, PCDDs, and PCDFs in Mammals. Pages 531-561 *in* W. N. Beyer and J. P. Meador (Eds.) Environmental Contaminants in Biota: Interpreting Tissue Concentrations. CRC Press, Boca Raton, FL, USA.

Figure 1. Small mammal sample collection locations.

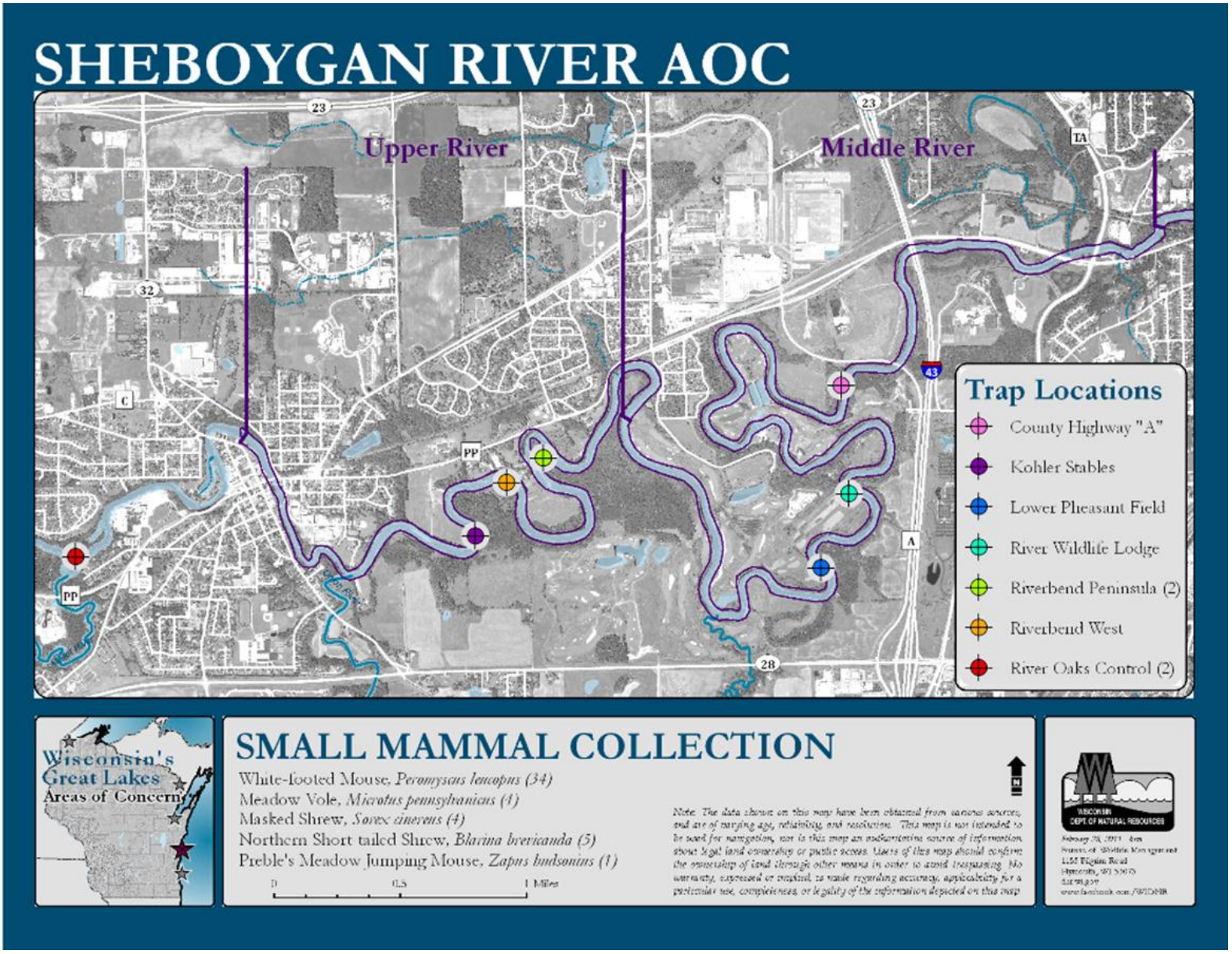

Figure 2. Mink and weasel sample collection locations.