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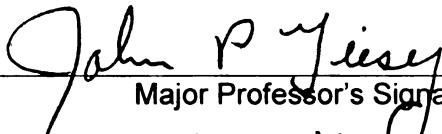
Exposure and Risk of PCBs to Mink (*Mustela vison*) at the
Kalamazoo River Superfund Site, Michigan

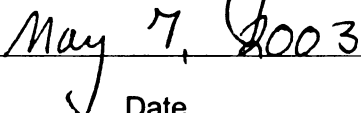
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**EXPOSURE AND RISK OF POLYCHLORINATED BIPHENYLS TO MINK
(*MUSTELA VISON*) AT THE KALAMAZOO RIVER SUPERFUND SITE, MICHIGAN**

By

Stephanie D. Pastva

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**Submitted to
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ABSTRACT

EXPOSURE AND RISK OF POLYCHLORINATED BIPHENYLS TO MINK (*MUSTELA VISON*) AT THE KALAMAZOO RIVER SUPERFUND SITE, MICHIGAN

By

Stephanie D. Pastva

125 kilometers of the Kalamazoo River, located in southwestern Michigan, has been designated a Superfund site with polychlorinated biphenyls (PCBs) as the contaminant of concern. Mink are of special concern due to their trophic status and sensitivity to PCBs. A top-down risk assessment was conducted by measuring concentrations of total PCBs and TEQs in tissues of mink collected from the Kalamazoo River area of concern (KRAOC). Mink were caught from areas within the KRAOC and from Fort Custer Recreation Area (FC), an upstream reference area on the same river system. Total PCB concentrations, in livers of mink, averaged 2.7 and 2.3 mg PCB/kg ww from the KRAOC and FC, respectively. Total TEQs in livers of mink averaged 300 and 110 pg TEQ/g ww from KRAOC and FC respectively. Previously conducted studies in which mink were fed PCB-contaminated diets were used to calculate a range of hazard quotients (HQs) based on the no observed adverse effect level (NOAEL) and the lowest observed adverse effect level (LOAEL). For mink livers from KRAOC, total PCB-based HQs ranged from 0.37 to 0.88 and total TEQ-based HQs ranged from 1.1 to 1.4 (based on a comparison of the mean exposure level and the LOAEL). For mink livers from FC, total PCB-based HQs ranged from 0.31 – 0.73 and total TEQ-based HQs ranged from 0.39 – 0.49 (based on a comparison of the mean exposure level and the LOAEL).

The extent to which mink (*Mustela vison*), were exposed to PCBs through their diet and the resulting potential risk was estimated using three different dietary models. Fish, crayfish, and mammalian species were collected from two sites: Trowbridge (TB), a former impoundment within the KRAOC and FC. Prey items were analyzed for total PCB and TEQ concentrations. Total PCB and TEQ concentrations were greatest in fish species. Identified contents from gastrointestinal tracts of mink collected from the sites yielded the following dietary composition: 72% mammals, 14% fish, and 14% crayfish, and was used as one of the dietary models. At TB, LOAEL-based HQs for total PCBs and TEQs are less than 1.0 for all dietary models except the literature-based model in which fish comprise 85% of the mink's diet. Calculated risk, based on dietary models, was slightly less than risk calculations based on site-specific mink tissue residues. Both approaches were in agreement that the degree of exposure to PCBs and TEQs were near the threshold for effects on reproduction in mink.

Multiple lines of evidence were considered, including the habitat quality, number of animals trapped per territory, age and sex distributions, gross morphology, liver histology, baculum weight, and body weight. Based on these multiple lines of evidence, the observed concentrations of PCBs measured in the livers of mink in the KRAOC are unlikely sufficient to cause a reduction in the number of mink inhabiting the KRAOC.

ACKNOWLEDGEMENTS

Funding was provided through a grant from the Kalamazoo River Study Group to Michigan State University. This research would not have been successfully completed without the assistance of the numerous people : Scott Fitzgerald - necropsies; Steve Bursian - necropsies and equipment; Kevin Allen, Jim Burns, Dan Keith, Daniel Villeneuve, and Alan Zwiernik - trapping; K. Kannan, Dong-Hoon Khim, George Klemolin, and Jamie Kober - laboratory support and analysis; Ryan Holem- organizational support. Additional thanks to the MSU Kellogg Biological Station for housing and laboratory space during sample collection. Special thanks are extended to Alan L. Blankenship, Patrick W. Bradley, Paul D. Jones, Denise Kay, Arianne M. Neigh, Karl D. Strause, Matthew J. Zwiernik who are coauthors on the published manuscripts. They have been involved in conducting the field research, laboratory work, quality assurance, and their input was extremely valuable during the writing process. Above all, my advisor John Giesy, deserves many thanks... for obtaining funding for the research, for all the words of encouragement along the way, and for all the great opportunities.

On a personal note, I need to thank my fiancé, Scott, for all the little things he did enabling me to work harder and longer and keeping me sane at the same time. My friends have also offered prayers, support, and encouragement through the process. Finally, thanks to God for putting me in the right place at the right time and surrounded by awesome coworkers and friends.

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CHAPTER ONE:

Risks of Polychlorinated Biphenyls to Mink (*Mustela vison*) at the Kalamazoo River Superfund Site, Michigan.

Introduction

In 1990, approximately eighty miles of the Kalamazoo River was designated a Superfund site, referred to as the Kalamazoo River Area of Concern (KRAOC). The site extends from Morrow Dam in Kalamazoo County to Lake Michigan (Figure 1). The release of polychlorinated biphenyls (PCBs), the contaminant of concern (COC), resulted primarily from PCB-contaminated waste discharged from the recycling and processing of carbonless copy paper (CDM 1999). During the period of 1957 – 1971, the ink solvent used in carbonless copy paper contained PCBs, primarily Aroclor 1242 (Durfee et al. 1976). A lesser amount of PCBs may have also been added to inks and other additives, primarily Aroclor 1254 (Durfee et al. 1976).

The mink (*Mustela vison*) is a semi-aquatic member of the mustelidae family commonly found in waterways throughout North America, including Michigan (U.S.Environmental Protection Agency 1993). Mink have been shown to be one of the more sensitive mammals to the effects of PCBs, especially to effects such as kit weight, litter size and, kit mortality (Aulerich et al. 1971; Aulerich et al. 1973; Brunstrom et al. 2001; Heaton et al. 1995; Hochstein et al. 1998; Kihlstrom et al. 1992; Restum et al. 1998). Due to their sensitivity to PCBs

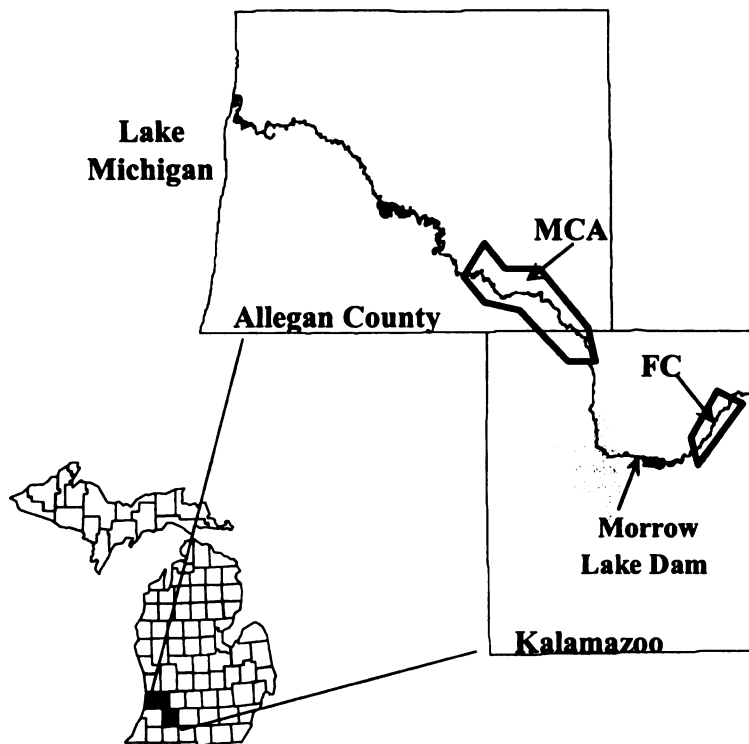


Figure 1. Map of Kalamazoo River Area of Concern (KRAOC). The inset map of Michigan indicates the locations of the two counties in which the study was conducted. The KRAOC extends from Morrow Lake dam to Lake Michigan. The mink collection area (MCA) in KRAOC is indicated by a box surrounding the river. Fort Custer (FC) is the upstream reference area and is also indicated by a box surrounding the river.

and their relatively great potential for exposure as piscivorous, top predators, mink often represent the “worst-case scenario” for the exposure of mammals to persistent contaminants in North American riverine systems. Mink are often used as a surrogate or sentinel species in risk assessments or monitoring programs, respectively. Therefore, the mink was selected as a sensitive, indicator of the effects of PCBs on animals residing in the KRAOC.

A congener-specific approach was utilized in this study since this method has been shown to more accurately predict adverse affects than Aroclor-based quantification methods for determination of total PCBs in environmentally weathered samples (Schwartz and Stalling 1987;Valoppi et al. 1998;Leonards et al. 1995;Tillitt et al. 1996). Furthermore, the most sensitive biological effects of PCBs have been attributed to planar congeners that resemble 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) and act through the aryl-hydrocarbon receptor (AhR). A congener-specific approach allows for the calculation of 2,3,7,8-TCDD equivalents (TEQs) by integrating the total concentrations and relative potencies of individual congeners identified in a mixture. The data collected for this study were utilized in two ways: first, all congeners were summed to obtain total PCB concentrations and secondly, key congeners were quantified and used to calculate TEQs.

There are two primary approaches for assessing risk to wildlife for bioaccumulative compounds like PCBs: bottom-up and top-down. In a bottom-up risk assessment, PCBs are measured at lower levels of the food chain (or even environmental matrices such as sediment). These models are used to predict an

average daily dose consumed by the species of interest. This estimated dose is then compared to previously determined effect level doses referred to as toxicity reference values (TRVs). Alternatively, in a top-down risk assessment, PCB concentrations are measured in target tissues of the species of interest. These site-specific tissue residue concentrations are then compared to published tissue residue-based effects to determine risk. Here we report the results of a top-down assessment in which concentrations of total PCBs and TEQs were measured in livers of mink from the KRAOC and Fort Custer recreation area (FC), an upstream reference area. The results of a bottom-up risk assessment are presented elsewhere (Pastva et al. 2003a).

Methodology

Sample Collection

Wild mink were trapped throughout the KRAOC and the upstream reference area, FC, during the winters from 2000-2002. Trapping was conducted by Michigan State University (MSU) personnel and by local trappers. Collection kits and oversight were utilized to ensure consistent sample handling and storage. Collection kits contained aluminum foil, plastic bags, labels, and datasheets for recording information including date and location of trapped specimen, and also detailed instructions regarding sample collection, handling, and storage. Skinned carcasses were frozen before shipment, under chain of custody, to MSU.

Necropsy

Mink carcasses were thawed and a gross necropsy was performed by a board certified pathologist (Dr. Scott Fitzgerald, MSU). The sex, weight (without pelt), and length (nose to base of tail) of each mink was determined. Organ condition was assessed during dissection to determine if there were gross abnormalities. Liver tissue was removed for PCB analysis and histology and a tooth was removed for dental cementum analysis to determine age. The jaws were also removed for histological analysis. Liver histology was performed by the MSU Animal Health Diagnostics Laboratory (East Lansing, MI), jaw histology was performed by Kerrie Beckett (MSU Department of Animal Science, East Lansing, MI), and dental cementum analysis was performed by Matson's Laboratory (Milltown, MT).

Chemical Analysis

The extraction method used was based on EPA method 3540 (Soxhlet extraction). A known quantity (approximately 5 g) of liver tissue was homogenized with anhydrous sodium sulfate (EM Science; Gibbstown, NJ) using a mortar and pestle. Surrogate standards, PCB #204 (IUPAC) and PCB #30 (AccuStandard, New Haven CT), were added to all samples, blanks, and matrix spikes before extraction. Samples were refluxed in a Soxhlet extraction apparatus (VWR Scientific, Plainfield, NJ) for 18 h using 400 ml of 3:1 dichloromethane/hexane (pesticide residue grade). After extraction, extracts were concentrated by rotary evaporation to a final volume of 11 ml. One ml of the hexane extract was used for lipid content determination. The remaining 10 ml of extract was passed through a neutral/acidic silica gel column to remove non-target analytes. Thirty cm glass columns, 15 mm in diameter, were packed with approximately 0.5 g anhydrous sodium sulfate, 2 g 100-200 mesh size silica gel (Aldrich; Milwaukee, WI), 2 g 40% sulfuric acid (JT Baker; Phillipsburg, NJ) impregnated silica gel, and 2 g silica gel. If the extract was particularly dirty, additional acid hydrolysis was performed. Briefly, 5 ml of concentrated sulfuric acid was added to the extract and shaken for at least 30 sec. After the separation of phases, the extract was transferred to another test tube and 5 ml of water was added to remove acid residues from the extract.

The extract was evaporated to a final volume of 1.0 ml under a stream of nitrogen. An aliquot of 0.5 ml was transferred to a GC vial for total PCB analysis while the remaining 0.5 ml was subjected to carbon column chromatography for

the separation of non-*ortho*-substituted (coplanar) PCB congeners, as described below.

PCBs including di- and mono-*ortho*-substituted congeners were quantified by use of a gas chromatograph (Perkin Elmer AutoSystem) equipped with a ^{63}Ni electron capture detector (GC-ECD). A fused silica capillary column (Zebron ZB-5; 5%phenylpolysiloxane, 30m $\times$ 0.25mm i.d.) having a film thickness of 0.25 μm was used (Phenomenex; Torrance, CA, USA). The column oven temperature was programmed to change from 120°C (1 min hold) to 160°C at a rate of 10°C/min (1 min hold) and then to 260°C at a rate of 2°C /min with a final holding time of 10 min. Injector and detector temperatures were kept at 225°C and 375°C, respectively. Helium and nitrogen were used as carrier and make up gas, respectively. A solution containing 100 individual PCB congeners with known composition and content was used as a standard. Congeners were identified by comparing sample peak retention times to those of the known standard. In sample extracts, concentrations of each congener were determined by comparing the peak area to that of the appropriate peak in the standard mixture. TurboChrom software (Perkin Elmer) was used to integrate the peaks. Concentrations of all resolved PCB congeners were summed to obtain total PCB concentrations.

Non-*ortho*-substituted (coplanar) PCB congeners #77, #81, #126, and #169 were separated from coeluting congeners and interferences by clean-up on a carbon column. Briefly, 30 cm glass columns, 15 mm in diameter, were packed with anhydrous sodium sulfate, carbon dispersed on silica gel (Waco

Chemical, Japan), and anhydrous sodium sulfate. 20 microliters of 50 ug/L radio-labeled ^{13}C coplanar PCB congener (#77, #81, #126 and #169) standard mix (Cambridge Isotope Laboratories, Andover MA) in isooctane were added to each extract. The first fraction, containing loading solvent, washing hexane, and column elutant of 100 ml 20% dichloromethane in hexane, was archived. After addition of isooctane as a keeper solvent, the second fraction was eluted with 200 ml of toluene, and contained non-*ortho* coplanar PCB congeners. The extract was then concentrated under a stream of nitrogen to a final volume of 20 μl .

Extracts were analyzed by GC-MS on a Hewlett Packard 5890 series II gas chromatograph equipped with an HP 5972 series mass selective detector. A fused silica capillary column (as described above) was used. Non-*ortho*-substituted PCB congeners were detected and confirmed by selected ion monitoring of the two ions of the molecular cluster. Congener concentrations were calculated based on ion ratios for the native and ^{13}C congener.

TEQ Computation

Concentrations of TEQs in mink livers were calculated by multiplying the concentration of individual PCB congeners by its respective World Health Organization toxic equivalence factor (TEF) (Van den Berg et al. 1998). Total TEQ concentrations were determined by summing the concentrations of TEQs of the individual congeners. The following PCB congeners were included for calculation of TEQ #77, #81, #105, #118, #126, #156, #157, #167, and #169. Although frequently used in the calculation of TEQ, congeners #114, #123, and

#189 were not included in the calculation of concentrations of TEQ as they could not be identified by the analytical method used. When a congener was not detected, a surrogate value of one half of the method detection limit was multiplied by the TEF to calculate TEQ. Congeners #156 and #157 frequently coeluted with congeners #171 and #200, respectively. In those instances, it was assumed that the entire concentration was due to congeners #156 and #157, providing the maximum estimate of TEQ concentration. By making this assumption congener #157 contributed < 5% of the total concentration of TEQ, whereas congener #156 contributed between < 0.01% and 50% of the total TEQs with a mean TEQ contribution of 10%.

Toxicity Reference Values

The PCB-based TRVs were calculated from two laboratory-based mink feeding studies (Bursian et al. 2002; Halbrook et al. 1999). Mink liver-based TRV values, reported by Halbrook et al. (Halbrook et al. 1999), for the LOAEL and NOAEL were 7.3 and 6.0 mg PCB/kg respectively, and the value based on the LOAEL could be used directly. However, the tissue-based NOAEL was not reported by the authors, but was estimated from the relationship between PCB concentrations in adipose and liver from the LOAEL diet. This adipose to liver relationship was then applied to the concentration of PCBs in adipose tissue from the NOAEL diet. The LOAEL reported by Halbrook et al., was based on a reduction of male kit weight after 6 wk (Halbrook et al. 1999). In a study by Bursian et al., the mink liver-based LOAEL was reported to be 3.1 mg PCB/kg (Bursian et al. 2002). However, the tissue residues associated with

concentrations of PCBs and TEQs values for the NOAEL were not specified (Bursian et al. 2002). Therefore, the NOAEL was estimated by using a LOAEL:NOAEL application factor derived from the known dietary-based LOAELs and NAOELs., This estimation resulted in a NOAEL TRV of 1.3 mg PCB/kg (Bursian et al. 2002). The LOAEL was based on reduced kit body weights after 3 wk and decreased kit survival after 6 wk (Bursian et al. 2002).

The TEQ-based TRVs were calculated from two laboratory-based mink feeding studies (Bursian et al. 2002;Heaton et al. 1995). The Bursian LOAEL and NOAEL mink liver-based TRVs used were 218 and 51 pg TEQ/g, respectively (Bursian et al. 2002). The NOAEL was estimated from the dietary-based LOAEL:NOAEL ratio. Heaton et al. (Heaton et al. 1995) used different TEF values to calculate TEQs. Therefore, the dietary concentrations of TEQ were recalculated based on the WHO TEFs so that they were consistent with those reported in this study. In addition, the LOAEL was based on the least tested dose. Therefore, the NOAEL was estimated by taking the geometric mean of the control and lowest dose. The resulting LOAEL and NOAEL mink liver-based TRVs based on the Heaton study were 273 and 70.9 pg TEQ/g, respectively (Heaton et al. 1995). The LOAEL was based on decreased kit survivability and decreased weights of kits 3 and 6 wk after whelping.

Results

Mink

Nine male and one female mink were collected within the KRAOC from D-Ave downstream to the former Trowbridge (TB) impoundment, a distance of approximately 25 river kilometers. Three mink (two male and one female) were collected from the Fort Custer upstream reference area, approximately 25 river kilometers upstream of D-Ave, in Kalamazoo, MI. The female collected from the KRAOC was classified as a ranch escapee. The fur from this female was black, which is rare in wild populations. The nearest mink ranch is located in Shelbyville (R. Aulerich, pers. com.), approximately 17 km north of the mink's capture site in Plainwell. The owners of this ranch acknowledge that they raise black colored mink and that mink occasionally do escape and they are not tagged. Because it may not represent conditions found at the KRAOC, this individual was excluded from further analyses and discussion. The concentration of total PCBs in liver for this mink was 0.03 mg PCB/kg ww. This value was considerably lower than the site mean. Therefore, elimination of this data results in increased estimates of risk.

Male mink from the KRAOC had an average weight and length of 1048 g and 40 cm respectively, while male mink from FC averaged 990 g and 39 cm respectively. The female mink from FC weighed 390g and was 34 cm long. Mink from KRAOC ranged in age from 1 to 3 y (average 1.6 y) and mink from FC ranged in age from juvenile (< 1 y) to 2 y (average 1.2 y). All of the mink

collected appeared to be in good condition and did not exhibit external or internal gross abnormalities. Baculum mass was not correlated to PCB concentrations ($r^2 = 0.09$, $p = 0.36$), even after correction for mink body weight. Based on a histological examination, the livers of the mink were normal. There were four individuals which exhibited lesions in the squamous epithelium tissue in the jaw bones (Kerrie Beckett, pers. com.), and three of these individuals also contained the greatest liver PCB concentrations.

Total PCB and TEQ concentrations

Total concentrations of PCBs in mink from the KRAOC ranged from 0.05 to 6.0 mg PCB/kg ww (average 2.7 mg PCB/kg ww) (Figure 2). Total PCB concentrations in livers of mink from FC ranged from 1.6 to 3.7 mg PCB/kg ww (average 2.3 mg PCB/kg ww) (Figure 2). There were no significant differences in PCB concentrations among the sites (Student's t-test unequal variance, $p=0.33$).

Concentrations of total TEQs in mink liver from KRAOC ranged from 1.1 to 1300 ng TEQ/kg ww (mean = 301 ng TEQ/kg ww) (Figure 3). In comparison, total TEQs in mink liver from FC ranged from 52 to 200 ng TEQ/kg ww (mean = 110 ng TEQ/kg ww) (Figure 3). There were no significant differences in concentrations of TEQ between sites (Student's t-test unequal variance, $p=0.10$). Total concentrations of PCBs were significantly correlated with total concentrations of TEQs ($r^2 = 0.76$, $p < 0.01$). Congener #126 contributed the greatest amount of the TEQs at both sites, averaging 77% at KRAOC and 69% at FC (Figure 4). Congeners #118 and #156 were the next greatest contributors but their relative rankings were different for the two sites.

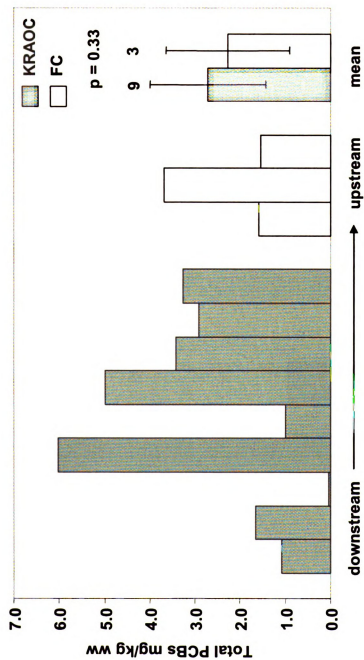


Figure 2. Total PCBs (mg/kg ww) in mink collected from Kalamazoo River Area of Concern (KRAOC) and the Fort Custer upstream reference area (FC).

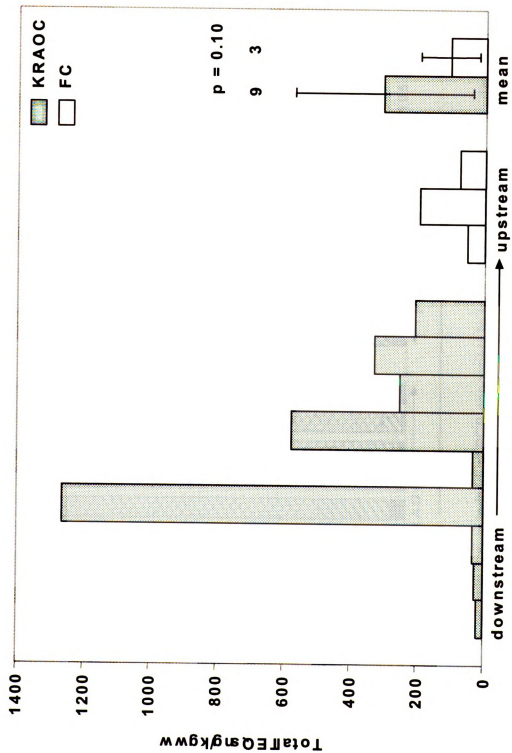


Figure 3. Total TEQ concentrations (pg/g ww) in mink from the Kalamazoo River.

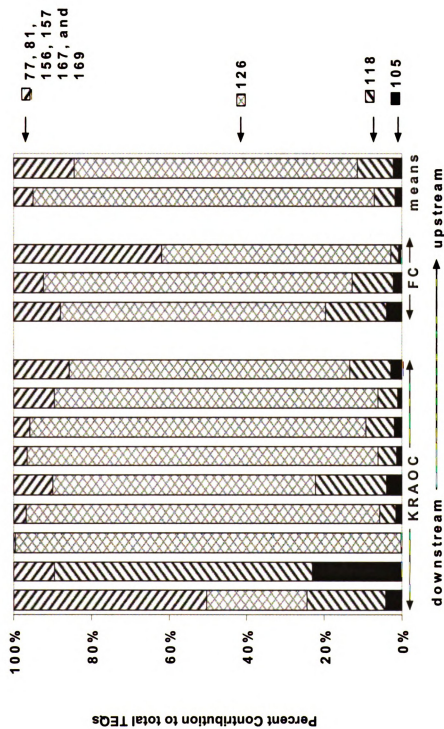


Figure 4. Relative contribution of individual PCB congeners to total TEQs in individual mink. The last two bars to the right of the figure and represent the arithmetic mean contribution of individual congeners to total TEQs in KRAOC mink livers (n=9) and FC mink livers (n=3).

Hazard quotients (HQs) were calculated based on both total concentrations of PCBs and on concentrations of TEQ in the liver (Table 1). HQs were also calculated based on both the LOAEL and NOAEL. HQ values were also calculated based on the results of two different laboratory exposure studies. Thus, there were two values presented for each combination of exposure measure and TRV. LOAEL-based HQ values based on mean PCBs concentrations in livers of mink ranged from 0.37 to 0.88 and from 0.31 to 0.7 for the KRAOC and FC, respectively. NOAEL-based HQs based on mean PCB concentrations and compared to the NOAEL values ranged from 0.45 to and 0.38 to 1.7 for mink from the KRAOC and FC, respectively. HQs based on the mean concentration of TEQs ranged from 1.1 to 1.4 and 0.39 to 0.49 for the KRAOC and FC, respectively. NOAEL-based HQ values for TEQs based on the NOAEL values ranged from 4.3 to 5.9 and from 1.5 to 2.1 for the KRAOC and FC, respectively.

Table 1. Hazard quotients calculated using Total PCB concentrations and Total TEQS in mink livers collected from the Kalamazoo River. The TRVs used to calculate HQs based on total PCBs are from feeding studies conducted by Halbrook et al. (Halbrook et al. 1999) and Bursian et al. (Bursian et al. 2002). The TRVs used to calculate total HQs based on TEQs are based upon mink feeding studies by Heaton et al. (Heaton et al. 1995) and Bursian et al. (Bursian et al. 2002).

	Total PCB HQ		Total TEQ HQ	
	NOAEL	LOAEL	NOAEL	LOAEL
KRAOC mean (n=9)	0.45 – 2.0	0.37 – 0.88	4.3 – 5.9	1.1 – 1.4
KRAOC 95% UCL (mean)	0.67 – 3.0	0.55 – 1.3	8.0 – 11	2.1 – 2.6
FC Mean (n=3)	0.38 – 1.7	0.31 – 0.7	1.5 – 2.1	0.39 – 0.49
FC 95% UCL (mean)	0.61 – 2.8	0.50 – 1.2	2.7 – 3.8	0.71 – 0.89

Discussion

Concentrations of PCB in livers of mink from this study, both KRAOC and FC, are greater than those reported from some other areas of North America. While tissue residues of PCBs in mink liver have been measured at relatively few locations, the only areas that had greater concentrations were from North Carolina, Connecticut and the Hudson River (Table 2).

It is difficult to compare the lengths and weights of mink from the Kalamazoo River to other wild mink due to differences in sampling methodology, or lack of methodology provided. Studies conducted in the Pacific Northwest using similar methods, found an average weight for males of 880g (Harding et al. 1999). This is less than the average weight of male mink collected in this study. PCB concentrations in the mink collected during that study (Harding et al. 1999) were less than those in livers of mink from the Kalamazoo River (Table 2). Although results reported by Harding et al (Harding et al. 1999) indicated a negative correlation between baculum mass and PCB concentrations, no such correlation was observed in mink from the Kalamazoo River. Lesions in the squamous epithelium tissue of jaws were observed. In laboratory studies, where mink were fed PCB 126 in the diet, eventually the teeth of mink exhibiting this lesion became displaced, loosened, with the end result being anorexia (Render et al. 2000). This is the first report of this lesion observed in wild mink. The presence of this lesion may be a good marker for PCB exposure. However, at this time, information on dose-response relationships and ecologically relevant endpoints are lacking so it is difficult to interpret these results. As the mink

Table 2. Range of total PCB concentrations (mg/kg ww) in mink liver reported in North America.

Location	Total PCBs (mg/kg ww)	Year(s) of mink collection	References
Rural western Maryland	0.62 - 2.4	1978 – 1979	(O'Shea et al. 1981)
South Carolina	0.042 – 1.5	1989 – 1991	(Osowski et al. 1995)
Georgia	ND – 1.4		
North Carolina	ND – 12		
Kalamazoo River, Michigan	0.05 – 6.0	2000 – 2002	This study
Illinois	0.04 – 0.86	1984 – 1989	(Halbrook et al. 1996)
Connecticut	0.57 – 10	1987 – 1988	(Major and Carr 1991)
Hudson River, New York	0.04 – 8.8	1998 – 2000	(Mayack and Loukmas 2001)
New York	0.10 – 0.60	1982 – 1984	(Foley et al. 1988)
Southern Ontario, Great Lakes	0.034 – 1.8	1988 – 1989	(Haffner et al. 1998)
Ontario	0.016 – 0.40		Unpublished data
St. Maurice River, LaTuque, Quebec	1.5 – 2.4	1991 – 1992 1994 – 1995	(Champoux 1996)
Oregon	0.52 – 3.5	1978 – 1979	(Henny et al. 1981)
British Columbia	<0.001 – 0.024	1990 -1992	(Elliot et al. 1999)
Washington	0.067 – 0.38		
Oregon	0.039 – 0.36		
Northwest Territories	0.007 – 0.07	1991 – 1993	(Poole et al. 1998)
British Columbia	0.07 – 0.08	1994 – 1996	(Harding et al. 1999)

collected from the Kalamazoo River were all of normal weight, the lesions do not appear to have progressed to a stage where fitness could be compromised. Overall, based on size and the gross and histological analysis, mink from the Kalamazoo River appeared to be in good physical condition.

This study was not designed to collect information on the size, age structure or sex ratio of the populations studied. It was expected that the sample sizes would be limited, and thus, the power to measure population dynamics is low. While the absolute numbers of mink collected were few, the number of mink collected is consistent with the numbers expected in an area of this size. Mink can have territories of over 2 km in river length (Gerell 1970), the FC sampling area was 10 km while the KRAOC sampling area was 25 km. In addition, mink habitat suitability was assessed along the Kalamazoo River and the floodplain areas were considered to have marginal habitat due to a lack of vegetative and structural complexity (Pastva 2003). The fact that most of the mink captured were adults and not juveniles as determined by dental cementum analysis suggests that they were likely resident animals. Dietary assessments conducted concurrently, within the KRAOC and FC study areas, concluded that risk based on dietary models, was slightly less than risk calculations based on site-specific mink tissue residues (Pastva et al. 2003a). Therefore, mink are most likely living in the study areas and are not recent immigrants. The sex ratio of collected mink was skewed toward males, this commonly occurs in trapping studies and harvest results, including those with mustelids (Buskirk and Lindstedt 1989; Elliot et al. 1999; Harding et al. 1999). This result may or may not represent the actual sex

ratio of the population but is most likely a sampling artifact. Males mustellids have larger territories and are more active than females (Buskirk and Lindstedt 1989), thereby increasing their likelihood of encountering a trap. Therefore, the sex ratio of mink captured here were not inconsistent with that which would be expected.

There was no attempt to estimate a site use factor, or determine the amount of time that mink would have spent in the areas studied. To do studies of this type would have required tagging and radio-telemetry studies that were beyond the scope of our study. This study was also not conducted to determine the duration of exposure that mink might receive. For this reason, we can not directly assess questions about immigration and emigration. This results in some uncertainties in addressing the potential impact of PCBs on mink populations. For instance, it has been suggested that exposure to PCBs might result in overt toxicity of adults or decrease reproduction to the point where the population is not self-sustaining, but rather dependent on immigration to maintain the population. While our data can not be used to assess this issue directly, the ages of the mink collected indicate that the mink were residents. Normally, it is juvenile mink that are émigrés. Thus, if the KROAC were acting as a “sink” for mink, one would expect that the population would be comprised mostly of individual less than one year old. This was not the case. No mink captured from KRAOC were less than one year old with approximately 45% of the individuals being 1 and 2 years old each, with one individual (~10%) being 3 years old.

There are two basic components to the HQ method of risk assessment, exposure and hazard, each with its own inherent uncertainties and variability. The measurement endpoint to assess exposure was the concentration of PCBs in liver, expressed either as total PCBs or TEQs. Hazard was assessed by use of TRVs based on ecologically relevant measurement endpoints such as survival, growth and reproduction as determined in controlled laboratory studies in which ranch mink were exposed to weathered mixtures of PCBs in the diet.

One of the null hypotheses being tested in this study was that there was no difference in concentrations between the KRAOC and the reference area. Use of measured instead of predicted estimates of exposure minimized the degree of uncertainty in the exposure portion of the risk assessment. However, the inherent nature of mink populations led to small sample sizes for statistical analyses. The contribution of sources of PCBs within the KRAOC relative to that occurring from other sources upstream of KRAOC was determined to be small relative to mean concentrations at the two study areas (16%). The difference in mean concentration between mink collected from KRAOC and FC was small (0.44 mg PCB/kg) and not statistically significant. Statistical power ($1 - \beta$) based on the variance sample sizes of the two populations, and type I error (α) of 0.05 and a type II error (β) of 0.2, was < 0.1 . If there had been a 2.5-fold difference in the mean PCB concentrations, it would have been possible to show a statistical difference between the means. Power would have been greater than 0.8, even with the same sample size and variability. Therefore, the likely reason for the lack of a significant difference was due primarily to the small difference between

the two populations, rather than to sample size or variance of the concentrations of PCBs in the two populations.

In this study, concentrations of PCBs in the liver were used as a measure of exposure that could be compared to a dose-response relationship to estimate the potential for effects. However, the PCBs, particularly the congeners contributing to effects mediated through the aryl hydrocarbon receptor (AhR), can be sequestered in the liver and not be biologically active. Thus, the use of concentrations of PCBs in the liver is a conservative measure of exposure and potential effects and likely overestimates the potential for effects. If organisms are exposed for a long period of time to low levels of AhR agonists, the ligands can be sequestered in the liver without leading to adverse effects. For instance, when mink were fed PCBs in the diet, hepatic cytochrome-450 1A activity, a measure of biochemical response was induced in a dose-dependent manner, but when the dietary exposure to PCBs was stopped, induction of the enzyme decreased to controls levels within a few weeks while concentrations of both total PCBs and TEQs in the liver did not decrease as rapidly (Shipp et al. 1998). The proposed reason for this observation is that induction of the cytochrome P-450 system requires free agonists that are able to interact with the AhR and dioxin response elements. Once exposure stops, agonists remain bound to the already induced P-450 enzymes, but there is insufficient free compound to result in further induction. Thus, the TEQs are present in the liver, but sequestered so that they are unavailable to interact further with biomolecules and cause adverse effects. For this reason, Shipp et al (Shipp et al. 1998) suggested that induction

of the cytochrome P-450 was a good indicator of current exposures to TEQ. In mink the critical mechanism of PCB toxicity has been suggested to be through the AhR mechanisms (Kannan et al. 2000). The congeners contributing to TEQ are known to be tightly bound to P450 1A1 and P4501A2 so that they are unavailable to interact with other molecules (Hahn et al. 1993). The ligands are in fact suicide substrates for these enzymes, one function of which has been postulated is protection from the effects AhR-ligands.

Since the mink used in this study were trapped and could not be recovered immediately or at the same time after death, it was not feasible to measure the specific CYP-450 activity in the liver. If activity could have been measured it would have been possible to estimate the proportion of the total concentration that was not bound to P4501A2 protein, which would constitute the biologically active fraction of the total concentrations of PCBs or TEQs in the liver. Since this was not possible, total concentrations of PCBs and TEQ in the liver were used as a conservative estimate of maximum exposure that would be expected to overestimate the actual or effective toxicologically relevant exposure and predictor of possible effects. The same properties of enzyme binding, which result in retention of the greatest concentrations of residues in the liver and make the liver a useful organ for monitoring exposure also complicate the interpretation of the toxicological significance for risk assessments.

Mink are one of the most sensitive organisms to the effects of PCBs (Kannan et al. 2000) and are unusual, among wildlife species, in that there is a large literature base of effects data from which to develop TRV information. In

particular, all the studies used to derive TRVs for this risk assessment were chronic exposures and examined ecologically relevant endpoints such as number of kits whelped, kit survival and kit weight. Furthermore, the endpoints on which the TRVs were based were the most sensitive endpoints. Also, the studies were not confounded by the potential effects of co-contamination. Thus, there was no need to apply uncertainty factors for differences in species sensitivity or acute-to-chronic adjustments. For these reasons, uncertainty related to the threshold for adverse effects has also been minimized.

In the study reported by Bursian (Bursian et al. 2002) AhR agonist co-contaminants, such as polychlorinated dibenzo-p-dioxin (PCDDs) and polychlorinated dibenzofuran (PCDF) congeners, were measured and considered to be minimal. Likewise, co-contaminants in fish from the Kalamazoo River were also considered negligible (1994). In addition, in both of these feeding studies the LOAEL dose was the critical dose. That is, there were at least two doses that were lower than the LOAEL, which minimizes bias due to experimental design and allows for an accurate estimate of the NOAEL. Although the Heaton study (Heaton et al. 1995) wasn't appropriate to use for PCB-based TRV derivation due to the presence of substantial co-contaminants, it was suitable for the estimation of TEQ-based TRVs.

An HQ of one or greater indicates that liver concentrations exceeded the level of effect on which the TRV was based. While a HQ of 1.0 or greater indicates the potential for risk, it does not mean that this level of effect would actually be observed at the population level. Due to the conservative nature of

measures of both exposure and hazard, population level impacts are frequently not observed until HQ values are above 10 (Giesy and Kannan 1998). The mean HQ-based LOAELs of total PCBs were less than 1.0, while the upper 95% confidence limit of those LOAEL-based HQ are less than 1.5 at both KRAOC and the upstream reference area (Table 1). When NOAEL-based HQs, for total PCBs, are considered, the mean HQs were 3.0 or less. HQs based on TEQs at both sites were greater when compared to their respective PCB-based HQs. HQ values based on total TEQs are approximately three times greater at KRAOC than those from FC (Table 1). HQ values based on total PCBs would indicate that exposure and subsequent risks to mink at both KRAOC and FC are similar. However, when based on TEQs, the risk to mink is greater, even though the exposure was not significantly greater. This may indicate an increased relative potency of the PCB exposure mixture at KRAOC compared to FC, or simply a large uncertainty in the conversion calculation.

The range of HQ values calculated from the studies considered to be relevant for deriving TRVs bracket 1.0 depending on the study and whether the TRV was based on total PCB or TRV concentrations or based on the NOAEL or LOAEL. Thus, it can be concluded that the current concentrations of PCBs in the livers of mink in the KRAOC are near the threshold for effects observed in laboratory studies of ranch mink. The LOAEL is the more accurate measure of potential effect because since the concentration associated with no effect is, in part a function of the experimental design and by definition an overestimate of hazard. All that is known is that the NOAEL is the greatest concentration tested

that did not cause a statistically significant effect. When TRVs from the Bursian study (Bursian et al. 2002) are used for comparison, concentrations of total PCBs were greater than the LOAEL in 44% of the individuals from KRAOC, while 67% of individuals from KRAOC had PCB concentrations that were greater than the NOAEL-based TRV. At FC, no individuals had concentrations greater than the LOAEL, while 100% of those individuals did have PCB concentrations greater than the NOAEL-based TRV derived from the Bursian study (Bursian et al. 2002). However, no individuals from KRAOC or FC have PCB concentrations greater than the NOAEL or LOAEL-based TRVs derived from the Halbrook study (Halbrook et al. 1999). Additionally, kit survival in treatment groups was not significantly different than in the control group.

The estimates of hazard based on concentrations of TEQ in the liver were similar to those based on total PCBs. Concentrations of TEQ in the liver of 33% of the mink from KRAOC exceeded TRVs derived from the LOAEL, while 56% exceeded the TRV based on NOAELs. The mean TEQ concentration from KRAOC was also greater than the NOAEL-based TRVs. No concentrations of TEQ exceeded the TRV values based on the LOAEL. However, depending on which TRV was used, between 67 and 100% TEQ concentrations exceeded the NOAEL-based TRVs.

Survival of kits reported by Bursian et al. (Bursian et al. 2002) was decreased by almost 50% at the LOAEL dose when compared to the control. Based on the number of individuals that had PCB concentrations exceeding this LOAEL, kit survival is possibly depressed at the KRAOC. However, since

information regarding compensatory mortality in the field is not available, it is not known how much this difference is likely to impact the population or ability of the population to be sustained. To address this issue would require employing an age-specific model of mortality and fecundity, the data for which is not available. However, the results from a similarly conducted study (Halbrook et al) indicate no such decreases of survival at concentrations greater than that reported in the Bursian study. Therefore, the results of the risk assessment based on concentrations in the liver are equivocal and near the threshold for adverse effects on the population. This conclusion is supported based the fact that PCB and TEQ-based HQs bracket 1.0.

Some uncertainty is still associated with the PCB and TEQ-based TRVs, because some values were extrapolated. For instance, the NOAELs from all three studies were estimated. As a result, HQs estimated using NOAEL-based TRVs have a greater amount of uncertainty associated with them than the LOAEL TRVs. However, the ranges of HQs derived from both the total PCB and total TEQ LOAEL-based TRVs are very similar, despite slight differences in experimental design. This indicates agreement among the different studies for effect concentrations. Finally, assumptions about co-eluting congeners and treatment of non-detects made during estimation of TEQ concentrations are conservative and lead to greater estimates of TEQ concentrations. Therefore, in general the TEQ-based HQs have a greater uncertainty associated with them and tend to be overestimates than do the PCB-based HQs.

In conclusion a risk assessment was performed to determine the risk of PCB exposure to mink residing along the Kalamazoo River. Uncertainties associated with the hazard of PCBs to mink were reduced, relative to estimates of exposure derived from measuring concentrations of PCBs in potential dietary items, by comparing concentrations of PCB in the liver with the results of suitable feeding studies to derive TRVs. Although PCB concentrations were measured in mink liver tissue, uncertainties still exist. However, these uncertainties would result in greater estimates of risk and therefore should be protective. A mink population is present at the KRAOC and data indicate that the mink were resident animals. PCB exposure at KRAOC is not great enough to cause direct mortality to adults or decrease number of kits born. However, based on results under laboratory conditions with ranch mink, the weight and/ or number of kits per litter six weeks post birth may be reduced at the present exposure level. It is uncertain if PCB exposure (expressed as total PCBs or TEQs) could cause adverse effects on the population. Information regarding the amount of kit survival to adulthood and recruitment necessary to maintain a viable population would have to be known to adequately determine risk at a population level. Multiple lines of evidence were considered, including the number of animals trapped per territory, age and sex distributions, gross morphology, liver histology, baculum weight, and body weight. Based on these multiple lines of evidence, the observed concentrations of PCBs measured in the livers of mink in the KRAOC are unlikely sufficient to cause a reduction in the number of mink inhabiting the KRAOC.

CHAPTER TWO:

Assessment of Risk Posed by Polychlorinated Biphenyls in the Diet of Mink (*Mustela vison*) at the Kalamazoo River Superfund Site, Michigan

Introduction

Located in southwestern Michigan, the main stem of the Kalamazoo River is approximately 195 km long and flows northwest, entering Lake Michigan near Saugatuck, Michigan (Figure 5). In 1990, approximately 123 km of the Kalamazoo River was designated a Superfund site. The site, referred to as the Kalamazoo River Area of Concern (KRAOC), and extends from Morrow Lake dam to Lake Michigan (CDM 1999). PCBs are the contaminant of concern, and studies are being conducted to determine the extent to which biota, specifically mink (*Mustela vison*), are being exposed to PCBs and the degree of risk posed by this potential exposure.

Due to the large size of the KRAOC, two areas were chosen for sample collection: the former Trowbridge impoundment (TB), as a worst-case scenario, and Fort Custer Recreational Area (FC), as an upstream reference area. TB was selected as a worst-case area within the KRAOC because it is the largest of the four former impoundments (~132 hectares of former sediments and ~70 hectares of existing impounded water), has the greatest estimated mass of PCBs, and has the greatest mean surficial concentration of PCBs in soils (~11 mg/kg, dw)

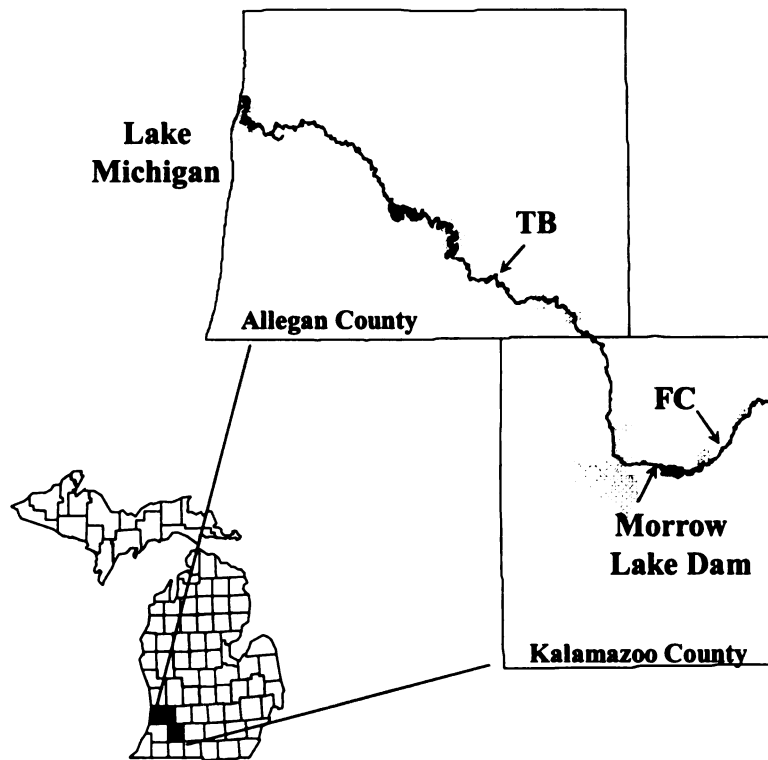


Figure 5. Map of Kalamazoo River Area of Concern. The inset map of Michigan indicates the locations of the two counties in which the study was conducted.

(Basland 2000). Since it was a former impoundment, both the aquatic and terrestrial ecosystems are exposed to PCBs. Fort Custer was chosen as the reference area because it is upstream of the KRAOC and has relatively little PCB contamination.

Mink are one of the receptors of concern at the KRAOC due to their sensitivity to PCBs and their relatively great potential for exposure as piscivorous, top predators. Mink are solitary, nocturnal, and have territories that average over 2 km of stream length. Mink are often considered to be a species of concern at other large sites, such as the Fox River (WI), Clinch River (TN), and Housatonic River (NY), where risk assessments have been conducted for PCBs. The study of mink exposure to PCBs and associated risks can incorporate top-down (based on concentrations of PCBs in the liver) and/or bottom-up (based on predicted dietary exposure) assessment methodologies.

The use of the top-down approach in which concentrations of PCBs are measured in the tissues of the receptor of concern is the most accurate measure of site-specific exposure. Results from the top-down approach applied to mink from the Kalamazoo River are presented in a companion paper from this study (Pastva et al. 2003b). In most cases, dietary models can be useful for predicting tissue residue measurements of mink are not or cannot be taken (U.S.Environmental Protection Agency 1998). Therefore, dietary models are often used to estimate exposure. This study was conducted to determine the accuracy of dietary exposure models.

One of the major factors in applying dietary exposure models is the dietary composition used to model exposure. Although mink are a semi-aquatic predator, they can occupy a wide range of habitat types including estuaries, rivers and streams, and wetlands (U.S.Environmental Protection Agency 1993). Studies of the diets of mink, including those conducted on Michigan rivers and streams, indicate that mink are opportunistic feeders and eat a variety of food items (aquatic and terrestrial animals) (Casson and Klimstra 1983;Hamilton, Jr. 1936;Sealander 1943;Whitman 1981). In addition, their food habits vary by habitat type and season. Therefore, using just one standard dietary composition may not be appropriate for every site. For this reason, several models, using different assumptions about the diet of mink were used to predict exposure and calculate risks. Specifically, in this study, the bottom-up assessment involved characterizing the dietary composition of mink from the site, collecting and measuring PCB concentrations in key prey items, and predicting dietary exposure. Here, we report the results of the bottom-up assessment and compare them to an assessment based on measured concentrations in the livers of mink.

Methods

Sample Collection and Preparation

Samples of muskrat (*Ondatra zibethicus*) and other mammals were collected during the period 2000 – 2002. Muskrats were trapped in the winter, using previously described methods, from locations where mink were also trapped (Pastva et al. 2003b). Muskrats were frozen with fur on. Later, muskrats were thawed, skinned, weighed, and the whole body was homogenized in a grinder (model 4732 SS; Hobart Corp., Troy, OH) which resulted in a coarse grind. This coarsely ground material was then further homogenized in a Waring Commercial Blender (model 36BL29, Waring Commercial Inc., New Hartford, CT), placed in cleaned glass jars (I-Chem, New Castle, DE) and stored at -20C until PCB analysis. Small mammals were sampled in June/July 2000 and again in August/September 2000 at 4 locations at TB and 3 locations at FC. Sampling occurred in a 35x35 m area in which alternating Sherman (XLF15) and pitfall (#10 canning tins) traps were placed ~5 m apart resulting in a total of 49 traps per grid. Traps were baited with rolled oats and peanut butter as needed. Traps were checked twice daily. Captured specimens were gently shaken out of traps into clear quart sized zip-lock bags, identified, and euthanized by cervical dislocation. Samples were then labeled, placed in a cooler, and transported back to the laboratory. Upon arrival at the laboratory, weights, lengths, visual health, sex, and species were recorded. Gut contents were removed, samples were wrapped whole in aluminum foil, and then frozen until homogenization for

chemical analysis. The following small mammal species were analyzed for PCB concentrations to use in the dietary assessment: eastern chipmunk (*Tamias striatus*), red squirrel (*Tamiasciurus hudsonicus*), meadow vole (*Microtus pennsylvanicus*), deer mouse (*Peromyscus maniculatus*), white-footed mouse (*Peromyscus leucopus*), and meadow jumping mouse (*Zapus hudsonius*).

Fish were collected by electro-fishing (Smith Root, Inc) in early winter 2000 (11/9/99-1/3/00) at TB and FC. Upon collection, fish samples were placed in one of two live wells. Upon completion of the day's sampling, or attainment of live well capacity, samples were categorized, euthanized using MS-222 (Western Chemical Inc, Ferndale, WA), placed in labeled pre-cleaned coolers and transported to the field laboratory where length and weight were determined. Fish were wrapped whole in aluminum foil, labeled, and transported on dry ice to the laboratory. Fish (whole body) were homogenized using a Waring Commercial Blender (model 36BL29). Homogenate was stored in cleaned glass jars (I-Chem, New Castle, DE) until PCB analysis was conducted. The following fish species were analyzed for PCB concentrations to use in the dietary assessment: carp (*Cyprinus carpio*), forage fish (collected based only on size, no species identification), smallmouth bass (*Micropterus dolomieu*), and suckers (*Catostomus commersoni*, *Hypentelium nigricans*, and *Moxostoma erythrum*).

Crayfish (*Scientific name*) were collected between June and September, 2000 from suitable habitat that was as proximal to the small mammal sampling grids as possible. Crayfish were sampled using wire minnow traps baited with dead fish that were captured within TB and FC. Traps were washed, allowed to

dry, then rinsed twice; first with acetone then hexane to avoid cross-contamination between sampling locations. Crayfish were wrapped in foil, labeled, placed in a cooler and transported to the secure field laboratory.

PCB Quantification and TEQ Computation

PCBs were analyzed using methods described previously (Pastva et al. 2003b). Briefly, after homogenization, samples were extracted with dichloromethane and hexane in a soxhlet apparatus, and prepared by use of silica gel column chromatography. The extract was concentrated to 1.0 ml and 0.5 ml was used for identification and quantification of individual PCB congeners using a gas chromatography- electron capture device (GC-ECD). The remaining 0.5 ml was further prepared using carbon column chromatography for identification and quantification of *mono*- and non-*ortho* PCB congeners with a GC- mass spectrophotometer device (MSD) (Pastva et al. 2003b).

Concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents (TEQs) were calculated by multiplying the concentration of individual PCB congeners by its respective World Health Organization (WHO) toxic equivalency factor (TEF) (Van den Berg et al. 1998). Congeners #114, #123, and #189 could not be detected in any of the samples analyzed, and therefore were not used to calculate TEQs. However, the TEQ for congeners #77, #81, #105, #118, #126, #156, #157, #167, and #169 were summed to predict a total TEQ. For instances in which one or more of these congeners was not detected, a proxy value equal to half of the method detection limit was used. Congeners #156, #157, and #167 frequently co-eluted with congeners #171, #200, and #185, respectively. In

those instances, it was assumed that the entire concentration was due to the coplanar congener, providing a maximum estimate of TEQ concentration.

Site-Specific Dietary Analysis

Stomachs and large intestines of mink were dissected from mink carcasses (Pastva et al. 2003b), and were stored frozen at -20°C. The stomach was cut open and contents removed. Contents from the intestinal tract were removed by squeezing. GI tract contents were rinsed through a stacking sieve (mesh numbers 5-230; Hubbard Scientific, Fort Collins, CO). After rinsing, the contents were dried at 90°C for 24 hours. Afterwards, the contents were hand separated and classified as either bone, feathers, exoskeleton, hair, teeth, scales, or miscellaneous (Litvaitis et al. 1996). From this, dietary composition was expressed as percent mammal, fish, bird, and crayfish (Lockie 1959). In instances in which no components were found, it was recorded as unknown and these individuals were not used to calculate the dietary composition for use with estimating dietary doses.

Average Potential Daily Dose Calculations

Average potential daily dose (ADD_{pot}) was used estimate the total dose of PCBs and TEQs present in food ingested (U.S.Environmental Protection Agency 1993) (Equation 1).

$$ADD_{pot} = \Sigma(C_k \times FR_k \times NIR_k) \quad (Eq\ 1)$$

Where:

C_k = Contaminant concentration in each prey item

FR_k = Fraction of time spent on site

NIR_k = Normalized Ingestion Rate = mean daily ingestion rate/mean body weight

Each of the dietary models assumed different relative proportions of each dietary component. Results from the site-specific dietary analysis were used as one model. These results represent what mink from the Kalamazoo River ate immediately prior to their capture. Due to the opportunistic nature of mink, two other dietary models were also chosen to estimate ADD_{pot} . These non-site specific dietary models assumed different proportions of dietary items based on values reported in the literature (Alexander 1977) or assumed and an equally opportunistic consumption of specified prey items. The proportion of prey in the literature-based diet was based on one study because it was a year-round study on a Michigan river and because it provided a maximum estimate of fish in diet compared to other studies (Alexander 1977). This literature-based diet consisted of 85% fish, 9% crayfish, and 6% small mammals. The equally opportunistic model gave equal weight (33.3%) to all three prey components. That model was chosen because it represents a median exposure of mink diet which can vary greatly, depending on prey availability. However, regardless of the model chosen, the portion of the diet that consisted of mammals was assumed to consist of 50% small mammals and 50% muskrat while the fish portion of the diet was assumed to consist of 25% carp, 25% forage fish, 25% smallmouth bass, and 25% sucker.

The concentrations of total PCBs and TEQs in site-specific prey items were then used to calculate the daily dose of PCBs and TEQs to mink from KRAOC and FC. Potential risks were estimated by use of a hazard quotient (HQ) approach (Equation 2), in which the ADD_{pot} for each dietary model was compared to appropriate toxicity reference values (TRVs) for PCB and TEQ TRVs from laboratory-based mink feeding studies (Brunstrom et al. 2001;Bursian et al. 2002;Halbrook et al. 1999;Heaton et al. 1995).

$$HQ = \frac{ADD_{pot} \text{ (mg PCB/kg/d or pg TEQ/kg/d)}}{\text{Dietary Toxicity Reference Value}} \quad (\text{Eq 2})$$

Toxicity Reference Values

The dietary TRVs applied in this assessment, for total PCBs and TEQ were based on studies in which mink had been chronically exposed to PCBs or TEQs in feed and ecologically relevant endpoints were evaluated. A summary of the results of these studies and justification for their use has been provided previously (Pastva et al. 2003b).

The TRV for total PCBs were based on two laboratory studies with mink (Bursian et al. 2002;Halbrook et al. 1999). The TRVs for weathered PCBs mixtures were determined to be 0.23 and 0.12 mg PCB/kg mink body weight/day, based on the LOAEL and NOAEL, respectively (Halbrook et al. 1999). In a second study (Bursian et al. 2002), where neither body weights nor ingestion rates were provided, a standard mean body weight for females of 1.1 kg and a standard ingestion rate of 0.13 kg/mink (S. Bursian, personal communication)

were used to derive a NIR of 0.11 kg food/kg bw/d. Based on these assumptions, TRVs were calculated to be 0.42 and 0.18 mg PCB/kg bw/d for the LOAEL and NOAEL, respectively (Bursian et al. 2002).

The TRVs for TEQs were based on three laboratory-based mink feeding studies (Brunstrom et al. 2001;Bursian et al. 2002;Heaton et al. 1995). Again, TRVs were calculated based on an estimated NIR if it was not given in the study. TRVs based on the LOAEL and NOAEL were estimated to be 7.8 ng TEQ /kg bw/d and 1.8 ng TEQ/kg bw/d, respectively (Bursian et al. 2002). Although a study-specific NIR could be calculated from one study (Heaton et al. 1995), the TEQ values in the mink diets of that study had to be adjusted to WHO TEFs rather than the H4IIE-potency factors that had been used to calculate TEQ in the original study (Heaton et al. 1995). In addition, the LOAEL was based on the lowest tested dose. Therefore, the NOAEL was estimated by taking the geometric mean of the control and lowest dose. The resulting TRVs based on the LOAEL and NOAEL were 4.5 and 1.1 ng TEQ/kg bw/d, respectively (Heaton et al. 1995). Finally, the TRVs from the third study (Brunstrom et al. 2001) were calculated using a study-specific NIR to yield LOAEL and NOAEL TRVs of 2.4 and 0.35 ng TEQ/kg bw/d, respectively. However, the TRV based on the NOAEL corresponds to a 2-4-*ortho*-CB fraction rather than a complete technical mixture. This fraction contained a seven-fold smaller concentration of TEQ than did the LOAEL (Brunstrom et al. 2001) and has the greatest difference in dose range of the studies used. TRVs are summarized in Table 3. Among the three studies described above, the study by Bursian et al. (Bursian et al. 2002) is strengthened

by the absence of adverse effects observed at 5 lower doses. This, coupled with the small dosing intervals provides confidence that this study bracketed the true threshold with the doses tested.

Table 3. Toxicity reference values (TRVs) used to calculate total PCB and TEQ
NOAEL and LOAEL-based hazard quotients.

	NOAEL TRV	LOAEL TRV	Reference
Total PCB (mg PCB/kg bw/d)	0.12	0.23	(Halbrook et al. 1999)
	0.18	0.42	(Bursian et al. 2002)
Total TEQ (ng TEQ/kg bw/d)	0.35	2.4	(Brunstrom et al. 2001)
	1.1	4.5	(Heaton et al. 1995)
	1.8	7.8	(Bursian et al. 2002)

Results

Concentrations of Total PCB and TEQ

Concentrations of total PCB at TB ranged from 0.01 in both muskrat and small mammals to 10 mg PCB/kg ww in smallmouth bass (Table 4). At FC, PCB concentrations in prey items ranged from 0.0014 in small mammals to 2.4 mg PCB/kg in carp (Table 4). PCB concentrations in prey items from TB were significantly greater (Student t-test $p < 0.05$) than those from the upstream reference area, FC (Figure 6).

Concentrations of TEQ in prey items at TB ranged from a minimum of 0.22 in small mammals to 111 ng TEQ/kg in smallmouth bass, while those at FC ranged from 0.08 in small mammals to 47 ng TEQ/kg ww in carp (Table 4). Prey items from TB contained significantly greater concentrations of TEQs than those from FC (Student t-test $p < 0.05$), with the exception of muskrat and carp which were not statistically different (Student t-test $p = 0.24$ and $p = 0.11$, respectively) (Figure 7).

Site-Specific Diet Composition

No individual mink had more than one identifiable prey category in its gastrointestinal tract. One mink contained scales, 5 contained fur, 1 contained crayfish exoskeleton, and 5 contained no major components in their GI tract. This resulted in the following site-specific dietary composition for mink on the Kalamazoo River: 72% mammals, 14% fish, and 14% crayfish.

Table 4. Total PCBs (mg PCB/kg ww) and TEQs (ng TEQ/kg ww) in prey items collected from two sites on the Kalamazoo River.

TB												FC			
		Mean	N	Std	range	Lipid	Mean	N	Std	range	Lipid				
				Dev		(%)			Dev		(%)				
PCB concentrations in prey															
43	Sucker	3.8	5	2.3	0.42 – 5.9	5.2	0.79	6	0.07	0.67 – 0.85	2.8				
	Small Mouth Bass	6.7	5	2.6	3.5 - 10	1.9	0.89	5	0.30	0.71 – 1.4	2.7				
	Carp	3.8	5	2.3	1.4 – 7.1	3.7	1.1	5	0.74	0.46 – 2.4	10				
	Forage fish	3.2	5	1.3	1.9 – 4.7	4.0	0.64	5	0.21	0.37 – 0.88	3.8				
	Crayfish	0.54	13	0.53	0.08 – 1.9	0.84	0.06	4	0.03	0.03 – 0.09	5.8				
	Muskrat	0.07	7	0.03	0.01 – 0.11	2.4	0.01	4	0.01	0.01 – 0.03	3.7				
	Small Mammals (non-shrew)	0.11	20	0.13	0.01 – 0.55	4.8	0.02	17	0.04	1.4x10 ⁻³ – 0.18	4.1				
TEQ concentrations in prey															
	Sucker	40	5	27	8.0 – 80	5.2	12	6	1.3	10 – 13	2.8				

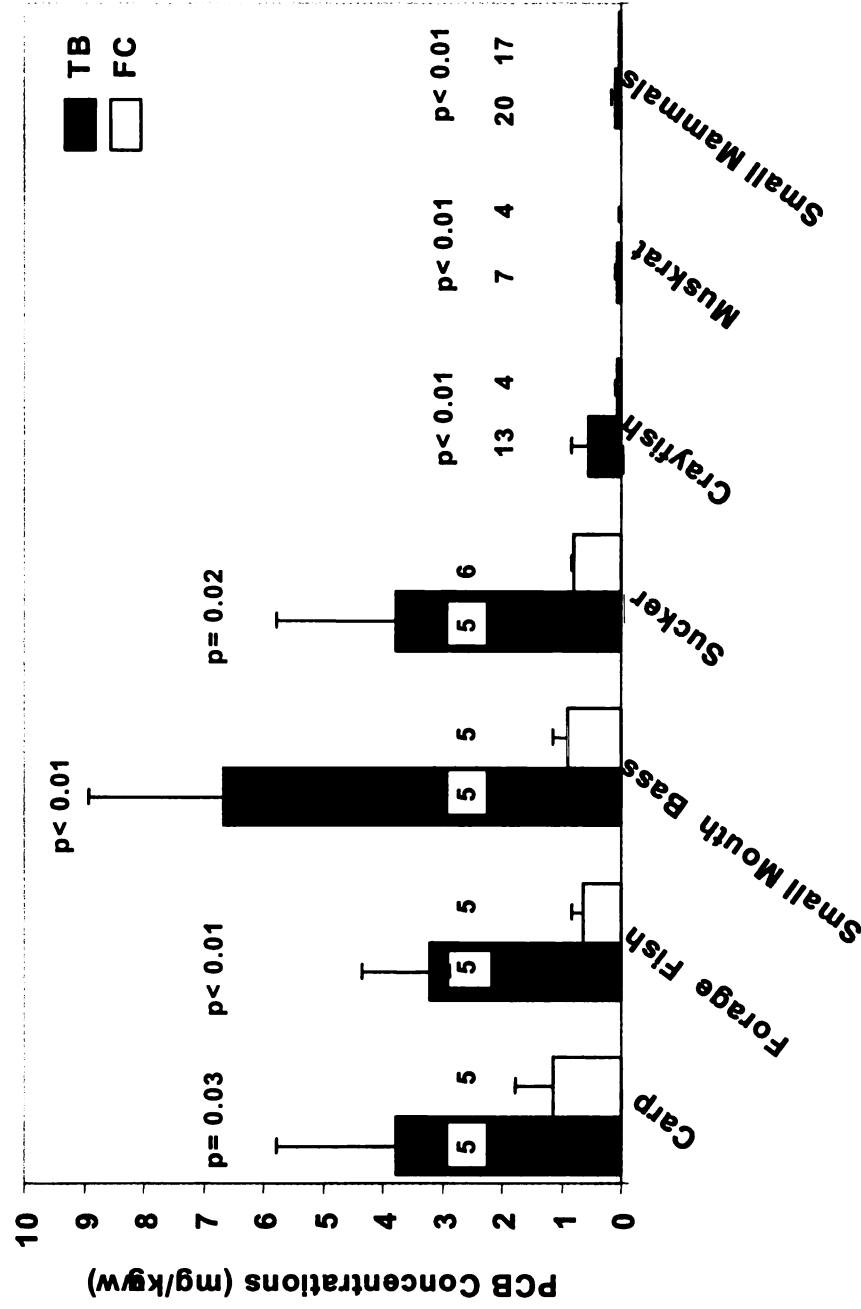


Figure 6. Mean total PCBs (mg/kg ww with 95% confidence intervals) in prey items collected from the Trowbridge (TB) and Fort Custer (FC), an upstream reference area, sections of the Kalamazoo River. Sample sizes and p-values (Student t-test) comparing mean PCB concentrations between sites are also presented.

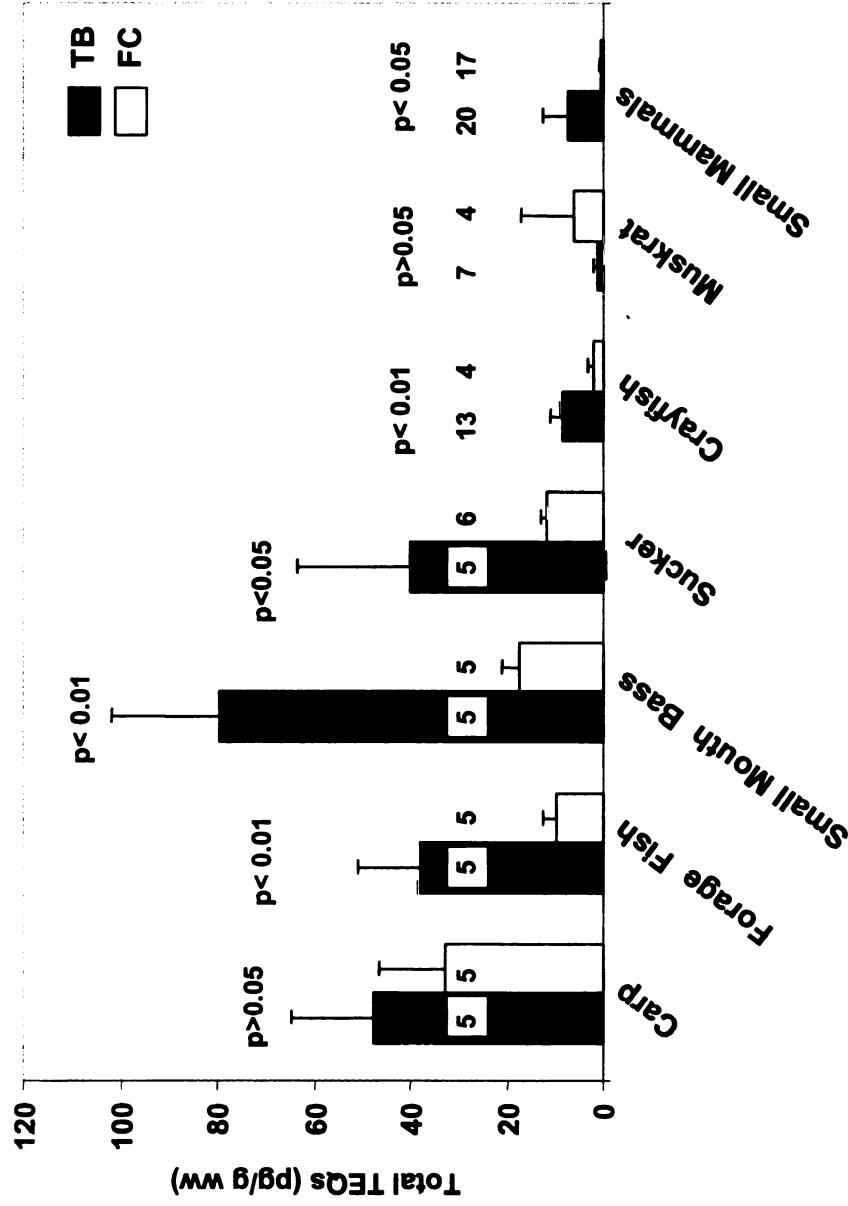


Figure 7. Mean concentrations of TEQs (ng/kg ww, with 95% confidence intervals) in prey items collected from Trowbridge (TB) and Fort Custer (FC), an upstream reference area, sections of the Kalamazoo River. Sample sizes and p-values (Student t-test) comparing mean PCB concentrations between sites are also presented.

Risk Calculations

Dietary exposures were reported as both concentrations of total PCBs and TEQs (Table 5). The rank order of ADDpot for the three different dietary models were literature-based > equally opportunistic > site-specific. The resulting dietary-based NOAEL and LOAEL HQs are presented (Table 6). A range of values are presented based on several TRV values. For both NOAEL and LOAEL-based HQs for PCBs, the TRVs derived from Bursian et al (Bursian et al. 2002) resulted in lesser HQs than those derived from Halbrook et al (Halbrook et al. 1999). Of the three studies used to calculate HQs based on TEQ, the TRV based on the study by Bursian et al (Bursian et al. 2002) resulted in the least HQs. Whereas, the TRVs based on the study by Brunstrom et al (Brunstrom et al. 2001) resulted in the greatest HQs, and the TRVs from Heaton et al. (Heaton et al. 1995) resulted in intermediate HQs.

Among the three diets assumed, mean LOAEL-based HQ values for PCB ranged from 0.20 to 1.8 at TB, while those based on concentrations in prey from FC ranged from 0.04 to 0.35. The NOAEL-based HQs ranged from 0.47 to 3.4 at TB and from 0.09 to 0.68 at FC. LOAEL-based HQ values for TEQ concentrations in prey ranged from 0.17 to 2.0 at TB, while those based on concentrations at FC ranged from 0.07 to 0.71. The mean NOAEL-based HQs of TEQ concentrations from TB and FC ranged from 0.73 to 14 and 0.31 to 4.9, respectively. Overall, there was relatively good agreement between HQ values based on total PCB and those based on TEQ. Among locations, PCB-based HQs were 4-5 fold greater at TB than FC, while TEQ-based HQs were 2-3 fold greater at TB than FC.

Table 5. Range of average potential daily doses (ADD_{pot}), based on mean and the upper 95% confidence limit (U95% CL) of prey items for total PCBs (mg PCB/kg bw/d and TEQs (ng TEQ/kg w/d), at both Trowbridge (TB) and Fort Custer (FC).

	TB		FC	
	PCB	TEQ	PCB	TEQ
Dietary Models				
<i>Site-specific^a</i>				
Mean	0.08	1.3	0.02	0.57
U95% CL	0.12	2.1	0.02	1.1
<i>Equally opportunistic^b</i>				
Mean	0.18	2.3	0.03	0.85
U95% CL	0.26	3.3	0.05	1.3
<i>Literature-based^c</i>				
Mean	0.41	4.9	0.08	1.7
U95% CL	0.59	6.7	0.11	2.3

a: Model is based on results from field collected mink GI tract contents

b: Model assumes that consumption of specified prey items is equal

c: Model based upon one mink diet reported in literature (Alexander 1977)

Table 6. Hazard quotients (HQ) values based on mean and the upper 95% confidence limit (U95% CL) of potential average daily doses of total PCBs and TEQs, when assuming three different dietary compositions for mink. The TRVs used to calculate HQs were based on feeding studies (Brunstrom et al. 2001;Bursian et al. 2002;Halbrook et al. 1999;Heaton et al. 1995). The range of calculated HQs is a result of using more than one TRV.

	TB		FC	
	LOAEL	NOAEL	LOAEL	NOAEL
PCB Based Dietary Models				
<i>Site-specific</i>				
Mean	0.20 – 0.36	0.47 – 0.70	0.04 – 0.07	0.09 – 0.13
U95% CL	0.29 – 0.52	0.67 – 1.0	0.05 – 0.10	0.12 – 0.18
<i>Equally opportunistic</i>				
Mean	0.43 – 0.79	1.0 – 1.5	0.08 – 0.15	0.19 – 0.29
U95% CL	0.62 – 1.1	1.5 – 2.2	0.11 – 0.20	0.26 – 0.39
<i>Literature-based</i>				
Mean	0.98 – 1.8	2.3 – 3.4	0.19 – 0.35	0.45 – 0.68
U95% CL	1.4 – 2.6	3.3 – 4.9	0.26 – 0.47	0.60 – 0.90
TEQ-Based Dietary Models				
<i>Site-specific</i>				
Mean	0.17 – 0.56	0.73– 3.8	0.07 – 0.24	0.31 – 1.6
U95% CL	0.26 – 0.86	1.1 – 5.9	0.14 – 0.46	0.61 – 3.2
<i>Equally opportunistic</i>				
Mean	0.30 – 0.97	1.3 – 6.7	0.11 – 0.35	0.46 – 2.4
U95% CL	0.42 – 1.4	1.8 – 9.5	0.17 – 0.54	0.71 – 3.7
<i>Literature-based</i>				
Mean	0.63 – 2.0	2.7 – 14	0.22 – 0.71	0.94 – 4.9
U95% CL	0.87 – 2.8	3.7 – 19	0.29 – 0.94	1.2 – 6.5

Discussion

Agreement in TRVs among studies

Among the four different feeding studies that were used to derive LOAEL-based TRVs for PCBs and TEQs, there is considerable agreement. For example, there was only a 2-fold and 3-fold difference among the LOAEL-based TRVs for PCBs and TEQs respectively. Furthermore, in three of the four studies, the LOAEL dose was one which caused decreased kit weight, but did not affect whelping rate or kit survival (Brunstrom et al. 2001; Bursian et al. 2002). Thus, kit weight after three or six weeks of birth appears to be the most sensitive endpoint.

NOAEL-based TRV values were also similar for the studies by Bursian et al. (Bursian et al. 2002) and Halbrook et al. (Halbrook et al. 1999). This is likely a result of a two-fold difference in dosing intervals and there being two tested doses that were less than the LOAEL for both studies. This experimental design minimizes bias and thus allows for an accurate estimate of the NOAEL. However, the NOAEL-based HQs for TEQs varied by five-fold among the three feeding studies. This greater uncertainty, relative to the other TRVs, is likely due to biases and uncertainties introduced by their respective study designs. The study conducted by Brunstrom et al. (Brunstrom et al. 2001) was designed to answer questions regarding effects of *ortho* vs non-*ortho* substituted PCB fractions, not as a typical dose-response study (there was a 7-fold difference in LOAEL and NOAEL TEQ concentrations) (Brunstrom et al. 2001). The study conducted by Heaton et al. (Heaton et al. 1995) NOAEL was not directly based on a tested

dose. Instead, it was estimated by taking the geometric mean of the control and lowest dose, which was the LOAEL (Heaton et al. 1995). As a result, HQs estimated using the NOAEL-based TRV for TEQs based on the study by Bursian et al. (Bursian et al. 2002) had the least uncertainty and thus were considered to be the most reliable.

Dietary modeling

The use of three different dietary models encompasses a wide range of potential mink feeding habits and also provides a measure of uncertainty when estimating risk to mink. The most conservative (greatest) risk estimates on the Kalamazoo River were obtained by using the literature-based dietary model because of the greater proportion of the diet that was assumed to be fish and the fact that concentrations of both PCBs and TEQs were greater in fish than in other prey items. It is noteworthy that the Great Lakes Water Quality Initiative assumes that fish comprise 85% of a mink's diet (U.S.Environmental Protection Agency 1995) for deriving water quality criteria protective of mammals. However, site-specific determination on the Kalamazoo River suggests that the diet of mink is comprised predominantly of mammalian species, especially during the winter.

The determination of risk to mink due to PCB concentrations in prey items, is dependant upon the dietary assumptions and which TRVs were used. At TB, LOAEL-based HQs for total PCBs and TEQs, based on mean estimated exposure concentrations, are less than 1.0 for all dietary models except the literature-based model in which fish comprise 85% of the mink's diet. Thus, if fish comprise 85% or more of the mink's diet, then exposure is potentially great

enough to cause risk to mink. If the most conservatively modeled diet (85% fish) is combined with the most conservative NOAEL-based dietary TRV, the mean PCB and TEQ-based HQs were 3.4 and 14, respectively. However, the true NOAEL lies somewhere between the LOAEL and NOAEL. At FC, total PCBs and TEQs pose minimal risk to mink regardless of the percentage of fish in the diet or which TRV was utilized.

Comparison of top-down and bottom-up methodologies

The results of the two basic approaches to determining exposure in risk assessments were compared to determine the accuracy of predicted versus measured exposures. This comparison was done by comparing resulting HQs based on a single study from which TRVs were derived. The study conducted by Bursian et al. (Bursian et al. 2002), was chosen to make this comparison for two reasons. First, it was the only feeding study from which estimates of both NOAEL- and LOAEL-based TRVs for both PCBs and TEQs could be derived. Secondly, the tested doses exhibited the full dose-response range, including both a NOAEL and LOAEL test dose.

When the upper and lower 95% confidence limits of mink HQs were compared to the mean dietary HQs, the bottom-up approach consistently estimated lesser exposure and thus risk of both PCBs and TEQ to mink (Figure 8 and Figure 9) This result was observed for all dietary models used, regardless of site, effect level, or PCB contaminant expression as total PCBs or TEQs. There did not appear to be a large difference among the LOAEL-based HQs for PCBs and TEQs (based on mean exposure) between the top-down and bottom-up

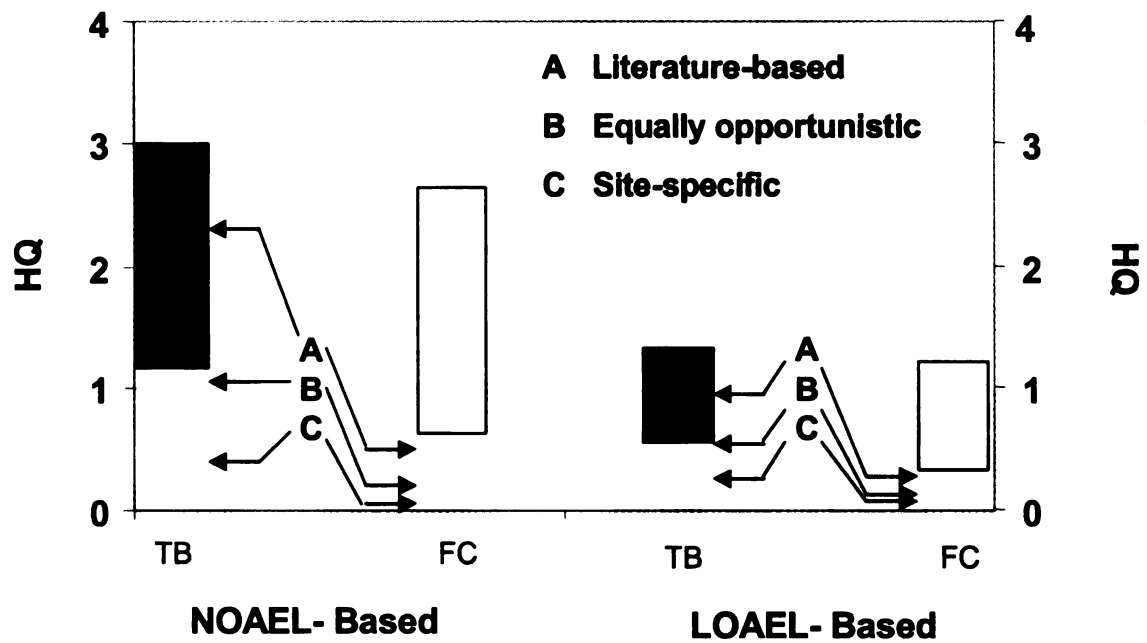


Figure 8. Comparison of HQ values based on predicted concentrations of total PCB determined based on dietary exposures or concentrations measured in the livers of mink at Fort Custer (FC) and Trowbridge (TB). The arrows represent the HQs of three mink dietary models. The solid vertical bars represent the upper and lower 95% confidence limits of HQs estimated from PCB concentrations in mink livers. The TRVs used to calculate HQs are based upon a mink feeding study conducted by Bursian et al. (Bursian et al. 2002).

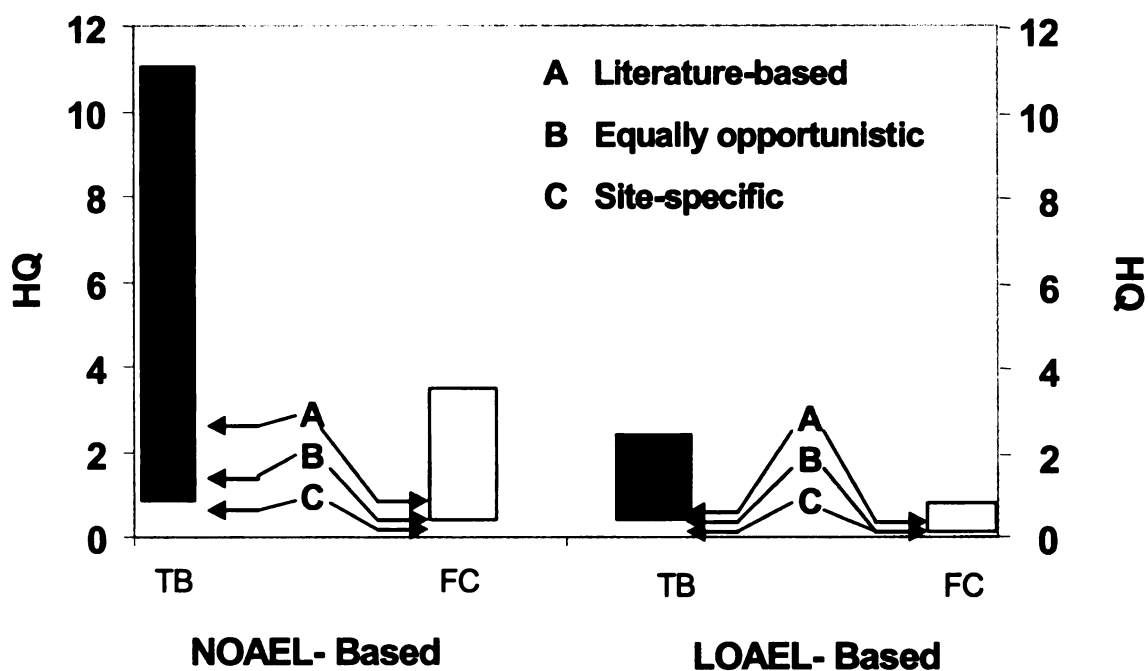


Figure 9. Comparison of HQ values based on predicted concentrations of TEQ determined based on dietary exposures to HQs based on TEQ concentrations measured in the livers of mink at Fort Custer (FC) and Trowbridge (TB). The arrows represent the HQs of three mink dietary models. The solid vertical bars represent the HQs estimated from TEQ concentrations in mink livers. The TRVs used to calculate HQs are based upon a mink feeding study conducted by Bursian et al. (Bursian et al. 2002).

approaches (Figures 8 and 9). For example, the LOAEL-based maximum HQs for PCBs and TEQs were 0.63 for the dietary model and 1.4 for the tissue residue-based approach. The range of LOAEL-based HQs was less than the range of NOAEL-based HQs for both top-down and bottom-up approaches, reflecting the greater uncertainty in the NOAEL-based TRVs.

One explanation of the underestimation of the bottom-up approach, is that the NIR of a wild mink is greater than that of a confined mink. Although wild male mink are of similar weight to ranch female mink, one would expect the wild mink to expend greater energy while hunting for food and defending a territory. Wild mink would have a greater NIR and therefore have a greater ADD than their ranch-raised mink counterparts. Alternatively, since the tissue residue-based TRVs were based on female mink liver and most of the mink captured were male, there are likely to be gender-specific differences in the toxicokinetics of PCBs in mink, specifically, from nursing-related elimination of PCBs.

In conclusion, mink on the Kalamazoo River appear to eat predominantly mammals, at least seasonally, than most conventional models assume. There is close agreement among different mink feeding studies in respect to effect levels, except for slightly more uncertainty with TEQ-based NOAELS. Overall, based on a bottom-up approach for estimating PCB exposure, mink from the KRAOC are expected to have minimal risk due to PCB exposure. Both the bottom-up and top-down approaches were in agreement that mink from TB have greater exposures to PCBs and TEQs than mink from FC. In the reference area, both exposures to PCBs and TEQs and the resulting estimates of risk were

approximately 3 to 5-fold less than in the area of concern, and ranged from 0.04 to 4.9. At TB, depending on the dietary model applied, NOAEL-based HQs ranged from 0.47 to 3.4 and from 0.73 to 14 for total PCBs and TEQ, respectively. LOAEL-based HQs for total PCBs and TEQ were less than 1.0 for all dietary models except the literature-based model in which fish are assumed to comprise 85% of the mink's diet where HQs were 1.8 and 2.0 for total PCBs and TEQs, respectively. Because the LOAEL-based HQ values are more certain and ecologically relevant, risks posed by PCBs in the diet of mink from the KRAOC were predicted to be minimal. Calculated risk based on dietary models was slightly less than risk calculations based on site-specific mink tissue residues. Both approaches were in agreement that the degree of exposure to PCBs and TEQs were near the threshold for effects on reproduction in mink, but unlikely to cause population-level effects on mink in the KRAOC.

CHAPTER THREE

Habitat Characterization and Mink (*Mustela vison*) Habitat Quality of the Kalamazoo River Superfund Site, MI.

Introduction

Located in southwestern Michigan, the main stem of the Kalamazoo River is approximately 195 km long and flows northwest, entering Lake Michigan near Saugatuck, Michigan. In 1990, approximately 125 km of the Kalamazoo River was designated a Superfund site. The site, referred to as the Kalamazoo River Area of Concern (KRAOC), and extends from Morrow Lake dam to Lake Michigan (CDM 1999). Polychlorinated biphenyls (PCBs) are the contaminant of concern, and studies are being conducted to determine the extent to which biota, specifically mink (*Mustela vison*), could be potentially adversely affected by this exposure. Mink are one of the receptors of concern at the KRAOC due to their sensitivity to PCBs and their relatively great potential for exposure as piscivorous, top predators (Alexander 1977;Aulerich et al. 1973;Restum et al. 1998). However, non-contaminant environmental stressors on the Kalamazoo River, including mink habitat availability and quality, have not been fully addressed in previous environmental risk assessments.

The mink (*Mustela vison*) is a semi-aquatic member of the mustelidae family commonly found in waterways throughout North America, including Michigan (U.S.Environmental Protection Agency 1993). There is considerable

variation in both the type of habitat used and dietary composition (Casson and Klimstra 1983; Hamilton, Jr. 1936; Sealander 1943; Whitman 1981). For instance, during the waterfowl breeding season in the prairie pothole region of Canada, mink had a large proportion of waterfowl in their diet (Arnold and Fritzell 1990). Mink diet can be very variable in similar areas depending on season. In southern Michigan, the dietary composition of mink have been reported to be primarily fish (Alexander 1977) and primarily mammals (Sealander 1943), depending on location and season.

Mink activity and dietary composition in an area have been shown to be correlated to prey availability (Loukmas and Halbrook 2001). Stream improvements such as the creation of pools, placement of logs, and other cover within the stream channel resulted increased mink activity (Burgess and Bider 1980). Along Lake Ontario, shoreline development for cottages resulted in decreased cover and vegetative complexity by the shores and mink activity was found to decrease (Racey and Euler 1983). Therefore, land uses and vegetation characteristics are likely to affect mink populations along the Kalamazoo River, regardless of contaminant stressors.

One tool that wildlife managers can use to predict the distribution and abundance of a species is a Habitat Suitability Index (HSI). In addition, HSIs can be used to quantify and evaluate the effects of human alterations of the environment. HSI scores are an index of theoretical carrying capacity and values can range from 1.0 for optimal habitat to 0.0 for completely unsuitable habitat. The HSI developed for mink by the U.S. Fish and Wildlife Service (Allen 1986)

assumes that if sufficient vegetation and cover is available in semi-aquatic habitats to support a prey base, then the habitat is suitable for mink. In addition, other assumptions include potential food availability, cover, and reproductive habitat requirements are described by the same set of habitat characteristics (Allen 1986).

The objective of this study was to quantify mink habitat quality along the Kalamazoo River (including the KRAOC) using the HSI model. In addition, habitat was characterized using land use and land cover data. A Geographic Information System (GIS) was created to interpolate HSI values from field-collected data to the entire river area. Habitat suitability data was used to determine which areas along the Kalamazoo River would unsuitable for mink. This data will also allow decision makers to visualize how river remediation alternatives could affect mink habitat availability.

Methods

The variables describing suitable mink habitat vary by habitat type (Allen 1986) and the riverine model was chosen as the most suitable model to use for the Kalamazoo River (Figure 10). The HSI for riverine habitat assumes that if both terrestrial food and aquatic food items are present, the area will be suitable for mink (Allen 1986). The variables to measure terrestrial food availability were canopy cover within 100m of the shore and shoreline cover. The permanence of water is the only variable that measures if aquatic food will be present. The measured variables are then used to create a score for each habitat component to define a total HSI. Water is present in the Kalamazoo River year-round, therefore, the terrestrial food availability component was assumed to limit habitat suitability. Shoreline cover and canopy cover were the variables used to calculate the suitability index scores (Figure 11). A geometric mean of the two variables were calculated to calculate the final HSI (Allen 1986).

$$\text{HSI} = (\text{canopy cover suitability index} * \text{shoreline cover suitability index})^{1/2} \quad \text{Eqn 1.}$$

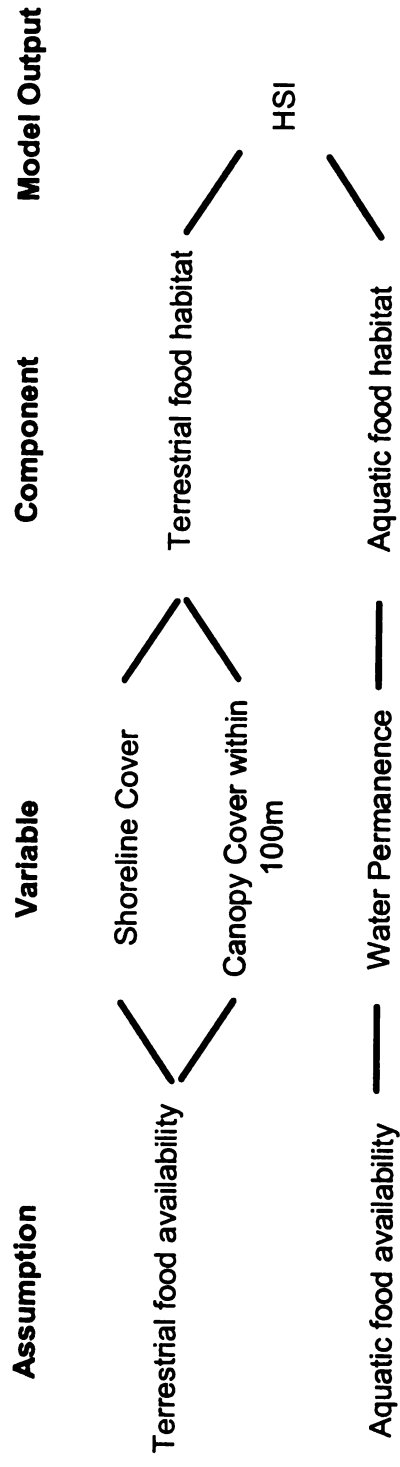


Figure 10. Description of the assumptions, variables, and components used to calculate HSI, as described by Loukmas (Loukmas 1994)

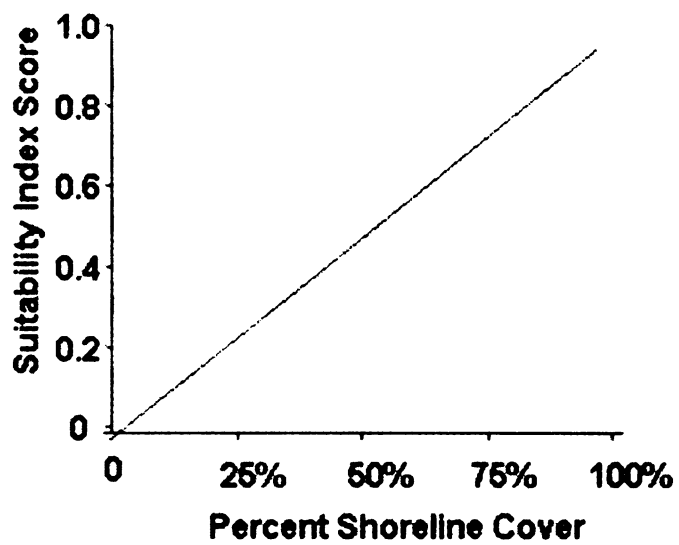
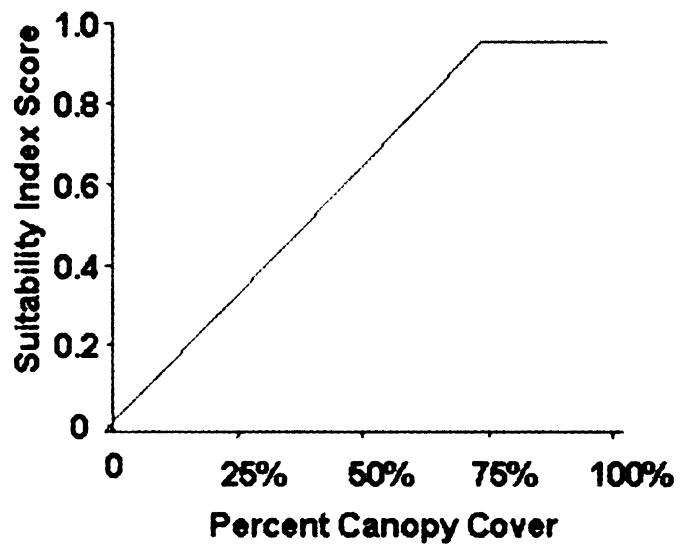


Figure 11. Suitability index scores for percent canopy and shoreline cover (Allen 1986).

The HSI is defined as:

$$\text{HSI} = \frac{\text{Habitat conditions at the site}}{\text{Optimum habitat conditions}}$$

In order to attain an HSI score of 1.0 for mink, the following variables must be present in a riverine system (Allen 1986):

75% (or more) of year with surface (river) water present

75% (or more) canopy cover of trees and shrubs within 100 m of river's edge

100% shoreline cover within 1 m of river's edge

Areas along the river that do not have sufficient canopy cover or shoreline cover result in lesser HSI values. On-site inspection was conducted to measure HSI components (percent canopy cover and shoreline cover). HSI components were measured on both river banks at 30 m x 100m transects along the river approximately 500 m from one another. Transects were reached by canoe (Old Town Canoe Co.; Old Town, ME, USA). Exact location was recorded with a GPS receiver (Garmin LTD, Loatha, KS, USA). At each transect, canopy cover was measured from 100 m perpendicular to the river's edge at 25 m intervals using a convex mirror densitometer (Forestry Suppliers Inc; Jackson, MS). Shoreline cover was ocularly estimated 30 m parallel to the riverbank, at 10 m intervals, according to the cover class scale developed by Daubenmire (Daubenmire 1959). Shoreline cover consisted of large and small woody debris, overhanging

vegetation, undercut banks, boulders, exposed roots, and emergent aquatic vegetation.

The data from each transect was imported into ArcView 3.2 (ESRI; Redland, CA, USA). The area between measured transects was interpolated with Spatial Analyst using the IDW method to provide HSI data for the entire area within 100m of the river. HSI was only interpolated in areas where HSI data was collected, not in areas where no data was collected (such as the City of Kalamazoo).

Results and Discussion

Shoreline and canopy cover was measured at 249 transects representing approximately 45km of river shoreline (Figure 12). HSI of individual transects ranged from 0.05 – 0.99 (average 0.76). 75% of transects were considered good habitat. When transect data was interpolated throughout the river stretches, 770 km² of riverine habitat was considered good habitat, while only 190km² was considered marginal or poor habitat (Figure 13 -Figure 15)

The section of river furthest downstream was located from Lake Allegan downstream to New Richmond, MI (Figure 13). This area was primarily considered good habitat and is mostly located within the Allegan State Game area (Figure 13). This section of river was characterized by forested areas that had relatively complex shorelines (personal observation). The middle section of the river is located from D-Ave in Kalamazoo, MI downstream to Allegan, MI (Figure 14). This area includes the portion of the KRAOC in which samples were collected for the environmental risk assessments described in Chapters 1 and 2. In this section, the areas described as marginal/poor habitat (Figure 14) typically correspond to former impoundment areas that were dominated by grasses, stinging nettle (*Urtica dioica*), and ragweed (*Ambrosia artemisiifolia*) which provides no canopy cover (personal observation). In addition, the shoreline in these areas tended to be very simple, with little shoreline cover (personal observation). The section of river furthest upstream, corresponds to Fort Custer (FC), the upstream reference area, used as a study site in Chapters 1 and 2 (Figure 15). In this section, marginal/poor habitat typically corresponded to

residential and industrial areas that are found at both the upstream and downstream sections of FC (Figure 15). The good habitat in the middle of the FC area corresponds to the Fort Custer State Recreation Area.

Because the HSI is so general, it produces very coarse results. The areas identified as poor habitat were primarily urban/residential or areas with little to no trees and simple shorelines. This level of assessment could be attained by a quick look at the site. When prey availability was studied, mink habitat suitability was correlated with prey availability (Loukmas and Halbrook 2001). Therefore, surveys of potential prey items would have to be conducted in order to provide more specific and detailed results regarding habitat quality.

The areas of the Kalamazoo River that were sampled indicate there is substantial habitat of good quality available to mink. While there are some areas with poor habitat quality, habitat quality in the sampled areas is not likely a limiting factor to mink populations residing along the Kalamazoo River. A previous risk assessment conducted on the Kalamazoo River determined observed levels of PCB exposure would not be expected to cause adverse population-level effects on mink. However, if remedial actions within the KRAOC are to be conducted, the effects upon mink habitat quality should be considered.

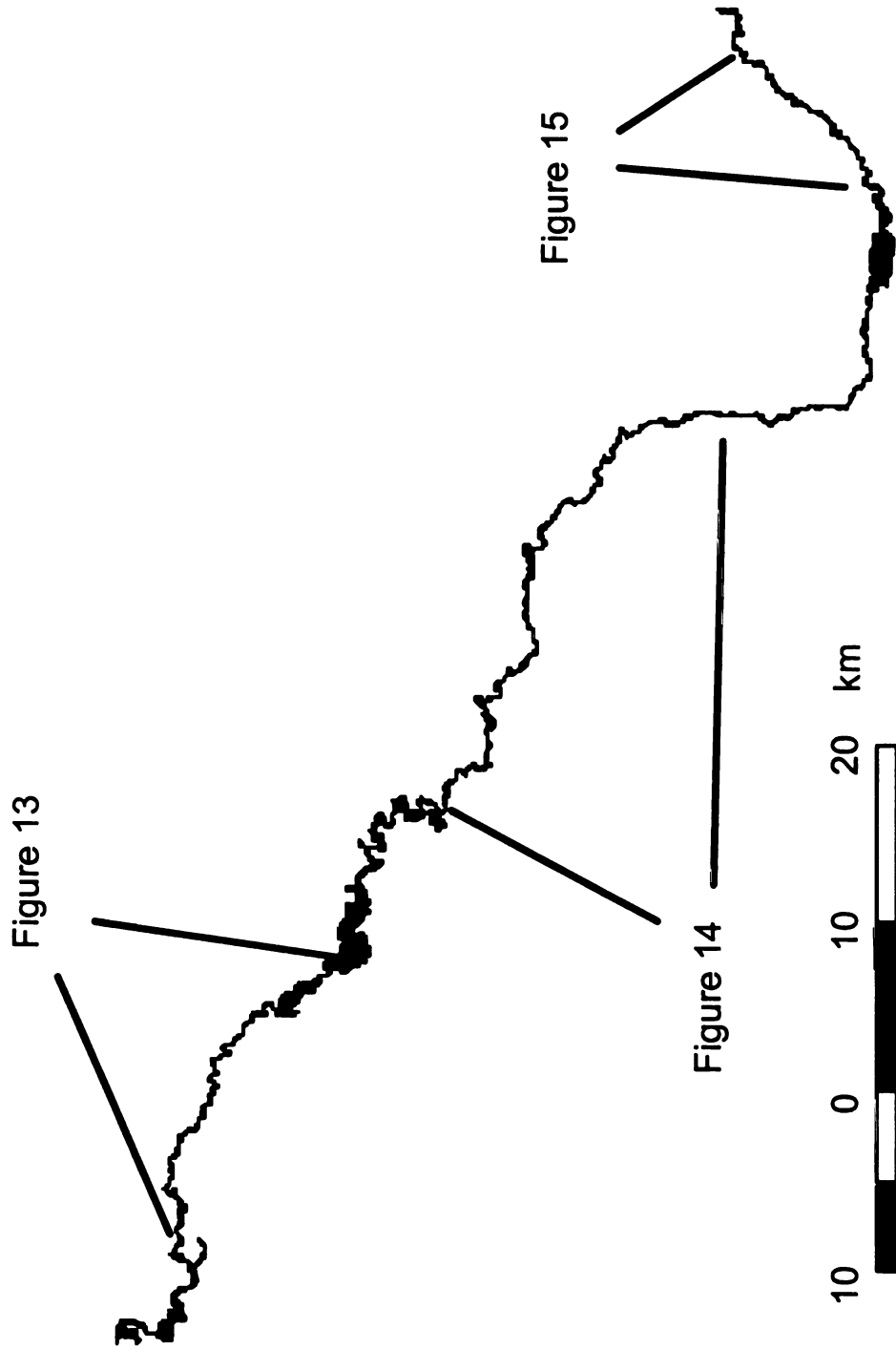


Figure 12. Extent of Kalamazoo River through Kalamazoo and Allegan counties with insets indicating close-up views of mink habitat suitability maps.

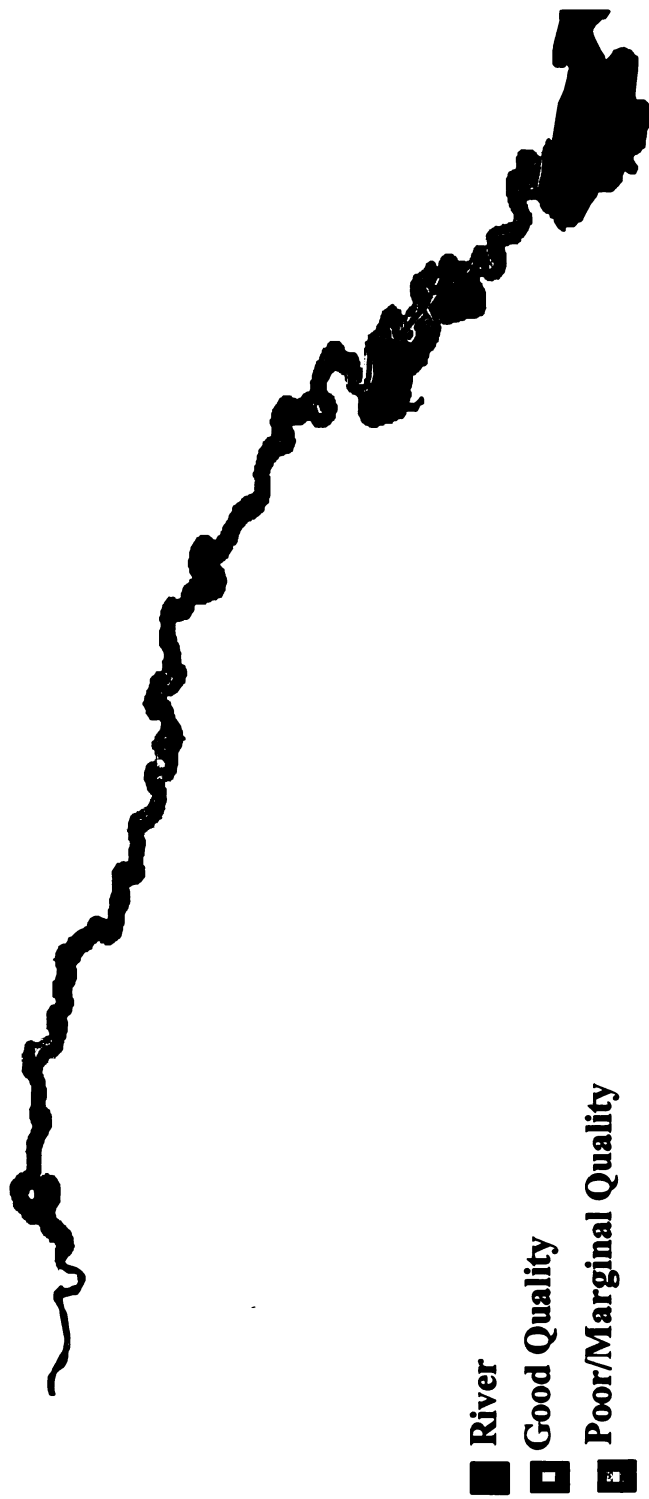


Figure 13. Mink habitat quality at the furthest downstream stretch of the Kalamazoo River. This area extends from Lake Allegan to New Richmond. This figure is presented in color.

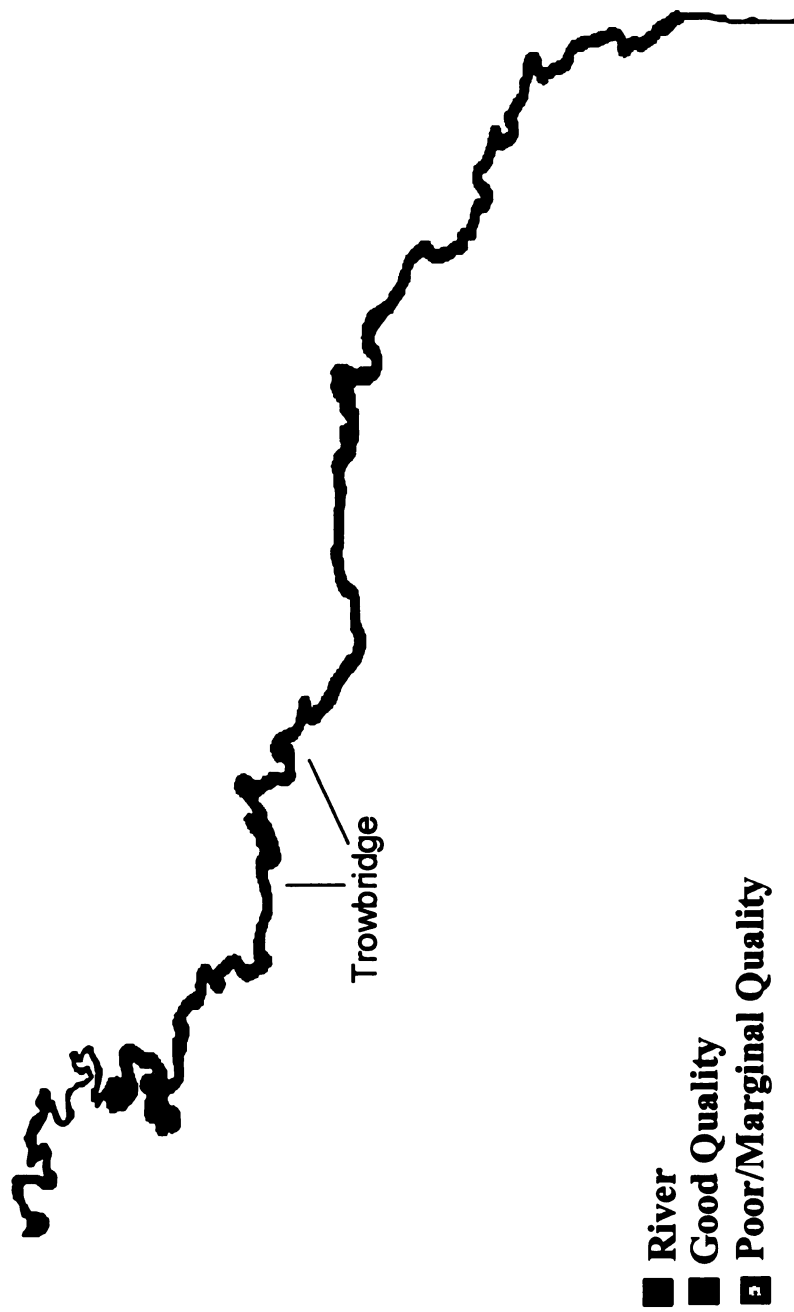


Figure 14. Mink habitat quality through the middle section of river, extending from D-Ave in Kalamazoo to the City of Allegan, and includes the Trowbridge (TB) impoundment. This figure is presented in color.

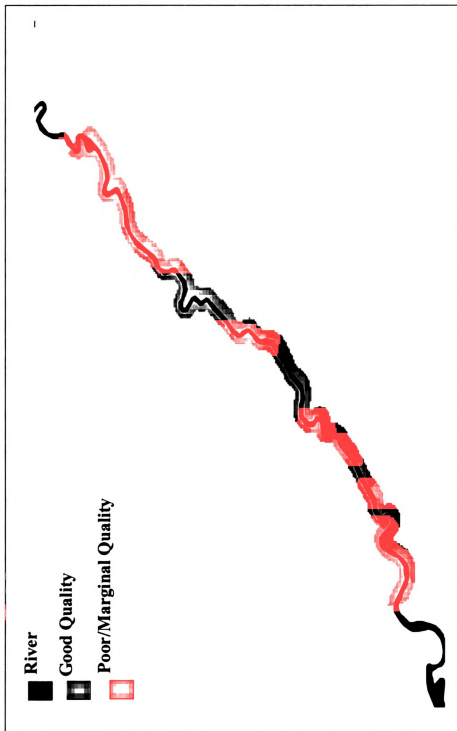


Figure 15. Mink habitat quality through the Fort Custer section of Kalamazoo River. This figure is presented in color.

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