Kalamazoo River Ecological Risk Studies Peer Review

293481

Charge and Supporting Materials

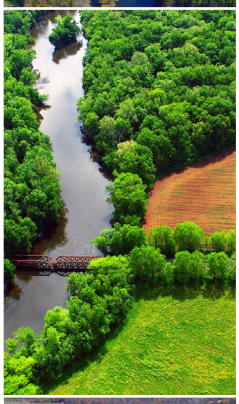
Volume 1

May 2008

Kalamazoo River Ecological Risk Studies Peer Review

Charge and Supporting Materials

table of contents



Volume 1

SECTION 1: General Information

- Meeting Agenda
  - Site Visit Tour Maps
- Summary of Process and Schedule
- List of Panel Members and Contact Information

SECTION 2: Final Charge for the Peer Review Panel

- Charge for the Peer Review Panel

SECTION 3: MSU Studies

- Summary of MSU Studies
- Copies of Published Manuscripts
  1. Blankenship, et al. 2005. Differential accumulation of PCB congeners in the terrestrial food web of the Kalamazoo River Superfund Site, Michigan, USA.
  2. Neigh, et al. 2006a. Exposure and Multiple Lines of Evidence Assessment of Risk for PCBs Found in the Diets of Passerine Birds at the Kalamazoo River Superfund Site, Michigan
  3. Neigh, et al. 2006b. Accumulation of Polychlorinated Biphenyls from Floodplain Soils by Passerine Birds
  4. Neigh, et al. 2007. Reproductive Success of Passerines Exposed to Polychlorinated Biphenyls Through the Terrestrial Food Web of the Kalamazoo River

(continued)

Charge and Supporting Materials
table of contents

5. Strause, et al. 2007a. Risk Assessment of Great Horned Owls (*Bubo virginianus*) Exposed to Polychlorinated Biphenyls (PCBs) and DDT along the Kalamazoo River, Michigan
6. Strause, et al. 2007b. Plasma to Egg conversion Factor for Evaluating Polychlorinated Biphenyl and DDT Exposures in Great Horned Owls and Bald Eagles
7. Strause, et al. 2008. Risk Assessment Methodologies for Exposure of the Great Horned Owls (*Bubo virginianus*) to PCBs on the Kalamazoo River, Michigan
8. Zwiernik, et al. 2007. Site-Specific Assessments of Environmental Risk and Natural Resource Damage Based on Great Horned Owls

SECTION 4: Supporting Information

- Formerly Impounded Areas Document (ARCADIS 2008)

Volume 2

SECTION 5: Additional Supporting Information

1. Final Revised BERA (CDM 2003)
2. SAP for MSU Studies with relevant SOPs attached
3. QAPP for MSU Studies

2008 Peer Review Panel Meeting
Kalamazoo, Michigan

Tuesday, May 13, 2008 — Wednesday, May 14, 2008

Hotel	Brook Lodge Hotel & Conference Resort 6535 North 42nd Street, Augusta, MI 49012
Hotel Phone Number	800.407.8486
Hotel Fax Number	269.731.2278
Hotel Website	http://www.hfs.msu.edu/brooklodge/about/index.html
Hotel Check In	4:00 p.m. (hotel will store luggage for early arrivals)
Hotel Check Out	11:00 a.m. (hotel will store luggage for late departures)
Transportation to/ from Airport	Estimated taxi fare from: Kalamazoo/Battle Creek International Airport (AZO): $40 (one way) Gerald R. Ford International Airport (GRR-Grand Rapids): $80 (one way)
Onsite Logistical Contact	Judy Williamson (mobile: 315.298.2690)
General Information	**Tuesday, May 13, 2008 - Upjohn Hall, Room A** 7:00 a.m. - 8:00 a.m. — Breakfast 8:00 a.m. - 11:45 a.m. — Morning Session 11:45 a.m. - 12:45 p.m. — Lunch 12:45 p.m. - 5:15 p.m. — Afternoon Session 5:15 p.m. — Meeting concludes for the day **Wednesday, May 14, 2008 – Site Visit** 7:00 a.m. - 8:00 a.m. — Breakfast 8:30 a.m. — Leave Brook Lodge for Site Visit 8:45 a.m. - 12:05 p.m. — Site Tour 12:05 p.m. - 1:30 p.m. — Lunch 1:30 p.m. - 2:30 p.m. — Site Tour continues 2:30 p.m. — Return to Hotel, Meeting Adjourns
Attire	Business casual
Directions to Hotel from Airport	**From Kalamazoo/Battle Creek Regional Airport:** North on Portage Road to I-94. Take I-94 East to 35th Street Exit (Galesburg/Augusta, Exit 85). Take left onto 35th Street to Battle Creek Street, right on Battle Creek Street. Follow Battle Creek Street through the light in Galesburg. After the light, the road becomes M-96. Follow M-96 over the railroad tracks and to the right. Take M-96 (parallel to tracks) to downtown Augusta. Turn left onto Webster Street at intersection in Augusta (by Shell station) – **SPEED LIMIT IS 25 mph.** Webster will turn into 42nd Street and Brook Lodge will be approximately 1.5 miles on the left. **From Kalamazoo/Chicago:** Take I-94 East to 35th Street Exit (Galesburg/Augusta, Exit 85). Take a left onto 35th Street to Miller Dr., right on Miller Dr. Follow Miller Dr. to 38th St., turn left. Follow 38th St. to E. Michigan Ave., turn left. Follow E. Michigan Ave. to McCollom, turn right. Follow McCollom to M-96, turn right. Take M-96 into downtown Augusta. Turn left onto Webster Street at intersection in Augusta (by Shell station) – **SPEED LIMIT IS 25mph.** Webster will turn into 42nd Street and Brook Lodge will be approximately 1.5 miles on the left.

Meeting Agenda

Tuesday, May 13, 2008

7:00 a.m. to 8:00 a.m.	Breakfast	All
8:00 a.m. to 8:30 a.m.	Welcome, introductions and review of agenda	Ken Dickson
8:30 a.m. to 9:00 a.m.	Overview of peer review process	Ken Dickson
9:00 a.m. to 9:30 a.m.	Presentation of Charge to Panel	Ken Jenkins Jim Chapman
9:30 a.m. to 10:00 a.m.	Questions and discussion of the Charge	Panel
10:00 a.m. to 10:20 a.m.	Break	
10:20 a.m. to 11:00 a.m.	Summary of baseline Ecological Risk Assessment (ERA)	Tony Gendusa
11:00 a.m. to 11:30 a.m.	Questions from Panel regarding baseline ERA	Panel
11:30 a.m. to 11:45 a.m.	Comments from BTAG and other attendees regarding the baseline ERA	Ken Dickson
11:45 a.m. to 12:45 p.m.	Lunch	
12:45 p.m. to 2:45 p.m.	Presentation of Michigan State University studies	John Giesy
2:45 p.m. to 3:00 p.m.	Break	
3:00 p.m. to 3:45 p.m.	Questions from Panel regarding MSU studies	Panel
3:45 p.m. to 4:30 p.m.	Comments from BTAG and other attendees regarding the MSU studies	Ken Dickson
4:30 p.m. to 5:00 p.m.	Additional questions from the Panel	Panel
5:00 p.m. to 5:15 p.m.	Discussion and acceptance of the Charge by Panel	Ken Dickson
5:15 p.m.	Meeting concludes	

Unless otherwise noted, all meeting activities will take place in Upjohn Hall, Room A

Site Visit Itinerary

Wednesday, May 14, 2008

7:00 a.m. to 8:00 a.m.	Breakfast
8:30 a.m. to 8:45 a.m.	Leave Brook Lodge Hotel and Conference Resort
8:45 a.m. to 9:15 a.m.	Arrive at Fort Custer; tour
9:15 a.m. to 10:00 a.m.	Depart Fort Custer for Trowbridge Impoundment
10:00 a.m. to 11:00 a.m.	Arrive at Trowbridge Impoundment; begin boat tour
11:00 a.m. to 11:10 a.m.	Disembark boat; depart for Otsego Impoundment
11:10 a.m. to 11:30 a.m.	Arrive at Otsego Impoundment; tour
11:30 a.m. to 11:45 a.m.	Leave for Otsego City Impoundment
11:45 a.m. to 12:05 p.m.	Arrive at Otsego City Impoundment; tour
12:05 p.m. to 1:30 p.m.	Lunch
1:30 p.m. to 1:45 p.m.	Leave for Plainwell Dam
1:45 p.m. to 2:05 p.m.	Arrive at Plainwell Dam
2:05 p.m. to 2:15 p.m.	Leave for Plainwell No.2 Impoundment
2:15 p.m. to 2:30 p.m.	Stop at Plainwell No.2 Impoundment
2:30 p.m.	Depart for Brook Lodge Hotel and Conference Resort

General Safety Information

Air Travel

- Plan to arrive at the airport at least 2 hours before domestic flights.
- Do not talk to strangers, or sit near supporting columns or glass windows in airports.
- While in flight, keep seatbelt fastened to counter unexpected turbulence.
- Listen to safety explanation and be aware of all emergency exit locations.

Ground Transportation/Car

- When renting a car, obtain maps in advance or from the rental car counter and clearly write the directions from the airport to the hotel.
- Stop to ask directions only in well-lit public areas. Keep the phone number of your destination with you.
- Obey posted speed limits.
- Always wear your seatbelt.
- If you have vehicle trouble on the road, raise the hood and turn on the hazard flashers. Stay inside the car with the doors locked and the windows up. If a motorist stops to help, open the window slightly and ask them to call the police if you do not have a cell phone.
- Pull over to a safe place on the side of the road and stop the car when using a cell phone.

Transportation

- Use only taxicabs from reputable/ recognizable companies and identify that a proper operator's license is posted inside cab.
- Arrive early at the station/stop to avoid having to run for a ride.
- During off hours, sit as close to the driver/ conductor as possible and stay awake.

Hotel Stay—

Arrival

- When registering, sign only your last name and first initial. Don't use titles or degrees.
- Be sure that your room number is not announced by the person checking you in. Instruct the desk staff not to give out your name and room number and ask for them to call you if someone inquires about you.

Checking In

- Verify that your room lock is functional. Check windows, outside doors, and adjoining doors to verify that they lock and operate properly. Keep the deadbolt on the door. If the room has a chain instead of a dead bolt or heavy-duty security clasp, twist it to take up the slack before latching it.

- Check the telephone to be sure that you know how to make an outside call and how to reach emergency services (e.g., do you dial 9-1-1 or 9 -9-1-1?).
- Plan your exit from the room and the floor in the event of a fire. Locate nearest fire exit and note how many doors away it is.
- Never open a hotel room door until you have confirmed the identity of the person asking entrance. If a person knocks on the door and identifies herself/himself as an employee of the hotel, call the front desk to confirm.

Leaving Your Room

- Take a minimum of cash and place credit cards and traveler's checks in separate pockets.
- Do not leave a sign on the hotel room door for maid services as it announces an empty room.
- Carry hotel stationary or a matchbook with the hotel's name and address to show to a cab driver or police officer if you become lost.

Hotel Security
→ 269.731.2200 (main desk, security after hours)

Kalamazoo County Sheriff (non-emergency)
→ 269.383.8821

Emergency
→ 911

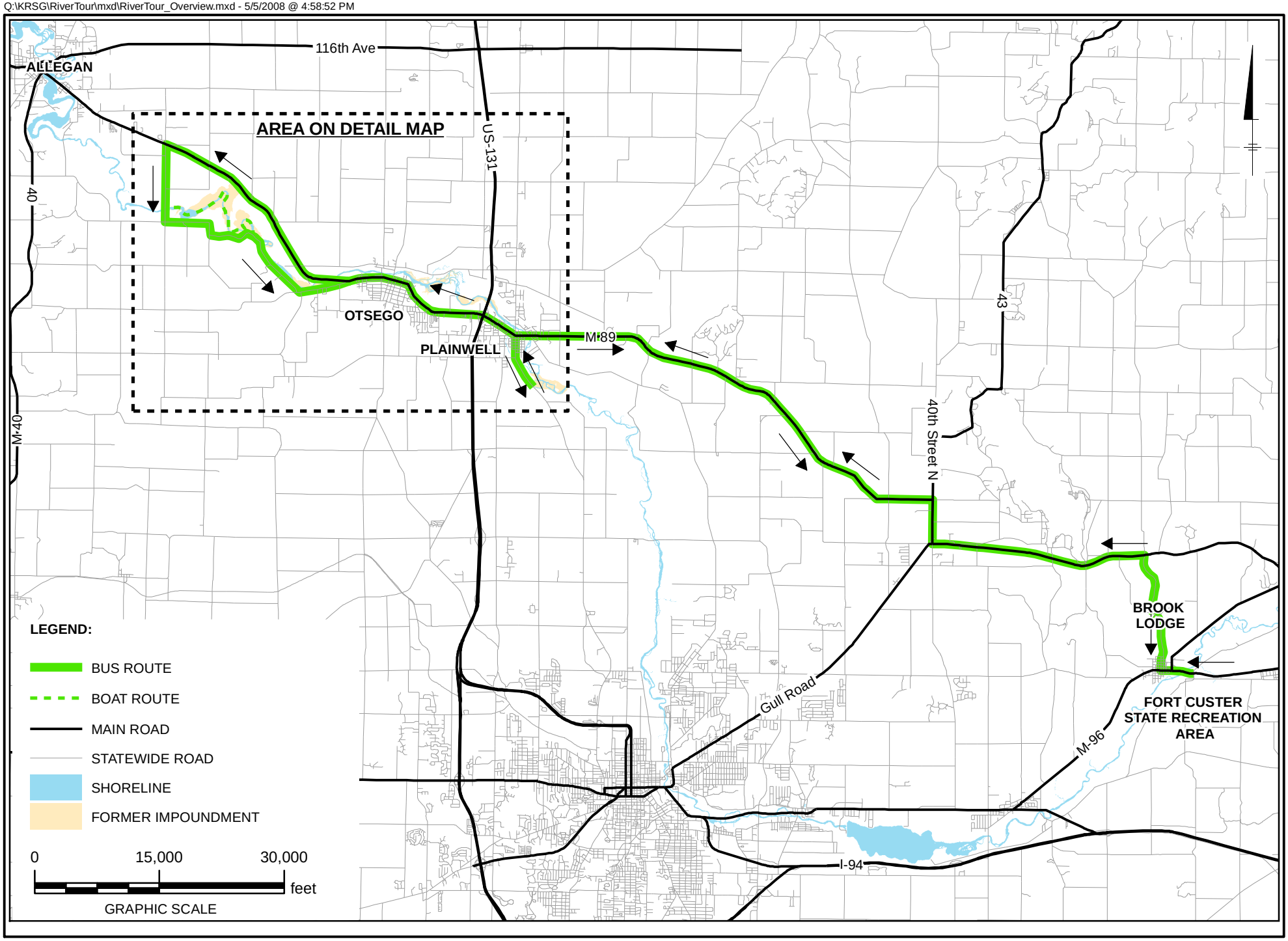
Onsite Support
→ Judy Williamson: 315.289.2690

Kalamazoo River Ecological Risk Studies Peer Review Panel

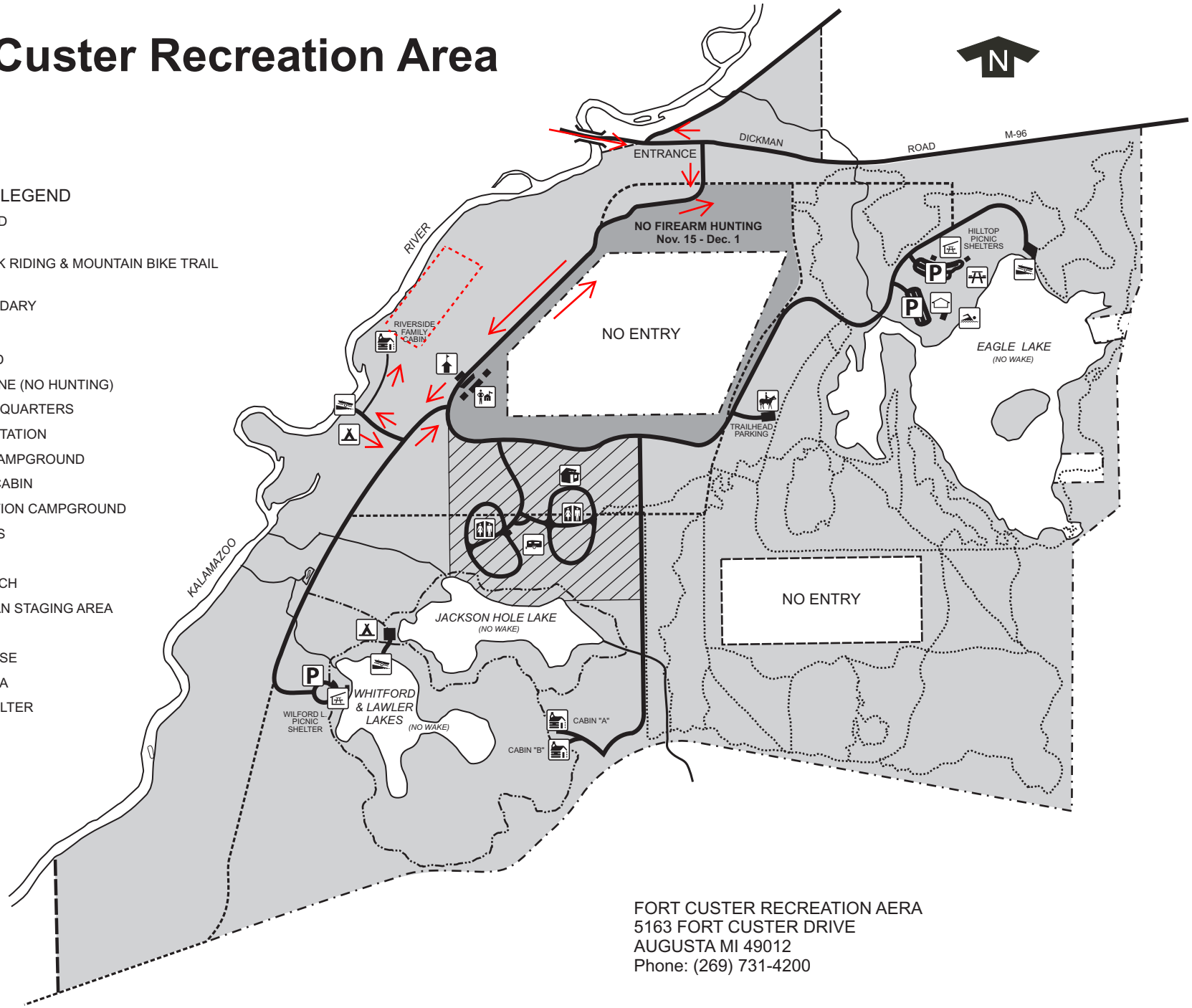
Site Visit Maps and Figures

May 2008

Q:\KRSG\RiverTour\mxd\RiverTour_Overview.mxd - 5/5/2008 @ 4:58:52 PM



Fort Custer Recreation Area



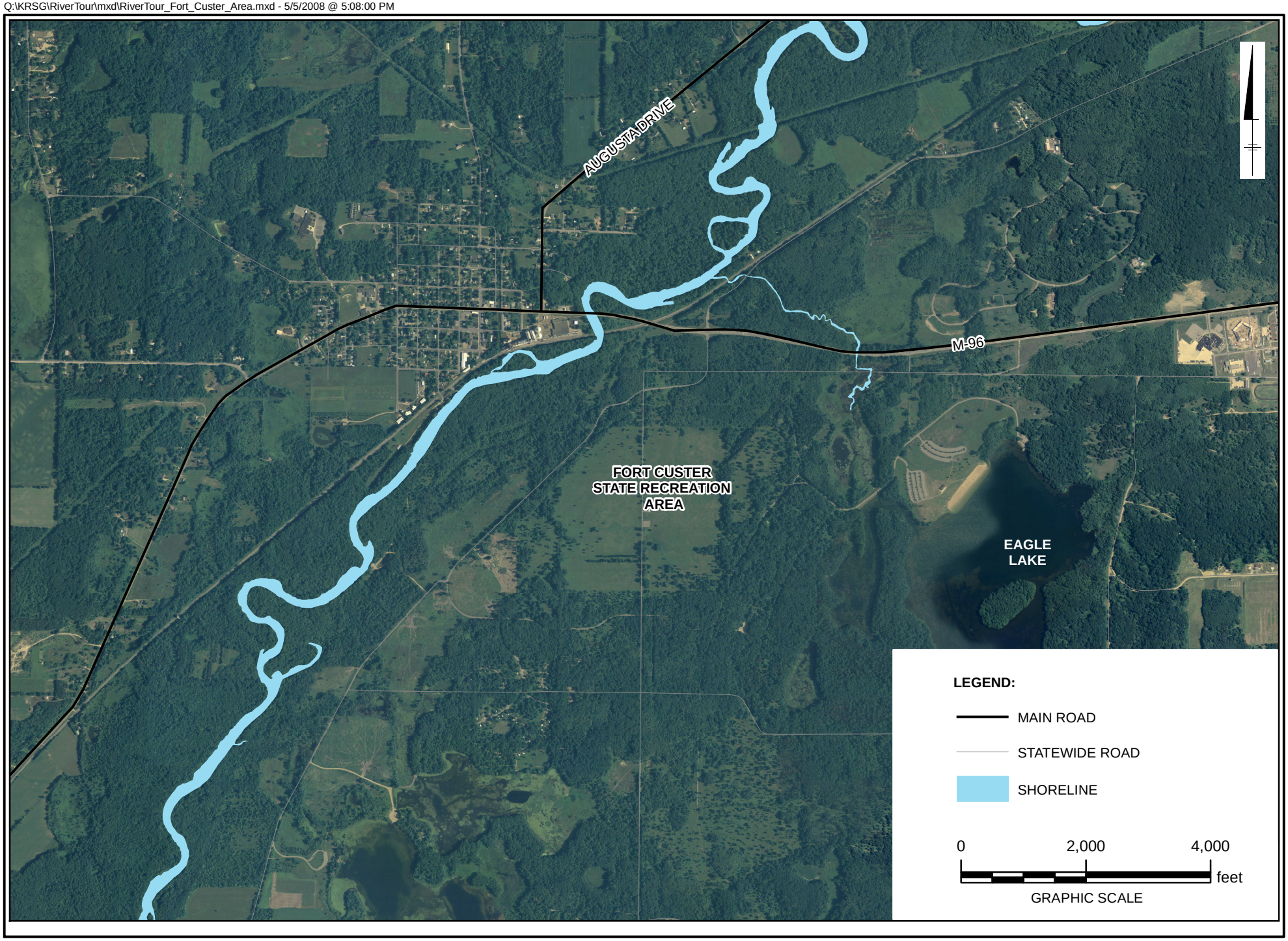
LEGEND

- PAVED ROAD
- BIKE TRAIL
- HORSEBACK RIDING & MOUNTAIN BIKE TRAIL
- FOOT TRAIL
- AREA BOUNDARY
- FENCE
- STATE LAND
- SAFETY ZONE (NO HUNTING)
- PARK HEADQUARTERS
- CONTACT STATION
- MODERN CAMPGROUND
- FRONTER CABIN
- ORGANIZATION CAMPGROUND
- MINI CABINS
- RESTROOM
- BOAT LAUNCH
- EQUESTRIAN STAGING AREA
- BEACH
- BEACHHOUSE
- PICNIC AREA
- PICNIC SHELTER
- PARKING

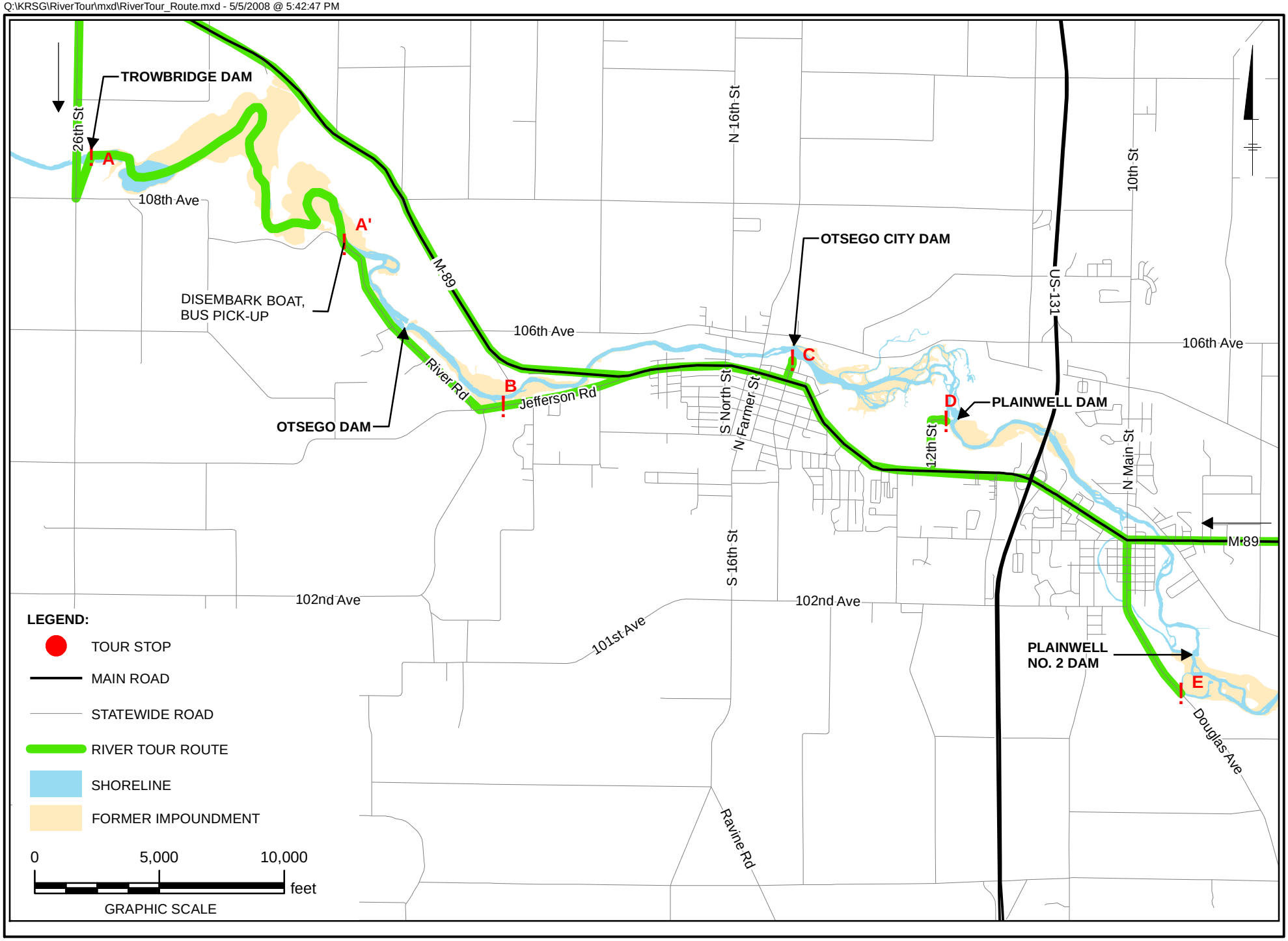
FORT CUSTER RECREATION AERA
5163 FORT CUSTER DRIVE
AUGUSTA MI 49012
Phone: (269) 731-4200

1/2006

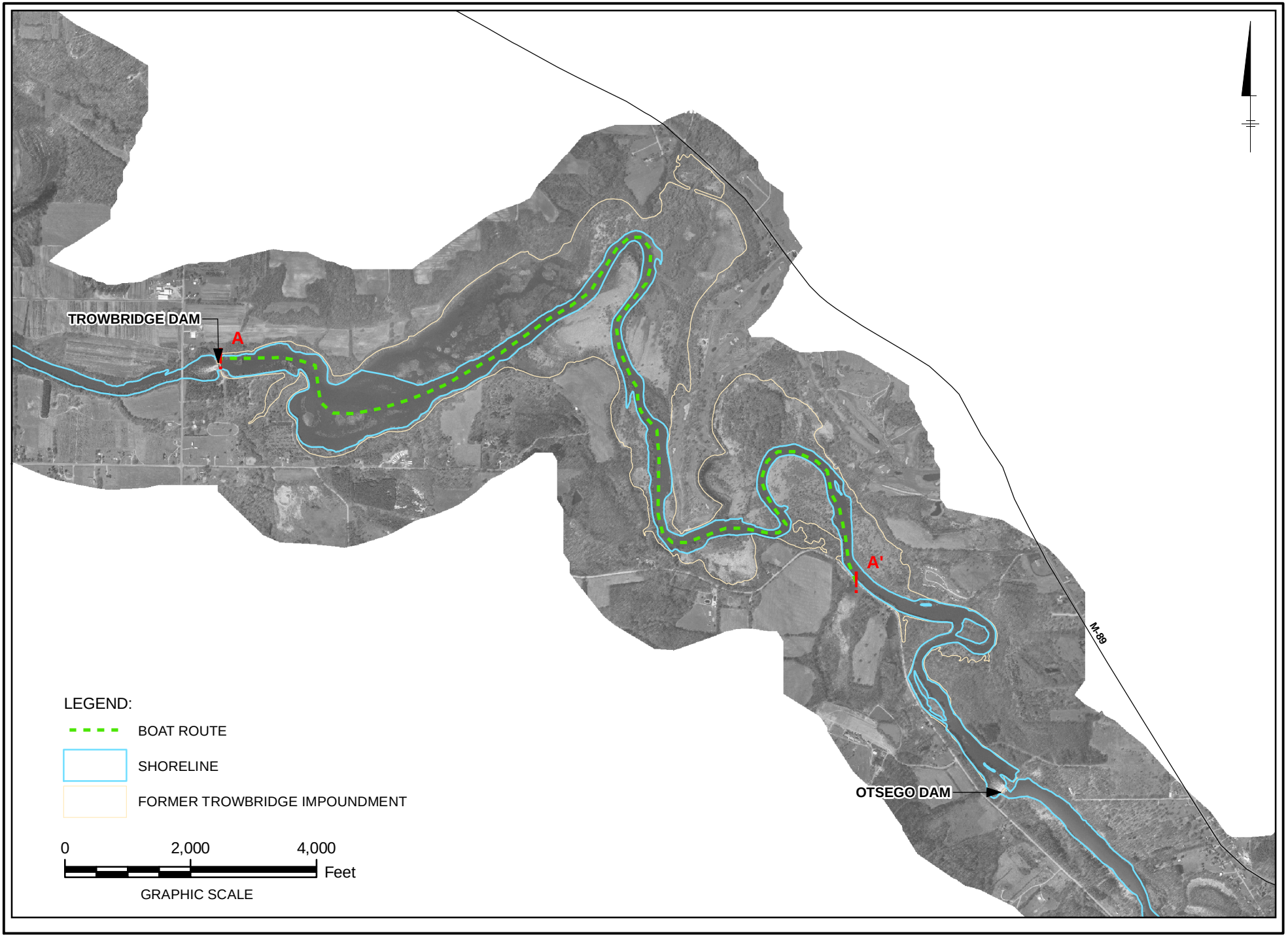
Q:\KRSG\RiverTour\mxd\RiverTour_Fort_Custer_Area.mxd - 5/5/2008 @ 5:08:00 PM



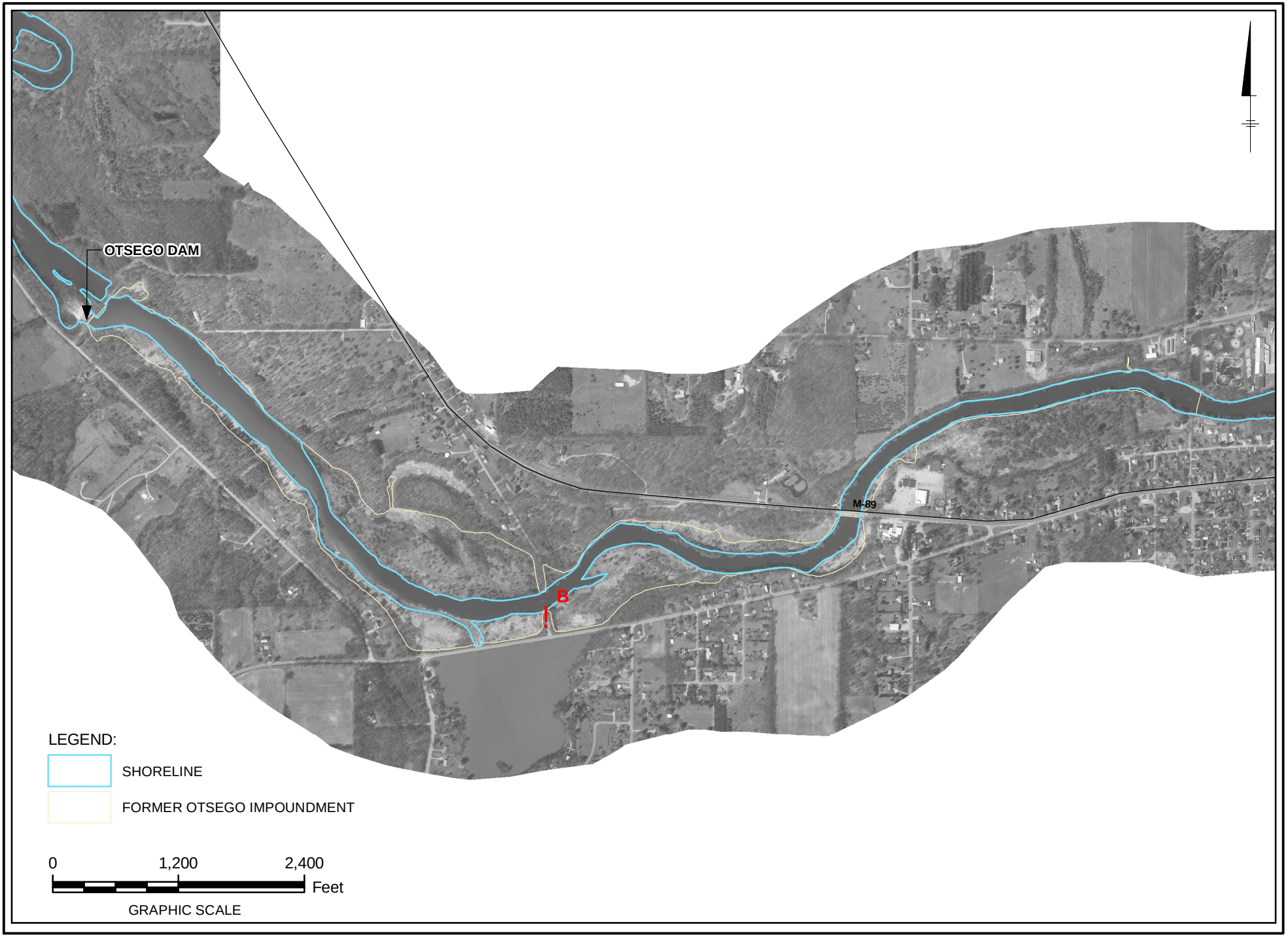
Q:\KRSG\RiverTour\mxd\RiverTour_Route.mxd - 5/5/2008 @ 5:42:47 PM



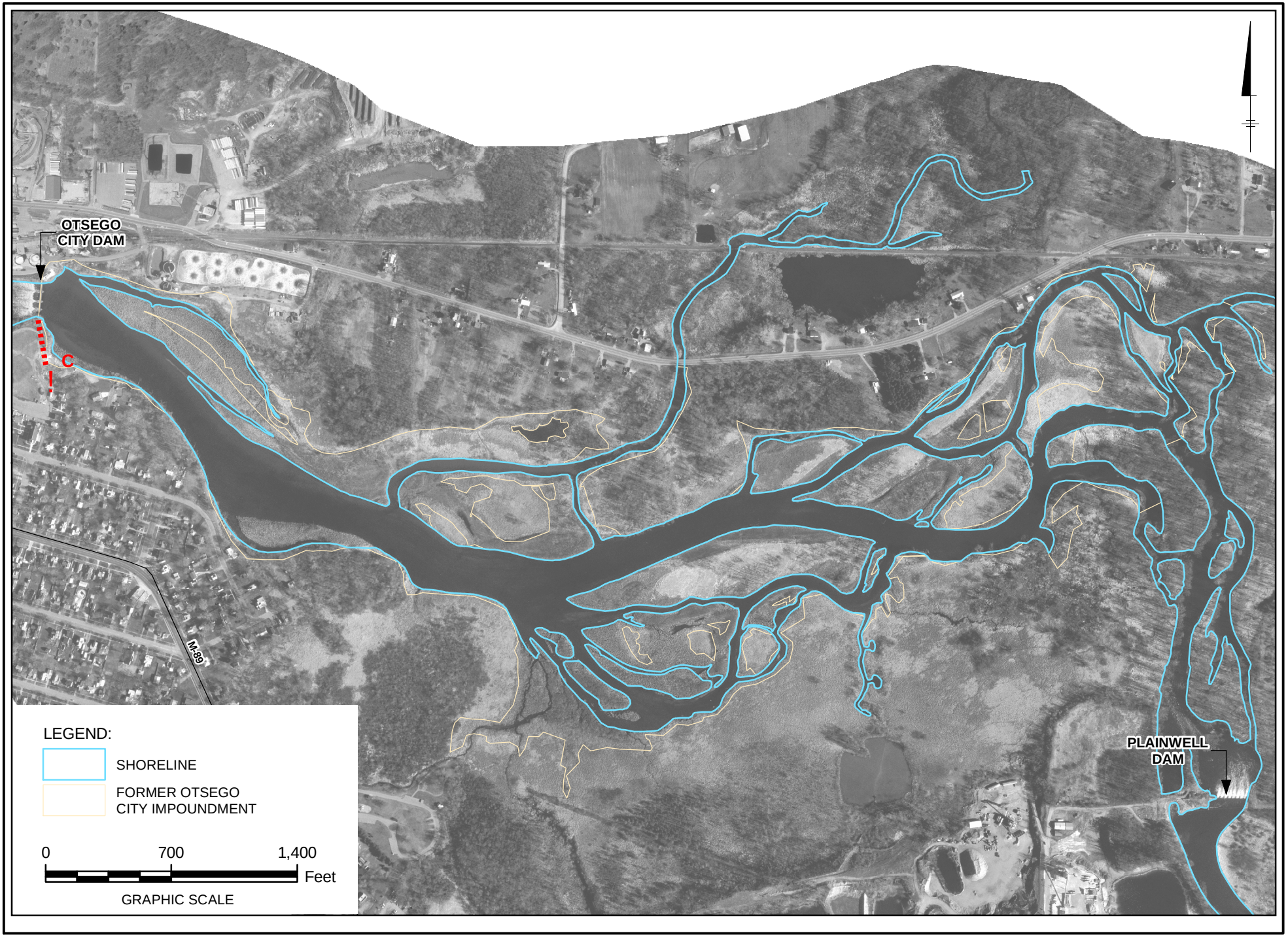
Q:\KRSG\RiverTour\mxd\RiverTour - Trowbridge Impoundment.mxd - 5/5/2008 @ 5:14:00 PM



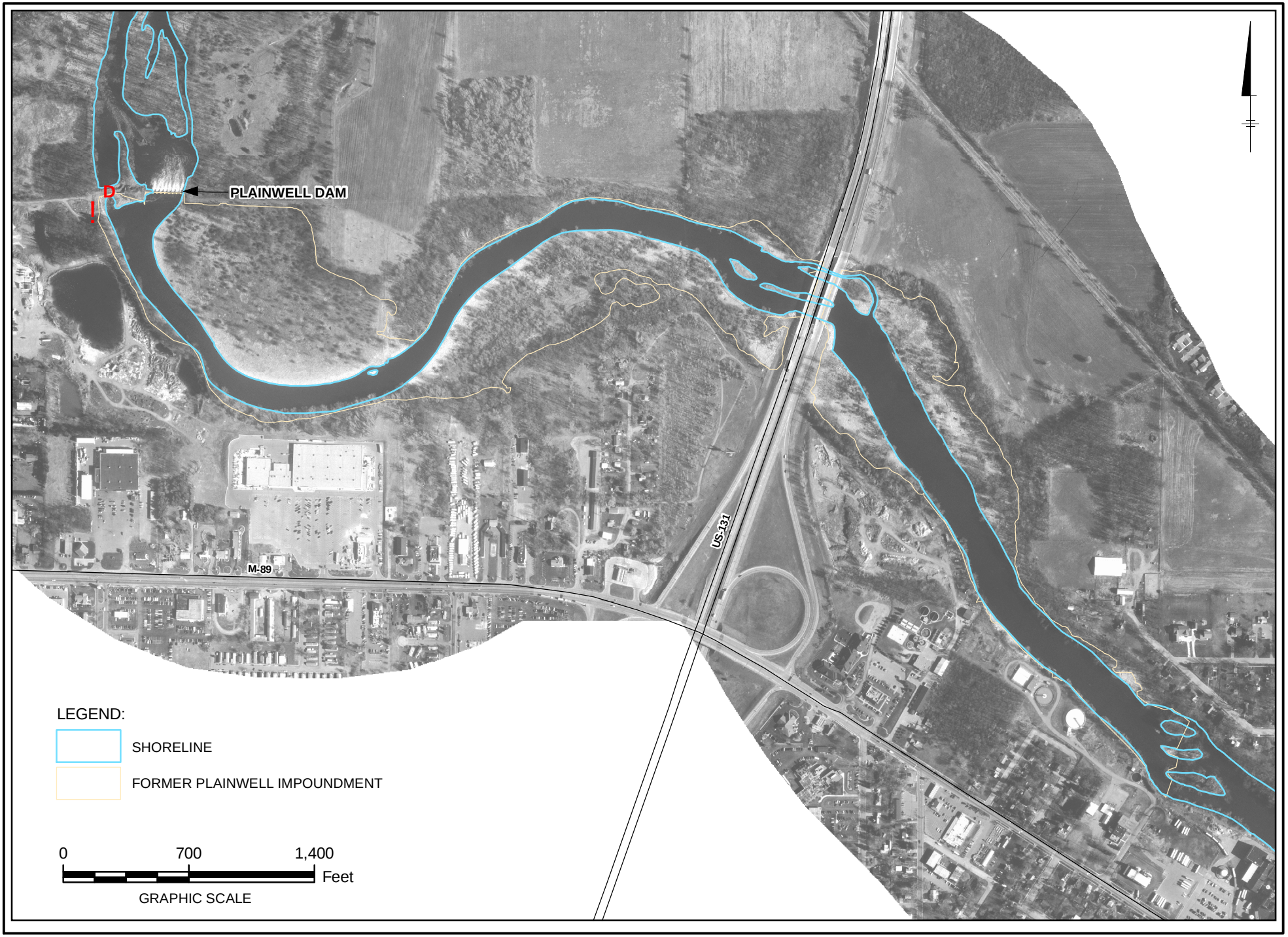
Q:\KRSG\RiverTour\mxd\RiverTour - Otsego Impoundment.mxd - 5/6/2008 @ 11:22:24 AM



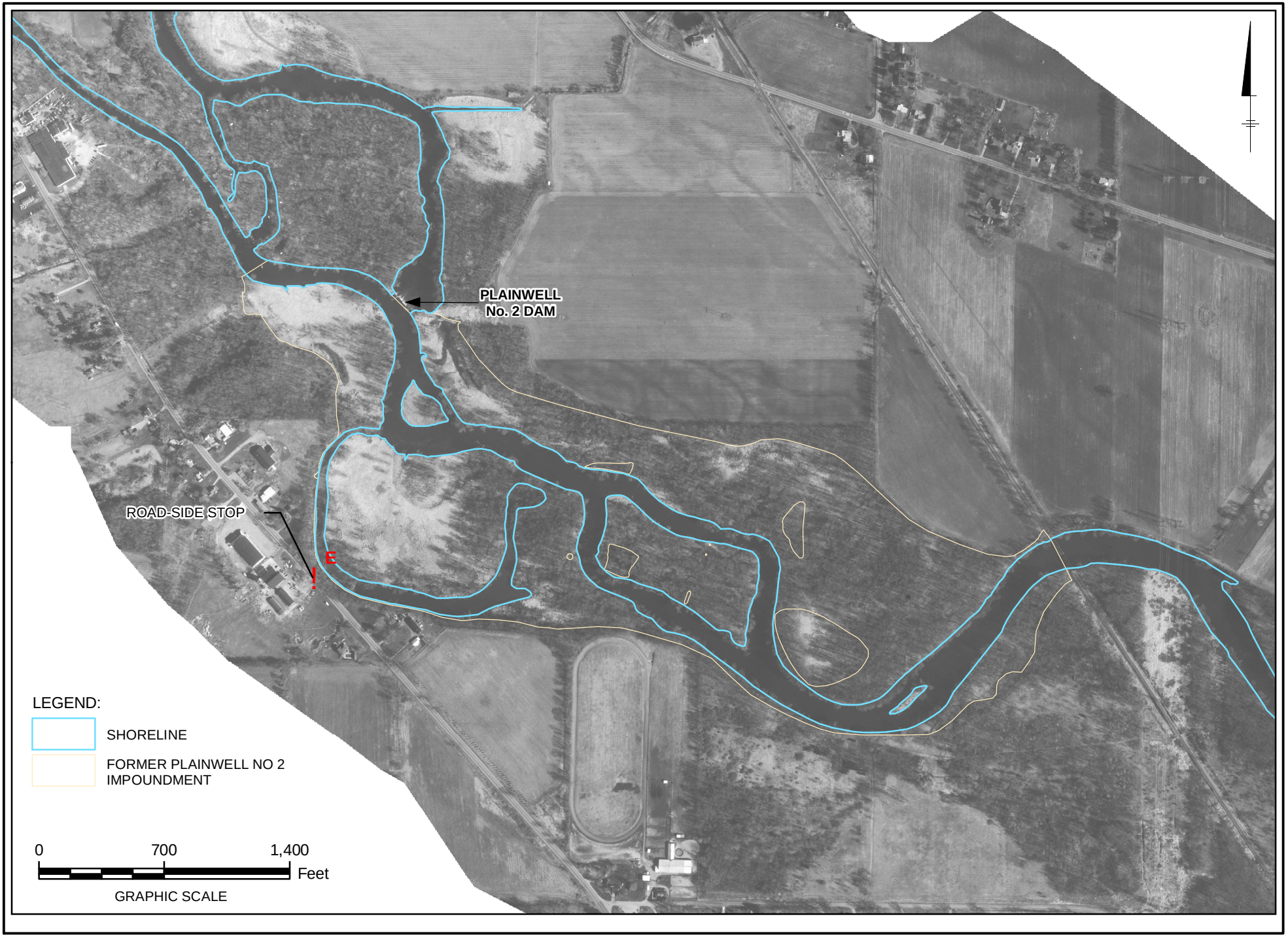
Q:\KRSG\RiverTour\mxd\RiverTour - Otsego City Impoundment.mxd - 5/5/2008 @ 5:19:35 PM



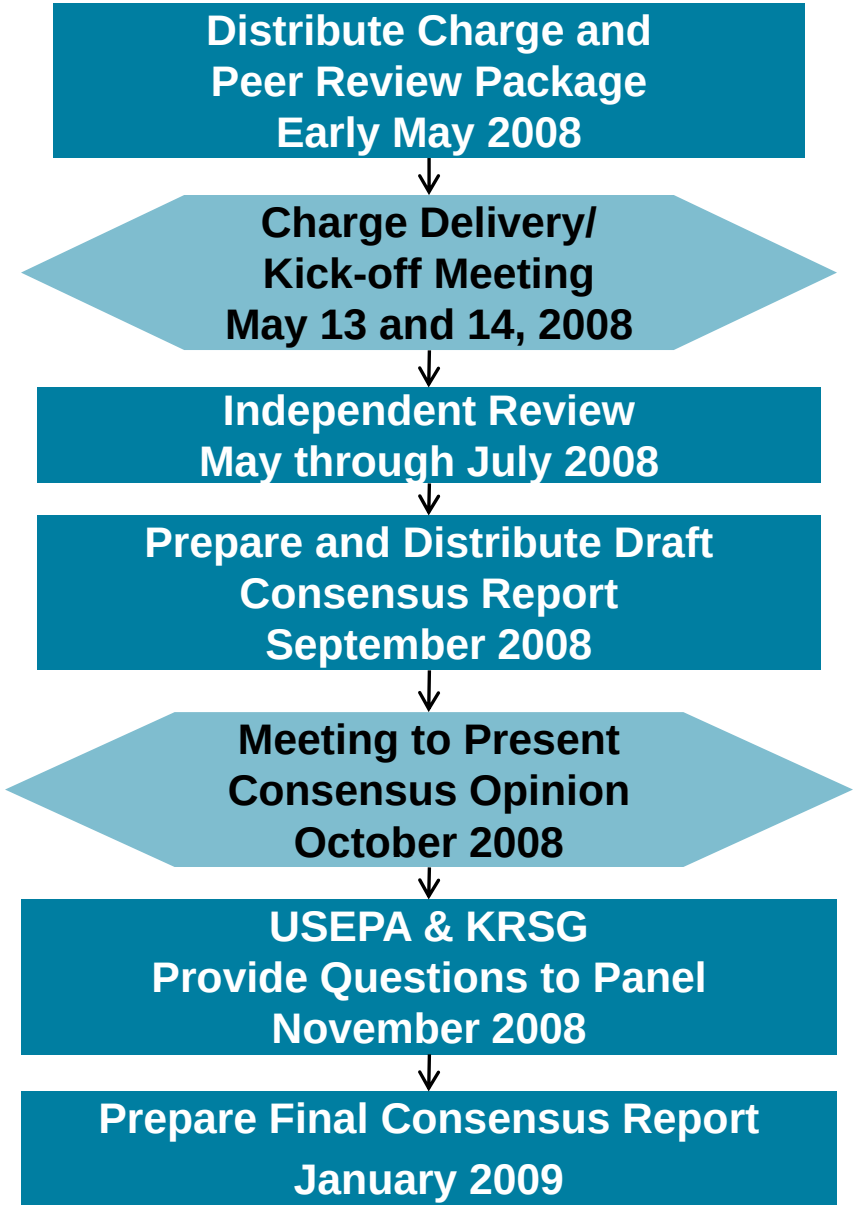
Q:\KRSG\RiverTour\mxd\RiverTour - Plainwell Impoundment.mxd - 5/5/2008 @ 5:22:03 PM



Q:\KRSG\RiverTour\mxd\RiverTour - Plainwell No2 Dam Impoundment.mxd - 5/6/2008 @ 11:23:20 AM



Peer Review Process and Schedule



Kalamazoo River Ecological Peer Review

Contact Information for Peer Review Panel

Name	Telephone / Email	Address
Peer Review Manager		
Dickson, Ken	940-391-3779 dickson@unt.edu	8065 Zackery Road Aubrey, TX 76227
Panel Members		
Barnthouse, Larry	513.894.4600 Barnthouse@lwb-env.com	LWB Environmental Services, Inc. 1620 New London Road Hamilton, OH 45013
Fairbrother, Anne	Tel: 425.458.6306 Cell: 425.941.2810 afairbrother@parametrix.com	Parametrix 411 108th Ave NE, Ste 1800 Bellevue, WA 98004
Grasman, Keith	616.526.6024 kag4@calvin.edu	Calvin College Biology Department, Devries Hall 103 1726 Knollcrest Circle SE Grand Rapids, MI 49546
Harwell, Mark	Office: 386.447.9050 Cell: 386.569.9216 mharwell@ecologicalrisk.com	Harwell Gentile & Associates, LLC 58 Seascape Drive Palm Coast, FL 32137-2451
McDonald, Lyman	Main: 307.634.1756 (recommended) Direct: 307.755.9727 Cell: 307.760.2342 lmcdonald@west-inc.com	West, Inc. 200 South Second St., Suite B Laramie, WY 82070 (for contract and hard copy to review)
Menzie, Charlie	Direct: 571.227.7240 Cell: 571.214.3648 camenzie@exponent.com	2 West Lane Severna Park, MD 21146 (for hard copy for review)
Warren-Hicks, William	Tel: 919.304.6029 Cell: 919.818.4308 billwh@mindspring.com	EcoStat, Inc. P.O. Box 425 Mebane, NC 27302-0425 (Shipping: 6429 Sykes Glen Trail, Mebane, NC 27302)

Section 2

Kalamazoo River Ecological Risk Studies

Charge for the Peer Review Panel

May 2008

Table of Contents

1. Background **1**

2. Objective and Scope of MSU Studies **3**

3. Charge to the Peer Review Panel **3**

3.1 Summary of Charge to the Panel 3

3.2 Specific Questions to be Addressed by the Panel 4

4. References **8**

Tables

Table 1 Summary of Conclusions of Baseline ERA by Receptor Group 1

Table 2 Data Types Available for Refining PCB Exposure Estimates 5

1. Background

On February 21, 2007 Georgia-Pacific Corporation and Millennium Holdings, LLC—collectively referred to as the Kalamazoo River Study Group, or KRSG—voluntarily entered into an Administrative Settlement Agreement and Order on Consent (AOC) (Comprehensive Environmental Response, Compensation, and Liability Act [CERCLA] Docket No. V-W-07-C-864) with the U.S. Environmental Protection Agency (USEPA) for the Allied Paper, Inc./Portage Creek/Kalamazoo River Superfund Site (Site or Superfund Site), located in Kalamazoo and Allegan counties in southwest Michigan. The AOC describes a series of activities associated with supplemental remedial investigations and feasibility studies (SRIs/FSs) that will be carried out over the next several years to address potential risks associated with polychlorinated biphenyls (PCBs) in seven defined Areas of the Kalamazoo River, including a stretch of Portage Creek from Alcott Street to its confluence with the Kalamazoo River. One of these activities is the completion of the Area-Specific Ecological Risk Assessment process for each of the seven defined Areas.

In 2003, Camp, Dresser & McKee (CDM) produced the *Final (Revised) Baseline Ecological Risk Assessment – Allied Paper, Inc./Portage Creek/Kalamazoo River Superfund Site* (Baseline ERA; CDM 2003) on behalf of the Michigan Department of Environmental Quality (MDEQ). CDM's work has been approved by MDEQ and USEPA. Table 1 presents a summary of the terrestrial receptors evaluated in the Baseline ERA and the associated conclusions (CDM 2003).

Table 1 Summary of Conclusions of Baseline ERA by Receptor Group

Receptor Group	Representative Species	Conclusion
Omnivorous Mammals	White-Footed Mouse and Deer Mouse	Unlikely to be at significant risk
Carnivorous Mammals	Red Fox	Unlikely to be at significant risk
Carnivorous Birds	Great-Horned Owl	May be at significant risk depending on diet [1]
Omnivorous/Vermivorous Birds	Robin	Moderate but significant risk

Note:
1. The conclusion for carnivorous birds (great-horned owls) was considered uncertain because of inconsistencies in estimates of risk based on modeled exposure, which indicated low risk, and measured concentrations of PCBs in owl eggs, which indicated high risk.

An initial Baseline Ecological Risk Assessment, prepared on behalf of the Michigan Department of Environmental Quality (MDEQ), was released in June of 1999 (CDM 1999). Subsequent to the release of that document, KRSG provided a series of grants to Michigan State University (MSU) for researchers there to conduct additional ecological studies (the MSU

studies). The MSU studies were not completed until after the Baseline ERA (CDM 2003) was released.

The KRSG has requested that data from the MSU studies be considered as additional lines of evidence for evaluation of ecological risks in the Area-Specific Ecological Risk Assessments and for subsequent risk management decisions. Information regarding the MSU studies has been provided to USEPA and MDEQ for review and consideration. However, since USEPA and MDEQ were not involved in the design or implementation of these studies, the Statement of Work (SOW; included as Attachment A to the AOC) calls for the MSU studies pertaining to floodplain soils to be subjected to a peer review process designed to assess their quality and utility as additional lines of evidence for evaluating potential ecological risk at the Site and informing risk management decisions. The specific charge developed to guide the peer review process is presented in Section 3 of this document.

Both the Baseline ERA (CDM 2003) and the MSU studies included assessments of submerged sediment-based pathways, but the focus of this peer review is specific to the terrestrial receptors whose potential risk is derived from the exposed sediments in formerly impounded areas of the Site. Portions of the floodplains in these areas contain sediments impacted by PCBs that were exposed when water levels in the impoundments were lowered. In this document and other documents related to this peer review, the term “floodplain” is used to refer to the areas of formerly impounded sediments (e.g., the extent of inundation prior to the lowering of water levels in the impoundments), and is not necessarily consistent with specific hydrological or zoning definitions of floodplain. The peer review is focused on these exposed sediments because KRSG and USEPA have agreed that exposures associated with the sediment-based ecological food web are unlikely to be the primary risk drivers for that medium. The peer review panel will receive the Baseline ERA (CDM 2003) for important background information, but will not conduct a peer review of that document.

Depending on the results of the peer review process, the information generated from the MSU studies will be evaluated, along with the information presented in the Baseline ERA (CDM 2003) and other relevant information, as independent lines of evidence in a weight of evidence approach to support the Area-Specific baseline assessments of ecological risks associated with exposure to floodplain soils within formerly impounded areas along the Kalamazoo River. The weighting of each line of evidence will incorporate a variety of technical considerations, including Site-specificity and relevance to assessment endpoints (e.g., protecting the sustainability of local populations). Preliminary Remediation Goals (PRGs) will be developed based on this approach and presented as a range of values along with the PRGs developed in the 2003 Baseline ERA to achieve a more complete understanding of the range of uncertainty associated with the data and provide important context for the risk managers.

2. Objective and Scope of MSU Studies

The KRSG grants to MSU supported the research of a team led by Dr. John Giesy (currently Professor emeritus at MSU, and Professor and Canada Research Chair at the University of Saskatchewan). With these grants, Dr. Giesy's team conducted numerous detailed field studies. For some receptors, multiple lines of evidence were evaluated to support a weight of evidence analysis and to reduce the uncertainties associated with each assessment endpoint. As described in the Peer Review Statement of Work to which this charge is attached (ARCADIS 2008), the Peer Review Panel has received a variety of materials associated with the MSU studies, including a summary of the results of the studies evaluating potential risk specific to exposed sediments in the former Trowbridge Impoundment, copies of publications generated as a result of these studies that have appeared in refereed scientific journals, and other relevant supporting information.

3. Charge to the Peer Review Panel

3.1 Summary of Charge to the Panel

The charge to the Peer Review Panel is to review the MSU studies with respect to their suitability for evaluating potential risks to terrestrial receptors exposed to PCBs in floodplain soils in the formerly impounded areas of the Kalamazoo River. A summary of the MSU studies and supporting information has been provided to assist the Panel in their understanding of the material to be reviewed. The Panel must also review the Baseline ERA (CDM 2003) for important supporting information and lines of evidence for future risk management decisions. The Baseline ERA (CDM 2003) provides context for the MSU studies, which were designed to provide additional lines of evidence for consideration in the final risk management decisions. However, the Baseline ERA (CDM 2003) is not subject to this peer review.

The primary objective of the peer review process is for the Panel to provide an independent, technical opinion regarding the extent to which the information in the MSU studies should be incorporated as independent lines of evidence, along with those presented in the Baseline ERA (CDM 2003), in a weight of evidence evaluation of ecological risks to terrestrial receptor species in formerly impounded areas and for subsequent risk management decisions. In reviewing the materials associated with the MSU studies, the Panel shall weigh the following general questions when addressing the specific questions presented in Section 3.2:

1) Are the methods employed in the MSU studies appropriate and consistent with the current state of the science and relevant guidance?

2) Have uncertainties associated with the MSU studies been clearly identified and discussed?

3) Do the data and analyses presented in the MSU studies constitute reasonable and appropriate lines of evidence to consider in the evaluation of risks to terrestrial receptors in future risk management decisions?

4) Do the MSU studies represent reasonable and appropriate lines of evidence for consideration in risk management decisions regarding the formerly impounded areas?

3.2 Specific Questions to be Addressed by the Panel

Each Panel member, consistent with his or her specific technical expertise, shall independently review each of the questions listed below and provide a thorough explanation of the response. Responses should be supported by citations or other background information, as appropriate. Answers to the primary questions should be supported by consideration of the supplemental issues. In evaluating each specific issue, the Panel members shall weigh the considerations described in the charge summary (Section 3.1).

Exposure Assessments

This section addresses specific issues regarding the evaluation and interpretation of levels of exposure to PCBs for receptors that use the floodplains of the formerly impounded areas. A summary of the types of data and strategies employed by MSU for the evaluation of exposure for the various receptor species is presented in Table 2 (included on the next page). Please address the following question regarding exposure and the supplemental issues.

Question 1. What are the relative strengths, limitations, and uncertainties associated with the methods employed by MSU to estimate the exposure of each receptor species to PCBs?

Supplemental Issues to Consider:

1a. Relative strength of various measures of exposure evaluated for each receptor when available individually and in combination. Examples of the types of data MSU considered include: a) literature based information on preferred prey; b) Site-specific data on receptor-specific prey items; c) site-specific bioaccumulation factor-based estimates of PCBs in prey; d) direct measures of PCBs in prey; and e) direct measures of PCBs in tissues/eggs of receptors.

1b. Effects of differing dietary preferences on extrapolating from the results of the MSU studies to other species. As an example, how may species-specific dietary preferences of the wrens or bluebirds evaluated in the MSU studies affect extrapolation of risk from these species to robins?

1c. The potential effects of future conditions, such as possible changes in habitat over time due to natural succession or anthropogenic changes to enhance recreational use. Some examples include lowered water table and reduced soil moisture content related to dam removal, transition to meadows including short grass habitat or succession to mature hardwood forest.

Table 2 Data Types Available for Refining PCB Exposure Estimates

Available Data	Proposed Use
Bird tissue data presented in Blankenship et al. 2005; Neigh et al. 2006b; Strause et al. 2007a, b, 2008; Zwiernik et al. 2007 and the summary of the MSU studies.	Develop estimate of avian body burden for use in dose model for upper trophic level species.
Shrew and other small mammal tissue data presented in CDM (2003), Blankenship et al. (2005), and the summary of the MSU studies.	Develop estimate of small mammal concentration for use in dose model for upper trophic level species.
Invertebrate tissue data presented in CDM (2003), Blankenship et al. (2005), and the summary of the MSU studies.	Develop estimate of invertebrate concentration for use in dose model for insectivores.
Egg concentrations from multiple avian species presented in CDM (2003), Neigh et al. 2006b, 2007; Strause et al. 2007a; Zwiernik et al. 2007 and the summary of MSU studies.	Compare to egg-based TRV.
Great horned owl pellet analysis and passerine nestling dietary composition analysis conducted as part of the MSU studies (Neigh et al. 2006a; Strause et al. 2008; Zwiernik et al. 2007).	Refine estimate of dietary composition for purpose of dose modeling.

Effects Assessment

This section addresses specific issues regarding the strategies employed in the MSU studies to evaluate potential effects of PCB exposure on receptors utilizing the floodplains of the formerly impounded areas. Please address the following questions regarding effects and the supplemental issues.

Question 2: What are the relative strengths, limitations, and uncertainties associated with the productivity assessments conducted by MSU on passerines and great horned owls (Neigh et al. 2006a, 2007; Strause et al. 2007a, 2008).

Supplemental Issues to Consider:

2a. Strengths and limitations of directly measuring productivity in the field compared to extrapolating from controlled laboratory studies.

2b. Extrapolation of results from field productivity studies to other species such as the American robin, which was the receptor species considered in the Baseline ERA (CDM 2003).

2c. Evaluation of potential causal factors (e.g. PCB concentrations, habitat differences, etc) associated with any difference in measures of productivity in passerines relative to the reference site.

Question 3: What are the relative strengths, limitations, and uncertainties associated with the hazard quotient calculations performed by MSU to evaluate potential risk to passerines, great horned owls, and shrews (Neigh et al. 2007; Strause et al. 2007a, 2008)?

Supplemental Issues to Consider:

3a. Choice of toxicity reference value (TRV), including relevance to receptor species and quality of study (e.g., duration, inclusion of sensitive life stages, exposure range, endpoints measured).

3b. Uncertainty resulting from extrapolating from laboratory study to field.

3c. Uncertainties in extrapolating from one species to another.

Applicability of the Investigations

This section addresses the overall quality of the data and the analyses presented in the MSU studies and their applicability for the evaluation of ecological risk and supporting risk management decisions for the floodplains of the formerly impounded areas. With this in mind please address the following questions.

Question 4: What are the relative strengths, limitations, and associated uncertainties that should be considered when evaluating the results of these studies as potential lines of evidence in future risk management decisions?

Supplemental Issues to Consider:

4a. Study designs including (but not limited to) sample size, replication, temporal duration, and aggregation of data.

4b. Data interpretation, including the choice and application of statistical methods.

4c. Approach for addressing natural variability.

4d. Identification and characterization of uncertainties.

4e. Adequacy of the data to support inferences on population-level effects.

Question 5: What are the relative strengths, limitations, and associated uncertainties that should be considered when extrapolating from the results of MSU studies conducted in the former Trowbridge Impoundment to the other formerly impounded areas of the Kalamazoo River?

Supplemental Issues to Consider:

5a. Numeric and spatial distributions of PCBs in floodplains of former impoundments.

5b. Habitat characteristics in floodplains of formerly impounded areas.

5c. Likely utilization of floodplains in formerly impounded area by the receptor species evaluated in MSU studies

Risk Management

This section addresses the potential usefulness of the MSU studies in supporting risk management decisions for the floodplains in the formerly impounded areas. It is possible that the results of the MSU studies would be incorporated as independent lines of evidence, along with data from the Baseline ERA (CDM 2003), in an Area-specific ecological risk assessment process. With this in mind please address the following question.

Question 6: Please comment on the applicability of the information presented in the MSU studies for informing risk management decisions.

4. References

ARCADIS. 2008. Baseline Ecological Risk Assessment Peer Review Scope of Work for the Allied Paper, Inc./Portage Creek/Kalamazoo River Superfund Site. March 2008.

Blankenship, A.L., M.J. Zwiernik, K.K. Coady, D.P. Kay, J.L. Newsted, K. Strause, C. Park, P.W. Bradley, A.M. Neigh, S.D. Millsap, P.D. Jones and J.P. Giesy. 2005. Differential Accumulation of Polychlorinated Biphenyl Congeners in the Terrestrial Food Web of the Kalamazoo River Superfund Site, Michigan. Environmental Science and Technology, Volume 39, pp. 5954-5965.

CDM. 1999. Initial Baseline Ecological Risk Assessment – Allied Paper, Inc./Portage Creek/Kalamazoo River Superfund Site. Prepared on behalf of the MDEQ Remediation and Redevelopment Division. June 1999.

CDM, 2003. Final (Revised) Baseline Ecological Risk Assessment – Allied Paper, Inc./Portage Creek/Kalamazoo River Superfund Site. Prepared on behalf of the MDEQ Remediation and Redevelopment Division. April 2003.

Neigh, A.M., M.J. Zwiernik, A.L. Blankenship, P.W. Bradley, D.P. Kay, M.A. MacCarroll, C.S. Park, P.D. Jones, S.D. Millsap, J.W. Newsted and J.P. Giesy. 2006a. Exposure and Multiple Lines of Evidence Assessment of Risk for PCBs Found in the Diets of Passerine Birds at the Kalamazoo River Superfund Site, Michigan. Human Ecological Risk Assessment, Volume 12, pp. 924-946.

Neigh, A.M., M.J. Zwiernik, P.W. Bradley, D.P. Kay, P.D. Jones, R.R. Holem, A.L. Blankenship, K.D. Strause, J.L. Newsted and J.P. Giesy. 2006b. Accumulation of Polychlorinated Biphenyls (PCBs) from Floodplain Soils by Passerine Birds. Environmental Toxicology and Chemistry, Volume 25, pp. 1503-1511.

Neigh, A.M., M.J. Zwiernik, C.A. Joldersma, A.L. Blankenship, K.D. Strause, S.D. Millsap, J.L. Newsted and J.P. Giesy. 2007. Reproductive Success of Passerines Exposed to PCBs Through the Terrestrial Food Web of the Kalamazoo River. Ecotoxicology and Environmental Safety, Volume 66, pp. 107-118.

Strause, K.D., M.J. Zwiernik, S.H. Im, P.W. Bradley, P.P. Moseley, D.P. Kay, C.S. Park, P.D. Jones, A.L. Blankenship, J.L. Newsted and J.P. Giesy. 2007a. Risk Assessment of Great Horned Owls (Bubo virginianus) Exposed to Polychlorinated Biphenyls (PCBs) and DDT along the Kalamazoo River, Michigan. Environmental Toxicology and Chemistry, Volume 26, pp. 1386-1398.

Strause, K.D., M.J. Zwiernik, S.H. Im, J.L. Newsted, D.P. Kay, P.W. Bradley, A.L. Blankenship, L.L. Williams and J.P. Giesy. 2007b. Plasma to egg conversion factor for evaluating polychlorinated biphenyl and DDT exposures in Great Horned Owl and Bald Eagles. Environmental Toxicology and Chemistry, Volume 26, pp. 1399-1409.

Strause, K.D., M.J. Zwiernik, J.L. Newsted, A.M. Neigh, S.D. Millsap, C.S. Park, P.P. Moseley, D.P. Kay, P.W. Bradley, P.D. Jones, A.L. Blankenship, J.G. Sikarskie and J.P.Giesy. 2008. Risk Assessment Methodologies for Exposure of Great Horned Owls (Bubo virginianus) to PCBs on the Kalamazoo River, Michigan. Integrated Environmental Assessment and Management. In Press, Volume 4 pp. 24-40.

Zwiernik MJ, Strause KD, Kay DP, Park CS, Blankenship AL, Giesy JP. 2007. Site-specific assessments of environmental risk and natural resource damage based on Great Horned Owls. *Human Ecol. Risk Assess.* 13:966-985.

Section 3

Kalamazoo River Ecological Risk Studies Peer Review Panel

Summary of MSU Studies

May 2008

Ecological Consequences of PCBs in the Exposed Sediments of Formerly Impounded Areas of the Kalamazoo River

Overview of Studies Conducted by Michigan State University

Prepared on behalf of the Kalamazoo River Study Group and the USEPA by:

Dr. John Giesy, Ph.D.
Professor Emeritus, Michigan State University

Dr. Matthew Zwiernik, Ph.D.
Assistant Professor, Michigan State University

Table of Contents

1 Introduction 1-1

1.1 Overview of MSU Studies 1-4
1.2 Quality Assurance and Quality Control Procedures 1-6
1.3 Report Overview 1-7

2 Assessment of Potential Risk to Terrestrial Omnivorous/Insectivorous Passerines (Eastern Bluebird and House Wren) 2-1

2.1 Measures of Reproductive Success 2-2
2.2 Hazard Quotient Analyses 2-4
2.2.1 Exposure Calculations 2-4
2.2.1.1 Dietary Exposure Estimates 2-5
2.2.1.2 Tissue Based Exposure Estimates 2-6
2.2.2 Effect Levels (i.e., Toxicity Reference Values) 2-7
2.2.2.1 Dietary TRVs 2-7
2.2.2.2 Egg-based TRVs 2-8
2.2.3 Results 2-11
2.2.3.1 Dietary HQs 2-11
2.2.3.2 Tissue Based HQs 2-11
2.3 Assessment of Terrestrial Passerine Risk Based on Multiple Lines of Evidence 2-12
2.4 Uncertainty 2-13
2.4.1 Spatial applicability of terrestrial passerine data 2-13
2.4.2 Representativeness of Selected Study Species 2-13

3 Assessment of Potential Risk to Carnivorous Terrestrial Birds (Great Horned Owl) 3-1

3.1 Hazard Quotient Analyses 3-2
3.1.1 Exposure Calculations 3-3
3.1.1.1 Dietary Exposure Estimate 3-3
3.1.1.2 Tissue Based Exposure Estimates 3-5
3.1.2 Effects Levels (i.e., Toxicity Reference Values) 3-5
3.1.2.1 Dietary TRVs 3-6
3.1.2.2 Egg-based TRVs 3-6
3.1.3 Results 3-7
3.1.3.1 Dietary HQs 3-7

3.1.3.2 Egg Based HQs 3-7
3.2 Measures of Reproductive Success 3-7
3.3 Summary and Discussion 3-9
3.4 Uncertainty 3-10
3.4.1 Dietary Exposures 3-10
3.4.2 Toxicity Reference Values (TRVs) 3-11
3.4.3 Productivity Assessment 3-11

4 Summary 4-1

5 References 5-1

Tables

Table 1-1 Terrestrial Receptors Considered in CDM 2003 and MSU's Studies
Table 2-1 Nest Productivity Measurements for Eastern Bluebirds and House Wrens at the Former Trowbridge Impoundment and the Ft. Custer Reference Area
Table 2-2 ΣPCB and TEQ Concentrations in Biota and Average Potential Daily Dose for Eastern Bluebirds and House Wrens from the Former Trowbridge Impoundment
Table 2-3 Mean Concentrations of ΣPCBs and TEQs in Passerine Tissues in the Former Trowbridge Impoundment
Table 2-4 Toxicity Reference Values for Dietary and Egg-based Exposure of Avian Species to ΣPCBs and TEQs
Table 2-5 Summary of Hazard Quotients for Terrestrial Passerines in the Former Trowbridge Impoundment
Table 3-1 Great Horned Owl Site-Specific Spring Diet Composition
Table 3-2 Geometric Mean and U95 CL Concentrations in Prey Items Collected from the Former Trowbridge Impoundment
Table 3-3 Range of Average Potential Daily Doses
Table 3-4 Measured Egg Concentrations in Resident Great Horned Owls
Table 3-5 Site-Specific Dietary Hazard Quotient Values for Great Horned Owls
Table 3-6 Relative Abundance and Productivity of Resident Great Horned Owls at Ft. Custer and the Former Trowbridge Impoundment

Figures

Figure 1-1 Formerly Impounded Areas of the Kalamazoo River and the Fort Custer Reference Area

Figure 1-2 Sample Locations in the Vicinity of the former Trowbridge Impoundment

Figure 2-1 Lines of Evidence Evaluated by MSU for Passerine Species

Figure 2-2 Dietary Composition of Passerine Species Evaluated

Figure 3-1 Lines of Evidence Evaluated by MSU for the Great Horned Owl

1 Introduction

The Kalamazoo River is located in southwestern Michigan, and flows in a northwesterly direction, through Kalamazoo and Allegan Counties to Lake Michigan. It drains approximately 2,000 square miles and is fed by more than 400 miles of tributaries. The Allied Paper, Inc./Portage Creek/Kalamazoo River Superfund Site (Site), includes 80 miles of the Kalamazoo River from Morrow Lake Dam downstream to Lake Michigan, including the river banks and formerly impounded floodplains, as well as a 3-mile stretch of Portage Creek and four areas used as disposal sites for paper-making residuals. The contaminants of concern for the Site are polychlorinated biphenyls (PCBs), which have been found in sediment, surface water, floodplain soils, and aquatic and terrestrial biota.

An initial Baseline Ecological Risk Assessment (Baseline ERA), prepared on behalf of the Michigan Department of Environmental Quality (MDEQ), was released in June of 1999 (Camp Dresser and McKee [CDM], 1999). Subsequent to the release of the initial Baseline ERA, the Kalamazoo River Study Group[1] (KRSG) began funding a series of studies by Michigan State University (MSU) to develop Site-specific information on exposure of representative receptor species to PCBs and the potential ecological risk posed by that exposure. Professor John P. Giesy, then a Distinguished Professor of Zoology at MSU, was the principal investigator for the MSU studies and Dr. Matthew Zwiernik was the project leader. The research was conducted through the auspices of the National Food Safety and Toxicology Center (NFSTC) and the Center for Integrative Toxicology (CIT) at MSU. Currently, Dr. Giesy is Distinguished Professor Emeritus of Zoology at MSU and Professor and Canada Research Chair in Environmental Toxicology at the University of Saskatchewan, Saskatoon, Saskatchewan, Canada. Professor Zwiernik is currently a Professor at the NFSTC and Adjunct Professor of Zoology and of Animal Science at MSU.

At MSU, we designed our studies to provide Site-specific information on exposure and effects of PCBs on selected wildlife species. The information was collected with the primary goal of addressing uncertainty in the Baseline ERA (CDM, 1999) by reducing the reliance on assumptions in lieu of Site-specific observations. Our intent was to develop multiple, Site-specific, independent lines of evidence to supplement those evaluated in the Baseline ERA (CDM, 1999). As an example, we determined dietary exposure for several avian species based on Site-specific observations of dietary composition and measurement of PCB concentrations in dietary items for the species being evaluated. In addition, we evaluated exposure directly by measuring the PCB concentrations in critical tissues (e.g., eggs) of the same ecological receptors. Hazard assessments were then

[1] Georgia-Pacific Corporation and Millennium Holdings, LLC are collectively referred to as the Kalamazoo River Study Group, or KRSG

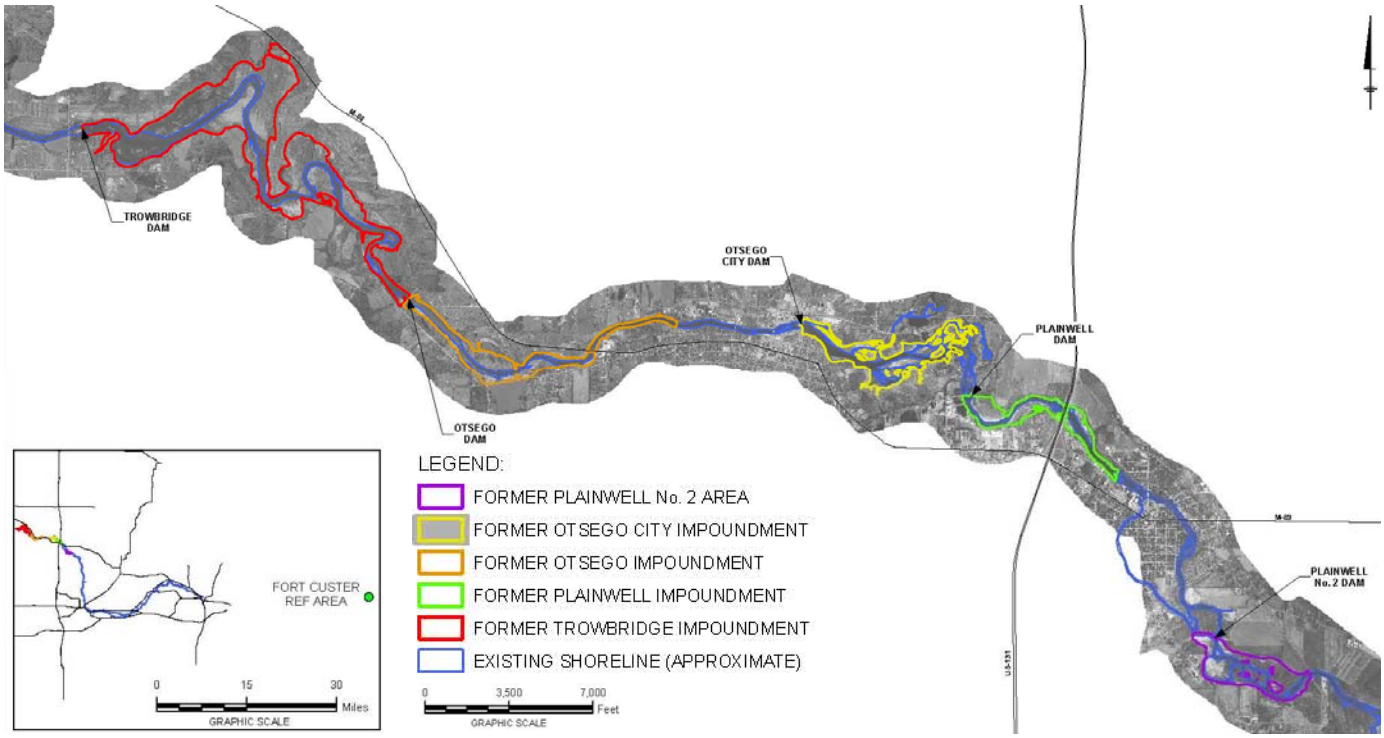
made for these species based on the two distinct estimates of exposure, and compared to Site-specific measures of effects including fecundity, growth, and productivity.

Our studies were conducted largely in the floodplains of the former Trowbridge Impoundment. There are five areas of the Site – the historically inundated area around the Plainwell No. 2 Dam and the formerly impounded areas associated with the dams at Plainwell, Otsego City, Otsego, and Trowbridge – where the current floodplains include historically submerged sediments that were exposed when the dams were opened, dismantled, or taken down to the sills. These exposed sediments, which had accumulated PCBs while submerged, became the soils of the newly exposed floodplains. The history of these impoundments, summaries of the PCB concentrations and distributions, habitat characterization, and descriptions of wildlife are presented in the report *Characteristics of the Formerly Impounded Areas* (ARCADIS 2008; included in Volume 1, Section 4 of the Charge and Supporting Materials Binder). We chose the former Trowbridge Impoundment because:

1) the available habitat types, presence of receptor species, and mixture of PCB congeners found there are generally consistent with those of the other formerly impounded areas;
2) PCB concentrations in exposed sediments were comparable to those of the former Otsego and Plainwell Impoundments; and,
3) it is the largest of the formerly impounded areas and consequently provides the opportunity to study populations of receptors whose foraging is most concentrated in habitats developed on exposed sediments.

In addition, we established a reference site in the Fort Custer State Recreational Area, which is some 40 kilometers (km) upstream of the former Trowbridge Impoundment and well upstream of any Site-related sources of PCBs. We selected the Fort Custer Recreation Area as a reference site because the results of historical investigations (CDM, 1993 and 1997; Blasland, Bouck & Lee, Inc. [BBL], 1993, 1994a, b, c; Giesy et al., 1994) showed low/background concentrations of PCBs and other contaminants. The reference site provided similar, but not identical, floodplain habitat with minimal exposure to PCBs. Parallel studies were conducted in the former Trowbridge Impoundment and the reference site to distinguish potential Site-related effects. The locations of the former Trowbridge Impoundment, the Fort Custer reference site, and the four other formerly impounded areas are shown on Figure 1-1.

Figure 1-1. Formerly Impounded Areas of the Kalamazoo River and the Fort Custer Reference Area



Although the studies were conducted to gather data that could be used to evaluate potential risks associated with PCB exposure in both aquatic (Millsap et al., 2004; Kay et al., 2005; Neigh et al., 2006c and d) and terrestrial food webs, the focus of this document is to provide a summary of only those efforts related to analyses of potential risks to terrestrial receptors who might come in contact with the exposed sediments of the floodplain habitat in the formerly impounded/historically inundated areas.

The results of our studies for terrestrial floodplains have been described in eight publications in the peer-reviewed literature (included in Volume 1, Section 3 of the Charge and Supporting Materials Binder) and have provided the basis for two Ph.D. dissertations. These results and all of the underlying data have been provided to the United States Environmental Protection Agency (USEPA) and MDEQ for review and consideration. However, USEPA and MDEQ were not involved in the design or implementation of these studies, and the Final Revised Baseline ERA (CDM, 2003; included in Volume 2, Section 5 of the Charge and Supporting Materials Binder) was completed in April 2003 before our studies were finalized. Therefore, the KRSG and USEPA have agreed to conduct a peer review of our ecological studies performed to date with respect to floodplain soils, prior to consideration of the results of these studies as independent lines of evidence in subsequent ecological risk assessments to be conducted in the floodplains of formerly impounded areas of the Kalamazoo River.

This document summarizes our studies evaluating risk to populations exposed to PCBs in soils of the formerly impounded areas. These include two passerine species, (eastern bluebird [*Sialia sialis*] and house wren [*Troglogytes aedon*]), (Neigh et al., 2006a, b, 2007) and the great-horned owl (*Bubo virginianus*) (Zwiernik et al., 2007; Strause et al., 2007a, b, 2008). Full details of the methods applied and the results observed can be found in the specific studies referenced, which are included in Volume 1, Section 3 of the Charge and Supporting Materials Binder.

1.1 Overview of MSU Studies

We performed a series of comprehensive field studies to evaluate potential risk to representative ecological receptors posed by elevated concentrations of PCBs in soils – specifically the exposed former sediments that are now in the floodplain – in the formerly impounded areas of the Kalamazoo River. These studies, which are included in Volume 1, Section 3 of the Charge and Supporting Materials Binder, were intended to support risk management decisions regarding these soils. As recognized by the USEPA (USEPA, 1994), site-specific field studies are important for sound decision making when complex systems are involved. This is due to the level of uncertainty involved in predicting wildlife contaminant exposure and the potential for effects in complex systems. Data were collected to address spatial and temporal considerations, receptor priority, and, utility as exposure- and effect-measurement endpoints.

Prior to our studies, substantial data on the concentrations and spatial distributions of PCBs in exposed sediments/floodplain soils of the formerly impounded areas were available (BBL, 1994a and b). However, we observed uncertainties that could not be resolved with the existing data sets and designed Site-specific studies to address these uncertainties and provide multiple lines of evidence for evaluation of risk. Each line of evidence was designed as a key measurement endpoint that could be directly associated with assessment endpoints established in the Baseline ERA (CDM, 1999). Although the research was conducted as a student-based research program, we administered a rigorous quality assurance (QA) program to ensure usability of the studies for both scientific publication and risk assessment.

As described above, our studies were designed to provide Site-specific data, encompassing multiple years and locations for the purpose of facilitating a weighted multiple line-of-evidence approach (Fairbrother, 2003; Hull et al., 2006). Studies included productivity measures for the two passerine species and the great horned owl, as well as measures of Site-specific dietary and tissue-based exposure. Site-specific dietary exposure was assessed by determining the Site-specific dietary composition of selected receptor species and then sampling those dietary items from where they were being consumed and analyzing them for PCBs. These data were then used

to reconstruct the Site-specific daily dietary exposure for each receptor for comparison to a dietary toxicity reference value (TRV). This comparison resulted in a numerical hazard quotient (HQ) that was used in conjunction with other lines of evidence to evaluate potential risk to the specified receptor. Likewise, the tissue-based assessment was evaluated based on direct measurements of PCBs in tissues compared to tissue-based TRVs. Dietary and tissue-based assessments were done on both total PCBs (ΣPCBs) and toxicity equivalency quotients (TEQ) basis for avian species (Neigh et al., 2006 a, b; Strause et al., 2008). Concentrations of TEQ in bird tissues were calculated by summing the product of the concentrations of individual PCB congeners and their respective, bird-specific World Health Organization (WHO) toxic equivalency factors (TEF) (Van den Berg et al., 1998). The results of these assessments are summarized in this document, and a more detailed discussion of each assessment can be found in the publications (Neigh et al., 2006a, b; Strause et al., 2008), which are included in Volume 1, Section 3 of the Charge and Supporting Materials Binder.

Individual receptor species were prioritized as to importance based on intensity of exposure, relative sensitivity to PCBs, prevalence of preferred habitat within the Kalamazoo River areas of concern (AOC), and appropriateness as a surrogate species, with special considerations given to those species previously identified (1999 initial and 2003 Final Revised Baseline ERA) as receptors of concern (see Table 1-1, below).

Table 1-1. Terrestrial Receptors Considered in CDM 2003 and MSU's Studies

Comprehensive Receptor List	Feeding Guild	Final Revised Baseline ERA (CDM 2003)	MSU's Studies
Omnivorous/Insectivorous Birds			
House Wren	Terrestrial insectivore (passerine)		√
Bluebirds	Terrestrial omnivore (passerine)		√
American Robin	Terrestrial omnivore (passerine)	√[1]	√
Carnivorous Birds			
Great Horned Owl	Terrestrial avian carnivore (raptor)	√	√
Small Insectivorous/Omnivorous Mammals			
Deer Mouse	Terrestrial omnivore/herbivore (mammal)	√[2]	
Carnivorous Mammals			
Red Fox	Terrestrial omnivore	√[2]	

Notes:
1. Receptor evaluated as a secondary representative species for receptor class
2. No significant risk identified in Final Revised Baseline ERA (CDM, 2003).

Assessments of exposure through the diet focused on six biological sampling areas (BSAs), each proximal to the Kalamazoo River, associated with similar habitats, entirely within the 100-year floodplain, and all on property

owned by the state of Michigan. The two reference site BSAs were located within the Fort Custer Recreation Area. The remaining four BSAs were located in the former Trowbridge Impoundment.

Terrestrial dietary items evaluated in our studies were collected from or in the vicinity of these BSAs to provide a site-specific measure of actual dietary exposures. Tissue-based exposure methodologies were used to directly quantify embryo (egg) and developmental (nestling) exposures, confirm dietary exposures, and address uncertainties associated with foraging range and residence time. Tissues analyzed included those of adults (birds and mammals), juveniles (birds and mammals), nestlings (e.g. passerine chicks, raptor plasma), and eggs (all avian receptors). Small mammals for analysis were collected entirely from within the BSAs, passerine tissues were collected from within or proximal to BSAs, and raptor tissues were collected within, proximal, and outside the BSAs. The sample locations for all data collected by MSU from the Site study area (i.e., the former Trowbridge Impoundment) are shown in Figure 1-2 (provided at the end of this section).

1.2 Quality Assurance and Quality Control Procedures

A QA program was developed and strictly applied to ensure that the methods used were consistent with the available guidance and state of the science so that the data would be of appropriate quality to support risk assessment and risk management activities. A Sampling and Analysis Plan (SAP) (included in Volume 2, Section 5 of the Charge and Supporting Materials Binder) was developed to govern our studies. In addition, before any work was conducted, detailed standard operating procedures (SOPs) (included in Volume 2, Section 5 of the Charge and Supporting Materials Binder) were developed for all phases of the research. The details of the methods, and in particular the QA guidance, are described in those documents. The SAP, supporting SOPs, and the Quality Assurance Project Plan (QAPP; included in Volume 2, Section 5 of the Charge and Supporting Materials Binder) were written based on USEPA guidance for studies at Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) sites. The results have been subject to extensive internal and external peer review through the processes outlined in the QAPP. In addition, all data were subject to the highest level quality assurance/quality control (QA/QC) evaluation. This was accomplished through oversight from ENTRIX Inc, which provided QA planning, conducted QA audits, and archived samples and data such that they could be applied to decision-making. The database for our studies, as well as all of the raw data including the QA reports for all of the analytical results have been provided to USEPA and the State of Michigan and are available upon request. These reports include all of the chromatograms, calculations, data reductions, and QA narrative reports, as well as data summaries. In addition, extensive pictorial documentation of locations and samples (including pictures of the sample, date and time, and global positioning system [GPS] location), field notes (scanned from field notebooks), field record sheets, subsequent sample tracking sheets, and

chain of custody records are available. For instrumental analyses, there are electronic and hard copy archives of all sample tracking sheets used to track the progress of a sample from the field, to archive, and through the analytical procedures. This additional information can be provided upon request. The SOPs (included in Volume 2, Section 5 of the Charge and Supporting Materials Binder) describe how these procedures and records were used.

1.3 Report Overview

Section 1: Introduction

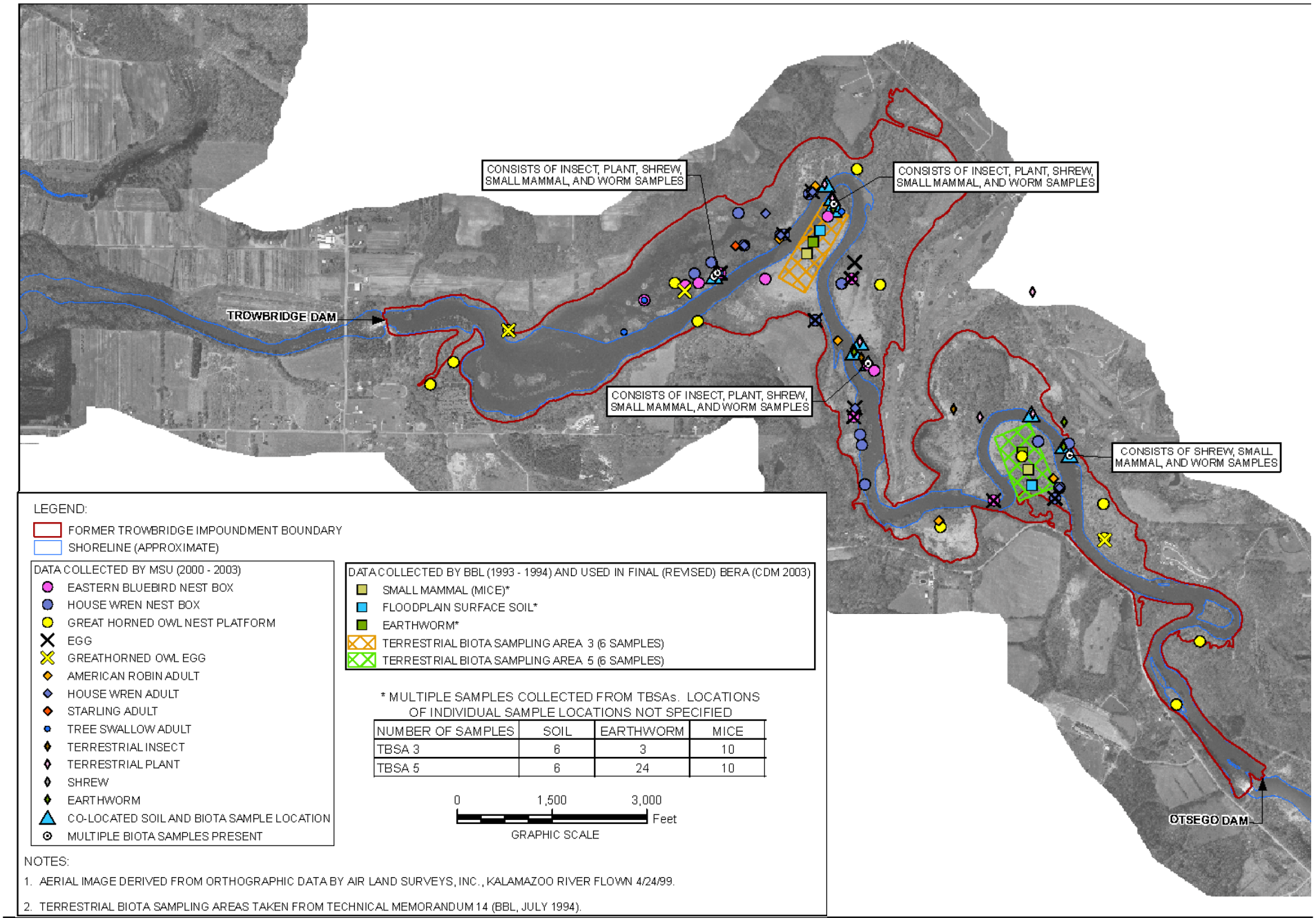
Section 2: Assessment of Potential Risk to Terrestrial Omnivorous/Insectivorous Passerine Birds (Eastern Bluebird and House Wren)

Section 3: Assessment of Potential Risk to Carnivorous Terrestrial Birds (Great Horned Owl)

Section 4: Conclusions

Section 5: References

Figure 1-2. Sample Locations in the Vicinity of the former Trowbridge Impoundment



NUMBER OF SAMPLES	SOIL	EARTHWORM	MICE
TBSA 3	6	3	10
TBSA 5	6	24	10

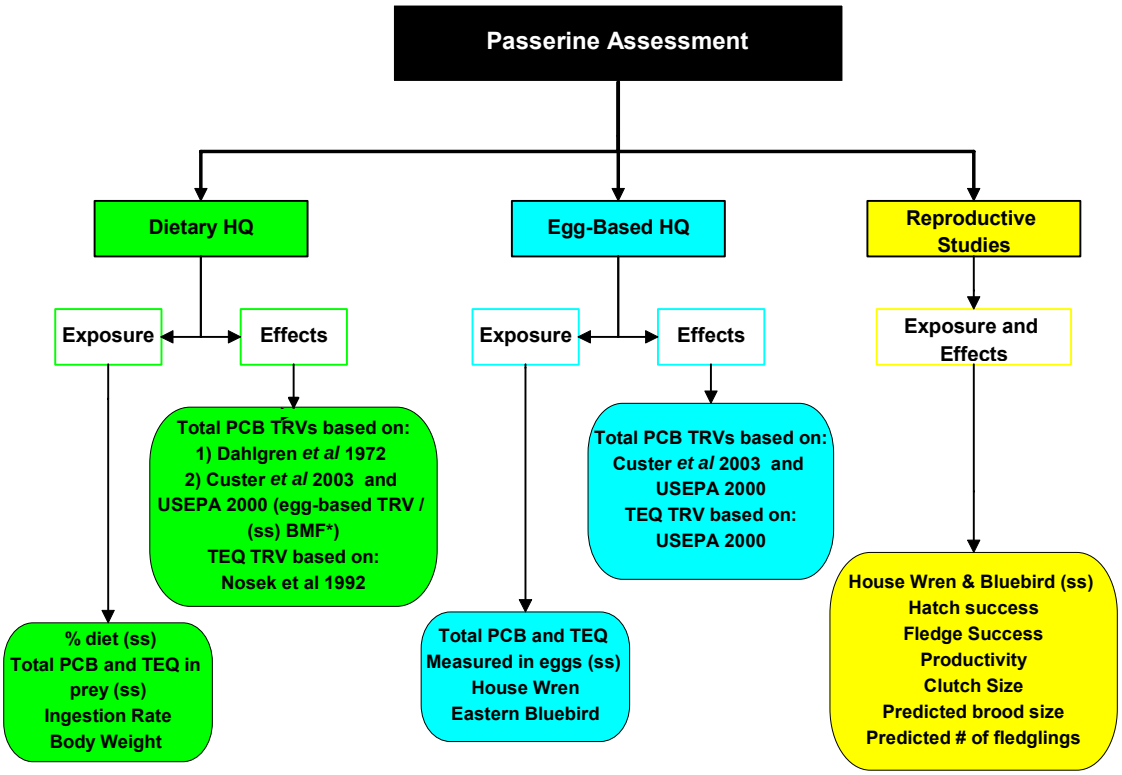
2 Assessment of Potential Risk to Terrestrial Omnivorous/Insectivorous Passerines (Eastern Bluebird and House Wren)

In the Final Revised Baseline ERA (CDM, 2003), the American robin was identified as one of the representative receptors for evaluation of terrestrial avian species, specifically for the omnivorous feeding guild. The Final Revised Baseline ERA (CDM, 2003) found that "omnivorous birds that consume substantial amounts of soil invertebrates (e.g., earthworms) would be at significant risk if foraging takes place in mostly contaminated areas." This conclusion was based primarily on a dietary HQ analysis that compared Site-wide measures of PCBs concentrations in plant and earthworm tissues to literature-derived TRVs based on domestic chickens.

While the results presented in the Final Revised Baseline ERA (CDM, 2003) were not complete at the time that our studies were undertaken, preliminary results presented in the Final Draft ERA (CDM, 1996) and in the initial draft of the Baseline ERA (CDM, 1999) were available and indicated that omnivorous birds feeding in the floodplain of the Kalamazoo River AOC would potentially be at risk from exposure to PCBs. The passerine studies were undertaken to provide additional Site-specific information regarding potential risk to omnivorous birds feeding in the floodplain to support risk management decision making. The available lines of evidence for this receptor group include direct measures of reproductive output of the eastern bluebird and house wren as well as HQs based on both dietary and tissue-based exposure (Neigh et al., 2006a and b, 2007).

The lines of evidence and associated inputs evaluated in our assessment are summarized in Figure 2-1 (below). Based on a number of species characteristics – including dietary composition, foraging characteristics, Site use, exposure potential, and ease of study – the house wren was selected as an example of an insectivore. The eastern bluebird was selected as the example omnivorous terrestrial passerine based on exposure potential, perceived sensitivity, and societal value. Both of these species are amenable to nesting in provided nest boxes, which allowed control of the feeding environment and facilitated collection of samples of adults, eggs, chicks, and food samples (boluses) from which dietary composition and PCB content could be determined.

Figure 2-1. Lines of Evidence Evaluated by MSU for Passerine Species



(ss) = site specific

* BMF = site- and species-specific biomagnification factor to estimate a dietary exposure from an egg concentration

2.1 Measures of Reproductive Success

Studies of reproductive performance of the house wren and eastern bluebird were conducted over the course of three consecutive years (Neigh et al., 2007). A total of 17 bluebird and 34 house wren nests were established at the Site study area (i.e., the former Trowbridge Impoundment) over the course of the study period, while 57 bluebird and 71 wren nests were completed at the reference area (Fort Custer). Nest boxes were monitored for clutch initiation, clutch size, hatch day, hatching success (percentage of eggs hatched), fledging success (percentage of nestlings fledged per nestling hatched), and productivity (percentage of nestlings fledged per egg laid). Additional comparisons included nest attentiveness and temporal comparisons of nestling growth.

House wrens made a greater number of nesting attempts than eastern bluebirds at both the Site and reference study areas. This difference was expected based on observations of species abundance and available habitat. Abandonment rates for both species ranged between 0% and 25%, which was similar to the range reported for unexposed bluebirds elsewhere (Rustad, 1972). Reproductive parameters for house wrens at each location were

not significantly different between years, therefore all years were combined. Over the duration of the study there were no differences between the Site study area and reference area for clutch size, hatching success, or overall productivity. The only statistically significant difference noted in house wren reproduction was a significant increase in fledging success for nests in the Site study area.

Clutch size, hatching success, and fledging success were also not different between the Site and reference study areas for eastern bluebirds over the duration of the study. However, eastern bluebird productivity was significantly less in the Site study area ($p<0.05$, Mann-Whitney U). This was largely based on a single female that twice attempted to produce a brood in 2001 without success but did not abandon the eggs. This one female accounted for a 40% decrease in productivity during that year, and if removed from the data set, the difference is not significant. Even when including this individual, the ranges of productivity (as identified by the mean and standard deviations) for the Site and reference study areas overlap. The results of the productivity analysis are summarized below in Table 2-1.

Other observational data for these studies included measures of individual condition. There were no gross physical abnormalities observed in adults (n=366) or nestlings (n=542) for any of the passerine species monitored during the study. There were no differences between the Site and reference study areas in adult nest attentiveness or in any of the nestling growth parameters for either species over the duration of the study. Moreover, there were no embryo abnormalities noted in any of the 305 eastern bluebird eggs and 607 house wren eggs examined. Additionally, there were no differences in egg mass for either species, or in egg length in house wrens. Egg lengths for the eastern bluebird were significantly greater at the reference site ($P<0.05$ Students t test); however, the difference in mean length was less than 1 millimeter (mm) which was less than 8% of the total length, and the egg length range of means plus one standard deviation overlapped. There were abnormalities of eggs noted in house wren eggs at both the Site and reference areas, including irregular shape, abnormal texture, and desiccated contents.

As discussed previously, measures of productivity were not significantly different between the Site and reference study areas for house wrens. Productivity for bluebirds was slightly less at the Site study area than at the reference area, largely due to small sample size at the Site study area and the resulting impact of the single female in 2001 that twice attempted to produce a brood without success but did not abandon the eggs. Measures of the condition of individuals were normal with the exception of the house wren egg abnormalities mentioned above. Given that no abnormalities were noted in any embryos or nestlings and that nestling growth was not affected, these egg abnormalities do not appear to have had an adverse impact on productivity.

Table 2-1. Nest Productivity Measurements for Eastern Bluebirds and House Wrens at the Former Trowbridge Impoundment and the Ft. Custer Reference Area

	Trowbridge[1]		Ft. Custer[1]	
	n	Mean	n	Mean
Eastern Bluebird				
Hatching success[2]	14	0.59	49	0.79
Fledging success[3]	9	0.83	45	0.96
Productivity[4]	13	0.47*	49	0.76
Clutch size	18	3.6	57	4.2
Predicted brood size	10	3.3	45	3.8
Predicted number of fledglings	8	3	43	3.9
House Wren				
Hatching success[2]	31	0.64	60	0.81
Fledging success[3]	25	1.0*	59	0.92
Productivity[4]	31	0.64	60	0.74
Clutch size	36	5.4	71	5.7
Predicted brood size	25	4.6	59	5
Predicted number of fledglings	25	4.6	56	4.8

[1] Sample size (n) and mean values are for all clutches. For information on early and late clutches, see Table 3 in Neigh et al. 2007
[2] Hatching success is calculated as the number of eggs hatched per egg laid
[3] Fledging success is calculated as the number of fledglings per nestling hatched
[4] Productivity is calculated as the number of fledglings per egg laid
* Mean of the Trowbridge population is statistically different from the Ft. Custer population ($P<0.05$)

2.2 Hazard Quotient Analyses

In addition to the direct measures of productivity discussed above, terrestrial insectivorous and omnivorous birds were assessed based on an HQ analyses as additional lines of evidence. The HQ analyses included the comparison of estimates of exposure to appropriate TRVs. Each of these elements and the resulting HQs are discussed in the following sections. Exposures and risks were calculated using both ΣPCBs and TEQ approaches to estimate exposures.

2.2.1 Exposure Calculations

Exposure of passerine birds to ΣPCBs was assessed in terms of daily dose for dietary exposure and direct measures of tissue concentrations in adults, nestlings, and eggs for tissue-based exposure. Corresponding TRVs are not available for adult and nestling tissues; however, such values are available for dietary exposure and eggs. Consequently, the exposure estimates described below focus on dietary and egg-based exposure. Details of other tissue measurements can be found in Neigh et al. (2006a and b).

2.2.1.1 Dietary Exposure Estimates

Dietary exposure for the house wren and eastern bluebird were estimated by calculating an average potential daily dose (APDD) for each species (Table 2-2). The dose model used is consistent with the model recommended in the USEPA's Wildlife Exposure Factors Handbook (USEPA, 1993) and is described in more detail in Neigh et al. (2006a). Site-specific inputs to the APDD for each receptor species include Site-specific dietary composition of each target species (Figure 2-2) and concentrations of ΣPCBs and TEQs in each identified dietary item (Table 2-2). Composition of the diet of each passerine was determined by quantifying stomach contents and bolus compositions. Identified prey items were collected from the locations where they were being consumed (i.e., from six individual BSAs that were co-located with passerine study areas) on sampling dates that roughly corresponded to spring, early summer, and late summer in 2000 and 2001. The nest box locations and prey tissue sample locations are provided in Figure 1-2. Additional details of the daily dose calculation and inputs can be found in Neigh et al. (2006a). Although these studies focused on house wrens and bluebirds as representatives of select feeding guilds, the Site-specific dietary item sampling data could be used to estimate dietary exposures for many potential receptors. The representativeness of these two species is discussed in more detail in Section 2.4.

Table 2-2. ΣPCB and TEQ Concentrations in Biota and Average Potential Daily Dose for Eastern Bluebirds and House Wrens From the Former Trowbridge Impoundment

	Number of Samples	Mean ΣPCBs (± std deviation)	Number of Samples	Mean Avian TEQs (± std deviation)
Biota Concentrations		**(mg/kg)[1]**		**(ng/kg)[1]**
Plants	28	0.023 ± 0.044	--	--
Terrestrial Invertebrates[2]	30	0.34 ± 0.57	30	80 ± 200
Fresh Earthworms	18	1.72 ± 1.75	18	238 ± 255
Depurated Earthworms	14	1.26 ± 1.13	14	154 ± 186
Average Potential Daily Dose		**(mg/kg/day)**		**(ng/kg/day)**
Eastern Bluebird APDD		0.51		70
House Wren APDD		0.13		31

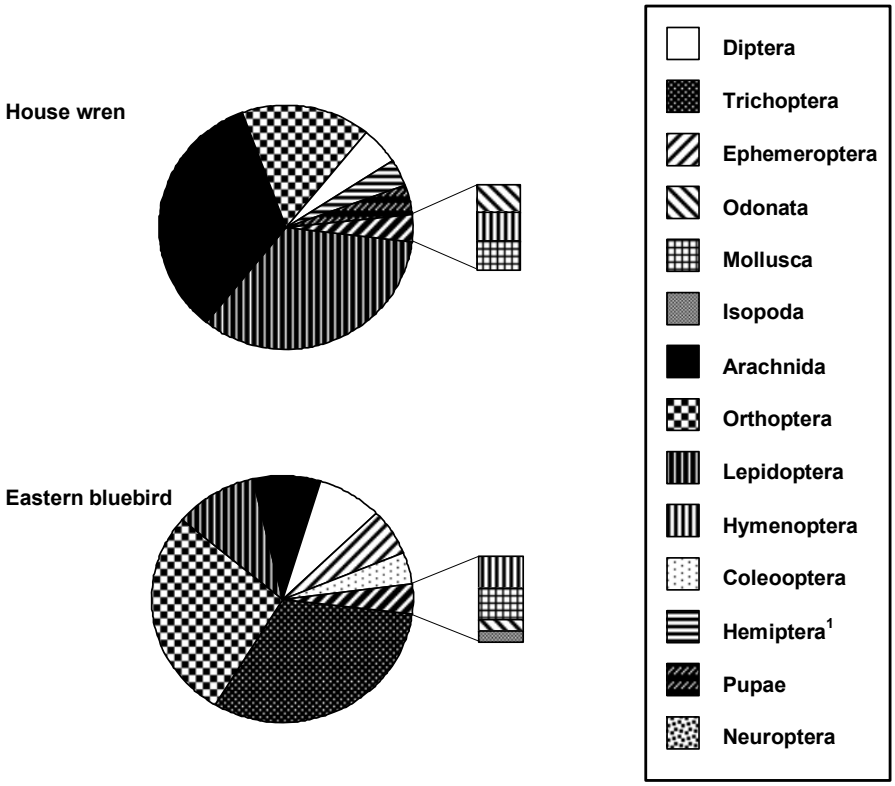
Notes:

1. PCBs concentrations are wet weight basis for biota
2. Earthworms were treated separately from other terrestrial invertebrates

mg/kg – milligrams per kilogram mg/kg/day – milligrams per kilogram per day

ng/kg – nanograms per kilogram ng/kg/day – nanograms per kilogram per day

Figure 2-2. Dietary Composition of Passerine Species Evaluated



Notes:
[1]The order Hemiptera also includes the order Homoptera.

2.2.1.2 Tissue Based Exposure Estimates

Samples of eggs were collected for eastern bluebirds and house wrens during the spring and summer of 2001, 2002, and 2003. A maximum of one tissue sample was collected randomly from each nest box for quantification of ΣPCB and TEQ concentrations. In addition, abandoned and addled eggs were taken for additional measurements of ΣPCB and TEQ concentrations. Eggs were sampled 7 to 10 days after they were laid. The detected concentrations of ΣPCBs in eggs and adult tissues are shown in Table 2-3. As previously discussed, no TRVs are available for comparison to concentrations in adults, therefore exposure estimates based on adult tissue were used only to assess relative exposure among the study species and other passerine species that use the Kalamazoo River floodplain (i.e., the American robin and tree swallow). The tree swallow had the greatest concentrations of ΣPCBs, followed by the house wren, and then the robin. This is likely a result of a greater proportion of the tree swallow's diet consisting of aquatic insects, which were found to have some of the highest PCB concentrations for insects measured (Kay et al., 2005).

Table 2-3. Mean Concentrations of ΣPCBs and TEQs in Passerine Tissues in the Former Trowbridge Impoundment

	Number of Samples	PCBs (mg/kg)	Number of Samples	Avian TEQ (ng/kg)
Eastern bluebird[1]				
Egg	7	8.3 (5.1)	5	77 (82)
House wren[1]				
Egg	14	5.8 (4.4)	11	360 (330)
Adult	9	3.2 (2.1)	9	110 (57)
Tree Swallows[2]				
Adult	9	8.7(9.7)	9	0.29 (0.23)
American Robin[3]				
Adult	17	0.92 (1.2)	17	3.9 (4.4)

Notes:
( ) - standard deviation
[1] Mean values taken from Neigh et al., 2006b
[2] Mean values taken from Neigh et al., 2006c
[3] Mean values taken from Blankenship et al., 2005

2.2.2 Effect Levels (i.e., Toxicity Reference Values)

TRVs were derived from information in the literature according to USEPA guidance. TRVs discussed in this report were based on concentrations of ΣPCBs and TEQs in the diet and eggs. The selection of TRVs was based on several criteria to determine their appropriateness for use in this study. These criteria included: 1) the use of wildlife species rather than a laboratory species where possible; 2) chronic exposure and sensitive life stages; 3) the evaluation of ecologically relevant endpoints; 4) minimal co-contamination; 5) multi-year studies; 6) and reported or calculated ΣPCB concentrations. There were few studies available for passerine species and there were no studies with a similar passerine exposure profile. Thus, a range of TRVs were developed to address possible variability in species-sensitivity and test conditions. The selection of TRVs for evaluation of terrestrial feeding passerines in the Kalamazoo River floodplain is described in the following two sections, and the selected values are summarized in Table 2-4, below.

2.2.2.1 Dietary TRVs

The dietary TRVs for ΣPCBs – based on the no observed adverse effect level (NOAEL) or lowest observed adverse effect level (LOAEL) selected for this evaluation, as well as for USEPA applications (USEPA, 1995; 2000) – were derived from a study in which ring-necked pheasants (*Phasianus colchicus*) were dosed with Aroclor 1254 and a threshold for effects was assessed for critical reproductive life stages (Dahlgren et al., 1972). The rationale for the TRV selection is described in Neigh et al. (2006a). Wild passerine birds, such as those

examined in this study, appear to be less sensitive to PCBs exposure (Thiel et al., 1997; Custer et al., 1998; Henning et al., 2003) than domesticated *Galliformes* on which the dietary TRVs are based (e.g. ring-necked pheasant; Dahlgren et al., 1972). To address this uncertainty, an alternative set of dietary TRVs were developed using studies conducted on wild passerine species exposed to PCBs (Custer et al., 2003; USEPA, 2000). Because these studies only provided egg tissue concentrations that can be associated with either no or few adverse effects (i.e., they did not estimate dietary exposure), a dietary TRV was extrapolated from the egg-based TRVs using Site- and species-specific biomagnification factors (BMFs). BMFs are calculated by dividing measured egg concentrations by Site- and species-specific dietary dose estimates. The diet and egg inputs and resulting BMFs and TRVs for the house wren and eastern bluebird are summarized (Table 2-4). The BMF calculation is described in more detail in Neigh et al. (2006a). The egg-based TRVs used in the BMF approach are described in Section 2.2.2.2.

No studies of the effects of TEQs in passerines were found in the literature. In the absence of such data, a dietary TEQ-based LOAEL of 140 nanograms TEQ per gram of body weight per day (ng TEQ/g bw/d) was derived (Neigh et al., 2006a) based on a sub-chronic laboratory study evaluating the effects of intra-peritoneal injections of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) at concentrations of 1000 nanograms TCDD per gram per week (ng TCDD/g/week) on ring-necked pheasants (Nosek et al., 1992). A NOAEL (14 ng TEQ/g bw/d) was derived as well by applying a safety factor of 10 (Strause et al., 2008). TEQ-based TRVs were also derived from Site-specific BMFs as described above and are presented in Table 2-4.

2.2.2.2 Egg-based TRVs

In addition to a dietary assessment, risk was assessed through HQs by comparing concentrations of ΣPCBs measured in eggs to tissue-based TRVs based on the NOAEL and the LOAEL for ΣPCBs. Concentrations of PCBs in eggs are the most direct and ecologically relevant measurement endpoint for predicting adverse effects on reproduction induced by ΣPCBs because they provide an empirical measure of exposure (i.e., the tissue burden) rather than a modeled exposure (i.e., dietary exposure), which even with Site-specific inputs still relies on a number of generic assumptions (i.e., assumed ingestion rate, absorption rate, etc). HQs calculated from the exposure of eggs to ΣPCBs are considered to be a maximum estimate of exposure to a sensitive life stage (Giesy et al., 1994).

Although appropriate toxicity data were not available for an exclusively terrestrial avian species, TRVs were available for the tree swallow, a wild passerine species. The egg-based TRVs selected for this assessment were derived from several studies conducted using tree swallows on the Hudson River and Housatonic River. On the

Hudson River, potential effects in tree swallows were monitored over the course of two years. Effects such as abnormal plumage, decreased hatching success, and increased abandonment were observed in one year but not the other. The NOAEL value of 26.7 mg ΣPCBs/ kg wet weight (ww) represents the lowest site-wide mean concentration of PCBs in eggs from any of the locations evaluated during the year in which no effects were observed. The LOAEL for ΣPCBs was derived from a Housatonic River study in which hatching success was impaired during two years of the study (Custer et al., 2003). The least average concentration of ΣPCBs in piping chicks from the two years (63 mg ΣPCBs/kg) was used as the LOAEL. This study was deemed appropriate because effects were observed in two consecutive years and sensitive reproductive endpoints were evaluated.

The TRV selected as a threshold for effects based on egg TEQ concentrations was also based on the Hudson River study (USEPA, 2000). The greatest TEQ concentration in the year without effects – 13,000 ng TEQ/kg ww – was selected as the NOAEL value. A LOAEL based on TEQs could not be derived from the literature (Neigh et al., 2006a and b).

Table 2-4. Toxicity Reference Values for Dietary and Egg-Based Exposure of Avian Species to ΣPCBs and TEQs

Reference	BMF	TRV
ΣPCBs		
Dietary TRVs (mg/kg/d)		
Dahlgren *et al*. (1972)	--	LOAEL = 1.8 mg/kg/d
		NOAEL= 0.6 mg/kg/d
	Eastern Bluebird BMF (16) = mean egg ΣPCBs concentration (8.3 mg/kg ww) / dietary exposure estimate (0.51 mg/kg/day)	LOAEL = 3.9 mg/kg/d[1]
		NOAEL = 1.6 mg/kg ww[2]
	House Wren BMF (45) = mean egg ΣPCBs concentration (5.8 mg/kg ww) / dietary exposure estimate (0.13 mg/kg/day)	LOAEL = 1.4 mg/kg/d[1]
		NOAEL = 0.6 mg/kg/d[2]
Egg-based TRVs (mg/kg wet weight)		
Custer et al. (2003)	--	LOAEL = 63 mg/kg wet weight
USEPA (2000)	--	NOAEL = 26.7 mg/kg wet weight
TEQs		
Dietary TRVs (mg/kg/d)		
Nosek et al. (1992)	--	LOAEL = 140 ng TEQ/kg/d
		NOAEL= 14 ng TEQ/kg/d
	Eastern Bluebird BMF (1.1) = mean egg TEQ concentration (77 ng/kg) / dietary exposure estimate (70 ng/kg/day)	LOAEL = NA
		NOAEL = 11,818 ng TEQ/kg/d[1]
	House Wren BMF (11.6) = mean egg TEQ concentration (360 ng/kg ww) / dietary exposure estimate (31 ng/kg/day)	LOAEL = NA
		NOAEL = 1119[2]
Egg-based TRVs (ng TEQ/kg ww)		
	--	LOAEL = NA
USEPA (2000)	--	NOAEL = 13,000 ng/kg ww

Notes:

1 Calculated from TRV selected from Custer et al. (2003) / species BMF.

2 Calculated from TRV selected from USEPA (2000) / species BMF.

NA Value not available

-- BMF not applicable

2.2.3 Results

Both dietary and egg-based HQs for ΣPCBs and TEQs are described below. These and other results are described in more detail in Neigh et al. (2006a and b).

2.2.3.1 Dietary HQs

HQs based on dietary ΣPCBs and mean APDD in the Site study area were less than 1.0 for both NOAEL and LOAEL TRVs for both passerine species based on Site-specific dietary items and Site-specific dietary composition for house wrens and eastern bluebirds. The HQs for the dietary TEQs and mean APDD were greater, with the NOAEL HQs greater than 1.0 (i.e., 5.0 and 2.2 for the eastern bluebird and house wren respectively) and LOAEL HQs less than 1.0 for both species (Table 2-5). One reason for the greater estimated TEQ HQs is that concentrations of TEQs are calculated by multiplying each congener by a relative potency expressed as a toxicity equivalence factor (TEF). TEF values are consensus values that were intended to be conservative estimates of risk (van den Berg et al., 1998) and thus, they tend to overestimate risk (Neigh et al., 2006a). In addition, the detection limit was used to represent congeners that were present at concentrations less than the method detection limit, which also results in an overestimate of risk (Neigh et al., 2006a). Species were also evaluated at the upper 95% upper confidence limit on the mean (U95 CL) for APDD to describe a range of ΣPCB and TEQ HQs that would represent the most sensitive portion of the population. NOAEL U95 CL HQ values for the eastern bluebird were greater than 1.0 but LOAEL HQs were less than 1.0. Both NOAEL and LOAEL U95 CL HQs for the house wren were less than 1.0. The dietary HQs based on the BMF approach were all less than 1.0 (i.e., for both species, for ΣPCBs and TEQs and for NOAEL and LOAEL TRVs). The mean and U95 CL concentrations for each species and the Site-specific diet are presented graphically in Neigh et al.'s Figure 5 (2006a). Additional details of the analyses described here can also be found in the publication (Neigh et al, 2006a).

2.2.3.2 Tissue Based HQs

HQs based on mean and U95 CL ΣPCB and TEQ concentrations for the Site study area were well below 1.0 for both species. Moreover, no sample (i.e., nest box) exceeded the NOAEL egg-based TRV of 26.7 mg/kg (i.e., the maximum detected ΣPCB concentration in eastern bluebird eggs was 15.6 mg/kg and in house wren eggs was 8.3 mg/kg). Mean HQ values are summarized Table 2-5.

Table 2-5. Summary of Hazard Quotients for Terrestrial Passerines in the Former Trowbridge Impoundment

	Mean ΣPCB HQs		Mean TEQ HQs	
Receptor	**NOAEL**	**LOAEL**	**NOAEL**	**LOAEL**
Dietary				
Eastern Bluebird	0.85	0.28	5.0	0.50
House Wren	0.22	0.072	2.2	0.22
Dietary using BMF				
Eastern Bluebird	0.31	0.13	0.006	NA
House Wren	0.22	0.092	0.028	NA
Egg-Based				
Eastern Bluebird	0.31	0.13	0.0059	NA
House Wren	0.22	0.092	0.028	NA

Notes: TRVs shown in Table 2-4 were used in the HQ calculations
NA = no toxicity reference value available. HQ could not be calculated.

2.3 Assessment of Terrestrial Passerine Risk Based on Multiple Lines of Evidence

A multiple lines of evidence approach for an ERA reduces uncertainty and provides the most accurate evaluation of potential hazard (Fairbrother, 2003; Hull et al., 2006). The approach includes various types of data and integrates the strengths and weaknesses of each into a conclusion that best describes the entire data set.

Based on our extensive multi-year, Site-specific exposure and effects assessment, there does not appear to be significant risk of adverse effects for terrestrial passerine birds exposed to PCBs (either as ΣPCBs or TEQs) originating from floodplain soils of the Kalamazoo River. This conclusion is based on data that suggest there is neither a significant implication for cause nor an identification of effects consistent with ecologically relevant risk.

Dietary and tissue-based exposure assessments demonstrated that terrestrial passerines in the former Trowbridge Impoundment are accumulating increased concentrations of ΣPCBs relative to the reference study area (Neigh et al., 2006b). However, multiple lines of evidence, taken together, indicate that this increased exposure to ΣPCBs in floodplain soils is not associated with increased risk to these species. Egg-based HQs were all well below 1.0 as were the majority of the dietary based HQs. The TEQ analyses tended to result in greater HQs than the ΣPCBs analyses. The TEQ calculations are considered less certain than the ΣPCBs analysis due to limitations in available toxicity data for avian species for TCDD (Giesy and Kannan, 1998; Giesy et al., 2000; Blankenship and Giesy, 2002; Giesy et al., 2006). Moreover, it has been suggested that TEQs based on PCBs may overestimate exposure relative to TCDD (Custer et al., 2005), so assessments based on TCDD exposure are

likely to overestimate risk when applied to PCB exposure. Measures of condition of individual house wrens and eastern bluebirds (i.e., physical attributes, nest attentiveness, nestling growth) were not different between the Site and reference study areas. Similarly, most measures of reproductive success, including clutch size, hatching success, fledging success, and overall productivity were not different between the Site and reference study areas. Differences were observed for house wren fledging success, which was greater in the Site study area than the reference study areas, and eastern bluebird productivity, which was reduced in the Site study area relative to the reference study area. However, both of these parameters fall within normal ranges compared to other studies (Neigh et al., 2007).

2.4 Uncertainty

These conclusions are based on substantial Site-specific data, which were collected over multiple years to support multiple independent lines of evidence, all of which lead to a similar conclusion. Thus, these results have a high degree of certainty relative to more generic assessments. However, some uncertainty associated with the assessments and conclusions remain, as discussed below.

2.4.1 Spatial applicability of terrestrial passerine data

Measured concentrations of ΣPCBs in passerine tissues, as well as supporting information on food web exposure, confirms the applicability of the selected study BSAs to other areas of floodplain soils. To provide insight into the relative exposures between the Site study area (defined as the former Trowbridge Impoundment) and other areas of floodplain soils along the river, adults and eggs of American robin were also collected from the former Plainwell Impoundment, which is a less expansive, but similarly impacted area of floodplain soils approximately 20km upstream of the passerine study area but downstream of most Site-related point sources of ΣPCBs. Mean concentrations of ΣPCBs in adult American robins from Plainwell were ~60% less (0.7 mg ΣPCBs/kg ww, n=9) than those for robins from the Site study area (former Trowbridge Impoundment, 1.1 mg ΣPCBs/kg ww, n=8). Moreover, congener profiles and thus dioxin-like relative potency were similar between the two areas, indicating similar exposure pathways. This apparent similarity in exposure supports the use of Trowbridge as a conservative model for other formerly impounded areas along the river.

2.4.2 Representativeness of Selected Study Species

The house wren and eastern bluebird were selected to represent terrestrial insectivorous and omnivorous avian guilds, respectively. As described previously, these two species were selected for a number of reasons, including species characteristics, dietary composition, foraging characteristics, Site use, exposure potential, and ease of study. To evaluate the representativeness of these two species for other omnivorous and insectivorous birds feeding in the floodplains – in particular the American robin, which was selected as the representative

omnivorous bird in the Final Revised Baseline ERA (CDM, 2003) – whole body ΣPCB tissue burdens were compared. The concentrations of ΣPCBs in adult American robins were substantially less than those of co-located house wrens (Table 2-3), indicating that robin exposure to ΣPCBs within the study area is less than other representative species.

While whole body eastern bluebird tissue data were not measured, the eastern bluebird is considered representative of other omnivorous birds, including the robin. Both in the family *Turdidae*, the American robin and eastern bluebird have similar dietary composition characteristics, feeding on a combination of plants and invertebrates. While their dietary composition is similar, the foraging patterns of the two species are slightly different, with the eastern bluebird using a perch and pounce method and the American robin tending toward communal short grass foraging, especially early in the year during egg laying and brooding. Based on Site-specific observations of *Turdidae* foraging, we noted that eastern bluebirds tended to forage on-site almost exclusively, while American robins appeared to spend significant time off-site, above the floodplain in managed short grass habitat (e.g., lawns and golf courses proximal to the Site study area). Thus, based on Site-specific habitat characteristics and Site-specific observations, it was deemed that the American robin's Site use was likely less than that of its eastern bluebird cohort, thereby reducing its exposure potential to floodplain contaminants. Another factor that could contribute to the observed greater exposure (as measured by tissue concentrations) of eastern bluebirds relative to American robins is the finding that the Site-specific bluebird diet included some aquatic insects that may contain greater PCBs concentrations than the terrestrial species consumed by the American robin (Kay et al., 2005).

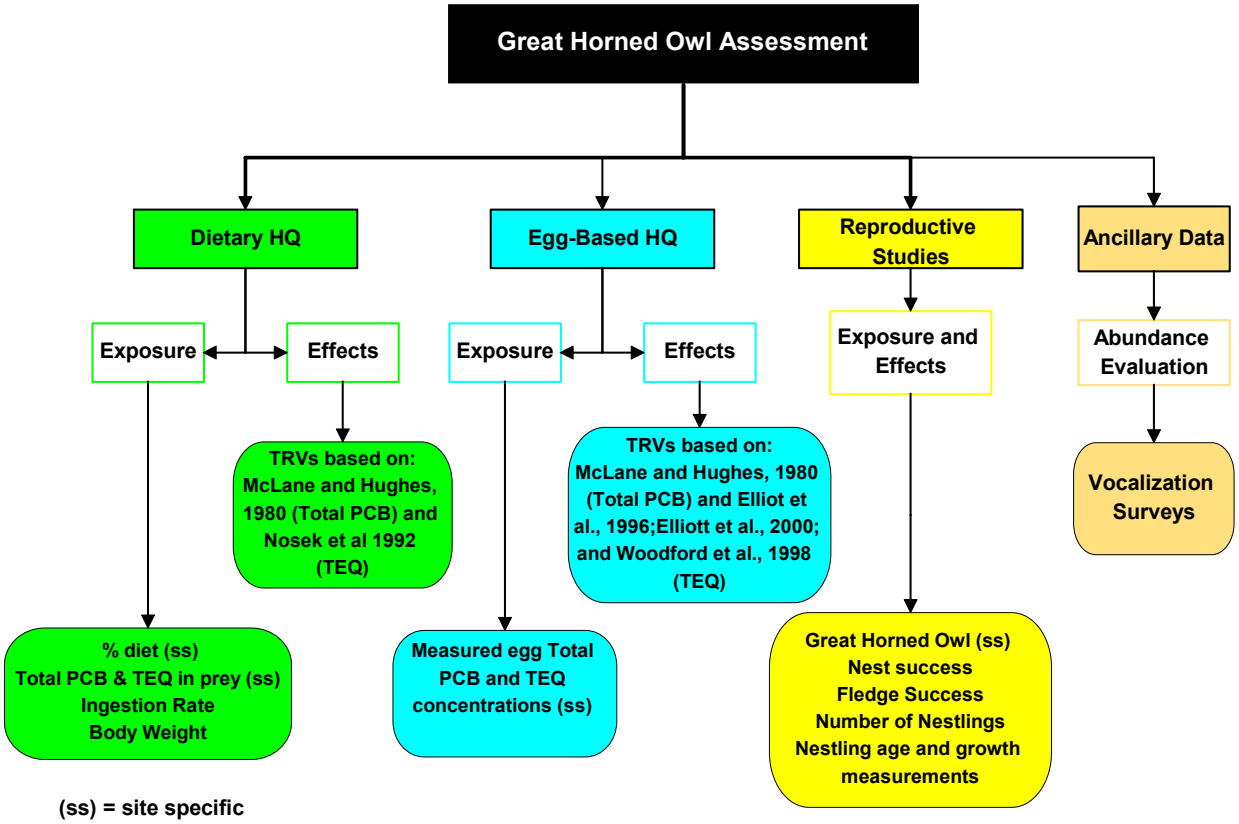
Given that these species are closely related, we hypothesized that they would have similar sensitivity to adverse effects from the same toxicant. Thus, based on an estimated greater exposure to ΣPCBs and similar sensitivity, individual and population measures were collected only for bluebirds, though we believe these date are conservatively representative of other passerines, including the American robin.

3 Assessment of Potential Risk to Carnivorous Terrestrial Birds (Great Horned Owl)

The great horned owl (GHO) was the avian species evaluated in the Final Revised Baseline ERA (CDM, 2003) to represent the carnivorous feeding guild. Risks to the GHO were based on two lines of evidence: dietary exposures estimated through a food web model, and comparisons of measured egg concentrations to tissue-based TRVs. The two approaches yielded conflicting results, with high risks predicted based on the egg concentration approach and comparatively low risks based on the food web model. In addition, CDM (2003) identified several uncertainties with the evaluated data. For example, there did not appear to be a clear link between the concentrations of PCBs in sediment and soil and the measured concentrations in egg tissues, particularly in the lower Kalamazoo River (i.e., downstream of Lake Allegan). With respect to the food web model, the dietary exposure estimate was based on a combination of measured (small mammal) and predicted (bird) prey tissue concentrations, however, Site-specific information about actual diet composition of the GHO within the study area was very limited. CDM (2003) concluded that the GHO might be at significant risk depending on the actual composition of the diet but that because "the primary of route of exposure of great horned owls to PCBs is poorly understood at this site, protection of great horned owls and other similar birds should not be the basis of PRGs [preliminary remediation guidelines] for floodplain sediment or surface soil."

Based on the preliminary results presented by CDM (1996; 1999), we designed a multiple-lines-of-evidence approach to evaluate potential risks to the GHO from exposure to PCBs (Strause et al., 2007a, 2008; Zwiernik et al., 2007). Specifically, the assessment included three lines of evidence including a Site-specific study of productivity and two HQ assessments based on Site-specific exposure estimates. The productivity assessment measured reproductive performance at both the Site study area (former Trowbridge Impoundment) and reference area (Fort Custer). The HQ assessments included a dietary-based assessment relying on measured prey tissue concentrations and a tissue-based assessment focused on comparisons of measured egg concentrations to egg-based TRVs. Exposures and risks were calculated using both a ΣPCBs and TEQ approach (Zwiernik, et al., 2007; Strause, et al. 2007a; 2008). The lines of evidence and associated inputs evaluated in our assessment are summarized in Figure 3-1 (below).

Figure 3-1. Lines of Evidence Evaluated by MSU for the Great Horned Owl



As with other receptors, the GHO study focused primarily on the former Trowbridge Impoundment with Fort Custer as the reference study area (Strause et al., 2008). For the GHO, studies of active nests were expanded to additional locations including several marshes south of Lake Allegan, referred to as the lower Kalamazoo River Study sites (LKRSS) (Strause et al., 2007a; Zwiernik, et al., 2007). However, the habitat in these marshes is quite different from that of the formerly impounded areas, which are the focus of the peer review. Given these habitat differences, it is unlikely that these data could be easily extrapolated to the formerly impounded areas. Therefore, we have not considered the data from the LKRSS in this summary.

3.1 Hazard Quotient Analyses

Risks to the GHO were evaluated through two HQ assessments, one based on a dietary food web model to estimate a daily dose for comparison to a dietary TRV, and the other based on comparison of measured and estimated egg concentrations to tissue-based TRVs. Each of these elements and the resulting HQs for both ΣPCBs and TEQ are discussed in the following sections. This summary focuses on the Site-specific information

only; information on the reference site (Fort Custer) can be found in Strause et al. (2007a; 2008) and Zwiernik et al. (2007).

3.1.1 Exposure Calculations

Exposure of GHO to ΣPCBs and TEQ was assessed in terms of daily dose for dietary exposure and direct measures of tissue concentrations in nestlings and eggs. Because TRVs are not available for nestling tissues, the exposure estimates described below focus on dietary and egg-based exposures. In addition, this summary focuses only on dose estimates derived from Site-specific data such as measured concentrations of ΣPCBs and TEQ in eggs, and Site-specific estimates of diet. The related publications (Strause, et al., 2007a, b, and 2008; Zwiernik, et al., 2007) also include discussions of egg concentrations predicted from the measured nestling blood plasma concentrations as well as dose calculations based on dietary compositions reported in the literature.

3.1.1.1 Dietary Exposure Estimate

Dietary exposure for GHOs was estimated by calculating APDD for ΣPCBs and TEQ. The dose model used is consistent with the model recommended in the USEPA's Wildlife Exposure Factors Handbook (USEPA, 1993) and is described in more detail in Strause et al. (2008). Site-specific inputs to the APDD include Site-specific measures of dietary composition and measured prey tissue concentrations. One of the key uncertainties noted in the Final Revised Baseline ERA (CDM, 2003) was lack of information about the GHO's diet; therefore, relative proportions of prey items in the Site-specific diet were determined by examining unconsumed prey remains as well as the skeletal remains in regurgitated pellets (Strause et al., 2008; Zwiernik et al., 2007). Based on this analysis as well as information reported in the literature for a separate population of Michigan GHO (Craighead and Craighead, 1956), six general categories of prey were identified: passerine birds, waterfowl, mice/voles, shrews, muskrats, and rabbit/squirrel. Site-specific dietary composition results are summarized in Table 3-1. Prey species identified in diet were subsequently collected for the purpose of developing Site-specific tissue concentrations. Specifically, whole-body prey item samples were collected from the Site study area as described in Strause et al., (2008) and Zwiernik et al. (2007) and evaluated for ΣPCBs, TEQ and lipid content. These data were combined with data previously collected, including 17 waterfowl samples, as well as data for chipmunk and red squirrel to develop geometric mean PCBs and TEQ concentrations (U95 CL) for each species represented in the diet of the GHO (Table 3-2).

Table 3-1. Great Horned Owl Site-Specific Diet Composition

Prey Type	Numeric Basis (percent occurrence)	Mass Basis (percent contribution)
Class Aves	**27.5**	**24.8**
Passerine[1]	25.8	22.0
Waterfowl[2]	1.7	2.8
Class Mammalia	**72.5**	**75.2**
Mice/Vole	49.0	6.0
Shrew	2.0	0.2
Muskrat	5.5	19.0
Rabbit[3]	16.0	50.0

Notes:
1. Passerine category includes all terrestrial birds and all unidentified bird ("unknown bird") remains.
2. Waterfowl category includes all aquatic birds.
3. Rabbit category includes squirrels and all unidentified medium-size mammal ("unknown mammal") remains.

Table 3-2. Geometric Mean and U95 CL Concentrations in Prey Items Collected from the former Trowbridge Impoundment

	Number of Samples	Range (ug/g ww)	Geometric Mean (ug/g ww)	U95 CL (ug/g ww)	Lipid (%)
	∑PCBs				
Passerines[1]	20	0.06 – 32	1.3[2]	3.1	4.90
Waterfowl[3]	17	0.13 – 28	0.89	1.8	3.80
Mice/Vole	20	0.03 – 0.6	0.07[2]	0.10	4.57
Shrew	17	0.03 – 3.2	0.85[2]	1.5	2.68
Muskrat	7	0.01 – 0.11	0.06[2]	0.09	2.07
Rabbit[4]	1	0.57	0.57	0.57	4.95
Crayfish	13	0.08 – 1.9	0.37[2]	0.60	1.62
	TEQ				
Passerines[1]	19	0.001 – 4.8	0.06[2]	0.19	0.005
Waterfowl[3]	17	0.04 – 7.7	0.24	0.72	0.004
Mice/Vole	20	0.00047 – 0.004	0.0008	0.001	0.005
Shrew	17	0.004 – 0.25	0.05[2]	0.08	0.003
Muskrat	7	0.0043 – 0.05	0.01	0.05	0.002
Rabbit[4]	1	0.07	0.07	0.07	0.005
Crayfish	13	0.003 – 0.37	0.06[2]	0.11	0.002

Notes:
1. Geometric mean ∑PCB concentrations in Trowbridge starling (1), house wren (6), tree swallow (5), and American robin (8 for total PCB, 7 for TEQ) used as representative of terrestrial passerine concentrations.
2. Trowbridge concentrations were significantly greater than those at Fort Custer, the reference site ($p \leq 0.01$)
3. Waterfowl samples were collected from 5 locations on the Kalamazoo River and were not divided between upstream and downstream sampling locations because of uncertain local residence status on the river.
4. Total PCBs and TEQ concentrations in Trowbridge chipmunk (1) used as surrogate value for rabbit concentration.

Using the Site-specific prey tissue concentrations and dietary composition information, the APDD for ΣPCBs and TEQ were calculated for GHOs (Strause et al., 2008; Zwiernik et al., 2007). It was assumed that GHOs at the study area will obtain 100% of their diet requirements from within the 100-yr floodplain (Site use factor = 1). In addition, incidental soil ingestion was calculated for the Site study area GHOs as described by Strause et al. (2008). A summary of the APPD developed is presented in Table 3-3.

Table 3-3. Range of Average Potential Daily Doses[1]

	Trowbridge PCB-Based Dietary Models	
	ΣPCB	TEQ
Site-Specific APDD (ug/g-day)		
Geometric Mean	0.03 – 0.04	0.002 – 0.004
U95 CL	0.059 – 0.061	0.005 – 0.007

Notes:
1. The range of calculated APDD results from using Site-specific diet estimations based on both total frequency (numeric-basis) and biomass contribution (mass-basis). Includes incidental ingestion of floodplain soils at the former Trowbridge Impoundment.

3.1.1.2 Tissue Based Exposure Estimates

Exposure of GHOs to ΣPCBs and TEQ were also evaluated by collecting and measuring ΣPCBs and TEQ concentrations in eggs from nests within the reference site (Fort Custer) and the Site study area (former Trowbridge Impoundment) (Strause et al., 2007a, b; Zwiernik et al., 2007). Nineteen eggs were collected from the Site study area and evaluated for PCBs. Fresh eggs were collected as soon as possible following confirmed initiation of incubation while addled eggs were collected four to six weeks post hatch or in instances when nest abandonment had occurred. A summary of the measured egg concentrations is provided in Table 3-4 (Strause et al., 2007a).

Table 3-4. Measured Egg Concentrations in Resident Great Horned Owls

	n	Range	Geometric Mean
ΣPCB (ug/g)	6	0.53 – 4.4	1.4
TEQ (ug/g)	5	0.000007 – 0.00003	0.000014

3.1.2 Effects Levels (i.e., Toxicity Reference Values)

TRVs were derived from information in the literature according to USEPA guidance for both dietary and egg-based exposures. The selection of TRVs for the GHO was based on the same criteria described in Section 2.1

for the passerine species but using data from controlled dose-response studies of screech owl (*Otus asio*) (McLane and Hughes, 1980).

3.1.2.1 Dietary TRVs

TRVs for ΣPCBs used in the dietary exposure assessment were based on values reported in the literature for NOAELs and LOAELs for ΣPCBs. The dietary NOAEL of 0.4 ug ΣPCBs/g/day for GHO was based on a controlled, laboratory study on the reproductive effects of ΣPCBs on the screech owl (McLane and Hughes, 1980). A LOAEL was not identified in this study. Therefore, a TRV was estimated by applying a NOAEL to LOAEL uncertainty factor of 3, resulting in a LOAEL TRV of 1.2 micrograms ΣPCBs per gram of body weight per day (ug ΣPCBs/g bw/day).

No studies of the effects of TEQ were available for deriving TRVs, and no studies were found in which there was a closely related test species to GHO. In the absence of such data, a dietary TEQ-based LOAEL of 0.0014 microgram TEC per gram of body weight per day (ug TEQ/g bw/d) for GHO was derived (Strause et al., 2008) based on a sub-chronic laboratory study evaluating the effects of intra-peritoneal injections of TCDD at concentrations of 0.0001 ug TCDD/g/week on ring-necked pheasants (Nosek et al., 1992). A NOAEL (0.000014 ug TEQ/g bw/d) was derived as well by applying a safety factor of 10 (Strause et al., 2008).

3.1.2.2 Egg-based TRVs

TRVs were used to evaluate the potential for adverse effects due to concentrations of ΣPCBs in GHO eggs. The TRV values selected were based on a feeding study with screech owls (McLane and Hughes, 1980). The NOAEL in this study was 7.0 ug ΣPCBs/g bw/day. A LOAEL was not determined in this study. Therefore, a TRV was estimated by multiplying the NOAEL value by an uncertainty factor of 3, resulting in a LOAEL TRV of 21 ug ΣPCBs/g bw/day.

No relevant studies on effects of TEQ in the eggs of owl species were available from which to derive a TRV. Therefore, a tissue-based NOAEL for TEQ in GHO eggs of greater than 0.001 micrograms TEQ per gram of egg (ug TEQ/g egg) (wet weight) was estimated (Strause et al., 2007a) based on the results of studies evaluating bald eagle chicks (presented on an egg basis (Elliot et al., 1996) and osprey egg exposures (Elliott et al., 2000; Woodford et al., 1998). A LOAEC concentration of 0.004 ug TEQ/g egg (wet weight) was applied, based on CYP1A induction in bald eagles (Strause et al., 2007a).

3.1.3 Results

Both dietary and egg-based HQs for ΣPCBs and TEQ are described below.

3.1.3.1 Dietary HQs

Dietary HQs were calculated based on the range of APDD values encompassing the geometric mean and associated U95 CL values for each respective prey item and the dietary proportion contributed by each prey item compiled on both a numeric- and mass-basis (Strause et al., 2008). All HQs determined for geometric mean and U95 CL exposures were less than 1.0 (Table 3-5, below).

Table 3-5. Site-Specific Dietary Hazard Quotient values for Great Horned Owls[1]

	HQ NOAEL	HQ LOAEL	HQ NOAEL	HQ LOAEL
	ΣPCBs		TEQ	
Geometric Mean	0.07 – 0.09[2]	0.02 – 0.03	0.17 – 0.27	0.02 – 0.03
U95 CL	0.14 – 0.15	0.05	0.38 – 0.50	0.04 – 0.05

Notes:
1. HQs calculated based on toxicity reference values provided in Strause et al. (2007a) and APDD based on results of field collected great horned owl pellets and prey remains from active nests at each Kalamazoo River study site.
2. The range of calculated Average Potential Daily Doses (APDD) results from using diet estimations based on both total frequency (numeric-basis) and biomass contribution (mass-basis) (see Strause et al., 2007a, Table 2).

3.1.3.2 Egg Based HQs

Egg-based HQs were calculated by dividing concentrations of ΣPCBs and TEQ in GHO eggs by the egg-based NOAEL and LOAEL TRVs. The measured geometric mean and U95 CL concentrations of ΣPCBs TEQ in the eggs from the Site study area were all below the egg-based NOAEL TRVs (greatest NOAEL based HQ = 0.05); therefore, no adverse effects were predicted by this assessment (Strause et al., 2007a).

3.2 Measures of Reproductive Success

The Site-specific GHO population was assessed through an evaluation of reproductive performance of GHO populations in both the Site study area (former Trowbridge Impoundment) and reference site (Fort Custer) (Strause et al., 2007a, 2008; Zwiernik et al., 2007). Information collected at each of seven active nests (one in Fort Custer, six in Trowbridge) included nest success, number of nestlings per nest, fledgling success, and nestling age and growth measurements.

Abundance of GHO was also estimated using a vocalization survey in which GHO hoots were broadcast to provoke responses from both adult and juvenile GHO. A total of 46 successful call-response surveys were

conducted from 2000 to 2002. A more detailed discussion of these methods is provided in Zwiernik et al., (2007) and Strause et al. (2007a, 2008).

Over the three-year study period from 2000 to 2002, productivity (fledglings/active nest) at active nests was monitored. Reproductive success was similar at both study areas, averaging 1.0 successful fledge per active nest at each location (Table 3-6, below; Strause et al., 2007a; 2008). These results are consistent with productivity measurements for natural Midwestern GHO populations residing in varied upland habitats (Holt, 1996), suggesting that despite the difference in PCBs exposure at the Site study area relative to the reference site, the reproductive success of the population does not appear to be impacted (Strause et al., 2007a; 2008). Similarly, Site-specific measures of abundance and use indicate the Site study area (the former Trowbridge Impoundment) GHO populations were near the expected carrying capacity (approximately one pair per 1600 hectares (ha) and a total of three pairs in the Trowbridge floodplain per Houston et al. [1998]). Furthermore, nest acceptance rates and nest fidelity of actively breeding GHOs across all nesting seasons included in the study were consistent with previous studies of artificial nest acceptance and habitat usage by *Strigiforms* in Midwestern forests (Strause et al., 2007a; 2008).

Table 3-6. Relative Abundance and Productivity of Resident Great Horned Owls at Ft. Custer and the Former Trowbridge Impoundment

Study Year	2000		2001		2002		All Years (2000 – 2002)	
Location	**Ft. Custer**	**Trowbridge**	**Ft. Custer**	**Trowbridge**	**Ft. Custer**	**Trowbridge**	**Ft. Custer**	**Trowbridge**
Relative Abundance[1,2]	N=4	N=7	N=9	N=7	N=11	N=8	N=24	N=22
Adults	Mean Response Rate[3]		Mean Response Rate[3]		Mean Response Rate[3]		Mean Response Rate[3]	
Total[4]	2.5	2.57	0.89	2.71	0.55	3	1.31	2.76
Foraging[5]	1.5	1.43	0.67	1.86	0.37	1.63	0.85	1.64
Paired[6]	1	1.14	0.22	0.86	0.18	1.38	0.47	1.13
Juveniles	Response Frequency[7] n, (%)		Response Frequency[7] n, (%)		Response Frequency[7] n, (%)		Response Frequency[7] n, (%)	
Fledgling[8]	0(0)	0(0)	1(11)	7(100)	0(0)	1(12)	1(04)	8(36)
Productivity[9,10]	N=0	N=1	N=1	N=2	N=0	N=3	N=1	N=6
Fledglings	0	1	1	4	0	1	1	6
Fledglings/ Nest	0	1	1	2	0	0.3	1	1

Notes:

1. Relative abundance estimates derived from hoot call/response surveys completed at dawn and dusk.
2. N=number of complete surveys.
3. Mean response rate is averaged across N completed surveys for each year. All years mean is the mean of the means.
4. Includes discrete responses from both individual and paired (male + female) owls.
5. Includes responses from unpaired individuals only.
6. Includes responses from paired (male + female) owls only.
7. Average response frequency of fledgling owls (n=number [percent] of completed surveys with at least one fledgling begging call response) expressed on a yearly basis, and averaged over all years.
8. A measure of current and successful breeding activity.
9. The total number of successful fledglings from all active nests (# fledglings/# active nests) per year within each sampling area, and averaged over all years/sum total of all active nests.
10. N=number of active nests.

3.3 Summary and Discussion

We employed multiple lines of evidence to minimize uncertainty in assessment endpoints and to provide a rigorous basis for remedial decision-making. Results of both the dietary and tissue based hazard assessments suggest that GHO populations residing in the reference and Site study areas are not at risk for effects induced by PCBs or TEQ in floodplain soil, (Strause et al., 2007a; 2008; Zwiernik et al., 2007). This conclusion is consistent with measurements of fledgling productivity, which indicated that reproductive success in the Site-specific population was consistent with that expected in healthy, Midwestern populations (Strause et al., 2007a). The results of these studies suggest that current concentrations of ΣPCBs and TEQ in the formerly impounded areas of the Site are not sufficient to pose a significant risk to GHO populations.

3.4 Uncertainty

As described for the passerines, the conclusions for the GHO are based on substantial Site-specific data, collected over multiple years, to support multiple independent lines of evidence, all of which lead to a similar conclusion. Thus, these results have a high degree of certainty relative to more generic assessments. However, some uncertainty associated with the assessments and conclusions remain and they are discussed below.

3.4.1 Dietary Exposures

This assessment relied on an analysis of Site-specific pellet and prey remains to provide an estimate of the dietary composition of the GHO residing along the Kalamazoo River. There are uncertainties associated with these methods. For example, it is possible to overestimate the frequency of occurrence of large prey items because of their tendency to be represented in more than one pellet or prey remain sample. To reduce this uncertainty, all pellet and prey remains were combined into two separate composite samples for each field season, one approximately 4-6 weeks post hatch and one post-fledge. Conversely, very small prey items may be under represented because they may be completely digested. However, small prey items are unlikely, on a mass basis, to comprise a significant proportion of the overall diet for most individuals. Additionally, many small prey items are likely to be very young animals, which are unlikely to have accumulated significant concentrations of PCBs prior to being consumed

GHO diets have been shown to vary by season, habitat, and prey availability. These variables were controlled by targeting riparian floodplain habitats that were buffered from most human disturbances and within 100 meters (m) of the river to provide uniformity in foraging habitat and available prey populations. In addition, pellets and prey remains were collected from multiple years and always during active nesting and brooding periods. Results for the Site and reference study areas did exhibit differences in the proportion of passerine versus terrestrial birds and in the proportion of rabbits (Strause et al., 2008). However, when considered on a class rather than species basis, these differences were less significant.

The daily dose estimates were based on concentrations measured in prey species collected from the Site study area. To the extent possible, prey species evaluated were based on the Site-specific dietary composition determined for the study area; however, there were a few species that could not be collected (i.e., rabbit, pheasant, and grey/fox squirrel) and data for surrogate species were used. All surrogate data used to address these data gaps were selected in a conservative manner, so it is likely that the estimated dose is greater than the actual dose to GHO at the study area.

3.4.2 Toxicity Reference Values (TRVs)

There were no available toxicity studies evaluating the effects of TEQ on GHO. For the egg-based TRV, studies focusing on the effects of TEQ on eagles and ospreys were used to derive estimated TRVs. Although these data are not specific to GHO, these species are carnivorous and therefore represent an appropriate trophic level. In addition, at the concentrations selected there were no adverse effects on developmental or any other ecologically relevant endpoint. Therefore, these concentrations are expected to be appropriately conservative to be protective of the GHO.

For the dietary exposures, a study evaluating the effects of intra-peritoneal injections of TCDD in ring-necked pheasants was used as the basis for the TRV. Use of a TRV based on TCDD exposure is likely to overestimate risks when applied to PCB exposure. In addition, tolerance to TEQ exposure by birds is species specific and available information indicates that raptors (e.g., American kestrel, osprey, and bald eagle) are less sensitive than *Galliformes* (e.g., ring-necked pheasants). Because GHOs are more similar to raptors, it is assumed that they would also be more tolerant than ring-necked pheasants. Based on these considerations, these TRVs were determined to be appropriately conservative to be protective of the GHO.

3.4.3 Productivity Assessment

The assessment of reproductive health is based on a relatively small sample size (i.e., seven active nests). However, the measures of fledgling success and species abundance were consistent with those reported in the literature for other healthy GHO populations.

4 Summary

We performed a series of comprehensive, Site-specific, multi-year, multiple lines of evidence studies to evaluate potential risk to representative ecological receptors posed by elevated concentrations of PCBs in floodplain soils in the formerly impounded areas of the Kalamazoo River. Terrestrial receptor groups evaluated included omnivorous (eastern bluebird), insectivorous (house wren) and carnivorous (great horned owl) birds. The information was collected with the primary goal of reducing uncertainty in the Final Revised Baseline ERA (CDM, 2003) by minimizing reliance on corollary assumptions.

Avian studies included productivity measures for the eastern bluebird, house wren, and GHO, as well as measures of Site-specific dietary and tissue-based exposure. Most measures of reproductive success, including clutch size, hatching success, fledging success, and overall productivity were not different between the Site and reference study areas for GHO, house wrens, and eastern bluebirds. Differences were observed for house wren fledging success, which was greater in the Site study area than the reference study areas, and eastern bluebird productivity, which was reduced in the Site study area relative to the reference study area. However, both of these parameters fall within normal ranges compared to other studies (Neigh et al., 2007). For GHOs, reproductive success in the Site-specific population was consistent with that expected in healthy, Midwestern populations (Strause et al., 2007a). The HQ analyses indicated that terrestrial passerines in the former Trowbridge Impoundment are accumulating increased concentrations of ΣPCBs relative to the reference study area (Neigh et al., 2006b), but multiple lines of evidence, taken together, indicate that this increased exposure to ΣPCBs in floodplain soils is not associated with increased risk to these species. The HQ analysis for the GHO indicated that GHO populations residing in the reference and Site study areas are not at risk for effects induced by PCBs (either as ΣPCB or TEQ) in floodplain soil (Strause et al., 2007a; 2008; Zwiernik et al., 2007).

5 References

ARCADIS. 2008. Characteristics of the Formerly Impounded Areas. April 2008.

BBL. 1993. Remedial Investigation/Feasibility Study Work Plan, July 1993.

BBL. 1994a. Technical Memorandum 14: Biota Investigation, July 1994.

BBL. 1994b. Addendum 1 to Technical Memorandum 14: Biota Investigation (turtle data), November 1994.

BBL. 1994c. Technical Memorandum 2: Results of Phase 1 TBSA Soil Sampling, February 1994.

Blankenship AL and JP Giesy. 2002. Use of biomarkers of exposure and vertebrate tissue residues in the hazard characterization of PCBs at contaminated sites: Application to birds and mammals. In G. I Sunahra, A. Y. Renoux, C. Thellen, C. L. Gaudet and A. Pilon. (Eds.). *Environmental Analysis of Contaminated Sites: Toxicological Methods and Approaches*. John Wiley and Sons, New York. Pp. 153-180.

Blankenship AL, Zwiernik M J, Coady KK, Kay DP, Newsted JL, Bradley PW. Neigh, AM, and J. P. Giesy. 2005. Differential accumulation of PCB congeners in the terrestrial food web of the Kalamazoo River Superfund Site, Michigan, USA. Environ. Sci. Technol. 39:5954-5963.

CDM. 1993. Biota Sampling Plan, October 1993

CDM. 1996. Draft Ecological Risk Assessment, March 1996

CDM. 1997. Technical Memorandum – Mink/Muskrat Biota Sampling, October 1997

CDM. 1999. Initial Baseline Ecological Risk Assessment, June 1999.

CDM. 2003. Final (Revised) Baseline Ecological Risk Assessment, April 2003.

Craighead JJ and Craighead FC. 1956. Hawks, owls and wildlife. Harrisburg (PA), USA: The Stackpole Co. p. 403.

Custer CM, Custer TW, Allen PD, et al. 1998. Reproduction and environmental contamination in tree swallow nesting in the Fox River Drainage and Green Bay Wisconsin, Environ Pollut 119:323-32

Custer CM, Custer TW, Dummer PM, Munner KL. 2003. Exposure and effects of chemical contaminants on tree swallows nesting along the Housatonic River, Berkshire County, Massachusetts, USA, 1998-2000. Environ Toxicol Chem 22:1605-1621

Custer TW, Custer CM, Rosiu CJ, et al. 2005. Exposure and effects of 2,3,7,8-tetrachloro-dibenso-*p*-dioxin in tree swallows (*Tachycineta bicolor*) nesting along the Woonasquatucket River, Rhode Island. Environ Toxicol Chem 24:93-109

Dahlgren RB, Linder RL, and Carlson CW. 1972. Polychlorinated biphenyls: Their effects on penned pheasants. Environ Health Perspect 1:89-101.

Elliott JE, Norstrom RJ, Lorenzen A, Hart LE, Philibert H, Kennedy SW, Stegemen JJ, Bellward GD, Cheng KM. 1996. Biological effects of polychlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls in bald eagle (*Haliaeetus leucocephalus*) chicks. Environ Toxicol Chem 15:782-793.

Elliot JE, Wilson LK, Henny CJ, Trudeau S, Leighton FA, Kennedy SW, Cheng KM. 2000. Biological effects of chlorinated hydrocarbons in osprey chicks. Environ Toxicol Chem 20: 866-879.

Fairbrother A. 2003. Lines of evidence in wildlife risk assessments. *Human & Ecological Risk Assessment* 9:1475-1491.

Giesy, J.P., Ludwig, J.P., and Verbrugge, D. 1994. Tree swallow reproductive health, a report to Pharmacia-Upjohn to MDEQ.

Giesy, J. P. and K. Kannan. 1998. Dioxin-like and Non-dioxin-like Toxic Effects of Polychlorinated Biphenyls (PCBs): Implications for Risk Assessment. *Crit. Rev. Toxicol.* 28:511-569.

Giesy, J. P., K. Kannan, A. L. Blankenship and P. D. Jones. 2000. Dioxin-like and Non-dioxin-like Toxic Effects of Polychlorinated Biphenyls (PCBs): Implications for Risk Assessment. Central European J. Pub. Health. 17:10-12.

Giesy, J. P., K. Kannan, P. D. Jones and A. L. Blankenship. 2006. PCBs and Related Compounds. Chapter 11, pp. 245-331. In J. Carr (Ed.) Endocrine Disruptors: Biological Basis for Health Effects in Wildlife and Humans. Oxford University Press, New York. 477 p.

Henning MH, Rosinson SK, McKay KJ, et al. 2003. Productivity of American robins exposed to polychlorinated biphenyls, Housatonic River, Massachusetts, USA. Environ Toxicol Chem 22:2783-8.

Houston, CS, DG Smith, C Rohner. 1998. Great Horned Owl (*Bubo birginianus*) in Poole A, Gill F, eds. *The Birds of North America*, No 372. The Birds of North America, Philadelphia, PA USA, pp 1-28.

Holt, JB. 1996. A banding study of Cincinnati area great horned owls. *J. Raptor Res.* 30:194-197.

Hull, R. N. and Swanson, S. 2006. Sequential Analysis of Lines of Evidence- An Advanced Weight-of-Evidence Approach for Ecological Risk Assessment. Integ. Environ. Assess. Manage. Vol 2, No 4 pp302-311.

Kay, D. P., A. L. Blankenship, K. K. Coady, A. M. Neigh, M. J. Zwiernik, S. D. Millsap, K. Strause, C. Park, P. Bradley, J. L. Nested, P. D. Jones and J. P. Giesy. 2005. Differential Accumulation of PCB Congeners in the Aquatic Food Web at the Kalamazoo River Superfund Site. Environ. Sci. Technol. 39:5964-74.

McLane MAR, Hughes DL. 1980. Reproductive success of screech owls fed Aroclor 1248. Arch Environ Contam 9:661-665.

Millsap, S A., A. L. Blankenship, P. Bradley, P. D. Jones, A. Neigh, K. Strause, M. Zwiernik and J. P. Giesy. 2004. Comparison of Risk Assessment Methodologies for Exposure of Mink to PCBs on the Kalamazoo River, Michigan. *Environ. Sci. Toxicol.* 38:6451-6459.

Neigh, AM, Zwiernik M J, A. L. Blankenship, P. W. Bradley, D. P. Kay, M.A. McCarroll, C.S. Park, P. D. Jones, S.D. Millsap, J. L. Newsted and J. P. Giesy. 2006a. Exposure and Multiple Lines of Evidence: Assessment of Risk for PCBs Found in the Diets of Passerine Birds at the Kalamazoo River Superfund Site, Michigan. Hum. Ecol. Risk. Assess. 12:924-946.

Neigh, A. M., M. J. Zwiernik, P. W. Bradley, D. P. Kay, P. D. Jones, R. R. Holem, A. L. Blankenship, K. D. Strause, J. L. Newsted and J. P. Giesy. 2006b. Accumulation of Polychlorinated Biphenyls from Floodplain Soils by Passerine Birds. Environ. Toxicol. Chem. 25:1503-1511.

Neigh, A., M. Zwiernik, P. W. Bradley, D. P. Kay, C. S. Park, P. D. Jones, J. L. Newsted, A. L. Blankenship and J. P. Giesy. 2006c. Tree Swallow Exposure to Polychlorinated Biphenyls at the Kalamazoo River Superfund Site, Michigan, USA. Environ. Toxicol. Chem. 25:428-437.

Neigh, A. M., M. J. Zwiernik, J. MacCaroll, J. L. Newsted, A. L. Blankenship, P. D. Jones, D. P. Kay and J. P. Giesy. 2006d. Productivity of Tree Swallows (*Tachycineta bicolor)* Exposed to PCBs at the Kalamazoo River Superfund Site. J. Toxicol. Env. Health. 69:395-415.

Neigh AM, Zwiernik MJ, Joldersma CA, Blankenship AL, Strause KD, Millsap SD, Newsted JL, Giesy JP. 2007. Reproductive Success of Passerines Exposed to Polychlorinated Biphenyls Through the Terrestrial Food Web of the Kalamazoo River. Ecotoxicol. Environ. Safe. 66:107-118.

Nosek, JA, Craven SR, Sullivan JR, Hurley SS, Peterson RE. 1992. Toxicity and reproductive effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in ring-necked pheasant hens. J. Toxicol. Environ. Health 35: 187-198.

Rustad OQ. 1972. An eastern bluebird nesting study in south central Minnesota. Loon 44, 80-84.

Strause, K.D., M.J. Zwiernik, S.H. Im, P.W. Bradley, P.P. Moseley, D.P. Kay, C.S. Park, P.D. Jones, A.L. Blankenship, J.L. Newsted and J.P. Giesy. 2007a. Risk Assessment of Great Horned Owls (Bubo virginianus) Exposed to Polychlorinated Biphenyls (PCBs) and DDT along the Kalamazoo River, Michigan. Environmental Toxicology and Chemistry, Volume 26, pp. 1386-1398.

Strause, K.D, M.J. Zwiernik, S. Hyeon Im, J.L. Newsted, D.P. Kay, P. W. Bradley, A.L. Blankenship, J. P. Giesy. 2007b. Plasma to egg conversion factor for evaluating Polychlorinated Biphenyl and DDT exposures in great horned owls and bald eagles. Environ. Toxicol. Chem. 26:1399-1409.

Strause, K.D., M.J. Zwiernik, J.L. Newsted, A.M. Neigh, S.D. Millsap, C.S. Park, P.P. Moseley, D.P. Kay, P.W. Bradley, P.D. Jones, A.L. Blankenship, J.G. Sikarskie and J.P. Giesy. 2008. Risk Assessment Methodologies for Exposure of Great Horned Owls (Bubo virginianus) to PCBs on the Kalamazoo River, Michigan. Integrated Environmental Assessment and Management. In Press, Volume 4 pp. 24-40.

Thiel DA, Martin SG, Duncan JW, et al. 1997. Evaluation of the effects of dioxin-contaminated sludges on wild birds. Proc TAPPI Env Conf 94-9.

USEPA. 1993. Wildlife exposure factors handbook. Washington (DC): USEPA. EPA 600/R-93/187b.

USEPA. 1994. ECO Update, Field Studies for Ecological Risk Assessment. Office of Emergency and Remedial Response, Hazardous Site Evaluation Division. EPA 540-F-94-014, PB94-963305, Sept, 1994.

USEPA. 1995. Great Lakes water quality initiative criteria documents for the protection of wildlife: DDT, Mercury, 2,3,7,8-TCDD, PCBs. EPA 820-B-95-0083. Washington, DC.

USEPA. 2000 .Revised baseline ecological risk assessment. Hudson River PCBs Reassessment. Phase 2 Report. Washington D.C.

Van den Berg M, Birnbaum L, Bosveld AT, Brunstrom B, Cook P, Freely M, Giesy JP, Hanberg A, Hasagawa R., Kennedy SW, Kubiak T, Larsen JC, Rolaf van Leeuwen FX, Djien Liem AK, Nolt C, Peterson RE, Poellinger L, Safe S, Schrenk D, Tillet D, Tysklind M, Younes M, Waern F, Zacharewski T. 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PDCFs for humans and wildlife. Environ Health Perspect. 106: 775-792.

Woodford, JE, Karasov WH, Meyer MW, Chambers L. 1998. Impact of 2,3,7,8-TCDD exposure on survival, growth, and behavior of ospreys breeding in Wisconsin, USA. Environ Toxicol Chem 17: 1323-1331.

Zwiernik MJ, Strause KD, Kay DP, Park CS, Blankenship AL, Giesy JP. 2007. Site-specific assessments of environmental risk and natural resource damage based on Great Horned Owls. *Human Ecol. Risk Assess.* 13:966-985.

Attachment 3

Michigan State University (MSU) Papers

Environ. Sci. Technol. **2005**, *39,* 5954−5963

Differential Accumulation of Polychlorinated Biphenyl Congeners in the Terrestrial Food Web of the Kalamazoo River Superfund Site, Michigan

ALAN L. BLANKENSHIP,[*,†,#] MATTHEW J. ZWIERNIK,[#,‡] KATHERINE K. COADY,[†] DENISE P. KAY,[†] JOHN L. NEWSTED,[†,#,‡] KARL STRAUSE,[†] CYRUS PARK,[‡] PATRICK W. BRADLEY,[†] ARIANNE M. NEIGH,[‡] STEPHANIE D. MILLSAP,[‡,§,∥] PAUL D. JONES,[#,‡] AND JOHN P. GIESY[#,‡,⊥]

ENTRIX, Inc., 4295 Okemos Road, Suite 101, Okemos, Michigan 48864, National Food Safety and Toxicology Center, Center for Integrative Toxciology, and Department of Zoology, Michigan State University, East Lansing, Michigan 48824, Department of Fisheries and Wildlife, Michigan State University, East Lansing, Michigan 48824, U.S. Fish and Wildlife Service, Grosse Ile, Michigan 48138, and Department of Biology and Chemistry, City University of Hong Kong, Kowloon, Hong Kong SAR, China

A series of field studies was conducted to determine the bioaccumulation of polychlorinated biphenyl (PCB) congeners in the terrestrial food web of the Kalamazoo River flood plain. Samples included colocated soils, native plants likely to be consumed by wildlife, several taxa of terrestrial invertebrates, small mammals, passerine bird eggs, nestlings, and adults, and great horned owl plasma and eggs. Mean concentrations of total PCBs in samples from the former Trowbridge impoundment were 6.5 mg/kg dry weight for soils and 0.023, 0.13, 1.3, 1.3, 1.6, and 8.2 mg/kg wet weight for plants, small herbivorous mammals, depurated earthworms, shrews, great horned owl eggs, and house wren eggs, respectively. Historical data from the Kalamazoo River have reported Aroclor-equivalent total PCB concentrations in the terrestrial food web; however, the degree of environmental weathering of the parent PCB mixtures was unknown. In this study, earthworms and composite samples of coleoptera exhibited PCB congener patterns that were similar to patterns in colocated soils. However, in plants, less chlorinated PCBs (e.g., mono-, di-, tri-, and tetrachlorinated biphenyls) were predominant, and in small mammals, there was a notable enrichment of PCBs 153, 180, 138, 118, and 99. In general, concentrations of PCBs were lower in most biota than in soil from the Kalamazoo River Area of Concern (KRAOC) although there was a modest biomagnification of PCBs from lower trophic level biota to higher trophic levels. As a consequence of environmental weathering of PCBs in the terrestrial food web of the KRAOC, the relative potency of the PCBs (expressed as mg TEQs/kg PCBs) decreased from soil to most biota. While there was a general trend, as expected, in which concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TEQs) increased with total PCBs, this relationship was rather poor ($R^2 = 0.13$). Taken together, these data suggest that the differential accumulation of PCB congeners in the terrestrial food web can be explained by congener-specific differences in bioavailability from soil, exposure pathways, and metabolic potential of each of the food web components.

* Corresponding author phone: (517)381-1434; fax: (517)381-1435; e-mail: ablankenship@entrix.com.

† ENTRIX, Inc.

National Food Safety and Technology Center and Center for Integrative Toxicology, Michigan State University.

‡ Department of Zoology, Michigan State University.

§ Department of Fisheries and Wildlife, Michigan State University.

∥ U.S. Fish and Wildlife Service.

⊥ Department of Biology and Chemistry, City University of Hong Kong.

Introduction

In 1990, approximately 80 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 primary contaminants of potential ecological concern (COPECs), resulted from PCB-contaminated waste discharged from the recycling and processing of carbonless copy paper (*1*). During the period from 1957 to 1971, the ink solvent used in carbonless copy paper contained mixtures of PCBs, primarily Aroclor 1242 (*2*). Aroclor 1254 may also have been added to inks and other additives in lesser amounts (*2*). The majority of PCBs in the Kalamazoo River watershed are associated with sediment deposits in a series of impoundments. However, partial removal of three dams (e.g., Plainwell, Otsego, and Trowbridge) to their sill levels in the 1970s allowed substantial amounts of sediments to be transported downstream, lowered the water levels to the sill of the dams' spillways, and exposed former sediments in the former impoundments. Much of the exposed former sediment is within the flood plain and thus becomes periodically inundated during high flow events.

PCBs are an environmentally ubiquitous, complex mixture of individual compounds that are chlorinated with 1−10 chlorines in various combinations of positions to create a total of 209 possible congeners. Historically, site-specific PCB data from samples collected from the KRAOC have been quantified as Aroclors 1016, 1242, 1248, 1254, and/or 1260 (*1*). However, the analytical methodologies used in these investigations (i.e., EPA Methods 8080 and 8081) do not measure "Aroclors" but rather a pattern of PCB congeners. The analyst then determines the Aroclor pattern that most closely approximates that mixture of PCB congeners. In some cases, the PCBs were quantified as a particular Aroclor simply because that Aroclor or mixture of Aroclors was used as the standard.

Each Aroclor is a complex mixture of numerous PCB congeners, and each congener behaves independently once released to the environment. Due to selective volatilization, degradation, accumulation, sorption, and metabolism (i.e., collectively termed "environmental weathering"), the relative concentrations of congeners in a mixture or matrix change as a function of time. Limitations associated with Aroclor-based determination of PCBs in environmental samples have been recognized for a long time (*3*−*6*) and have been acknowledged by the U.S. EPA (*7*, *8*) and the National

10.1021/es0483185 CCC: $30.25 © 2005 American Chemical Society
Published on Web 07/16/2005

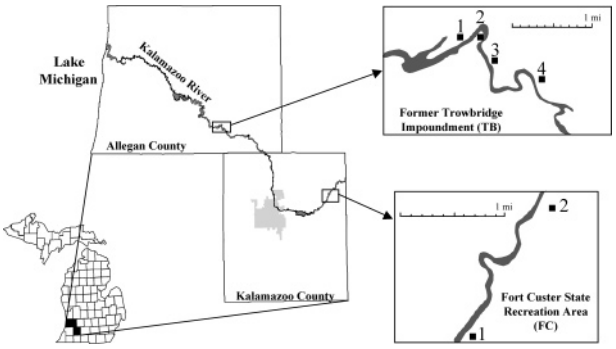


FIGURE 1. Site location map for the sampling grids at the former Trowbridge impoundment (TB) and the Fort Custer (FC) reference area along the Kalamazoo River, Michigan.

Research Council (*9*). In addition, there are difficulties and uncertainties with assessing the toxicity of environmentally weathered PCB mixtures that are quantified as Aroclors. Congener-specific analysis, including coplanar PCB congeners combined with a calculation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents (TEQs), is generally thought to correlate better with toxicity than measures of total PCBs (*10*−*12*). However, recent work by Custer et al. (*13*) calls into question whether toxic equivalency factors (TEFs) developed for PCBs are appropriate to predict effects in some bird species.

While considerable work has been conducted on the environmental fate and bioaccumulation of PCB congeners in aquatic habitats (*14*−*16*), very little is known about the environmental fate and bioaccumulation of PCB congeners in terrestrial systems. Therefore, the specific focus of the current study is the riparian, seasonally terrestrial/flood plain habitat along the Kalamazoo River. These flood plains are generally broad and covered with lowland forest or located within marshy wetlands. Specifically, wetland habitats within the Kalamazoo River watershed include marsh, wet meadow, wooded swamp, and bottomland forest. Associated water regimes range from permanently flooded for some marsh habitats to temporarily or seasonally flooded for the wet meadow and bottomland forest habitats. Wooded vegetation along the Kalamazoo River and flood plain consists of varying mixtures of willow, cottonwood, silver maple, and ash, with sycamores scattered throughout the lowland areas. Extensive wetlands along the river contain varying amounts of purple loosestrife, cattails, sedges, rushes, and aquatic species such as pond weeds and water lilies. In the former impoundments, there are large open areas dominated by *Ambiosia trifida* (giant ragweed), *Urtica dioica* (stinging nettles), *Elytrugia repens* (quack grass), *Conium maculatum* (poison hemlock), *Alliarria petiolata* (garlic mustard), and *Silica alba* (white campion). Riparian habitats along the Kalamazoo River provide food and forage for diverse species of wildlife.

These areas of intermittently flooded wetland soils have substantially different ecological functions compared to true sediments in permanently flooded areas and also have substantial differences in chemical fate, transport, bioavailability, and wildlife exposure. Delineation between soil and sediment within wetland habitats becomes arguably more difficult in such areas and based on traditional criteria may or may not coincide with a delineation that is most appropriate for the management of risk for receptors of concern. One approach for delineation can be based on the habitat it supports and other factors such as duration of water inundation, exposure potentials for key receptors, bioavailability, concentrations of PCBs, concentrations of organic matter, vegetation types, and prevalence of invertebrate type (benthic or terrestrial). Taken together, site-specific wildlife exposure data and site-specific conceptual site models support the conclusion that key receptor exposures likely diverge at the invertebrate level within wetland habitats under the premise that terrestrial invertebrates are associated with soils and benthic invertebrates are associated with sediments.

The focus of the current investigation was the intermittently flooded wetland soils and the accumulation of PCBs in the associated terrestrial food web. The purposes of this study were to (1) determine congener-specific PCB concentrations and relative patterns of congeners in terrestrial samples collected from the Kalamazoo River flood plain, (2) compare congener concentrations and patterns spatially and temporally between locations, (3) examine and contrast congener concentrations and patterns among trophic levels to gain a better understanding of the dynamics and accumulation of PCBs in a terrestrial food web, and (4) compare the relationship between concentrations of total PCBs and TEQs across multiple levels of the terrestrial food web. The results presented here are part of a larger and more comprehensive set of field investigations that were designed to gather site-specific data on the concentrations of individual PCB congeners. Taken together, the results from these studies will better define PCB exposures by determining site-specific dietary composition of key receptors, PCB concentrations in site-specific prey items, tissue residue levels in key receptors, and data necessary to develop a food web model that can be used to address movement of PCBs within the KRAOC food web.

Experimental Section

Study Area Locations. This study focused on two locations for food web analysis. Within the KRAOC, the former Trowbridge impoundment was selected as a study site since it is the largest of the three former impoundments (e.g., containing approximately 132 hectares of former sediments and 69 hectares of existing impounded water), has the greatest mass of PCBs, and has the greatest surficial mean concentration of PCBs in soils (approximately 11 mg/kg, dry weight (dw)) relative to the other former impoundments. Within the former Trowbridge impoundment (TB), four sampling grids were established within the flood plain (Figure 1). Fort Custer State Recreation Area (FC) was selected as a reference site for this study because it is upstream of the KRAOC and is relatively uncontaminated with PCBs. Specifically, FC is about 30 miles upstream of TB. Within the reference area at FC, two sampling grids were established within the flood plain (Figure 1).

Sampling Grid Design and Collection Methods. Most samples of plants, soils, above-ground invertebrates, subterranean invertebrates (earthworms), and small mammals were collected simultaneously (within a specified sampling period) within a 30 × 30 m^2 sampling grid. Specifically, plants, earthworms, and colocated soils were collected from a randomly selected 1 m^2 area within the sampling grid. A few samples were collected in close proximity but outside of specified sampling grids based on targeted species of plants and birds. For plant collections, at least 10 g of plant material was collected above the root crown. Also, seeds and/or fruits likely to be consumed by songbirds and those plant species previously identified as PCB bioaccumulators (Dave Charters, personal communication) were targeted. Soil samples were collected using a Wildco stainless steel core sampler (2 in. diameter; 20 in. length) or a bucket auger (2 in. diameter; 6 in. length). For earthworms, two composite samples of approximately 20 g were collected from each sampling grid during every sampling event. One earthworm composite sample (termed "nondepurated") was rinsed with water and analyzed for PCBs. The other earthworm composite sample ("depurated") was rinsed with water and allowed to depurate for 24−48 h.

All samples were collected under approved state and federal permits and in accordance with Michigan State University's All-University Committee on Animal Use and Care. Above-ground terrestrial invertebrates were collected by hand picking and sweep nets. Invertebrates were sorted and classified into orders before analysis. Small mammals, including the white-footed deer mouse (*Peromyscus leucopus*), red squirrel (*Tamiasciurus hudsonicus*), Eastern chipmunk (*Tamias striatus*), meadow jumping mouse (*Zapus hudsonicus*), meadow vole (*Microtus pennsylvanicus*), short-tailed shrew (*Blarina brevicauda*), and masked shrew (*Sorex cinereus*) were collected by setting 49 alternating Sherman live traps and pitfall traps that were placed within each sampling grid. Eggs and nestlings of house wren (*Troglodytes aedon*) and Eastern bluebird (*Sialia sialis*) were collected from nest boxes within the study areas. Adult house wrens and American robins (*Turdus migratorius*) were collected by either mist-netting or gunshot (*17*−*18*). Addled eggs and plasma from great horned owls (*Bubo virginianus*) were collected from natural nests or artificial nesting platforms within the TB and FC study areas.

Sampling Rounds. Samples were collected during the years of 2000, 2001, and 2002, with most samples being collected during 2000 and 2001. To address seasonal trends in concentrations of PCBs, samples were collected during distinct spans of time or "periods" during the spring and summer of 2000. Time periods for sample collections were as follows: period 1 for soils, plants, terrestrial invertebrates, and earthworms, May 22−June 21, 2000; period 2 for soils, plants, terrestrial invertebrates, and earthworms, July 17−Aug 16, 2000; period 3 for soils, plants, terrestrial invertebrates, and earthworms, Aug 24−Sept 19, 2000; period 1 for small mammals and shrews, June 7−July 20, 2000; period 2 for small mammals and shrews, Aug 26−Sept 19, 2000.

Analytical Methodology. Surrogate standards, PCB 204 (IUPAC) and PCB 30 (AccuStandard, New Haven, CT), were added to all samples, blanks, and matrix spikes before Soxhlet extraction. Each analytical batch consisted of 20 samples, at least one extraction blank, a matrix spike and matrix spike duplicate, laboratory spike, and standard reference material. PCBs including di- and mono-ortho-substituted congeners were quantified by use of a gas chromatograph (Perkin-Elmer AutoSystem) equipped with a J&W DB-5 30-m capillary column and a ^{63}Ni electron capture detector (GC-ECD). A solution containing 98 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 after an initial five-point calibration assured instrument linearity. PCB congeners were quantified by use of a gas chromatograph (Perkin-Elmer AutoSystem or Hewlett-Packard 5890 SII) equipped with a ^{63}Ni electron capture detector. Concentrations of all resolved PCB congeners were summed to obtain total PCB concentrations. The concentrations of the surrogate standards were calculated and used to determine extraction efficiency for each sample. A conservative estimate of the method detection limit for individual congeners is 1 ppt. This method detection limit is based on the injection volume of 1 μL, the signal-to-noise ratio of a 25 ppt standard, and the mass of sample extracted.

Non-ortho-substituted (coplanar) PCB congeners 77, 81, 126, and 169 were separated from coeluting congeners and interferences by cleanup on a carbon-impregnated silica gel column. Extracts were analyzed by GC-MS on a Hewlett-Packard 5890 series II gas chromatograph equipped with a Phenomenex ZB-5 30-m capillary column and a HP 5972 series mass-selective detector. Non-ortho-substituted PCB congeners were detected and confirmed by selected ion monitoring of the two largest ions of the molecular cluster. Congener concentrations were calculated based on ion ratios for the native and ^{13}C congener.

Calculation of TCDD Equivalents. Concentrations of TEQs in samples were calculated by multiplying the concentration of individual PCB congeners by its respective World Health Organization (WHO) toxic equivalency factor (TEF) (*19*). Total TEQ concentrations were determined by summing the concentrations of the TEQs of congener IUPAC numbers 77, 81, 105, 118, 126, 156, 157, 167, and 169. When a congener was not detected, a surrogate value of one-half of the limit of detection was multiplied by the TEF to calculate the congener-specific TEQ. The potential impact of assigning a proxy value of one-half the detection limit for the TEQ calculations was found to be minimal for samples from Trowbridge (data not shown). To test this, a ratio was calculated between the maximum estimated TEQ concentration (using the full detection limit for congeners that were not detected) and the minimum estimated TEQ concentration (using a value of zero for congeners that were not detected). As expected, with samples from Fort Custer, there was more uncertainty in the estimated TEQ concentrations as there were more congeners that were not detected. Due to the differing sensitivities of mammals, birds, and fish toward PCBs and other dioxin-like chemicals, three sets of TEF values have been developed for TEQ calculations that apply to mammalian, avian, and fish receptor species (*19*). When mono-ortho congeners coeluted with other PCB congeners, the total concentration for that coeluting pair was considered to be entirely due to the mono-ortho congener. Thus, for a sample with coeluting congeners, the TEQ concentration was overestimated.

Statistical Analysis. The Kolmogorov−Smirnov one-sample test with Lillifor's transformation was used to assess whether total PCB data sets were normally distributed. When data were normally distributed, analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) was used to detect significant differences among data sets (*20*). When data sets were not normally distributed, the nonparametric Kruskal−Wallis test was used to detect differences among data sets. When total PCBs between the TB and the FC locations were compared, the two-group t-test or the Mann−Whitney U-test was used if the data was normally distributed or nonnormally distributed, respectively. The criterion for significance that was used in all statistical tests was $p < 0.05$.

Results and Discussion

Ranges of PCB and TEQ Concentrations in Terrestrial Food Web Components. Concentrations of total PCBs and TEQs were significantly greater in terrestrial food web components from TB in the KRAOC than those from the upstream reference site at FC (Tables 1 and 2). Maximum observed concentrations were 36.3 mg/kg wet weight (ww) in a single house wren egg from TB and 0.47 mg/kg (ww) in a single great horned owl egg from FC. For avian TEQs, the maximum observed concentrations were 5500 ng/kg (dw) in soil at TB and 49 ng/kg (ww) in a shrew from FC. Average concentrations of total PCBs in soils from TB were approximately 700-fold greater than those of soils from the reference site. However, average concentrations of total PCBs in orthoptera (i.e., crickets and grasshoppers), plants, and small mammals (excluding shrews) from TB were only approximately 2-, 7-, and 6-fold greater than those of the reference area, respectively. Taken together, these data suggest that plants do not readily bioaccumulate PCBs from soil and that exposure to PCBs via soil and plant ingestion is likely minimal to herbivores relative to carnivores (discussed below). Some of the plants that were collected included those previously characterized as PCB bioaccumulator species such as grasses,

TABLE 1. Total PCB Concentrations (mg/kg, ww) in Soil and Soil-Associated Biota from the Former Trowbridge Impoundment (TB) and the Fort Custer State Recreational Area Reference Site (FC)

	TB location				FC location			
	N	mean % lipid[a]	mean total PCBs (mg/kg)[b]	geometric mean (mg/kg)[b]	*N*	mean % lipid[a]	mean total PCBs (mg/kg)[b]	geometric mean (mg/kg)[b]
soil[a]	21	5.4	6.5 ± 4.7[e]	4.7	8	4.9	0.009 ± 0.009	0.005
plants	28	0.70	0.023 ± 0.044[e]	0.009	10	3.0	0.003 ± 0.003	0.002
terrestrial invertebrates[c]	30	5.6	0.34 ± 0.57[e]	0.10	18	4.2	0.023 ± 0.032	0.007
fresh earthworms	18	2.0	1.7 ± 1.8[e]	1.1	9	1.9	0.003 ± 0.001	0.002
depurated earthworms	14	2.2	1.3 ± 1.1[e]	0.86	7	1.8	0.006 ± 0.004	0.005
small mammals[d]	21	4.8	0.13 ± 0.16[e]	0.074	18	4.1	0.021 ± 0.042	0.007
shrews	17	2.9	1.3 ± 0.94[e]	0.85	16	4.2	0.009 ± 0.005	0.008
house wren eggs	21	10	8.2 ± 8.3[e]	5.8	15	13	0.12 ± 0.12	0.067
house wren nestlings	18	6.1	1.4 ± 2.7[e]	0.70	13	5.3	0.020 ± 0.020	0.015
house wren adults	9	5.4	3.2 ± 2.0[e]	2.6	8	4.6	0.072 ± 0.032	0.064
bluebird eggs	10	9.6	7.4 ± 5.3[e]	5.2	15	8.1	0.16 ± 0.10	0.13
bluebird nestlings	7	4.5	1.7 ± 2.33[e]	0.72	19	4.8	0.015 ± 0.013	0.012
American robin adults	17	4.3	0.92 ± 1.2[e]	0.30	4	2.3	0.091 ± 0.15	0.030
great horned owl plasma	7	0.49	0.045 ± 0.022	0.040	1	0.46	0.008	0.008
great horned owl eggs	4	7.2	1.6 ± 0.87	1.4	1	5.8	0.47	0.47

[a] Mean percent organic carbon reported for soils; mean ± standard deviation. [b] Concentrations of total PCBs (mg/kg, wet weight basis for biota and dry weight basis for soils) based on PCB congeners as described in text. [c] Earthworms were treated separately from other terrestrial invertebrates. [d] Shrews were treated separately from other small mammals. [e] Indicates a significant difference from the FC reference site at $p < 0.05$.

TABLE 2. Average Concentrations of Avian and Mammalian TEQs among Various Trophic Levels in the Terrestrial Food Web at Both the Former Trowbridge Impoundment (TB) and the Fort Custer State Recreational Area Reference Site (FC)

	TB location					FC location				
		avian TEQs (ng/kg)[a]		mammalian TEQs (ng/kg)[a]			avian TEQs (ng/kg)[a]		mammalian TEQs (ng/kg)[a]	
	N	mean	geometric mean	mean	geometric mean	*N*	mean	geometric mean	mean	geometric mean
soil	21	1240 ± 1410[d]	303	77 ± 50[d]	55	8	0.79 ± 0.73	0.42	0.40 ± 0.49	0.24
terrestrial invertebrates[b]	30	80 ± 200[d]	12	7.5 ± 12[d]		18	1.6 ± 2.0	0.34	0.61 ± 0.74	
fresh earthworms	18	238 ± 255[d]	117	13 ± 8.1[d]	3.0	9	0.65 ± 0.48	0.54	0.41 ± 0.37	0.18
depurated earthworms	14	154 ± 186[d]	93	10 ± 9.5[d]	10	7	1.8 ± 4.0	0.47	1.1 ± 2.4	0.31
small mammals[c]	21	9.8 ± 19[d]	4.6	9.4 ± 20[d]	7.2	18	0.89 ± 1.0	0.58	0.70 ± 0.90	0.29
shrews	17	72 ± 69[d]	47	60 ± 56[d]	4.0	16	4.2 ± 12	1.3	3.7 ± 12	0.41
house wren eggs	21	423 ± 587[d]	250	*e*	*e*	15	6.0 ± 4.5	0.94	*e*	*e*
house wren nestlings	18	89 ± 117[d]	58	*e*	*e*	13	1.4 ± 0.96	1.2	*e*	*e*
house wren adults	9	107 ± 57[d]	91	*e*	*e*	8	7.1 ± 5.7	5.8	*e*	*e*
bluebird eggs	10	57 ± 60[d]	38	*e*	*e*	15	2.9 ± 4.7	1.3	*e*	*e*
bluebird nestlings	7	6.7 ± 4.7[d]	5.3	*e*	*e*	19	1.6 ± 1.3	1.3	*e*	*e*
American robin adults	17	3.9 ± 4.4	2.4	*e*	*e*	4	1.1 ± 0.55	0.96	*e*	*e*
great horned owl plasma	2	0.69 ± 0.79	1.6	*e*	*e*	1	*e*	*e*	*e*	*e*
great horned owl eggs	4	13 ± 10	11	*e*	*e*	1	8.4	8.4	*e*	*e*

[a] Concentrations of TEQs (ng/kg, wet weight basis for biota and dry weight basis for soils) based on PCB congeners as described in text; mean ± standard deviation. [b] Earthworms were treated separately from other terrestrial invertebrates. [c] Shrews were treated separately from other small mammals. [d] Indicates a significant difference from the FC reference site at $p < 0.05$. [e] Not calculated.

quackgrass, sumac, and aspen (Dave Charters, personal communication). However, in this study, there were no statistically significant differences in concentrations of total PCBs among these bioaccumulating species and any other plant samples that were analyzed.

Average concentrations of PCBs in coleoptera (i.e., beetles), house wren eggs, shrews, and depurated earthworms were approximately 50-, 70-, 150-, and 200-fold greater at Trowbridge than those from the reference area, respectively. The coleoptera samples included a considerable quantity of June bugs (*Phyllophaga* sp.) and Japanese beetles (*Popillia japonica*), the larval stage of which resides in soil as grubs. In addition, the larval stages of some coleopteran species are very rich in lipids (*21*), which combined with the contact with soil during this life stage may account for the greater accumulation of PCBs in coleopteran species as compared to that in orthopteran species, which are not as closely linked to the soil environment.

As no reports could be found in the literature comparing PCB uptake between fresh and depurated earthworms, concentrations of PCBs were compared in samples matched for the same sampling grid and time point. While variable and not statistically significant, the concentrations of PCBs at the TB sampling area tended to be greater in the fresh earthworms compared to those of depurated earthworms, whereas at Fort Custer concentrations of PCBs were all less in the fresh earthworms than the depurated earthworms (data not shown). At each sampling area, the relationship was found to be poor between concentrations of PCBs in soil (on a dry weight or organic-carbon-normalized basis) and fresh or depurated earthworms (on a wet weight or lipid-normalized basis; data not shown).

Due to its relatively great ingestion rate, relatively small home range (varies from 0.39 to 0.96 ha (*22*–*23*)), and its relatively short lifespan (approximately 1 year (*24*–*25*)), the short-tailed shrew has been recommended as a sentinel species of contaminant exposure based on trophic level and value as a bioindicator (*26*). At TB, shrews tended to have approximately 10-fold greater concentrations of total PCBs than other small mammals, such as white-footed mice, moles,

TABLE 3. Bioaccumulation Factors of Total PCBs (Organic-Carbon- and Lipid-Normalized) among the Terrestrial Food Web at the Former Trowbridge Impoundment (TB) and the Fort Custer State Recreational Area Reference Site(FC)[a]

trophic transfer	TB location	FC location
Biota−Soil Accumulation Factor (BSAF)		
soil → terrestrial plant	0.016	2.5
soil → terrestrial invertebrates[b]	0.022	2.0
soil → earthworms (nondepurated)	0.66	0.99
soil → earthworms (depurated)	0.48	2.4
soil → small mammals	0.018	1.6
soil → shrews	0.35	1.6
soil → robin adults	0.080	10
soil → house wren eggs	0.76	6.1
soil → bluebird eggs	0.74	13
soil → great horned owl eggs	0.23	64
Biomagnification Factor (BMF)		
plant → small mammals	1.1	0.65
terrestrial invertebrates[b] → shrews	16	0.83
earthworms (depurated) → shrews	0.74	0.69
earthworms (depurated) → robin adults	0.17	4.4
terrestrial invertebrates[b] → house wren eggs	35	3.1
terrestrial invertebrates[b] → bluebird eggs	34	6.6
small mammals → great horned owl eggs	13	40

[a] Biomagnification calculations were made based on the assumption that total PCB concentrations in soils and biota and bioavailability of PCBs were homogeneous throughout each location. Animal data were normalized by lipid content, while soil data were normalized by organic carbon content. Calculations were based on geometric means. [b] Earthworms were treated separately from other terrestrial invertebrates.

and voles. This trend has been observed with other persistent, bioaccumulative compounds such as dichlorodiphenyltrichloroethane (DDT) (*27*). Thus, these data support the selection of the short-tailed shrew and similar species as ecological receptors of concern due to their relatively great potential for exposure relative to other terrestrial mammalian species.

When data for individual sampling grids within each location were compared, there were no statistically significant differences in concentrations of PCBs or TEQs among sampling grids within either the TB or the FC sampling areas (data not shown). In addition, there were no statistically significant temporal differences.

Bioavailability and Bioaccumulation. Except for eggs of house wrens and Eastern bluebirds, average concentrations of total PCBs in all food web items from TB were less than the average concentration of total PCBs in soil. When PCB concentrations were normalized to organic carbon for soils and lipids for biota, the accumulation of PCBs from soil (defined as the biota−soil accumulation factor or BSAF) was less than unity for all of the TB sample types (Table 3). Limited evidence suggests that bioavailability decreases over time as chemicals age in soil (*28*). This may be due in part to the relatively great binding affinity of many PCB congeners to particulate matter and the diffusion of PCB congeners into the soil lattice and Donnan spaces. Also, at TB, concentrations of total organic carbon in flood plain soils (with an average of approximately 5−6%) may further reduce bioavailability of PCBs. On the basis of available life history information, most of the target biota at TB would be expected to reside and forage within the contaminated flood plain. However, some of the species may not be year-round residents (e.g., passerine species). Thus, there is some uncertainty regarding what their exposures might be when these species are not at TB. The slightly greater concentrations of PCBs in passerine bird eggs compared to other sample types are likely due to a 13−15-fold biomagnification from terrestrial invertebrates (Table 3). Similar trophic-transfer biomagnification factors (BMFs) of approximately 8−10-fold were observed for insects to shrews and small mammals to great horned owls. While it should be noted that these are apparent BMFs since the actual diet is unknown, these data demonstrate the potential for biomagnification of PCBs in a terrestrial food web. Similar observations have been made for simple terrestrial food webs (*29*−*31*). The BSAF for earthworms at Trowbridge is similar to that observed for earthworms in the Rhine Delta flood plains, whereas the BSAF for shrews at Trowbridge is lower than those from the Rhine Delta flood plains (*31*).

However, in areas where PCB concentrations in soil are relatively low, such as with the FC reference area, PCB concentrations in some biota samples were greater than the concentrations in soil. In addition, the BSAFs for Fort Custer were greater than unity for several matrixes. In part, this may be due to a relatively greater influence of atmospheric input and other potential exposure pathways at the reference area. Alternatively, it may be possible that there are concentration-dependent differences in the efficiency of biota to uptake PCBs from soil. Taken together, these observations demonstrate that caution should be exercised when applying BSAFs across sites with greatly different PCB concentrations. Similar cautions have been raised in regards to the application of BSAFs from one site to another since there are potential differences in soil and sediment characteristics that may affect bioavailability (*32*).

PCB Congener Composition in Terrestrial Food Web Components. If differential environmental weathering of PCB congeners did not occur, then one might expect somewhat similar patterns of PCB congeners between soil and components of the terrestrial food web. However, there were clear qualitative differences (discussed in detail below) in the patterns of relative PCB congener concentrations in most components of the food web compared to that of soil (Figure 2). Exceptions to this generality included earthworms and coleoptera composite samples, which exhibited remarkably similar PCB congener patterns to colocated soils (Figure 2). As discussed earlier, the coleoptera samples included a considerable quantity of June bugs (*Phyllophaga* sp.) and Japanese beetles (*Popillia japonica*), the larval stage of which reside in soil as grubs. The intimate contact with soil for earthworms and the larval stages of the coleoptera samples coupled with their relative inability to metabolize PCBs are likely factors to explain the similar congener patterns that were observed.

For most of the other food web components, the PCB congener patterns were dramatically different from that of soils. In plants, for example, there was a marked shift toward lesser chlorinated congeners with a marked decrease in more chlorinated congeners. Taken together with the very low concentrations of total PCBs in plants relative to soil, these data are consistent with the fact that PCBs are not readily translocated into above-ground plant tissue. While it is possible that some of the PCBs in the plant tissue could be due to deposition of PCB-containing silt and particulates during times of inundation, the most likely mechanism is aerial deposition of lighter, more volatile congeners followed by absorption in the waxy cuticle (*33*).

For shrews, there was a dramatic shift in PCB patterns compared to that of soil (Figure 3). The lesser chlorinated PCB congeners were noticeably diminished whereas there was a notable enrichment of PCBs 153, 180, 138, 118, and 99. A similar pattern was observed for small mammals excluding shrews (data not shown) and passerine samples, indicating that this phenomenon may occur with a diverse array of species, at least those species with a functional Ah-receptor-mediated pathway leading to inducible cytochrome P450 enzyme activity and therefore greater metabolic capabilities.

One way to evaluate the potential role of metabolism on the relative differences in congener patterns in a food web is to group PCB congeners by structural features that are

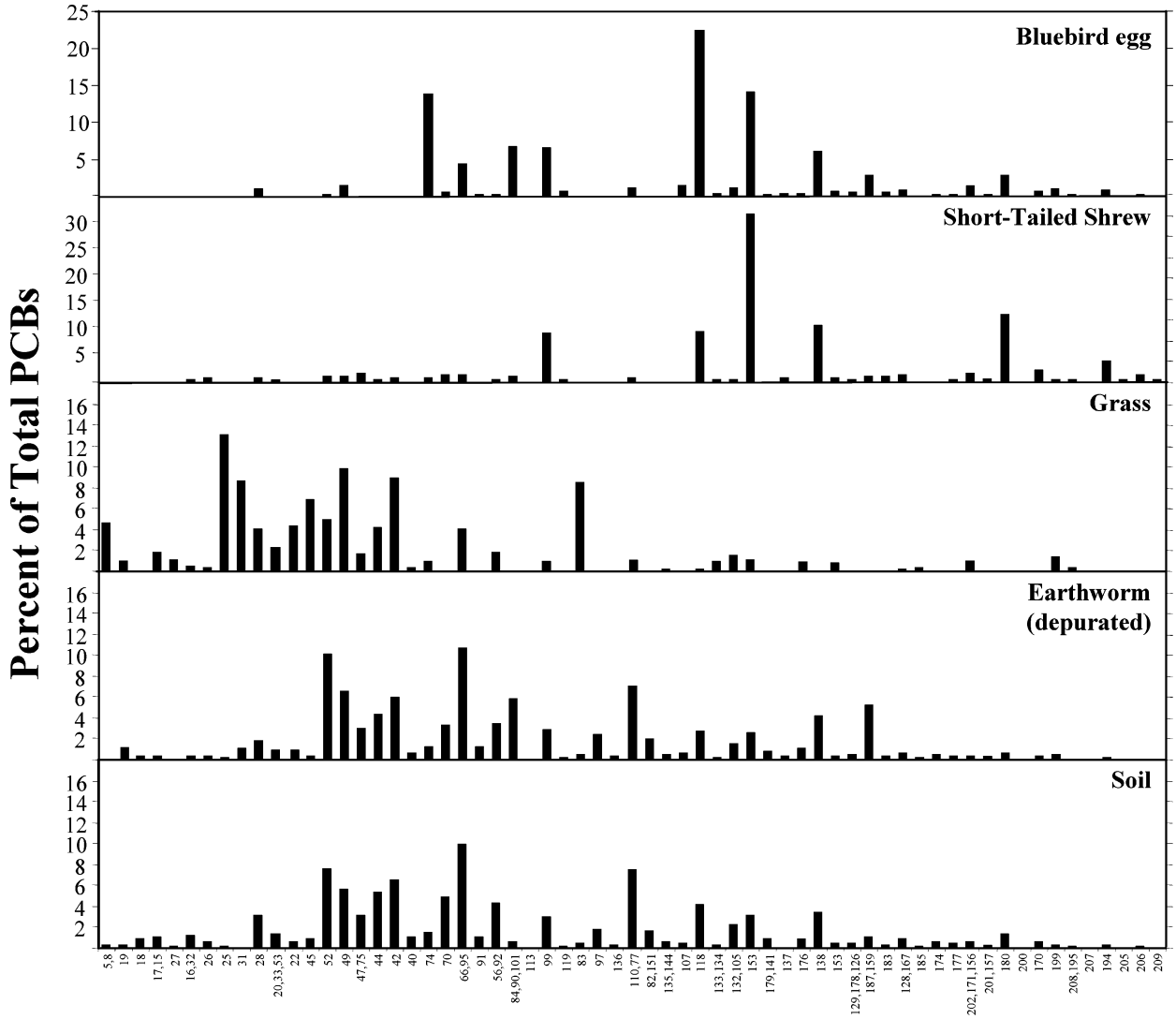


FIGURE 2. PCB congener pattern for representative samples of soil, grass, depurated earthworm, short-tailed shrews, and bluebird eggs collected from the former Trowbridge impoundment. Data (expressed as a weight percent relative to total PCBs) are from Trowbridge grid 1 in 2000.

related to potential susceptibility to catabolic metabolism (*34*−*35*). For example, many species of vertebrates are capable of enzymatically modifying congeners with adjacent unsubstituted meta and para carbons (i.e., lacking chlorines in these positions). Some species, including some marine mammals, can also enzymatically modify congeners with adjacent unsubstituted ortho and meta carbons (*36*). Such congeners can potentially be hydroxylated and excreted. If one evaluates the ratio of congener concentrations in earthworms from Trowbridge to that of colocated soil, often termed the biota−soil accumulation factor (BSAF), the ratio for the most abundant congeners ranged from 0.2 to 0.7, regardless of ortho-, meta-, and para-substitution patterns, with the exception of the coeluting pair of PCB 159/187 (Figure 3). In contrast, there were considerable differences in BSAFs among congeners for shrews. For example, average concentrations of PCB congeners with adjacent unsubstituted meta and para carbons (groups C and D in Figure 3) were diminished in shrews relative to soils, whereas congeners were generally stable or enriched with no adjacent unsubstituted meta and para sites (groups A and B in Figure 3). Thus, shrews, which occupy trophic positions higher up on the food chain and have relatively great metabolic capacity, appear capable of metabolizing certain congeners (e.g., generally those with adjacent unsubstituted meta and para carbons). This has been shown previously in aquatic systems and selected terrestrial species (*34*, *36*−*37*), but to our knowledge this has not been demonstrated in a complex terrestrial food web.

In general, it is not valid to apply a BSAF or BMF to transfer TEQs from one trophic level to another. This is because TEQs include a fractional contribution from several congeners that have unique toxicokinetic profiles. For this reason, each individual PCB congener needs to be corrected not only for its relative toxic potency but also its accumulation potential. In particular, PCB congeners 77 and 81, which may account for a substantial portion of the predicted avian TEQ concentration, are much more susceptible to metabolism than other congeners such as PCB congeners 126 and 169 (*11*, *37*−*39*). Even when such toxicokinetic differences are accounted for, there remains additional uncertainty regarding WHO avian TEFs. Recent work by Custer et al. (*13*) calls into question whether TEFs developed for PCBs are appropriate to predict effects in birds. For example, on the basis of tree swallow studies on the Woonasquatucket River, Custer et al. (*13*) derived a LD_{50} concentration of 1700 pg/g of TEQs in field-collected tree swallow eggs (primarily due to TCDD). However, if one compares this LD_{50} to concentrations of TEQs (calculated from PCBs) between 1730 and 12700 pg/g ww in tree swallow eggs from the Hudson River, then one would expect considerable population-level effects due to mortality. However, there were minimal effects on subtle endpoints at

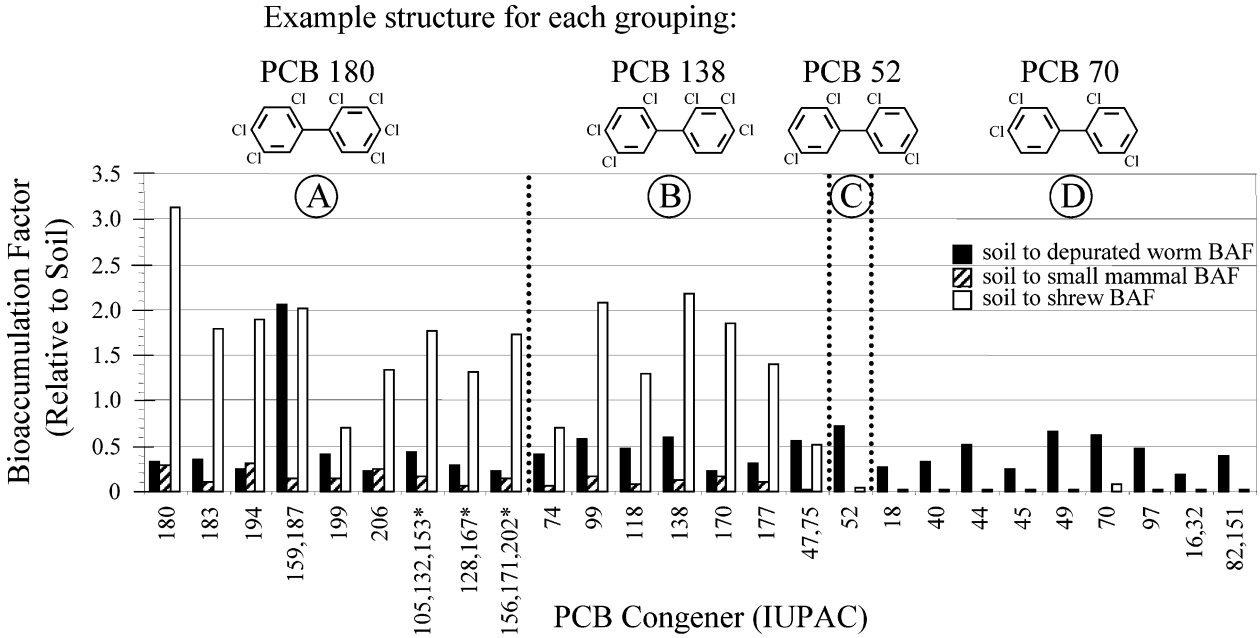


FIGURE 3. Relative accumulation of PCB congeners from soil to earthworms, shrews, and small mammals (excluding shrews) from the former Trowbridge impoundment (TB). Average PCB congener concentrations were adjusted to organic carbon content in soil and percent lipid in biota. PCB congeners are grouped as follows based on structural similarities: group A, no adjacent unsubstituted ortho−meta or meta−para sites; group B, adjacent unsubstituted ortho−meta but not meta−para sites; group C, adjacent unsubstituted meta−para but not ortho−meta sites; group D, adjacent unsubstituted ortho−meta and meta−para sites. The asterisk indicates that these coeluting congeners include congeners from both group A and group B.

TEQ concentrations (based on PCBs) in tree swallow eggs from the Hudson River (*40*). In other words, on the basis of this one comparison, a concentration of TCDD may not be toxicologically equivalent to the same concentration expressed as TEQs calculated from avian TEFs when PCBs contribute substantially to the calculated TEQs.

The relative potency of a PCB congener mixture can be calculated by dividing the concentration of TEQs (ng TEQs/kg) by the concentration of PCBs (mg PCBs/kg) in the same sample. The ratio of such relative potencies among different trophic levels is a useful measure of how the relative potency changes at each trophic level (*38*). Taken together with the BMF for total PCBs, a holistic picture can be developed that shows how both total PCB concentrations and dioxin-like potency of the PCB congener mixture changes by trophic level (Figure 4). From this analysis, it is clear that at TB there is a general reduction of relative potency (e.g., less than unity) at most trophic levels regardless of whether PCBs are biomagnified or not. For example, there is an apparent biomagnification of total PCBs going from terrestrial invertebrates to shrews (BMF = 16) whereas the relative potency ratio is only 0.23. For this example, the reduction in relative potency in shrews is, in part, due to lower concentrations of PCB 77, a congener that has been found to be susceptible to metabolism (*11*). One exception was for soil to terrestrial invertebrates, which is understandable since terrestrial invertebrates have a relatively low metabolic capacity. Another exception was the trophic transfer from adult robins to great horned owl eggs, in which there was an increase in both relative potency and in total PCB concentration. Since it is unclear how much of the great horned owl diet may consist of robins and similar passerine species, this apparent BMF may be an artifact. Also, since great horned owl samples are more limited in number and difficult to obtain compared to other sample types, the conclusions that have been drawn for this sample type are relatively limited.

Finally, in ecological risk assessments there is often a desire to predict concentrations of TEQs from total PCB data. This desire stems from the general thought that toxicity is better

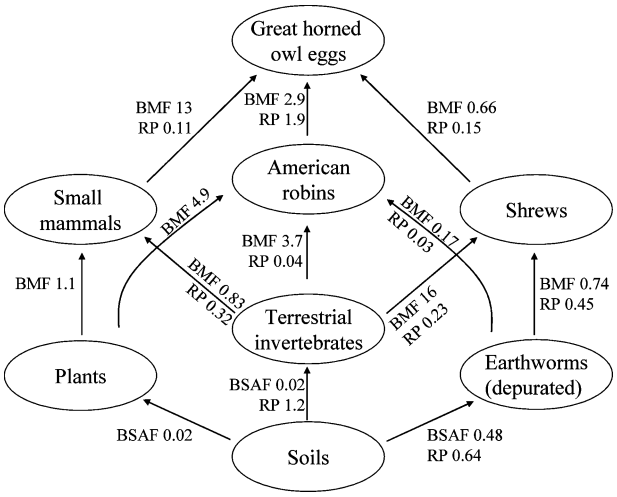


FIGURE 4. Lipid-normalized biota−soil accumulation factors (BSAFs), biomagnification factors (BMFs), and relative potency ratios (RPs) of the PCB congener mixtures at each trophic level using data from the former Trowbridge impoundment (TB).

correlated to TEQs than PCBs. However, there is often a lack of appropriate congener-specific data due to cost or other constraints at PCB-contaminated sites. EPA guidance suggests that for a given site it may be cost-effective and appropriate to analyze a subset of site-related samples for both total PCBs and PCB congeners to derive a correlation from which concentrations of TEQs may be estimated (*8*). Therefore, we evaluated the relationship between concentrations of PCBs and avian TEQs. While there was a general trend, as expected, in which concentrations of TEQs increased with total PCBs, this relationship was rather poor ($R^2 = 0.13$ using samples from TB and $R^2 = 0.059$ using samples from FC) when evaluated across all sample types. If one restricts the comparison among discrete sample types (using data from TB), then the correlation improves somewhat for certain sample types such as passerine adults ($R^2 = 0.50$), small

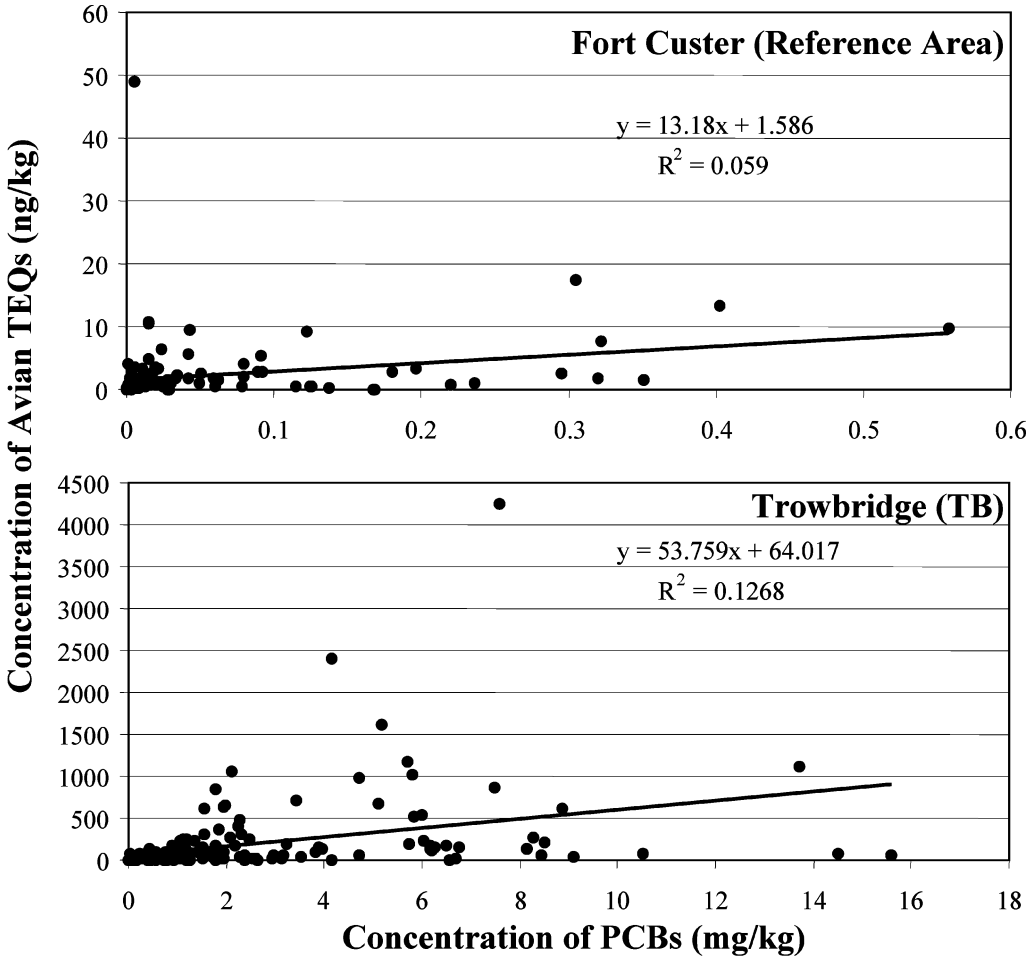


FIGURE 5. Scatter plot of PCB and avian TEQ concentrations for all individual samples from Fort Custer (top panel, 131 samples) and the former Trowbridge impoundment (lower panel, 173 samples). Concentrations are based on dry weight for soils and wet weight for biota.

mammals ($R^2 = 0.40$), terrestrial invertebrates ($R^2 = 0.49$), and worms ($R^2 = 0.42$), but the correlation was relatively poor for sample types such as soil ($R^2 = 0.16$), shrews ($R^2 = 0.0012$), and passerine nestlings ($R^2 = 0.0024$) and eggs ($R^2 = 0.0077$). Therefore, any attempts to estimate concentrations of TEQs from total PCB data should be evaluated carefully on a sample-specific basis.

On the basis of these results, if it is necessary to understand the concentration of TEQs at a site in a given sample type, then the most technically defensible approach would be to simply quantify individual PCB congeners. This would reduce uncertainty relative to estimating TEQ concentrations from total PCB data. However, the reduction in uncertainty would have to be weighed along with considerations of budgetary constraints, data quality objectives, and risk management options. Depending on the intended use of the data (e.g., delineation of the nature and extent of contamination, source evaluation, exposure assessment, and/or risk characterization), several factors should be evaluated to balance needs for information gained from congener-specific PCB analysis relative to uncertainty reduction and cost-effectiveness. For example, in the cases of nature and extent evaluation and screening-level ecological risk assessments, there may be little need to conduct congener-specific analysis when total PCB quantification can be conducted at a fraction of the cost and be sufficient to achieve the objectives. However, if there are multiple PCB sources that may need to be evaluated or if there is a relatively great likelihood of wildlife exposure, then congener-specific analysis of at least a subset of samples would be advisable. On the basis of the analyses of how well (or poorly) concentrations of TEQs relate to concentrations of total PCBs, it may be advisable to analyze all wildlife samples with congener-specific analysis. Finally, in the case of relatively rare samples of certain receptor tissues (e.g., peregrine falcon eggs, etc.), it is highly recommended that a congener-specific analysis with ultralow detection limits for coplanar PCB congeners be performed to maximize the information gained from such a limited sample.

In conclusion, concentrations of PCBs were lower in biota than those the in soil from the KRAOC although there was a modest biomagnification of PCBs from certain lower trophic level biota to higher trophic levels (e.g., terrestrial invertebrates to shrews, small mammals to great horned owls, etc.). Furthermore, environmental weathering of PCBs in the terrestrial food web of the KRAOC led to a decrease in the relative potency of PCBs (expressed as mg TEQs/kg PCBs) from soil to most biota. Taken together, these data suggest that differential accumulation of PCB congeners in the terrestrial food web can be explained by congener-specific differences in bioavailability from soil, exposure pathways, and metabolic potential of each of the food web components.

Acknowledgments

Funding was provided through a grant from the Kalamazoo River Study Group to Michigan State University. We thank Ryan Holem, Carrie Ruppert, George Klemolin, Pamela Moseley, and Monica Macarroll for their assistance in the field collections and Karen Smyth and Melissa Shotwell for their assistance preparing this manuscript.

Supporting Information Available

Congener-specific data for the Trowbridge and Fort Custer sites (all grids combined). This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Michigan Department of Environmental Quality (MDEQ) and CDM. *Final Baseline Ecological Risk Assessment Allied Paper, Inc./Portage Creek/Kalamazoo River Superfund Site*; 2003.

(2) Durfee, R. L.; Contos, G.; Whitmore, F. C.; Barden, J. D.; Hackman, E. E.; Westin, R. A. *PCBs in the United States: Industrial Use and Environmental Distributions*; EPA560/6-76-005; Environmental Protection Agency, Office of Toxic Substances: Washington, DC, 1976.

(3) Alford-Stevens, A. L. Analyzing PCBs. *Environ. Sci. Technol.* **1986**, *20*, 1194−1199.

(4) Schwartz, T. R.; Stalling, D. L.; Rice C. L. Are Polychlorinated Biphenyl Residues Adequately Described by Aroclor Mixture Equivalents? Isomer-Specific Principal Components Analysis of Such Residues in Fish and Turtles. *Environ. Sci. Technol.* **1987**, *21*, 72−76.

(5) Eganhouse, R. P.; Gossett, R. W. Sources and Magnitude of Bias Associated with Determination of Polychlorinated Biphenyls in Environmental Samples. *Anal. Chem.* **1991**, *63*, 2130−2137.

(6) Schwartz, T. R.; Stalling. D. L. Chemometric Comparison of Polychlorinated Biphenyl Residues and Toxicologically Active Polychlorinated Biphenyl Congeners in the Eggs of Forster's Terns (*Sterna fosteri*). *Arch. Environ. Contam. Toxicol.* **1991**, *20*, 183−199.

(7) USEPA. *PCBs: Cancer Dose−Response Assessment and Application to Environmental Mixtures*; EPA600/600/P-96/001F; 1996.

(8) USEPA, Region 9, Biological Technical Advisory Group. *Use of PCB Congener and Homologue Analysis in Ecological Risk Assessments*; 1998.

(9) National Research Council (NRC). *A Risk-Management Strategy for PCB-Contaminated Sediments*; National Academy Press: Washington, DC, 2001.

(10) Giesy, J. P.; Kannan, K. Dioxin-like and Non-Dioxin-like Toxic Effects of Polychlorinated Biphenyls (PCBs): Implications for Risk Assessment. *Crit. Rev. Toxicol.* **1998**, *28*, 511−569.

(11) Blankenship, A. L.; Giesy, J. P. In *Environmental Analysis of Contaminated Sites: Tools to Measure Success or Failure*; Sunahara, G. I., Renoux, A. Y., Thellen, C., Gaudet, C. L., Eds.; John Wiley & Sons, Ltd.: London, 2002.

(12) USEPA. *Framework for Application of the Toxicity Equivalence Methodology for Polychlorinated Dioxins, Furans and Biphenyls in Ecological Risk Assessment (External Review Draft)*; 630/P-03/002A; Washington, DC, 2003.

(13) Custer, C. M.; Custer, T. W.; Rosiu, C. J.; Melancon, M. J.; Bickham, J. W.; Matson, C. W. Exposure and Effects of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin in Tree Swallows (*Tachycineta bicolor*) Nesting along the Woonasquatucket River, Rhode Island. *Environ. Toxicol. Chem.* **2005**, *24*, 93−109.

(14) Metcalfe, T. L.; Metcalfe, C. D. The Trophodynamics of PCBs, Including Mono- and Non-Ortho Congeners, in the Food Web of North-Central Lake Ontario. *Sci. Total Environ.* **1997**, *201*, 245−272.

(15) Custer, C. M.; Custer, T. W.; Dummer, P. M.; Munney, K. L. Exposure and Effects of Chemical Contaminants on Tree Swallows Nesting Along the Housatonic River, Berkshire County, Massachusetts, USA, 1998−2000. *Environ. Toxicol. Chem.* **2003**, *22*, 1605−1621

(16) Naito, W.; Jin, J.; Kang, Y.; Yamamuro, M.; Masunaga, S.; Nakanishi, J. Dynamics of PCDDs/DFs and Coplanar-PCBs in an Aquatic Food Chain of Tokyo Bay. *Chemosphere* **2003**, *53*, 347−362.

(17) Neigh, A. M.; Zwiernik, M. J.; MacCarroll, M. A.; Newsted, J. L.; Blankenship, A. L.; Kay, D. P.; Giesy, J. P. Tree Swallow (*Tachycineta bicolor*) Exposure to Polychlorinated Biphenyls at the Kalamazoo River Superfund Site. *Environ. Toxicol. Chem.*, submitted for publication.

(18) Neigh, A. M.; Zwiernik, M. J.; Bradley, P. W.; Kay, D. P.; Park, C. S.; Jones, P. D.; Newsted, J. L.; Blankenship A. L.; Giesy, J. P. Productivity of Tree Swallows (*Tachycineta bicolor*) Exposed to PCBs at the Kalamazoo River Superfund Site. *Arch. Environ. Contam. Toxicol.*, submitted for publication.

(19) Van den Berg, M.; Birnbaum, L.; Bosveld, B. T. C.; Brunstrom, B.; Cook, P.; Feeley, M.; Giesy, J. P.; Hanberg, A.; Hasegawa, R.; Kennedy, S. W.; Kubiak, T.; Carsen, J. C.; van Leeuwen, F. X. R.; Liem, A. K. D.; Nolt, C.; Peterson, R. E.; Poellinger, L.; Safe, S.; Schrenk, D.; Tillitt, D.; Tysklind, M.; Younes, M.; Waern, F.; Zacharewski, T. Toxic Equivalency Factors (TEFs) for PCBs, PCDDs, PCDFs for Humans and Wildlife. *Environ. Health Perspect.* **1998**, *106*, 775−792.

(20) Morrison, D. F. *Multivariate Statistical Methods*, 2nd ed.; McGraw-Hill Series in Probability and Statistics; McGraw-Hill: New York, 1976.

(21) Barker, D.; Fitzpatrick, M. P.; Dierenfeld, E. S. Nutrient Composition of Selected Whole Invertebrates. *Zoo Biol.* **1998**, *17*, 123−134.

(22) Buckner, C. H. Populations and Ecological Relationships of Shrews in Tamarack Bogs of Southeastern Manitoba. *J. Mammal.* **1966**, *47*, 181−194.

(23) Faust, B. F.; Smith, M. H.; Wray, W. Distances Moved by Small Mammals as an Apparent Function of Grid Size. *Acta Theriol.* **1971**, *11*, 161−177.

(24) Russell, J. S. Evaluation of Liver Cytochromes P450 Induction in the Short-Tailed Shrew and White-Tailed Mouse. M.S. Thesis, Southern Illinois University, Carbondale, IL, 1998.

(25) Russell, J. S.; Halbrook, R. S.; Woolf, A.; French, J. B., Jr.; Melancon, M. J. Blarina Brevicauda as a Biological Monitor of Polychlorinated Biphenyls: Evaluation of Hepatic Cytochrome P450 Induction. *Environ. Toxicol. Chem.* **2004**, *23*, 1867−1873.

(26) Talmage, S. S.; Walton, B. T. Small Mammals as Monitors of Environmental Contaminants. *Rev. Environ. Contam. Toxicol.* **1991**, *119*, 47−108.

(27) Forsythe, D. J.; Peterle, T. J. Species and Age Differences in Accumulation of ^{36}Cl-DDT by Voles and Shrews in the Field. *Environ. Pollut., Ser. A* **1984**, *33*, 327−340.

(28) Alexander, M. How Toxic are Toxic Chemicals in Soil? *Environ. Sci. Technol.* **1995**, *29*, 2713−2717.

(29) Winter, S.; Streit, B. Organochlorine Compounds in a Three-Step Terrestrial Food Chain. *Chemosphere* **1992**, *24*, 1765−1774.

(30) Hebert, C. E.; Weseloh, D. V.; Kot, L.; Glooschenko, V. Organochlorine Contaminants in a Terrestrial Foodweb on the Niagara Peninsula, Ontario, Canada 1987−89. *Arch. Environ. Contam.Toxicol.* **1994**, *26*, 356−366.

(31) Hendriks, A. J.; Ma, W. C.; Brouns, J. J.; Deruiterdijkman, E. M.; Gast, R. Modeling and Monitoring Organochlorine and Heavy Metal Accumulation in Soils, Earthworms, and Shrews in Rhine-Delta Floodplains. *Arch. Environ. Contam. Toxicol.* **1995**, *29*, 115−127.

(32) Menzie, C. A.; Burke, A. M.; Grasso, D.; Harnois, M.; Magee, B.; McDonald, D.; Montgomery, C.; Nichols, A.; Pignatello, J.; Price, B.; Price, R.; Rose, J.; Shatkin, J. A.; Smets, B.; Smith, J.; Svirsky, S. An Approach for Incorporating Information on Chemical Availability in Soils into Risk Assessment and Risk-Based Decision Making. *Hum. Ecol. Risk Assess.* **2000**, *6*, 479−510.

(33) Paterson, S.; Mackay, D.; McFarlane, C. A Model of Organic Chemical Uptake by Plants from Soil and the Atmosphere. *Environ. Sci. Technol.* **1994**, *28*, 2259−2266.

(34) Bright, D. A.; Dushenko, W. T.; Grundy, S. L.; Reimer, K. J. Effects of Local and Distant Contaminant Sources: Polychlorinated Biphenyls and Other Organochlorines in Bottom-Dwelling Animals from an Arctic Estuary. *Sci. Total Environ.* **1995**, 160−161, 265−283.

(35) Li, H.; Boon, J. P.; Lewis, W. E.; van den Berg, M.; Nyman, M.; Letcher, R. J. Hepatic Microsomal Cytochrome P450 Enzyme Activity in Relation to in Vitro Metabolism/Inhibition of Polychlorinated Biphenyls and Testosterone in Baltic Grey Seal (*Halichoerus grypus*). *Environ Toxicol Chem.* **2003**, *22*, 636−44.

(36) Boon, J. P.; van der Meer, J.; Allchin, C. R.; Law, R. J.; Klungsoyr, J.; Leonards, P. E. G.; Spliid, H.; Storr-Hansen, E.; McKenzie, C.; Wells, D. E. Concentration-Dependent Changes of PCB Patterns in Fish-Eating Mammals: Structural Evidence for Induction of Cytochrome P450. *Arch. Environ. Contam. Toxicol.* **1997**, *33*, 298−311.

(37) Smith, L. M.; Schwartz, T. R.; Feltz, K.; Kubiak, T. J. Determination and Occurrence of AHH-Active Polychlorinated Biphenyls, 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and 2,3,7,8-Tetrachlorodibenzofuran in Lake Michigan Sediment and Biota—The Question of Their Relative Toxicological Significance. *Chemosphere* **1990**, *21*, 063−1085.

(38) Froese, K. L.; Verbrugge, D. A.; Ankley, G. T.; Niemi, G. J.; Larsen, C. P.; Giesy, J. P. Bioaccumulation of Polychlorinated Biphenyls from Sediments to Aquatic Insects and Tree Swallow Eggs and Nestlings in Saginaw Bay, Michigan, USA. *Environ. Toxicol. Chem.* **1998**, *17*, 484−492.

(39) Schlummer, M.; Moser, G. A.; McLachlan, M. S. Digestive Tract Absorption of PCDD/Fs, PCBs, and HCB in Humans: Mass Balances and Mechanistic Considerations. *Toxicol. Appl. Pharmacol.* **1998**, *152*, 128−137.

(40) McCarty, J. P.; Secord, A. L. Reproductive Ecology of Tree Swallows (*Tachycineta bicolor*) with High Levels of Polychlorinated Biphenyl Contamination. *Environ. Toxicol. Chem.* **1999**, *18*, 1433−1439.

Received for review October 28, 2004. Revised manuscript received May 23, 2005. Accepted June 8, 2005.

ES0483185

Supplemental Data Table for Fort Custer (Terrestrial Food Web)

Type and Sample size (n)	Soil (n=8)		Plants (n=10)		Terr. Inverts. (n=18)		Fresh Earthworms (n=9)		Depurated Earthworms (n=7)		Small Mammals (n=18)		Shrews (n=16)		House Wren Eggs (n=15)		House Wren Nestlings (n=13)		House Wren Adults (n=8)	
	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.
Lipid or TOC (%)	4.93	4.22	3.05	6.58	4.17	2.79	1.88	0.318	1.79	0.192	4.08	1.95	4.25	2.25	13	13.7	5.33	2.32	4.6	0.927
Total PCBs (mg/kg)	0.00907	0.00922	0.00331	0.00298	0.0233	0.0324	0.0025	0.000966	0.00611	0.00404	0.0206	0.0419	0.0089	0.00464	0.118	0.117	0.0203	0.0202	0.0716	0.0329
Total PCBs (pg/g)	9070	9220	3310	2980	23300	32400	2500	966	6110	4040	20600	41900	8900	4640	118000	117000	20300	20200	71600	32900
PCB Congener																				
4, 10	1120	1940	329	372	3990	4680	481	338	791	1120	11000	11600	326	INT	1	0	INT	INT	INT	INT
5, 8	168	254	184	251	3.06	8.72	17.2	48.7	80	100	660	832	348	242	186	718	39.5	139	156	239
6	122	168	2.63	4.6	3440	7290	58.6	173	334	541	1650	2960	358	512	9910	20100	45.5	161	641	1280
9	105	241	7.33	11	261	606	73.6	93.2	163	229	3690	8240	71.5	99.7	1	0	265	363	289	233
15,17	7.6	17	7.3	19.9	4580	13000	12.9	35.7	17	41.2	35.2	83.7	232	336	1	0	2600	4390	1	0
16,32	47.2	106	13.7	22.5	23.8	96.9	15.1	40	62	150	1	0	124	188	1	0	1	0	1	0
18	308	545	399	1130	879	2680	40.7	78.8	1	0	3.64	9.89	95.9	200	1	0	1130	4080	1	0
19	49.6	77	50.9	55.9	183	444	1	0	11	26.8	3710	14000	28.3	77.2	297	1150	777	1420	60.6	84.1
20,33,53	180	227	76.9	110	1	0	52.9	137	40	48.3	741	2900	87	111	232	893	1	0	140	263
22	2.84	4	37.4	37.4	875	2440	13.9	38.7	83	92.9	108	255	158	299	1	0	1	0	1	0
25	50	96	37	60.1	64.5	238	62.3	123	243	325	398	1640	11.7	41.6	1	0	224	332	207	332
26	51.8	112	65.9	93.3	184	561	1	0	42	108	2020	2170	91.5	122	6100	14900	177	326	267	703
27	1090	1550	29.4	58.8	4.44	10.3	1	0	150	295	43.3	106	36.6	116	1	0	1	0	72.5	202
28,31	206	290	55.8	53.5	24.6	61.4	24.7	34.4	428	549	14.2	34.9	25.2	53.7	2	0	2	0	649	879
37,41,42	206	248	148	145	76.8	318	21.7	59.4	94	235	20.4	56.2	96.2	139	306	1180	2	0	288	808
40	21.9	38	55.7	145	106	445	1	0	1	0	2	4.12	312	419	1	0	1	0	1	0
44	108	152	30.9	52	663	2360	33.3	59.3	70	97	206	588	108	243	5120	19800	172	311	117	328
45	73	127	80	64.3	1070	3180	1	0	17	26.6	227	892	32.4	46.3	497	1400	47.3	116	13.4	35
47,75	2230	5700	26.3	29.7	193	353	177	266	490	440	1880	4620	48.3	94.5	5700	14000	124	326	530	529
49	113	178	28.8	37.7	2.11	4.71	12.1	31.5	89	88.7	99.7	209	138	180	1	0	142	510	73.1	124
52	126	236	40.3	84.6	37.5	100	15.9	42.1	116	116	29.6	54.7	204	294	1	0	1	0	21	52.9
56,92,84,90,101,113	253	300	211	188	120	322	137	92.1	553	610	476	773	1050	862	4.8	0.775	46.5	116	897	585
66,95,96	263	335	56.5	63.1	107	224	53	99.9	199	257	496	1670	71	174	1	0	92.4	175	849	810
70	137	202	132	86.6	245	973	29.1	44.5	364	599	2900	3360	178		1	0	757	917	468	1150
74	13.8	23	36.9	52.9	112	348	56.4	61.8	221	226	377	868	14.2	31.8	1	0	486	750	468	1000
77, 85, 110, 120	242	251	319	611	847	2680	140	86.7	330	380	9.25	22.8	25.4	54.3	20000	43700	2870	6430	8500	14600
81,87,117	58.3		27.9	27.6	1	0	INT		94	186	72.1	301	56.8	74.9	1	0	21.1	72.4	150	141
82, 151	5.36	11	9.2	13	574	1570	32	41.2	68	148	3.06	4.48	11.9	21.4	989	2190	2	0	98.1	273
83	8.69	12	23.5	37.7	51	212	28.4	42.1	78	114	161	328	51.3	58.5	1	0	18.4	62.7	1	0
91	27.5	37	1.5	1.58	1	0	8.44	22.3	44	63.5	4.27	12.7	54.3	134	1	0	1	0	13.4	35
97	22.7	46	28.9	52.9	2.33	5.66	21.4	29.3	159	103	1	0	56.1	157	1	0	1	0	1	0
99	83.4	83	90.6	127	493	1500	68.1	39.3	125	81	28.4	46.5	207	206	759	1590	173	305	966	594
105, 132, 153	461	460	119	123	1110	2820	275	130	360	250	947	863	1620	1020	14500	13500	2650	3310	12500	7450
107	17.7	28	12.3	23.2	28.1	96.7	13.2	20.2	16	23	1	0	4.33	12.9	1	0	62.3	152	281	247
118	208	266	9.7	8.99	896	3490	66.2	62.9	227	407	113	114	243	231	4150	5650	1030	1460	4350	3460
119	10.8	14	37.3	93.2	324	1370	1	0	21	35.2	9.06	26.7	1	0	1	0	53.1	127	56.6	157
126, 129, 178	40.2	62	18.5	41.3	1	0	12.7	14.8	11	12.9	32.9	62.9	1.47	0.516	785	1570	105	210	912	666
128, 167,185	94.4	121	109	160	1650	5280	27.7	41.9	12	16	82.7	130	142	206	3780	7150	507	487	3300	1990
133, 134	70.8	142	19.9	26.7	1350	3850	6.78	17.3	1	0.378	83.1	289	50.7	129	694	1460	50.8	121	564	191
135, 144	30.5	45	3.4	4.95	129	537	4.56	10.7	113	176	95.9	139	3.93	7.74	1	0	71.6	208	84.8	178
136	2.87	4	6	11	209	614	7.67	20	20	36.6	60.9	164	89.7	192	1	0	8.05	25.4	26.6	72.5
137,176	279	256	30.7	30.1	38.9	90	118	74	119	76.9	20.9	58.9	105	308	548	2110	109	267	916	679
138,158	357	475	42.2	52.6	108	237	279	148	224	182	789	1170	685	672	7590	7730	1480	1770	7590	5020
141, 179	32.6	64	9.2	12.2	5.22	13.9	6.11	8.98	37	76.5	27.7	108	9.13	20.2	1.47	0.516	87.2	251	232	251
156, 171, 202,157,201	53.4	105	169	288	220	716	2	0	40	99.4	273	381	190	195	6440	16200	541	629	1790	1500
159, 187	139	165	1.9	1.91	116	399	245	139	202	174	252	327	296	291	13600	15100	1720	1490	6170	4250
170	35.3	63	8.6	17.6	400	1440	1	0	1	0	118	177	269	317	837	1610	230	399	1320	873
174	36.5	52	23.1	45.9	1	0	3.56	7.67	5	9.83	112	148	58.1	140	1	0	184	324	829	744
177	24.9	40	131	292	1	0	1	0	1	0	183	220	72.4	94.8	672	1400	46.8	129	675	484
180	100	107	61.2	134	314	1070	56.1	47.5	31	23.8	474	519	575	682	11900	10600	1610	1700	6960	4270
183	17.8	32	28.8	53.5	68.3	155	7.67	13.2	17	38.5	151	347	157	136	2020	3960	456	389	1530	948
194	6.64	15	1.44	1.33	1	0	1	0	1	0	85.4	120	234	293	3070	6800	397	573	2670	2080
195, 208	70.7	153	31.7	60	1580	4310	19.6	42.3	15	34.8	36.9	57.7	9.87	14	2860	6200	110	251	1440	1690
200	2.36	3	4.6	8.93	9.61	36.5	1	0	1	0	141	290	41.9	132	1	0	67.9	108	1	0
199	89.7	95	17.7	26.9	33.3	137	19.1	28.5	13	22.6	159	260	63.5	58.6	11800	30500	935	1360	3940	4100
205	2.84	4	49.2	69.2	333	905	1	0	1	0	18.3	34.4	12.3	40.8	1330	5140	50.8	99.1	83.9	132
206	107	215	58.1	146	68.3	246	1	0	15	36.7	67.8	111	185	211	7820	20700	552	956	4420	9520
207	1.73	1	4.4	7.66	16.4	65.3	1	0	1	0	1.94	4.01	10.2	18.6	230	888	12.9	43	189	383
209	206	365	9.3	14.5	152	463	20.4	58.3	34	86.6	65.1	116	184	172	1200	2400	103	151	1420	1890
77	8.88	9	2	0	18.6	26.3	4.78	2.17	14	31.2	4.22	6.43	8.56	3.76	64.9	24.8	9.38	7.96	23.3	20.3
81	1.75	1	1.5	0.707	19.5	29.3	1.78	1.09	2	1.13	3.06	3.52	5.63	5.56	60.6	28.7	8.92	9.09	8.13	4.64
126	7.22	10	3	2.83	10.7	11.3	5.56	3.28	11	23.3	8.11	9.36	36.9	117	77.4	39.3	11.4	4.66	34.8	18.9
169	3.95	2	3	1.41	9.83	9.08	4.67	2.74	4	1.89	4.39	7.7	15.2	41.7	69.6	23.5	13	6.61	29.8	16.6

Notes: (1) PCB congeners were identified in accordance with IUPAC nomenclature.
(2) A value of "1" indicates that this congener was not detected at a detection limit of 1 pg/g.
(3) INT indicates that a interference was encountered when trying to quantify the congener.
(4) Units are expressed as pg/g on a wet weight basis for all biota samples and on a dry weight basis for soil samples (arithmetic mean and standard deviation are shown).
(5) Congeners 77, 81, 126, and 169 were separated from coeluting congeners and interferences by clean-up on a carbon impregnated silica gel column and then analyzed by GC-MS.
(6) Grouping of PCB congeners indicates that those congeners co-eluted in one or more samples. In some cases, due to slighlty different co-elution patterns (usually in different matrices), some congener data were mathematically co-eluted (i.e., summed together) in order to present the data in one coherent table.
(7) Earthworms were treated separately from other terrestrial invertebrates and were analyzed as either fresh (exterior rinse with water) or depurated (rinsed with water and allowed to depurate for 24-48 h).
(8) Shrews were treated separately from other small mammals.

Supplemental Data Table for Trowbridge (Terrestrial Food Web)

Type and Sample size (n)	Soil (n=21)		Plants (n=28)		Terr. Inverts. (n=30)		Fresh Earthworms (n=18)		Depurated Earthworms (n=14)		Small Mammals (n=21)		Shrews (n=17)		House Wren Eggs (n=21)		House Wren Nestlings (n=18)		House Wren Adults (n=9)	
	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.
Lipid or TOC (%)	5.37	1.79	0.698	0.381	5.61	3.17	2.02	0.541	2.17	0.978	4.82	1.66	2.92	1.22	9.67	6.37	6.1	2.17	5.36	1.49
Total PCBs (mg/kg)	6.53	4.7	0.0232	0.0444	0.342	0.573	1.72	1.75	1.26	1.13	0.13	0.161	1.31	0.941	8.23	8.31	1.39	2.69	3.21	2.05
Total PCBs (pg/g)	6530000	4700000	23200	44400	342000	573000	1720000	1750000	1260000	1130000	130000	161000	1310000	941000	8230000	8310000	1390000	2690000	3210000	2050000
PCB Congener																				
4, 10	1250	3530	115	68.6	4820	11000	5050	3210	1810	530	505	474	4970	5140	1	0	4980		INT	
5, 8	13500	12600	203	285	54.7	214	362	358	757	1660	45.8	73.4	722	1790	1	0	1	0	295	278
6	4550	3780	96	233	1260	2100	400	565	693	1040	275	567	529	556	1	0	902	1760	1720	2450
9	1080	1760	523	1030	1990	3120	303	456	306	553	236	400	38.1	102	17200	58500	13800	16100	1020	655
15,17	78800	64500	256	563	3630	9810	7490	8690	5480	10600	1	0	1460	4070	1	0	2190	5680	17.2	48.7
16,32	93100	98800	311	540	932	1930	9150	9640	6760	13500	198	345	639	935	1	0	1	0	28.6	72.9
18	75600	73700	321	583	1420	2440	11900	13000	8180	15900	38.2	112	480	736	1	0	532	2250	41	106
19	11900	11700	69.5	88.1	1240	2540	770	484	876	1560	103	375	23.4	52.4	1	0	202	606	28.7	83
20,33,53	67000	51400	389	769	1420	3050	10300	9480	7660	13700	113	354	430	703	1	0	14.6	57.7	239	386
22	42500	41700	152	210	1220	2080	6080	6030	4650	5590	71.6	209	290	304	4360	12300	623	1490	1	0
25	17600	18000	501	2100	1100	1600	2310	2910	2090	2900	85.5	208	236	576	417	1500	175	408	936	970
26	43500	42700	231	398	1770	2970	7170	8200	5410	6800	53.6	142	2050	1800	2190	5390	809	721	85.6	160
27	10500	14900	44.1	66.1	519	2060	1650	1800	1340	2400	31.9	77.5	142	250	375	1420	1	0	329	983
28,31	323000	321000	1340	2780	17900	40600	74400	87400	59800	60700	1500	2050	15700	21600	323000	607000	31500	35100	39900	22700
37,41,42	575000	577000	1780	4010	11500	20200	140000	185000	86400	80100	378	561	7180	6530	43600	74700	3830	3400	5550	7770
40	64900	49600	216	411	1460	2360	12100	12000	8240	11100	404	604	495	758	227	671	306	395	125	245
44	356000	285000	1050	2250	7340	14000	116000	138000	73500	73600	128	236	2820	3150	3940	8000	648	865	441	519
45	63200	54400	221	390	2390	3040	8360	9020	5930	10000	6.62	25.7	407	810	6810	18000	222	798	20	57
47,75	182000	154000	684	1350	13700	26500	52700	63400	37700	37400	1860	2850	45600	62800	242000	365000	35700	54400	50800	31200
49	345000	256000	1240	2560	9880	16100	123000	145000	90100	90000	280	275	3950	3750	87400	136000	11100	10200	14100	23500
52	454000	343000	1700	3140	14800	23600	167000	188000	126000	115000	529	579	9610	8580	35100	55000	3850	3520	3930	5410
56,92,84,90,101,113	565000	411000	1990	4100	28700	55600	179000	181000	112000	88300	1740	1450	15700	13800	366000	442000	58400	82400	67500	55200
66,95,96	634000	500000	2000	3950	30500	64900	174000	190000	122000	104000	3340	3900	60900	77800	982000	1330000	139000	230000	178000	56100
70	294000	243000	1050	2150	11500	19700	98500	119000	72200	78400	541	561	11900	16400	153000	302000	13300	11900	9860	12900
74	106000	97000	406	805	9330	20300	27900	37200	17100	18100	5060	15700	36400	51500	515000	710000	83900	171000	118000	49300
77, 85, 110, 120	400000	296000	1410	2800	16900	34500	111000	100000	96900	88100	740	674	14200	17700	729000	1350000	70100	109000	81200	99900
81,87,117	116000	91700	239	600	6750	15900	42400	30700	17900	22400	784	952	5590	4520	160000	157000	10700	13600	35700	24100
82, 151	76900	43400	247	531	2690	5410	19200	15300	12300	14000	104	227	972	740	18500	24100	2550	3340	1270	1220
83	28300	22500	136	148	1140	1750	5000	4670	3360	3810	9.29	38	330	380	640	1850	261	617	126	236
91	53000	32500	199	350	1590	2700	14100	13800	10200	10700	43.4	78.2	971	802	10600	14600	1690	1950	1240	1490
97	96200	71000	338	703	3490	6770	28700	23300	18100	16500	99	134	871	918	5410	7390	636	793	913	1130
99	151000	104000	696	1150	11700	21700	41500	39200	35100	31600	19500	37000	163000	124000	584000	522000	98800	208000	189000	137000
105, 132, 153	243000	157000	837	1870	24300	51800	52700	40300	42000	42000	25000	34200	234000	156000	1210000	909000	228000	470000	685000	560000
107	26200	18600	83.6	212	2480	5020	5030	3670	4710	5280	181	300	1960	2800	65300	51300	13400	30900	30200	21900
118	202000	153000	529	1270	20800	38600	38100	31300	36000	34900	10500	17600	132000	102000	1270000	1080000	226000	503000	590000	406000
119	12700	9410	33.3	82.5	1450	2790	2380	1730	1850	1770	68.9	127	2680	3500	20800	18200	3430	5740	4530	2670
126, 129, 178	18400	14300	59.8	159	1100	1580	2240	1070	3260	3360	857	1070	3420	3030	32700	23100	7580	17200	14200	10200
128, 167,185	48200	31800	317	683	8830	35700	6260	4080	5740	6630	2620	3310	31200	35200	127000	133000	20300	47700	67400	52900
133, 134	29000	22200	142	267	2960	11200	3680	4690	1620	1470	714	925	8320	9220	35800	37800	8180	18600	14500	12100
135, 144	40400	27900	117	214	1650	2610	6510	5870	4820	4580	22.7	45.1	536	481	19300	21600	3450	4460	2200	1780
136	17100	13000	69.6	103	352	897	2410	1190	1930	1540	7	27.5	280	725	1	0	62.6	200	73.7	140
137,176	39100	24800	114	292	5990	23700	5180	2030	6190	6650	1310	1820	12600	13500	62500	45800	10800	23500	25700	18900
138,158	196000	155000	512	1110	19200	44700	56600	55000	44900	38600	17900	23100	214000	168000	670000	478000	127000	267000	411000	373000
141, 179	37000	20200	122	324	1940	4270	6820	4340	6170	6200	83.8	96.3	1030	686	36000	26700	6440	11900	8010	6240
156, 171, 202,157,201	37300	23800	121	248	1950	4290	4750	4060	3300	4200	4220	4290	29900	33600	86400	51700	20900	52000	58500	48200
159, 187	45700	32800	122	282	8750	17900	40300	20100	35500	25300	5410	8600	44800	65900	392000	289000	80900	182000	217000	167000
170	32200	20500	77	226	1320	1620	3060	1920	2910	4160	3860	4860	31000	24900	57900	34300	12000	27300	42100	31400
174	29700	18900	89.3	180	8420	37300	4350	2690	3850	3990	492	620	12100	31400	31100	35700	6660	10700	5790	4230
177	22100	13000	107	273	3360	12800	2460	1340	2740	3290	1960	1500	16000	30900	35800	30200	7540	14700	15500	11600
180	51500	36000	133	298	3890	6630	4810	3210	6670	8680	7570	9480	84800	61600	205000	127000	46100	109000	167000	174000
183	21000	17100	62.5	164	2980	9220	1680	1460	2370	2080	1380	1750	16300	19100	46600	32300	8730	17900	23700	18900
194	13800	11600	21	61.6	1380	3000	1090	620	1290	1900	2200	2380	14900	14000	38600	22100	8830	19700	33600	31000
195, 208	10600	10400	47.5	154	1440	3190	845	823	569	820	835	899	3020	2730	11500	7870	2100	4520	5010	3080
200	1970	1580	27.2	102	113	385	238	297	792	2010	366	375	33.9	69.4	2360	3430	324	978	270	295
199	18200	12200	60.6	107	1060	1930	3210	1430	2940	2860	2230	2920	6600	12200	50900	32500	10900	24100	36700	32400
205	1140	1420	30.9	78.1	1500	7090	55.8	83.4	39.8	87.9	209	276	979	927	2080	3590	720	1660	1060	910
206	7780	5450	35.4	88.3	3670	18200	555	363	633	994	1140	1260	5580	4780	12500	8800	2600	5410	8040	6890
207	790	901	2.89	5.92	351	1700	189	678	93.4	187	46.6	90.1	308	472	152	327	13.8	54.4	533	375
209	6470	9370	28.3	53.4	2400	12000	302	353	255	454	458	694	2380	4000	3730	4640	379	987	2610	1450
77	30800	27200	237	343	1760	4120	4880	4880	2880	3540	32.8	33.1	503	471	4020	6780	785	630	1190	1480
81	321	319	4.25	2.87	38.5	49.7	66	62.6	42.4	49.1	7.5	9.34	48.1	37.8	925	1360	146	192	176	186
126	381	326	8	5.35	55.8	56.3	41.1	27.4	30.8	32.1	79.9	183	1320	4290	1020	955	277	590	603	365
169	6.95	6.57	2.5	1.29	25.4	23.8	16.5	22.4	5.64	2.24	4.8	4.92	16.2	16.4	66.3	37.7	23.1	27.9	54.8	35.4

Notes: (1) PCB congeners were identified in accordance with IUPAC nomenclature.
(2) A value of "1" indicates that this congener was not detected at a detection limit of 1 pg/g.
(3) INT indicates that a interference was encountered when trying to quantify the congener.
(4) Units are expressed as pg/g on a wet weight basis for all biota samples and on a dry weight basis for soil samples (arithmetic mean and standard deviation are shown).
(5) Congeners 77, 81, 126, and 169 were separated from coeluting congeners and interferences by clean-up on a carbon impregnated silica gel column and then analyzed by GC-MS.
(6) Grouping of PCB congeners indicates that those congeners co-eluted in one or more samples. In some cases, due to slighlty different co-elution patterns (usually in different matrices), some congener data were mathematically co-eluted (i.e., summed together) in order to present the data in one coherent table.
(7) Earthworms were treated separately from other terrestrial invertebrates and were analyzed as either fresh (exterior rinse with water) or depurated (rinsed with water and allowed to depurate for 24-48 h).
(8) Shrews were treated separately from other small mammals.

Human and Ecological Risk Assessment, 12: 924–946, 2006
Copyright © Taylor & Francis Group, LLC
ISSN: 1080-7039 print / 1549-7680 online
DOI: 10.1080/10807030600826995

Exposure and Multiple Lines of Evidence Assessment of Risk for PCBs Found in the Diets of Passerine Birds at the Kalamazoo River Superfund Site, Michigan

Arianne M. Neigh,[1] Matthew J. Zwiernik,[1] Alan L. Blankenship,[1,2] Patrick W. Bradley,[1] Denise P. Kay,[1] Monica A. MacCarroll,[1] Cyrus S. Park,[1] Paul D. Jones,[1,2] Stephanie D. Millsap,[1] John W. Newsted,[2] and John P. Giesy[1,2,3]
[1]Zoology Department, Center for Integrative Toxicology, National Food Safety and Toxicology Center, Michigan State University, E. Lansing, Michigan, USA; [2]ENTRIX, Inc., Okemos, Michigan, USA; [3]Biology and Chemistry Department, City University of Hong Kong, Kowloon, Hong Kong, SAR, China

ABSTRACT

Dietary exposures of passerine birds at the Kalamazoo River, Michigan, were examined due to the presence of polychlorinated biphenyls (PCBs) in the terrestrial and aquatic food webs. Average potential daily doses in diets were 6- to 29-fold and 16- to 35-fold greater at a contaminated location than at a reference location for PCB exposures quantified as total PCBs and 2,3,7,8–tetrachlorodibenzo-*p*-dioxin equivalents (TEQs), respectively. Birds with diets comprised of primarily aquatic insects had greater dietary exposure than birds with diets of primarily terrestrial insects. Risk associated with dietary exposure varied with the selection of the threshold for effects including hazard quotients, which exceeded 1 in instances where the most conservative toxicity reference values were utilized. Risk based on concentrations of PCBs in the tissues indicated little risk to avian species, and co-located studies evaluating reproductive health did not suggest that observed incidences of diminished reproductive success were related to PCB exposure. Measures of risk based on comparison to toxicity reference values (TRVs) were consistent with direct measures of ecologically relevant endpoints of reproductive fitness, but uncertainty exists in the selection of threshold values for effects in these species especially based on TEQs. This is largely due to the absence of species-specific, dose-response relationships. Therefore, the best estimate of risk is through the application of multiple lines of evidence.

Key Words: insects, birds, bioaccumulation, dietary exposure, risk assessment, trophic level.

Received 31 January 2005; revised manuscript accepted 11 May 2005.
Address correspondence to Matthew Zwiernik, 218C National Food Safety and Toxicology Blvd. Michigan State University, East Lansing, MI 48824, USA. E-mail: zwiernik@msu.edu

INTRODUCTION

The purpose of this study was to describe the exposure pathways for passerine birds exposed to polychlorinated biphenyls (PCBs) at the Kalamazoo River and to estimate risk associated with PCBs through those pathways. A secondary goal was to compare the two established methodologies for estimating exposure and subsequent risk. Finally, the results of the risk assessments based on the two methods of estimating exposure were compared to measures of reproductive fitness at the more PCB-contaminated site and the less contaminated, upstream reference location. The first method for estimating exposure, the "top-down" approach, measured concentrations of PCBs in the eggs, nestlings, and adults of each species. The other approach, which predicts exposure based on concentrations of PCBs in dietary items, is referred to as "bottom-up" (Fairbrother 2003). The two approaches, although inherently linked, have become disjointed in the risk evaluation process. The reason for this separation is often related to constraints on funding and time, but the assessor cannot be unequivocally assured that their selection of methodology does not overlook some important interaction in determining risk, or for that matter, does not over or underestimate risk based on uncertainties due to the adjustment of models from the given data.

It has been suggested that several lines of evidence be used to evaluate risk instead of implementing only one type of methodology (Fairbrother 2003). However, it may not always be possible to apply multiple lines of evidence simultaneously. For this reason, the levels of risk estimated by the two methods were compared so that in the future, assessors will have an estimate of the similarity of the two methods. Based on this concept, a system of evaluating representative species in all trophic levels of the ecosystem was used to describe the complex dynamics present. This broad-based trophic level approach generates a bottom-to-top description of contaminant exposure and effects in the system. Detailed results of the top-down approach, including tissue concentrations and measurements of reproductive fitness, are presented elsewhere (Neigh *et al.* 2006a,b), but the degree of concordance between the top-down and bottom-up approaches is evaluated in this article. Specific measurement endpoint comparisons include (1) estimate of exposure and subsequent risk calculation for four passerine species based on dietary exposure; (2) comparisons of measured site-specific dietary exposure to dietary exposures derived from a literature based diet; (3) the contributions of terrestrial and aquatic food web–based exposures as related to generalized feeding guilds; (4) tissue-based exposure assessments; (5) species productivity; (6) and hazard quotients based on the total concentration of PCBs and 2,3,7,8–tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents (TEQs) of the PCB exposure mixture.

Exposures to PCBs and the potential effects of these exposures on passerine birds have been examined in several aquatic ecosystems (Custer *et al.* 1998; Bishop *et al.* 1999; McCarty and Secord 1999; Custer *et al.* 2003), but the effects on species exposed through terrestrial food webs have been less well documented. Even fewer studies have evaluated differential accumulations of PCBs by wildlife from contaminated media with the same point of origin but environmentally weathered in substantially different ways. This study was conducted to determine the differential sources of PCB dietary exposure, aquatic and terrestrial, in order to quantify risk based on exposure parameters. The demarcation between terrestrial and aquatic food webs within

riverine systems is indistinct because of the interaction between the two exposure pathways. In this manuscript, the aquatic system was defined as the food web based on invertebrates with aquatic life stages exposed through in-stream sediments. Species in a food web based primarily on plants and invertebrates with essential terrestrial life stages will be referred to as the terrestrial system.

The Kalamazoo River, Michigan, was designated a Superfund site in 1990 due to PCB contamination released during the paper recycling process at multiple locations along its banks (MDEQ 2003). Clays, inks, and paper fiber stripped from recycled paper pulp were originally deposited over in-stream sediment, but the area of contamination is now partitioned between in-stream sediment, floodplain soils, and former impoundment sediments. Floodplain soils became exposed through the frequent and regular flooding of the river over its banks; the floodwaters exposing, carrying, and depositing sediments over broad areas of the 100-year floodplain. An even greater source of terrestrial PCB exposure originated from the removal of three dams from the river's watercourse, which led to the establishment of a terrestrial ecosystem when water levels were lowered and former sediment exposed. Kalamazoo River wildlife can be exposed to PCBs via both aquatic and terrestrial food chains due to this ubiquitous contamination of sediments and vegetated former lake bottom soil.

Risks posed by exposure of insectivorous and omnivorous birds to PCBs within the Kalamazoo River Superfund Site were evaluated through the use of four passerine birds. These species were selected because they represent exposure to upper-trophic level predators via different routes in the terrestrial and the aquatic food web. PCBs in the diet are known to be persistent and bioaccumulative (Kannan *et al.* 1989) and can cause reproductive impairment (Dahlgren *et al.* 1972), behavioral anomalies (Halbrook *et al.* 1998), and physiological abnormalities in offspring (Ludwig *et al.* 1993). The tree swallow (*Tachycineta bicolor*) was selected to monitor dietary exposure to passerines primarily through the aquatic food web because it had been used in other studies and found to be suitable (Ankley *et al.* 1993; Jones *et al.* 1993; Froese *et al.* 1998; Bishop *et al.* 1999; McCarty and Secord 1999; Harris and Elliott 2000; Custer *et al.* 2002), it tolerates handling (Rendell and Robertson 1990), and individuals are relatively abundant along the river. In a novel approach to risk assessment, the house wren (*Troglodytes aedon*) was selected as an indicator of terrestrial food web exposure. Its ability to colonize much of the habitat in the river basin and its abundance were important determinants for choosing the species. The American robin (*Turdus migratorius*), another terrestrial species, was chosen to represent omnivorous species and was also considered to be maximally exposed to soil contaminants because of the large proportion of earthworms, which are in direct contact with contaminated soil, in their diet. Finally, the eastern bluebird (*Sialia sialis*) was selected for study because little is known about PCB accumulation by this species, although there is a very large body of literature on its biology.

MATERIALS AND METHODS

Site Details

Two sites within the Kalamazoo River floodplain were selected for studies of passerine birds, the Fort Custer State RecreationArea (FC) and former Trowbridge

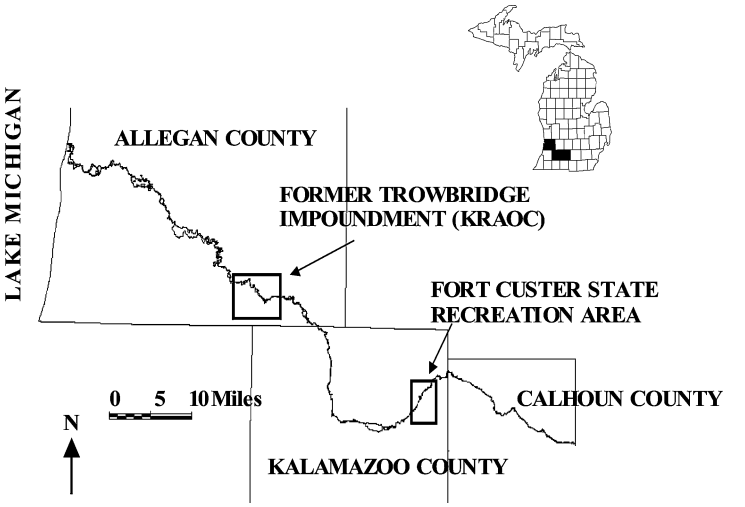


Figure 1. The PCB contaminated Trowbridge Impoundment within the Kalamazoo River Area of Concern and an upstream reference location at the Fort Custer State Recreation Area.

Impoundment (TB). FC was selected as the upstream reference area based on a number if criteria. FC is an undeveloped area of similar habitat to TB. FC is ~7 km upstream of the initiation of the Superfund site and ~40 km upstream of TB. FC has an established nest box trail (~20 years) with an associated historical database concerning tree swallow tissue contaminant content and productivity. This database suggests background contaminant exposure and normal productivity. TB lies downstream of the point sources and is the most PCB contaminated location within the Kalamazoo River Area of Concern (KRAOC) (Figure 1). The Trowbridge impoundment includes 132 ha of former sediments that are now under natural vegetation. Nest boxes in both locations were limited to the 100-year floodplain.

Bolus and Tissue Collection

Bolus samples were collected from individual nestlings (day 3 to day 14) during late morning and early afternoon at TB and FC to coincide with peak feeding periods (Kuerzi 1941). However, it has been suggested that the time of day does not strongly influence the number of feeding visits by the parent, and the number of visits are comparable across the breeding season (McCarty 2002). Ligatures were placed on all nestlings in each nest according to Johnson *et al.* (1980) for 1 to 2 hours to limit the effect of food deprivation on growth. After placement of ligatures, nestlings were observed for several minutes to determine if the ligature was affecting behavior of the adults or nestlings. Ligatures were removed from nestlings that appeared to be disturbed by their presence. Each hour, the nestlings' throats and the inside of the nests were checked for boluses. The packaging of the bolus in saliva by the adult prevented the loss of small dietary items through the ligature. Once a bolus was collected from a nestling, the ligature was removed from that nestling. Bolus sampling did not exceed four separate events or 3 g of bolus material at each nest. Sampling

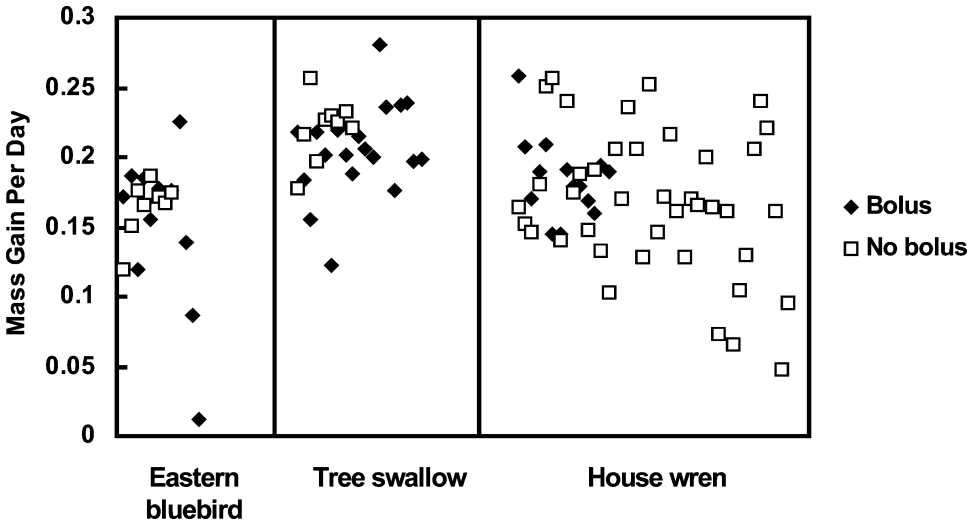


Figure 2. Comparison of mass gained per day of life for nestlings in nests from which boluses were collected (bolus) or not collected (no bolus) at the Kalamazoo River.

was discontinued when nestling tree swallows and eastern bluebirds were 14 days or house wrens were 10 days to eliminate premature fledging. This method is thought to provide an accurate representation of the nestling dietary composition (Johnson *et al.* 1980). There were no cases of abandonment at nests in which bolus sampling took place. Bolus collection did not statistically effect the growth of nestlings, and there were no discernible differences in growth of nestlings between nests from which a bolus was collected or not collected (Figure 2).

The stomachs of the birds in the study were collected to describe dietary composition in more detail. Tree swallow, eastern bluebird, and house wren nestlings were collected from randomly chosen nests, euthanized by cervical dislocation, and frozen until processing. Stomach contents were removed from nestlings and pooled based on proximity to insect sampling grids. Detailed descriptions of sampling methods are described elsewhere (Neigh *et al.* 2006a).

Average Potential Daily Dose

The amount of PCBs ingested by passerine birds was calculated using the wildlife dose equation for dietary exposures in the U.S. Environmental Protection Agency's (USEPA's) Exposure Factors Handbook (USEPA 1993). Average potential daily doses (APDD), calculated for total PCBs and TEQs based on avian-specific World Health Organization (WHO—Avian) toxic equivalency factors (van den Berg *et al.* 1998), were based on site-specific and literature-based diets for tree swallows, eastern bluebirds, house wrens, and American robins at the Kalamazoo River (Equation 1). Site-specific diets based on bolus content and literature-based diets were used to estimate dietary composition of the nestlings during the nestling period. Due to the lack of knowledge about the feeding habits of adults and nestlings after leaving the nest box, dietary dose only estimates site-specific exposure to nestlings during

the nesting period.

$$\text{APDD} = \sum (C_k \times FR_k \times NIR_k) \quad (1)$$

where C_k = Concentration of PCBs (totals or $TEQs_{WHO\text{-}Avian}$, ww) in the kth prey item in the passerine diet, FR_k = Fraction of the passerine diet based on mass represented by the kth prey item, NIR_k = Normalized ingestion rate of kth prey item (g prey/g body weight/day, ww).

Concentrations of PCBs in prey items were determined for insects collected in the bolus of nestlings and during site-specific sampling of insect orders; the results of which are reported elsewhere (Blankenship *et al.* 2005; Coady *et al.* 2005). FR_k was determined based on the dietary composition of each bird species. NIR_k was derived from ingestion rates reported for the American robin (0.89 g/g/d) and marsh wren (*Cistothorus palustris*) (0.99 g/g/d) (USEPA 1993). Dietary composition was determined from bolus samples based on occurrence and were converted to composition based on mass using the order-specific weights of individual insects from each location within KRAOC.

Comparisons of risk based on dietary exposure to PCBs were based on hazard quotients (HQs). Hazard quotients were calculated as the APDD (mg PCB/kg/d or ng TEQ/kg/d) divided by the corresponding toxicity reference value (TRV).

Selection of Toxicity Reference Values

TRVs from the literature were developed for the effects of PCBs based on both total PCB concentrations and $TEQs_{WHO\text{-}Avian}$ in the diet and eggs. The selection of TRVs was based on several criteria to determine their appropriateness for use in this study. These criterion included: (1) the use of wildlife species rather than traditional laboratory species; (2) chronic exposure over sensitive life stages; (3) the evaluation of ecologically relevant endpoints; (4) minimal co-contamination; (5) multiyear studies; (6) and total PCB or $TEQ_{WHO\text{-}Avian}$ values were reported or could be calculated. There were few studies available for passerine species. Due to the uncertainty in the dose-response of passerine birds to PCB exposure, a range of TRVs matching the given criteria have been reported (Table 1).

For this study as well as recent USEPA applications (USEPA 1995, 2000), a ring-necked pheasant (*Phasianus colchicus*) feeding study was selected as the most appropriate for the determination of a threshold for effects due to dietary exposure to passerine species (Dahlgren *et al.* 1972). The ring-necked pheasant study evaluates PCB toxicology during critical reproductive life stages, is of high quality, utilizes a wild species, and was found to be one of the most sensitive bird species examined. Chick survival and egg production were adversely affected in the 50 mg PCB/wk group, and hatchability was reduced by 14% in the 12.5 mg PCB/wk dose group compared to the control. The calculated daily dietary dose based on the 12.5 mg PCB/wk dose group and an adult pheasant weight of 1 kg (USEPA 1995) was 1.8 mg PCB/kg/d, and this value was considered the lowest observed adverse effect level (LOAEL). The no observed adverse effect level (NOAEL) of 0.6 mg PCB/kg/d was derived by dividing the LOAEL by a safety factor of three. A safety factor of three was determined to be acceptable because the LOAEL was established near the threshold for effects. Several species of wild passerine birds, such as the ones examined in this

Table 1. Toxicity reference values based on the no observed adverse effect level (NOAEL) and lowest observed adverse effect level (LOAEL) for dietary exposure of avian species to PCBs at the Kalamazoo River.

	Reference	Dietary TRV
Total PCBs (mg PCB/kg/d)		
	Dahlgren *et al.* (1972)	LOAEL = 1.8 mg PCB/kg/d
		NOAEL= 0.6 mg PCB/kg/d
	Calculated from BMFs	LOAEL = 14.7 mg PCB/kg/d[a]
		NOAEL = 1.9 mg PCB/kg/d[b]
Total TEQ (ng TEQ/kg/d)		
	Nosek *et al.* (1992)	LOAEL = 140 ng TEQ/kg/d
		NOAEL = 14 ng TEQ/kg/d
	Calculated from BMFs	NOAEL = 1000 ng TEQ/kg/d[c]

[a] Calculated from TRV selected from Custer *et al.* (2003).
[b] Calculated from TRV selected from Henning *et al.* (2003).
[c] Calculated from TRV selected from USEPA (2000).

study, appear to be less sensitive to PCB exposure (Thiel *et al.* 1997; Custer *et al.* 1998; Henning *et al.* 2003) than domesticated galliformes on which dietary TRVs could be based (*e.g.*, domestic chickens, ring-necked pheasant; Dahlgren *et al.* 1972).

In addition to the laboratory study, TRVs for dietary exposure to total PCBs were calculated from site-specific biomagnification factors (BMFs) between the diet and egg at the Kalamazoo River. Decreased hatching success in pippers of tree swallows has been reported to occur at 63 mg PCB/kg, ww in eggs (Custer *et al.* 2003), but no reproductive impairment of American robins was observed at 83.6 mg PCB/kg, ww in eggs (Henning *et al.* 2003). These studies are applicable to the current study because they fulfill the criteria for TRV selection, and they are conducted on wild passerine birds. TRVs for dietary exposure were calculated using biomagnification factors (BMFs) calculated by dividing concentrations of PCBs in eggs at the Kalamazoo River by the weighted average concentration of PCBs in the diet at the Kalamazoo River. For example, the site-specific BMF from diet to egg of tree swallows is 4.3 (calculated from egg concentration of 5.1 mg PCB/kg, ww / concentration in diet of 1.2 mg PCB/kg, ww). When the biomagnification factor (4.3) was applied to the LOAEL selected for eggs (63 mg PCB/kg), a TRV of 14.7 mg PCB/kg for the diet was calculated. No LOAEL could be established for terrestrial species, so the LOAEL calculated for tree swallows was used. This same concept was applied to arrive at the most conservative estimate for all avian species based on the NOAEL established for terrestrial passerine tissues, which was 83.6 mg PCB/kg, ww in eggs (Henning *et al.* 2003). The most conservative estimate of a TRV for dietary exposure when BMFs were applied was 1.9 mg PCB/kg, ww diet.

Few studies were available to derive TRVs based on $TEQ_{WHO\text{-}Avian}$ concentrations in the diet of wildlife species, whereas even fewer studies exist for passerine birds. A laboratory study by Nosek *et al.* (1992) found that intraperitoneal injections of 2,3,7,8 –TCDD at concentrations of 1000 ng TCDD/kg/wk (140 ng TCDD/kg/d) caused a 64% decrease in fertility and a 100% increase in embryo mortality in pheasants.

The study was a subchronic exposure (10 wk exposure period), and the length of the study was greater than the length of time an adult passerine may spend on site before nesting (~5 wk) (estimated from Adams 1979). Limitations of the study include the use of injections of TCDD instead of feeding TCDD contaminated prey to the test species and the evaluation of TCDD exposure and not PCB exposure. TEQs based on PCBs may overestimate exposure relative to TCDD (Custer *et al.* 2005), so TRVs based on TCDD exposure are likely to be conservatively estimate risk when applied to PCB exposure. TRVs derived from Nosek *et al.* (1992) are likely conservative because the galliformes used in the study are among the more sensitive species to the effects of TCDD (Hoffman *et al.* 1996). The NOAEL (14 ng TEQ/kg/d) was calculated by applying a safety factor of 10 to the LOAEL because effects due to the exposure are pronounced in the test subjects.

TRVs for $TEQ_{WHO\text{-}Avian}$ were also derived from site-specific BMFs. The NOAEL selected for $TEQ_{WHO\text{-}Avian}$ in the tissues of birds was 13000 ng TEQ/kg (see Neigh *et al.* 2006b). This field study was based on tree swallows and fulfilled the criteria for TRV selection. Based on the greatest BMF between diet and egg for the species examined in this study (BMF = 13 for house wrens, Neigh *et al.* 2006b), the TRV for dietary exposure to TEQs was determined to be 1000 ng TEQ/kg.

Little information on the toxicity of PCBs to passerine birds was available for terrestrial diets; thus there was uncertainty associated with the selection of an appropriate TRV to compare to Kalamazoo River dietary exposure. Several species of wild passerine birds, such as the ones examined in this study, appear to be less sensitive to PCB exposure (Thiel *et al.* 1997; Custer *et al.* 1998; Henning *et al.* 2003) than domesticated galliformes on which dietary TRVs could be based (*e.g.*, domestic chickens, ring-necked pheasant; Dahlgren *et al.* 1972). Thus, the dietary HQs based on domesticated avian species are likely an overestimate of hazard potential. In order to calculate a more realistic HQ based on similar species, site-specific and species-specific BMFs were applied to TRVs chosen for tissue exposure at the Kalamazoo River (Neigh *et al.* 2006a,b). The laboratory studies on galliformes and the toxicity reference values based on Kalamazoo River BMFs are intended to give a range of hazard quotients that expresses the most conservative estimate of risk and also reports a more appropriate estimate of the true risk of passerine birds exhibiting population level effects due to current contaminant levels at the Kalamazoo River. Due to the uncertainties in selecting TRVs, this study uses an examination of dietary exposure as part of a multiple lines of evidence approach to evaluate risk.

Assessment of Risk Using Multiple Lines of Evidence

In order to assist in the evaluation of risk, other lines of evidence were examined for agreement with the analysis and calculation of risk from dietary exposure (Fairbrother 2003) (Figure 3). Previously published studies on passerine birds inhabiting the riparian area of the Kalamazoo River have examined other lines of evidence to quantify site-specific risk to passerines (Neigh *et al.* 2006a,b,c,d). Two of the lines of evidence characterize exposure using the "bottom-up" approach, which is discussed here and describes dietary exposure, and using the "top-down" approach, which quantifies concentrations of PCBs present in the eggs, nestlings, and adults of the tree swallow, eastern bluebird, and house wren. A third and ancillary line of evidence investigates reproductive success of the species under investigation by

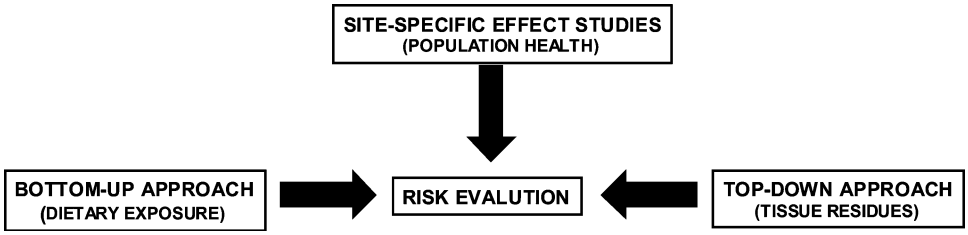


Figure 3. Multiple lines of evidence used to assess risk in Kalamazoo River passerine species.

comparing endpoints of reproductive success for birds at the contaminated location to the same endpoints of success for birds at a reference location. By evaluating these three lines of evidence together, it is possible to minimize the uncertainties associated with each approach and to make a more decisive conclusion of risk, especially when there is an agreement about risk from two or more lines of evidence.

RESULTS

Dietary Composition

The greatest number of bolus samples was collected during 2002. The most items were collected from tree swallows and the fewest from house wrens. A total of 1476 items from 64 nests were collected during the two years of diet collection in 2002 and 2003. Data for dietary composition were combined between years and between grids to increase the sample size and to provide a more representative account of passerine diets on the Kalamazoo River over the course of the study. A total of 11, 9, and 11 insect and invertebrate groups were represented in the bolus of the tree swallow, house wren, and eastern bluebird, respectively. There were a total of fourteen invertebrate groups taken by adult passerines: diptera, trichoptera, ephemeroptera, orthoptera, neuroptera, hymenoptera, araneae, odonata, hemiptera, lepidoptera, coleoptera, plecoptera, mollusca, and isopoda. For the purpose of analysis, homoptera was combined with hemiptera. Other items identified in the bolus were stones, glass, and pupae.

Aquatic insects made up the largest portion of insects in the bolus of tree swallows and were present in the bolus of all species in the following order: tree swallow (76.6%) > eastern bluebird (46.73%) > house wren (6.25%). Dipteran species represented 60.6%, 5.0%, and 8.0% of the total diet of tree swallows, house wrens, and eastern bluebirds (Figure 4). In contrast, diptera represented 79.1% and 80.0% of the aquatic species taken in the diet for tree swallows and house wrens, respectively, whereas diptera only comprised 17.1% of all aquatic species in the eastern bluebird. All dipterans were lumped into the aquatic category because the majority of dipterans captured as adults were of aquatic origin. Eastern bluebirds at TB also had a large aquatic component in the diet, but this was largely due to 1 bolus with 63 trichopterans collected from TB. When this was removed from the analysis, aquatic insects comprised 22.3% of the eastern bluebird diet.

The avian species examined differed in the occurrence of terrestrial insects in the diet relative to aquatic insects. Unlike the eastern bluebird and house wren, tree

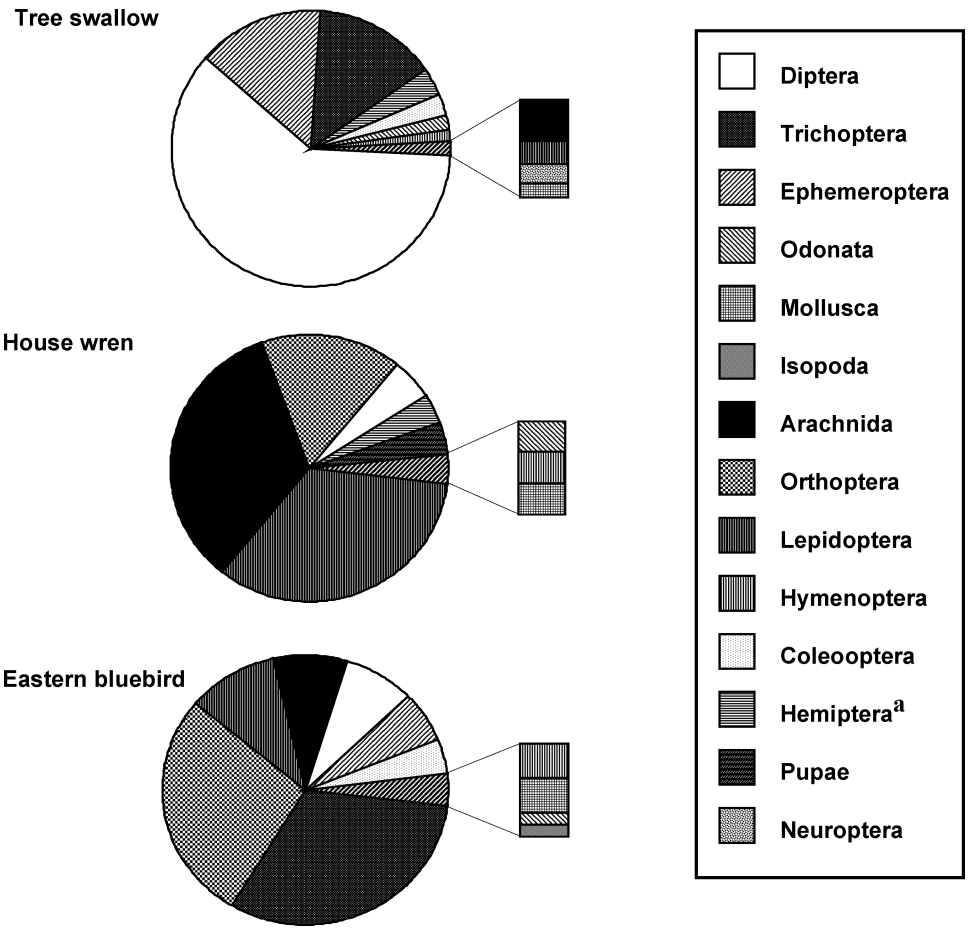


Figure 4. Dietary composition based on occurrence at the Kalamazoo River. The order hemiptera also includes the order homoptera.

swallows contained a greater proportion of aquatic insects than terrestrial insects, and only the terrestrial insect orders hemiptera and coleoptera represented more than 2% of the total diet. The order orthoptera was important in the diet of both house wrens and eastern bluebirds (house wrens = 16%; eastern bluebirds = 28%) but never occurred in tree swallow boluses. Lepidoptera were infrequently taken by tree swallow adults (<0.05% of diet), but comprised 34% and 10% of the house wren and eastern bluebird diet, respectively.

The contents of nestling stomachs were also identified. Much of the content could not be distinguished, but the indigestible portions, such as the legs, heads, and exoskeleton of orders were identifiable. The purpose of identifying stomach contents was not to quantify the diet using this method but to identify portions of the diet that were not observed during bolus sampling. Describing diet solely through this method could lead to bias because orders with indigestible portions would be easily identified, and therefore, stomach contents would appear to contain a larger proportion of orders with indigestible exoskeletons such as coleoptera (Wheelwright

Table 2. Concentration of total PCBs and $TEQs_{WHO\text{-}Avian}$ calculated for dietary exposure in avian species at the Kalamazoo River based on a site-specific diet and a literature-derived diet.

	Total PCB (mg/kg/d)		TEQ (ng/kg/d)	
	Fort Custer	Trowbridge	Fort Custer	Trowbridge
Eastern bluebird (*Sialia sialis*)				
Kalamazoo Diet	0.017	0.51	2.7	70
Pinkowski (1978)	0.015	0.39	2.3	70
Tree swallow (*Tachycineta bicolor*)				
Kalamazoo Diet	0.12	1.2	5.4	190
Johnson and Lombardo (2000)	0.11	0.33	5.6	50
McCarty and Winkler (1999)	0.070	0.49	1.9	95
House wren (*Troglodytes aedon*)				
Kalamazoo Diet	0.022	0.13	2.5	31
Kale (1965)	0.052	0.50	2.1	89
American robin (*Turdus migratorius*)				
Howell (1940)	0.030	0.41	0.97	52

1986). Sampling of stomach contents from 2001 and 2002 revealed that stones and mollusks were frequently found in the stomachs of nestlings, especially at FC, but large pieces of grit were not observed as frequently in nestlings from TB. Of the nestlings examined at FC ($n = 50$), 64% contained at least one stone and 10% contained at least one mollusk shell, while at TB ($n = 11$), 36% of the nestlings examined contained at least one stone and 18% contained a mollusk shell. Seeds of the garlic mustard plant (*Alliaria petiolata*) were also found in the stomachs of eastern bluebird and house wren nestlings from FC ($n = 3$).

Average Potential Daily Dose (APDD)

APDDs for passerine birds were calculated for both total PCB and $TEQ_{WHO\text{-}Avian}$ concentrations of each insect order for a diet based on dietary composition at the Kalamazoo River. Based on the site-specific diet, dietary ingestion of total PCBs and $TEQs_{WHO\text{-}Avian}$ were greatest for the tree swallow and least for house wren at TB (Table 2). Calculations of APDD for total PCBs were 10-, 29-, and 6-fold greater at TB than at FC, and $TEQs_{WHO\text{-}Avian}$ were 35-, 26-, and 16-fold greater at TB than FC in tree swallows, eastern bluebirds, and house wrens.

Soil ingestion based on literature values was also factored into the calculation of the APDD for total PCBs, but $TEQ_{WHO\text{-}Avian}$ concentrations in soils were not measured at the site and could not be factored into the APDD. Concentrations of total PCBs in soils were considered to be 85% bioavailable and contain 65% moisture (estimated from Studier and Sevick 1992) to make the dry weight (dw) concentrations comparable to wet weight concentrations in prey. The mass percent of grit in the diet consumed by the bird species were calculated based on the average mass of grit in the stomach of nestlings over the nestling period (tree swallow = 17.2 mg, dw; house wren = 6.2 mg, dw) (Mayoh and Zach 1986). The APDD calculated with soil was not greatly different from the APDD calculated with insects alone, but APDD

calculated with soil was greater in all cases. The APDD value for house wrens at TB was the most different with a 4% increase in concentration when soil was added to the calculation of APDD. All other APDD values did not change or increased by only 1–2% when soil was included.

The APDD was also calculated based on dietary composition found in the literature and compared to site-specific calculations (Table 2). Soil ingestion was factored into the calculation of APDD in both the literature diet and the site-specific diet for total PCBs but not for $TEQs_{WHO\text{-}Avian}$. The APDDs based on total PCBs and $TEQs_{WHO\text{-}Avian}$ for the site-specific diet were no more than 4-fold greater than in the literature diet, which suggests that the Kalamazoo River diet was similar to literature diets. The tree swallow diet was the most different from the literature diet in APDD, and the house wren was the only species in which the literature-derived diet yielded APDDs greater than those calculated for the Kalamazoo River. The site-specific dietary composition of the American robin was not quantified in this study, but an APDD was calculated using PCB concentrations of site-specific dietary items combined with a literature-based dietary composition. The APDD of total PCBs and $TEQs_{WHO\text{-}Avian}$ for the American robin were 0.41 mg PCB/kg, ww and 52 ng TEQ/kg, ww, respectively. This suggests that the exposure of the American robin is less than that of the tree swallow and eastern bluebird but more than in the house wren. It should be noted that the APDD calculation for the American robin does not include $TEQs_{WHO\text{-}Avian}$ ingested during the consumption of plant. This is because plant PCB concentrations are extremely low resulting in less than 4% of the total dietary PCB exposure. Similarly, American robin $TEQs_{WHO\text{-}Avian}$ exposure derived from plant consumption should be of an equally low proportion. A subset of plant samples analyzed for coplaner PCBs resulted in concentrations at or below limits of detection. However it should be noted that the American robin diet utilized contains 29% plants (Howell 1940), thus the APDD for $TEQs_{WHO\text{-}Avian}$ in the American robin diet may be slightly underestimated.

Assessment of Risk

Hazard quotients (HQs) were calculated for each location based on the literature-derived APDD and the site-specific APDD. Two different estimates of the NOAEL and LOAEL were calculated from TRVs derived from a laboratory study and from field studies. All HQs at FC for total PCBs were less than 1.0 based on both the NOAELs and the LOAELs. Therefore, only HQs at TB are discussed. Mean values of HQs based the NOAELs and LOAELs for total PCBs were less than 1.0 for all species, except for the tree swallow site-specific diet, which had a HQ of 2.0 based on the most conservative NOAEL (Figure 5). Species were also evaluated based on the upper 95% confidence limit (U95 CL) in order to describe a range of HQs that could be expected for a population. HQ values for all species at the U95 CL ranged from 4.8 for house wrens based on the most conservative NOAEL to 0.17 for house wrens based on the less conservative NOAEL. The HQs for all species based on the U95 CL for the LOAEL ranged from 1.6 for house wrens to 0.02 for house wrens based on the less conservative LOAEL.

HQs based on $TEQ_{WHO\text{-}Avian}$ concentrations followed similar patterns as HQs based on total concentrations of PCBs. HQs for FC were less than 1.0 for all comparisons,

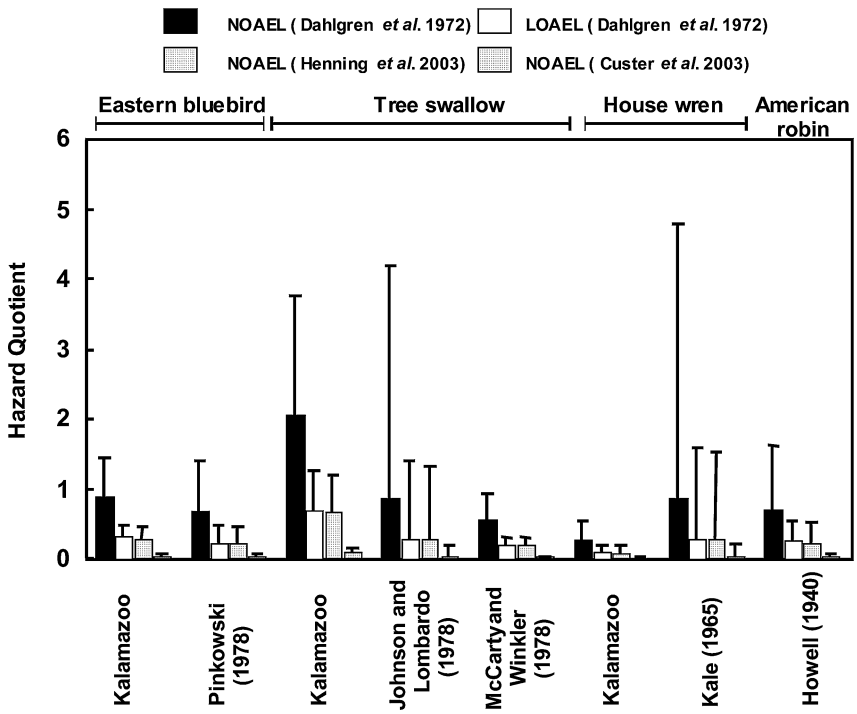


Figure 5. Passerine bird dietary hazard quotients for the Trowbridge Impoundment (Kalamazoo) based on the total PCB no observed adverse effect level (NOAEL) and the lowest observed adverse effect level (LOAEL). Dietary hazard quotients were calculated based on the Kalamazoo site-specific diet and literature-derived diets. Error bars represent the upper 95% confidence limit. HQs range from approximately 0 to 5.

so only values for TB are discussed. HQ values were greatest for tree swallows, and HQs for only the tree swallow based on LOAEL exceed 1.0 (Figure 6). HQ values based on the most conservative laboratory NOAEL (Nosek *et al.* 1992) exceeded 1.0 in all species, and in tree swallows, the HQ value exceeded 10.0. The mean HQ did not exceed 0.20 based on a less conservative NOAEL calculated from field studies on the tree swallow (calculated from USEPA 2000). Based on the U95 CL of the NOAEL derived from Nosek *et al.* (1992), HQs were as great as 70 in the tree swallow, but the greatest HQ calculated from USEPA (2000) based on the U95 CL was 0.99 for tree swallows.

DISCUSSION

Dietary Composition

The bolus sampling strategy at the Kalamazoo River seemed to accurately predict the general dietary feeding guilds of the species that were being evaluated, but it also identified some unique feeding characteristics of each species. Tree swallows were

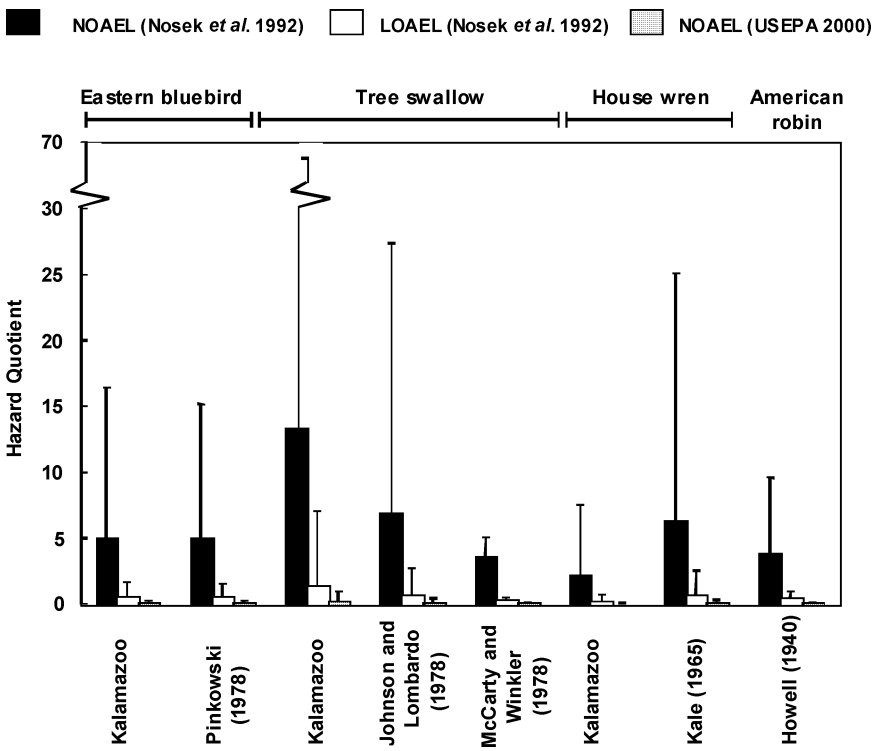


Figure 6. Passerine bird dietary hazard quotients for the Trowbridge Impoundment based on the $TEQ_{WHO\text{-}Avian}$ no observed adverse effect level (NOAEL) and the lowest observed adverse effect level (LOAEL) based on toxicity reference values calculated from the literature. Dietary hazard quotients were calculated based on the Kalamazoo site-specific diet and literature-derived diets. Error bars represent the upper 95% confidence limit. HQs range from approximately 0 to 70.

expected to feed primarily near the water's surface on emergent aquatic insects (Quinney and Ankney 1985; McCarty 1997). The tree swallows at the Kalamazoo River were observed feeding in riparian areas and at the water's surface, and the diet was comprised of a majority of aquatic insects (76.6%). Dipteran species comprise a large portion of the diet in tree swallows (60.6%), which is similar to a tree swallow population in New York (57%) (McCarty and Winkler 1999) and Michigan (58%) (Johnson and Lombardo 2000). Eastern bluebirds fed primarily on insects from terrestrial origins, as was expected (Pinkowski 1978), but there were some deviations from the diet not predicted by the literature. Trichoptera was found in the diet of both tree swallow and eastern bluebird populations at the Kalamazoo River, but was not represented in any of the literature-derived diets (Figure 7). This likely resulted from the close proximity of Kalamazoo populations to an aquatic ecosystem. There is also a notable absence of earthworms in bolus contents of eastern bluebirds inhabiting riparian areas of the Kalamazoo River, which is maybe a result of unsuitable soil conditions for earthworms at study locations. Previously, little was known about

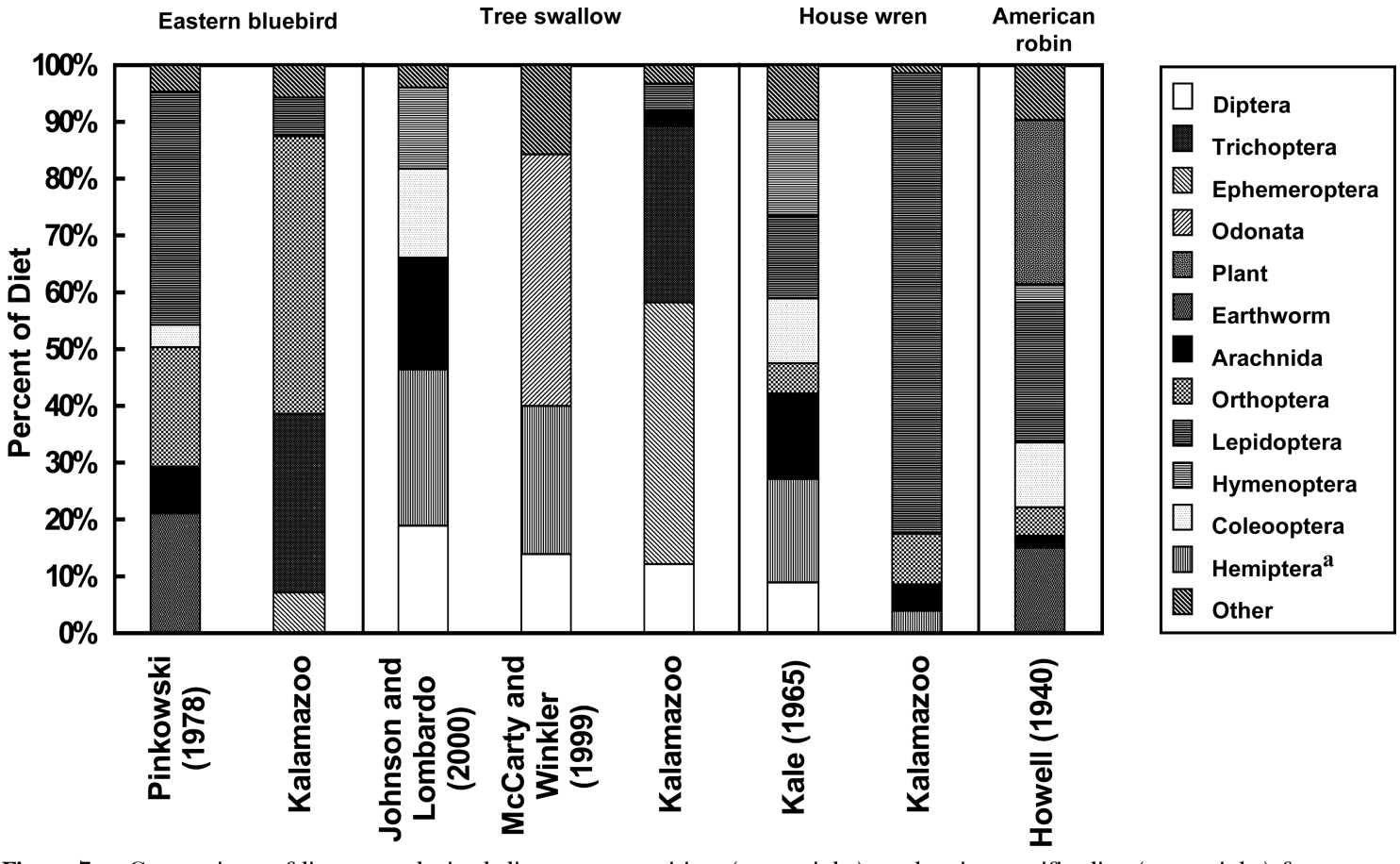


Figure 7. Comparison of literature-derived dietary composition (wet weight) to the site-specific diet (wet weight) for eastern bluebirds, tree swallows, house wrens, and American robins at the Trowbridge Impoundment on the Kalamazoo River. The order hemiptera also includes the order homoptera.

specifics of the house wren diet, but they also fell within the terrestrial feeding guild as predicted.

The differences between the diet of the Kalamazoo River and other studies suggest that although diets of each species may be predictably composed primarily of aquatic or terrestrial prey items, there may be important site-specific interactions and opportunistic feeding events that alter the general diet of a population. In the stomachs of nestlings, there were stones, mollusk shells, bits of glass, and the seeds of the garlic mustard plant, and likewise, metal shavings and plastic have been reported in the stomach of tree swallows (Mayoh and Zach 1986). It is believed that all these items were opportunistically accumulated as sources of grit. Ephemeroptera and trichoptera were found in some boluses of eastern bluebirds at TB, which possibly shows that this species will feed opportunistically. Diets may differ regionally or temporally depending on the amount of opportunistically selected prey. Although these changes did not result in large differences in exposure predictions at our site it is foreseeable that sites where the contaminant dispersion among proximal aquatic and terrestrial habitats are dissimilar, the unique composition of a site-specific diet may contribute significant concentrations to the overall assessment of exposure, and therefore, pose significant risk that may be overlooked when applying literature-based dietary composition to the assessment of risk.

Habitat dynamics may be a potential cause or even a tool to predict site-specific differences in the diet of passerine birds. Habitat is a critical factor in the determination of the diet of key receptors and the eventual determination of risk to those receptors. For example, Blancher and McNicol (1991) examined tree swallow diet in relation to wetland acidity. In wetlands with a high pH, tree swallows predominately preyed on mollusks and ephemeroptera, but in wetlands with low pH, they preyed on a greater percentage of diptera. The adult tree swallows also fed nestlings fewer aquatic orders relative to terrestrial orders in areas of wetland acidity, which was possibly linked to the inability of low calcium aquatic prey to satisfy the calcium requirements of nestlings (St. Louis and Barlow 1993). In a case such as this, the exposure of tree swallows through terrestrial pathways may be much more important than in a typical area. When diets are predicted instead of measured, interactions between habitat and prey availability must be identified. Only by measuring site-specific dietary composition or prey availability can these interactions be taken into account.

Average Potential Daily Dose

The intake of grit, or soil ingestion, was factored into calculations of the APDD as a conservative approach to calculating risk. Concentrations present in the soil are greater than concentrations in insects (soil = 4.3 mg PCB/kg, ww; insect = 0.55 mg PCB/kg, ww), so the contribution of soil to overall exposure could be substantial depending on the amount of soil ingested and the bioavailability of the PCBs through absorption in the gut. The majority of the exposure to PCBs through grit ingestion is not expected to be from grit itself, which is composed of stones, mollusk shells, and sand (Mayoh and Zach 1986), but from soil associated with the surface of the grit. Tree swallows feed aerially, so the only soil present in the diet would exist as incidental particles on prey or would be consumed in conjunction with grit particles.

In addition to grit ingestion, eastern bluebird, house wrens, and American robins may ingest some additional soil particles from the ground as they are feeding, but American robins likely have the greatest soil ingestion of all species due to the ingestion of earthworms with soil in the gut and soil adhering to the surface of the earthworms. This exposure pathway could potentially yield extensive exposure in species consuming large amounts of soil, so soil intake should be carefully considered when evaluating exposure in various species.

The APDD based on a theoretical diet derived from the literature was an underestimate of the site-specific diet in most species. The underestimation can be attributed to the proportion of aquatic insects in the diet at the Kalamazoo River, relative to that of the literature diet. The aquatic insects contain some of the greatest concentrations of PCBs for insects of the Kalamazoo River (Kay *et al.* 2005), which results in the greater concentrations of PCBs in the site-specific diet. Only for the house wren did the literature diet contain greater concentrations than in the diet of the Kalamazoo River. Lepidoptera and orthoptera contained lesser concentrations of total PCBs and $TEQs_{WHO\text{-}Avian}$ than all insect orders (Blankenship *et al.* 2005). Because the site-specific diet for house wrens was comprised of a much greater proportion of lepidoptera and orthoptera (90% by mass) than did the literature diet (20% by mass), the APDD calculated from the site-specific diet was also less than the literature diet. The differences in the proportion of insects may be a result of the fact that a literature diet could not be located for the house wren, so a published diet for the marsh wren was used (Kale 1965). The differences between the diets does not greatly effect the estimate of risk based on the mean concentration in the diet, but based on the U95 CL, the most conservative HQ is 0.53 from the site-specific diet and 4.8 from the literature diet. Depending on the weight given to this line of evidence during the risk evaluation process, a very different estimate of risk may be reached, so it is critical to establish a diet specific to the species.

Relationship Between Risk Estimates Based on Total PCBs and TEQs

Hazard quotients based on $TEQs_{WHO\text{-}Avian}$ were greater than those based on total concentrations of PCBs. HQs based on the total PCBs is thought to be a more accurate estimate of possible risk because the concentration can be compared directly to values reported in the studies from which TRVs were derived. There are difficulties and uncertainties with assessing the toxicity of environmentally weathered PCB mixtures that are quantified as Aroclors. Congener-specific analyses, including coplanar PCB congeners combined with a calculation of TEQs, are generally thought to correlate better with toxicity than measures of total PCBs (Giesy and Kannan 1998; Blankenship and Giesy 2002). However, recent work by Custer *et al.* (2005) calls into question whether toxic equivalency factors (TEFs) developed for PCBs are appropriate to predict effects in some bird species. One reason for the possible overestimate of risk posed by complex mixtures of PCBs is that concentrations of TEQs are calculated by multiplying each aromatic hydrocarbon receptor (AhR)–active PCB congener by a relative potency expressed as a TEF. TEF values are consensus values that were rounded up to be conservative estimates of risk (van den Berg *et al.* 1998). Thus, they tend to overestimate the risk. This coupled with the use of proxy values for congeners that were present at concentrations less than the

method detection limit were the most likely reasons that HQs estimated based on $TEQs_{WHO\text{-}Avian}$ were much greater than those estimated based on total PCBs. For example, based on tree swallow studies on the Woonasquatucket River, an LC50 based on TEQs was estimated to be 1700 pg TEQ/g, ww (primarily due to TCDD) (Custer *et al.* 2005). However, if one compares this LC50 to concentrations of TEQs (calculated from PCBs) between 1,730 and 12,700 pg TEQ/g, ww in tree swallow eggs from the Hudson River, one would expect considerable population-level effects due to mortality. However, there were minimal effects on subtle endpoints at TEQ concentrations (based on PCBs) in tree swallow eggs from the Hudson River (McCarty and Secord 1999). In other words, a concentration of TEQ was not toxicologically equivalent to the same concentration expressed as calculated TEQs (based only on PCBs).

Assessment of Risk Using Multiple Lines of Evidence

The study presented here was part of a larger study to examine PCB exposure and associated risk in aquatic and terrestrial ecosystems. In particular, risk associated with dietary exposure to total PCBs and TEQs, or the "bottom-up" approach, determined that based on the most conservative HQs, there appears to be risk to passerine species exposed through the diet due to dietary concentrations of TEQs being greater than the threshold for effects. These values suggest that effects may occur due to exposure to non-*ortho* and mono-*ortho* PCBs at the Kalamazoo River (USEPA 1998). When the more realistic, field-based TRVs are applied, the HQs are near to or less than 1.0, which suggests little risk of the population exhibiting reproductive effects. The lack of knowledge about sensitivity of passerine birds compared to other well-studied species suggests that for understudied passerines in particular, HQs based on total PCBs may be a more appropriate estimate of risk than those based on TEQ concentrations. As suggest by the multiple lines of evidence approach (Fairbrother 2003), additional lines of evidence besides dietary exposure should be evaluated in order to arrive at the best estimate of risk, especially given the uncertainty in selecting appropriate TRVs.

In addition to the "bottom-up" approach, a secondary line of evidence used the "top-down" approach to evaluate egg, nestling, and adult tissue concentrations in tree swallows, eastern bluebirds, house wrens, and American robins from the Kalamazoo River. This line of evidence suggested little risk to passerines based on tissue concentrations of total PCBs and TEQs at the site. Similar field studies completed at other sites found no effects on the reproductive fitness of passerine birds at concentrations of PCBs as great or greater than those measured in birds of the Kalamazoo River (Secord and McCarty 1997; Custer *et al.* 2003).

A third line of evidence was used to investigate population health of passerine species in contaminated portions of the Kalamazoo River compared to an upstream reference location. Studies of reproductive performance did not find statistically significant decreases in reproductive fitness of tree swallows at the more contaminated TB location relative to the FC reference location. However, there were some measures of reproductive success in the eastern bluebird (productivity) and house wren (clutch size, brood size, and hatching success) that were statistically less at TB than FC, but these differences were not found consistently across all of the reproductive

measures or throughout the entire nesting season (Neigh *et al.* 2006d). Also, samples sizes were small for eastern bluebirds, and so, a 10% decrease in reproductive success at the contaminated location during the study can be linked to a single female who made two unsuccessful reproductive attempts during one year.

Guidelines based on criteria established for describing the chemical causation for effects in ecoepidemiological studies were applied to this study to determine whether PCBs likely caused the observed reproductive effects. They include temporality, strength of association, consistency, and biological plausibility (Hill 1965). PCB contamination remained constant throughout the study but reproductive success varied over the course of the field season, among years, and among species, likely due to normal population variability, so the first criterion requiring consistent trends over the course of exposure (temporality) was not satisfied. Also, strength of association between PCB exposure and reproductive effects could not be identified. Some individuals with comparatively great concentrations of PCB reproduced successfully, whereas other individuals with background levels of contaminants failed to reproduce. Consistency between results of available studies is also needed in order to assign causality to PCBs for reproductive dysfunction in passerine birds. Few studies have investigated dosing in a laboratory setting in these species, but there are several studies available describing PCB concentrations in the field. Other field studies of passerine species did not find significant reproductive effects when concentrations of PCBs in tissues were similar or greater than in the birds at the Kalamazoo River (McCarty and Secord 1999; Harris and Elliot 2000; Henning *et al.* 2003), which suggests that PCBs are not the primary causative agent of effects. It is biologically plausible based on laboratory and field studies that PCBs deposited in the sediments can bioaccumulate up the food chain and elicit effects on the reproduction of upper trophic level species (Giesy *et al.* 1994). PCBs seem unlikely to be the primary cause of the observed reproductive effects based on the criteria described due to the lack of temporality, strength of association, and consistent findings of no effects at concentrations similar or greater than those present at the Kalamazoo River. Although it cannot be denied that PCBs cause reproductive impairments at certain concentrations, the below-threshold concentrations present in the tissues and diet at the Kalamazoo River suggest that other factors such as co-contamination by DDT and its metabolites, habitat quality, inclement weather, or prey abundance may be affecting reproduction.

There are several potential reasons for different conclusions between the lines of evidence. The evaluation of risk from tissue and dietary concentrations depends heavily on the selection of the TRV. Little data for these species exist based on concentrations of total PCBs, but even less data exist on the dose-response of $TEQs_{WHO\text{-}Avian}$ in the tissue or diet and their relation to reproductive effects. Upon selection of a TRV, uncertainty factors are often applied to compensate for unknown differences between species, exposure time, or exposure route (Chapman *et al.* 1998). The application of these factors is often an inexact science and can introduce a negative bias into the proper calculation of TRVs, which would then result in an overestimate of risk. In particular, in the case of PCBs, the use of total PCB concentrations as a measure or exposure, instead of TEQs seems to more accurately represent the actual risk based on field measures of effects. A strength of this study is that it used measured concentrations of PCBs to quantify exposure in the diet and tissues instead of predicting exposure based on concentrations in other matrices and then applying

biomagnification factors to predict other trophic level or life stage concentrations. As for the population health line of evidence, observations of reproductive performance may also be affected by environmental stressors, which may be confounded with exposure to PCBs.

CONCLUSION

The main purpose of this study was to quantify exposure pathways for four passerine birds and determine risk associated with exposure. As expected, the diet of the tree swallow consisted of primarily aquatic insects, whereas the diets of the eastern bluebird and house wren consisted of primarily terrestrial insects. Aquatic insects contained greater concentrations of total PCB and TEQs than terrestrial insects, and therefore, dietary exposure to PCBs was calculated to be the greatest in tree swallows. Risk varied with the TRV selected, but risk for total PCBs was generally below the threshold for effects. Risk associated with TEQs was below the threshold for effects when the most appropriate TRV was applied, but when the most conservative TRV was selected, all species were above the threshold for effects. Risk based on dietary exposure to passerine species is uncertain due to the lack of sound laboratory derived effects data in the literature for passerine species. There is a serious need to fill these data gaps in the literature, especially given the frequent use of bird species in risk assessment. Another primary goal of the study was to test the assumption that "top-down" and "bottom-up" assessments arrive at similar estimates of risk. The study also was an exercise in the application of a multiple lines of evidence approach to risk evaluation. Many factors can play a role in the calculation of risk, and by selecting only one method, there is a possibility of missing an important interaction and improperly characterizing a site. Three lines of evidence based on reproductive health, tissue concentrations, and dietary exposure arrived at differing conclusions of risk. Overall, the various lines of evidence suggest that the inconsistent differences in reproductive performance observed for some species of passerine birds at the more contaminated site were caused by factors other than exposure to PCBs. In addition, the top-down approach (concentrations measured in the tissues of the birds) suggested little risk to any species, but the bottom-up approach (exposure predicted based on the diet) arrives at different conclusions of risk depending on the TRV selected and whether they were based on concentrations of total PCBs or TEQs. When exposures were predicted through application of biomagnification factors, uncertainty factors, or if the appropriateness of the TRV is in question, the actual dynamics at the site can become even more difficult to characterize. As suggested by Fairbrother (2003), the application of several lines of evidence in multiple matrices seems to be the best approach to gather the most complete and appropriate information on which to gauge important risk decisions. By considering all of the lines of evidence simultaneously, it was concluded that the current concentrations of PCBs in the aquatic and terrestrial food chains were not causing population-level adverse effects on the populations of passerine birds studied here.

ACKNOWLEDGEMENTS

The authors extend a special thanks to the students and employees at the Michigan State Aquatic Toxicology Laboratory, Michigan State University Kellog Biological

Station, Entrix, Inc., and the Kalamazoo Nature Center. We also thank the Kalamazoo River Study Group for funding this study.

REFERENCES

Adams RJ. 1979. Seasonal distribution and abundance of birds in the Kalamazoo, Michigan, area. Jack-Pine Warbler 57:91–105

Ankley GT, Niemi GJ, Lodge KB, *et al.* 1993. Uptake of planar polychlorinated biphenyls and 2,3,7,8-substituted polychlorinated dibenzofurans and dibenzo-*p*-dioxins by birds nesting in the Lower Fox River and Green Bay, Wisconsin, USA. Arch Environ Contam Toxicol 24:332–44

Bishop CA, Mahony NA, Trudeau S, *et al.* 1999. Reproductive success and biochemical effects in tree swallows (*Tachycineta bicolor*) exposed to chlorinated hydrocarbon contaminants in wetlands of the Great Lakes and St. Lawrence River Basin, USA and Canada. Environ Toxicol Chem 18:263–71

Blancher PJ and McNicol DK. 1991. Tree swallow diet in relation to wetland acidity. Can J Zool 69:2629–37

Blankenship AL and Giesy JP. 2002. Use of biomarkers of exposure and vertebrate tissue residues in the hazard characterization of PCBs at contaminated sites: Application to birds and mammals. In: Sunahara GI, Renoux AY, Thellen C, *et al.* (eds), Environmental Analysis of Contaminated Sites, pp 153–80. John Wiley & Sons, Ltd., West Sussex, UK

Blankenship AL, Zwiernik MJ, Coady KK, *et al.* 2005. Differential accumulation of PCB congeners in the terrestrial food web at the Kalamazoo River, Michigan, USA. Environ Sci Technol 39:5954–63

Chapman PM, Fairbrother A, and Brown D. 1998. A critical evaluation of safety (uncertainty) factors for ecological risk assessment. Environ Toxicol Chem 17:99–108

Custer CM, Custer TW, Allen PD, *et al.* 1998. Reproduction and environmental contamination in tree swallows nesting in the Fox River Drainage and Green Bay, Wisconsin, USA. Environ Toxicol Chem 17:1786–98

Custer TW, Custer CM, and Hines RK. 2002. Dioxins and congener-specific polychlorinated biphenyls in three avian species from the Wisconsin River, Wisconsin. Environ Pollut 119:323–32

Custer CM, Custer TW, Dummer PM, *et al.* 2003. Exposure and effects of chemical contaminants on tree swallows nesting along the Housatonic River, Berkshire County, Massachusetts, USA, 1998–2000. Environ Toxicol Chem 22:1605–21

Custer CM, Custer TW, Rosiu CJ, *et al.* 2005. Exposure and effects of 2,3,7,8–tetrachlorodibenzo-p-dioxin in tree swallows (*Tachycineta bicolor*) nesting along the Woonasquatucket River, Rhode Island. Environ Toxicol Chem 24:93–109

Dahlgren RB, Linder RL, and Carlson CW. 1972. Polychlorinated biphenyls: Their effects on penned pheasants. Environ Health Perspect 1:89–101

Fairbrother A. 2003. Lines of evidence in wildlife risk assessments. Hum Ecol Risk Assess 9:1475–91

Froese KL, Verbrugge DA, Ankley GT, *et al.* 1998. Bioaccumulation of polychlorinated biphenyls from sediments to aquatic insects and tree swallow eggs and nestlings in Saginaw Bay, Michigan, USA. Environ Toxicol Chem 17:484–92

Giesy JP and Kannan K. 1998. Dioxin-like and Non-dioxin-like Toxic Effects of Polychlorinated Biphenyls (PCBs): Implications for Risk Assessment. Critical Rev Toxicol 28:511–69

Giesy JP, Ludwig JP, and Tillitt DE. 1994. Deformities in birds of the Great Lakes region: Assigning causality. Environ Sci Technol 28:128A–35A

Halbrook R, Woolf A, and Arenal C. 1998. European starling (*Sturnus vulgaris*): Avian model and monitor of contaminant and remedial effects at Crab Orchard National Wildlife Refuge. RR-E84. Waste Management and Research Center, Champaign, IL, USA

Harris ML and Elliott JE. 2000. Reproductive success and chlorinated hydrocarbon contamination in tree swallows (*Tachycineta bicolor*) nesting along rivers receiving pulp and paper mill effluent discharges. Environ Pollut 110:307–20

Henning MH, Robinson SK, McKay KJ, *et al.* 2003. Productivity of American robins exposed to polychlorinated biphenyls, Housatonic River, Massachusetts, USA. Environ Toxicol Chem 22:2783–8

Hill AB. 1965. The environment and disease: Association or causation? Proc R Soc Med 58:295–300

Hoffman DJ, Rice CP, and Kubiak TJ. 1996. PCBs and dioxins in birds. In: Beyers WN, Heinz GH, Redmon-Norwood AW (eds), Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations, pp 165–207. CRC Press, Inc, Boca Raton, FL, USA

Howell JC. 1940. Spring roosts of the robin. Wilson Bull 52:19–23

Johnson EJ, Best LB, and Heagy PA. 1980. Food sampling biases associated with the "ligature method." Condor 82:186–92

Johnson ME and Lombardo MP. 2000. Nestling tree swallow (*Tachycineta bicolor*) diets in an upland old field in western Michigan. Am Midl Nat 144:216–9

Jones PD, Giesy JP, Newsted JL, *et al.* 1993. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin equivalents in tissues of birds at Green Bay, Wisconsin, USA. Arch Environ Contam Toxicol 24:345–54

Kale HW II. 1965. Ecology and bioenergetics of the long-billed marsh wren *Telmatoidytes palustris griseus* (Brewster) in Georgia salt marshes. Publ Nuttall Ornith Club No. 5

Kannan N, Tanabe S, and Tatsukawa R. 1989. Persistency of highly toxic coplanar PCBs in aquatic ecosystems: Uptake and release kinetics of coplanar PCBs in green-lipped mussels (*Perna viridis Linnaeus*). Environ Poll 56:65–76

Kay DP, Blankenship AL, Neigh AM, *et al.* 2005. Differential accumulation of PCB congeners in the aquatic food web at the Kalamazoo River Superfund Site, Michigan, USA. Environ Sci Technol 39:5964–74

Kuerzi RG. 1941. Life history studies of the tree swallow. Proc Linn Soc NY 52–53:1–52

Ludwig JP, Auman HJ, Kurita H, *et al.* 1993. Caspian tern reproduction in the Saginaw Bay ecosystem following a 100-Year flood event. J Great Lakes Res 19:96–108

Mayoh KR and Zach R. 1986. Grit ingestion by nestling tree swallows and house wrens. Can J Zool 64:2090–3

McCarty JP. 1997. Aquatic community characteristics influence the foraging patterns of tree swallows. Condor 99:210–3

McCarty JP. 2002. The number of visits to the nest by parents is an accurate measure of food delivered to nestlings in tree swallows. J Field Ornithol 73:9–14

McCarty JP and Secord AL. 1999. Reproductive ecology of tree swallows (*Tachycineta bicolor*) with high levels of polychlorinated biphenyl contamination. Environ Toxicol Chem 18:1433–9

McCarty JP and Winkler DW. 1999. Foraging ecology and diet selectivity of tree swallows feeding nestlings. Condor 101:246–54

MDEQ (Michigan Department of Environmental Quality). 2003. Final (Revised) Baseline Ecological Risk Assessment, Allied Paper, Inc., Portage Creek, Kalamazoo River Superfund Site. Report April 2003. 1-6-12. Prepared by Camp, Dresser, and McKee for Michigan Department of Environmental Quality, Remediation and Development Division, Lansing, MI, USA

Neigh AM, Zwiernik MJ, Bradley PW, *et al.* 2006a. Tree swallow (*Tachycineta bicolor*) exposure to polychlorinated biphenyls at the Kalamazoo River Superfund Site. Environ Toxicol Chem 25:428–37

Neigh AM, Zwiernik MJ, Bradley PW, *et al.* 2006b. Accumulation of polychlorinated biphenyls (PCBs) from floodplain soils by passerine birds. Environ Toxicol Chem 25:1503–11

Neigh AM, Zwiernik MJ, MacCarroll MA, *et al.* 2006c. Productivity of tree swallows (*Tachycineta bicolor*) exposed to PCBs at the Kalamazoo River Superfund Site. J Toxicol Environ Health Part A 69:395–415

Neigh AM, Zwiernik MJ, Joldersma CA, *et al.* 2006d. Reproductive success of passerines exposed to PCBs through the terrestrial food web of the Kalamazoo River. Ecotoxicol Environ Safety (*submitted*)

Nosek JA, Craven SR, Sullivan JR, *et al.* 1992. Toxicity and reproductive effects of 2,3,7,8 –tetrachlorodibenzo-*p*-dioxin in ring-necked pheasant hens. J Toxicol Environ Health 35:187–98

Pinkowski BC. 1978. Feeding of nestling and fledgling eastern bluebird. Wilson Bull 90:84–98

Quinney TE and Ankney CD. 1985. Prey size selection by tree swallows. Auk 102:245–50

Rendell WB and Robertson RJ. 1990. Influence of forest edge on nest-site selection by tree swallows. Wilson Bull 102:634–44

Secord AL and McCarty JP. 1997. Polychlorinated biphenyl contamination of tree swallows in the upper Hudson River valley, New York. Effects on breeding biology and implications for other bird species. United States Fish and Wildlife Service, Cortland, NY, USA

St. Louis VL and Barlow JC. 1993. The reproductive success of tree swallows nesting near experimentally acidified lakes in northwestern Ontario. Can J Zool 71:1090–7

Studier EH and Sevick SH. 1992. Live mass, water content, nitrogen and mineral levels in some insects from south-central lower Michigan. Comp Biochem Physiol 103A:579–95

Thiel DA, Martin SG, Duncan JW, *et al.* 1997. Evaluation of the Effects of Dioxin-Contaminated Sludges on Wild Birds. Proc TAPPI Env Conf 94–9

USEPA (US Environmental Protection Agency). 1993. Wildlife Exposure Factors Handbook Volumes I, II, and III. EPA/600/R-93/187B. Office of Research and Development, Washington, DC, USA

USEPA (US Environmental Protection Agency). 1995. Great Lakes Water Quality Initiative Criteria Documents for the Protection of Wildlife: DDT, mercury, 2,3,7,8 –TCDD, PCBs. EPA/820/B-95/008. Office of Water, Washington, DC, USA

USEPA (US Environmental Protection Agency). 1998. Guidelines for Ecological Risk Assessment. EPA/630/R-95/002F. National Center for Environmental Reassessment, Washington, DC, USA

USEPA (US Environmental Protection Agency). 2000. Further Site Characterization and Analysis, Revised Baseline Ecological Risk Assessment, Hudson River PCBs Reassessment RI/FS Volume 2E. Prepared for USEPA Region 2 and USACE, Kansas City District by von Stackelberg K, Driscoll SB, Menzie C at Menzie-Cura & Associates, Inc., and TAMS Consultants, Inc. December 2000

van den Berg M, Birnbaum L, Bosveld ATC, *et al.* 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ Health Perspect 106:775–92

Wheelwright NT. 1986. The diet of American robins: An analysis of U.S. Biological Survey records. Auk 103:710–25

Environmental Toxicology and Chemistry, Vol. 25, No. 6, pp. 1503–1511, 2006
© 2006 SETAC
Printed in the USA
0730-7268/06 $12.00 + .00

Environmental Toxicology

ACCUMULATION OF POLYCHLORINATED BIPHENYLS FROM FLOODPLAIN SOILS BY PASSERINE BIRDS

ARIANNE M. NEIGH,† MATTHEW J. ZWIERNIK,*† PATRICK W. BRADLEY,† DENISE P. KAY,‡ PAUL D. JONES,†‡ RYAN R. HOLEM,‡ ALAN L. BLANKENSHIP,†‡ KARL D. STRAUSE,† JOHN L. NEWSTED,‡ and JOHN P. GIESY†‡§
†Zoology Department, Center for Integrative Toxicology, National Food Safety and Toxicology Center, Michigan State University, East Lansing, Michigan 48824, USA
‡ENTRIX, Okemos, Michigan 48864, USA
§Biology and Chemistry Department, City University of Hong Kong, Kowloon, Hong Kong, Special Administrative Region, China

(*Received* 18 *January* 2005; *Accepted* 21 *September* 2005)

Abstract—Eggs, nestlings, and adults of the eastern bluebird (*Sialia sialis*) and house wren (*Troglodytes aedon*) were collected at a polychlorinated biphenyl (PCB)–contaminated site and a reference location on the Kalamazoo River (MI, USA). Eggs and nestlings of eastern bluebirds at the more contaminated location contained concentrations of 8.3 and 1.3 mg/kg, respectively, of total PCBs and 77 and 6.3 ng/kg, respectively, of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TEQs). Eggs, nestlings, and adults of house wrens from the contaminated location contained 6.3, 0.77, and 3.2 mg/kg, respectively, of PCBs and 400, 63, and 110 ng/kg, respectively, of TEQs. Concentrations of total PCBs and TEQs in tissues at the more contaminated location were significantly greater than concentrations in tissues at the reference site for all tissue types of both species. Exposures of the two species studied were different, which suggests that terrestrial-based insectivorous passerine species, foraging in the same area, may have differential exposure to PCBs depending on specific foraging techniques and the insect orders that are targeted. Despite the greater accumulation of PCBs at the more contaminated location, the risk of exposure to PCBs did not exceed the threshold for adverse effects at either location.

Keywords—Bioaccumulation Dioxin equivalents House wren Eastern bluebird Kalamazoo River

INTRODUCTION

The Kalamazoo River in southwestern Michigan, USA, was contaminated with polychlorinated biphenyls (PCBs) when carbonless copy paper was inadvertently mixed into the paper recycling process from 1957 to 1971. In 1986, three dams were removed to their sill, which drained approximately 132 ha of formerly impounded sediment to create a large, contiguous landmass of lowland forest and marsh. Previous studies of the Kalamazoo River quantified concentrations of PCBs in multiple matrices, including soil, sediment, plants, mink, and tree swallows [1–3]. Surveys of in-stream surface sediment (depth, 0–10 cm) indicate that concentrations of PCBs range from less than 0.001 to 153 mg/kg dry weight, with a mean PCB concentration of approximately 3.0 mg/kg dry weight [4,5]. Passerine birds with terrestrial diets were identified as receptors of concern because of the contamination in the formerly impounded floodplain soils, but little is known about the trophic transfer and bioavailability of organochlorines to upper-food-web insectivores. Preliminary sampling quantified PCB concentrations in surficial floodplain soils (depth, 0–25 cm) of the former impoundment to be less than 0.001 to as great as 85 mg/kg dry weight, with mean values of approximately 11 mg/kg dry weight [6–8].

The house wren (*Troglodytes aedon*) and eastern bluebird (*Sialia sialis*) were selected as upper-trophic-level monitors of exposure to PCBs derived from contaminated soil at the Kalamazoo River Superfund site. The American robin (*Turdus migratorius*) was identified as a receptor of concern during a baseline ecological risk assessment [1] because of modeled contamination levels in its omnivorous diet. Likewise, eastern bluebirds, which also belong to the Turdidae family, feed on invertebrates in close contact with contaminated soil during portions of their life cycles [9]. Thus, it was hypothesized that PCB concentrations in bluebird tissue would be analogous to concentrations in American robin tissues. Primarily an insectivorous species, the house wren was used in the present study to determine risk to avian species with entirely insectivorous diets.

Multiple lines of evidence were employed to characterize risk of exposure to PCBs in terrestrial passerine populations. Concentrations of PCBs were significantly greater in the diet of birds at the more contaminated location than in the diet of birds from the upstream reference location [10]. Concentrations of total PCBs were deemed to be less than a threshold at which effects would be expected, but concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TEQs) based on avian World Health Organization (WHO-Avian) toxic equivalency factors [11] may exceed a level of effect. Colocated studies (Kalamazoo River) of reproductive performance of passerine species also were conducted during the same time period and applied to the overall evaluation of risk as an ancillary line of evidence [12,13]. These studies of reproductive performance over a two-year period indicated that house wren fledging success was significantly greater at Trowbridge Impoundment (TB; MI, USA) compared to fledging success at Fort Custer State Recreation Area (FC; MI, USA). Eastern bluebirds had significantly decreased productivity at the more contaminated location relative to the reference location during a three-year study, but 30% of the decrease in productivity between locations can be linked to a single female. Although

* To whom correspondence may be addressed (zwiernik@msu.edu).

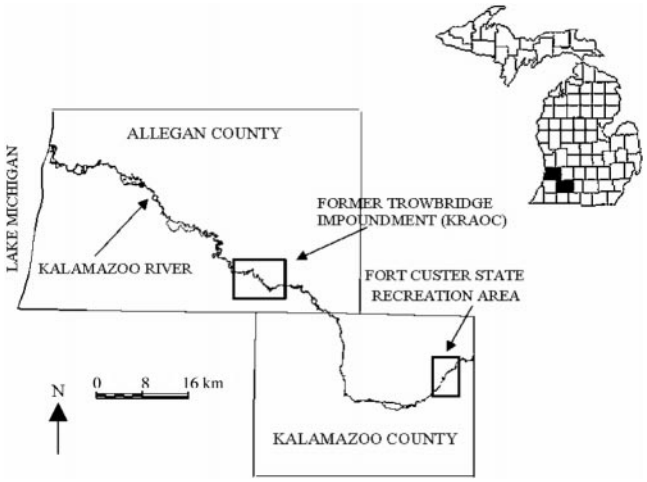


Fig. 1. Map of the Kalamazoo River Area of Concern (KRAOC) and reference site. The inset describes the location of the two counties in Michigan, USA, where studies were conducted. A box designates the boundaries of the upstream reference location, the Fort Custer State Recreation Area, and the Trowbridge Impoundment, located within the KRAOC. (Reprinted from Neigh et al. [3]. Copyright © 2006 Society of Environmental Toxicology and Chemistry. Reprinted by permission of Alliance Communications Group, a division of Allen Press.)

reproductive abnormalities were seen in both species, the cause of the depressed reproductive success was unlikely to be exposure to PCBs and more likely to be differences in habitat suitability, co-contaminants, or prey availability. The final line of evidence examined in the present study was the measured concentration of PCBs in tissues, which was compared to toxicity reference values (TRVs). The primary objectives of the present study were to determine if total PCB and $TEQ_{WHO\text{-}Avian}$ concentrations in passerine tissues were different between locations, if all avian species examined in the present study were equally exposed, and if the PCB concentrations in the tissues of passerine birds exceeded concentrations that, based on TRVs, would be expected to result in population-level adverse effects.

MATERIALS AND METHODS

Site details

Eggs, nestlings, and adults were collected from nest boxes between 2001 and 2003 at a location minimally contaminated with PCBs within FC [3] and at TB, a more contaminated location 67 km downstream (Fig. 1). Nest boxes were examined every 1 to 3 d to determine date of clutch initiation and date of hatch. Further physical description of the study sites and nest box locations have been described elsewhere [13].

Tissue sampling

Samples of eggs, nestlings, and adults were collected during the spring and summer of 2001, 2002, and 2003. One predetermined sample was collected from each nest box for quantification of total PCB and TEQ concentrations. In addition, abandoned and addled eggs, dead adults, and dead nestlings were salvaged for additional measurements of PCB and TEQ concentrations. Eggs were sampled 7 to 10 d after laying, and nestlings were sampled 6 to 8 d before fledging. Adult house wrens were sampled during 2002 and 2003 by mist net.

Chemical analysis

The present paper applies a ''top-down'' methodology based on measured PCB concentrations in eggs, nestlings, and adults to establish exposure in avian species of the Kalamazoo River. Exposure was quantified by congener-specific analysis of approximately 100 PCB congeners, including non-*ortho*-substituted (coplanar) and mono-*ortho*-substituted congeners and was reported as total PCBs. In addition, TEQs were calculated for PCBs based on relative potencies for birds [11]. Concentrations of total PCBs were intended to quantify potential exposure based on all measurable congeners in the environment, whereas concentrations of non-*ortho*-substituted and mono-*ortho*-substituted congeners, reported as $TEQ_{WHO\text{-}Avian}$, evaluated exposure based on the additive toxicity of the PCB congeners known to interact with the aryl hydrocarbon receptor. It has been postulated that the most sensitive measure of toxic effects of PCBs is through the aryl hydrocarbon receptor–mediated pathway and that, because of weathering of the total PCB mixture, the TEQ approach is a more accurate method to estimate exposure and potential toxic effects [14].

Eggs were prepared by removing the eggshell; the yolk and albumen remained for chemical analyses. Nestlings and adults were processed before chemical analyses by removing feathers, beaks, wings, legs, and stomach contents and then homogenizing the whole body in a solvent-rinsed grinder.

Concentrations of PCB congeners and *o,p′*- and *p,p′*-isomers of DDT were determined by previously described methods [3,15]. All samples were analyzed for PCB congeners, and a randomly selected subset of egg samples ($n = 5$ samples/species/site) were analyzed for DDT and DDT isomers. Chemical analyses included pertinent quality-assurance practices, including surrogate spikes, blanks, and duplicates. Acceptable surrogate recoveries ranged from 65 to 135%. Congeners were Soxhlet-extracted and separated by liquid chromatography. Total concentrations of PCBs were quantified by a Perkin-Elmer AutoSystem (Boston, MA, USA) and a Hewlett-Packard 5890 series II gas chromatograph (Wilmington, DE, USA) equipped with ^{63}Ni electron-capture detectors (5% phenylpolysiloxane; length, 30 m; inner diameter, 0.25 mm; film thickness 0.25 μm; Zebron ZB-5, Phenomenex, Torrance, CA, USA). The method detection limit for PCBs was estimated to be 1×10^{-3} mg/kg wet weight. Total PCB concentrations were calculated as the sum of all resolved PCB congeners and coeluting congeners. Non-*ortho*-substituted PCB congeners (International Union of Pure and Applied Chemistry nos. 77, 81, 126, and 169) were separated from coeluting congeners and interferences by carbon-column chromatography (30-cm × 15-mm glass columns). Extracts containing the non-*ortho*-substituted (coplanar) congeners were analyzed by a gas chromatograph mass-selective detector (Hewlett-Packard 5890 series II gas chromatograph) equipped with a Hewlett-Packard 5972 series detector. Detection limits varied among samples, but the mean detection limit for all samples was less than 100 ng/kg wet weight.

TEQ computation

Toxic equivalents in bird tissues were calculated by multiplying individual concentrations of aryl hydrocarbon receptor–active PCB congeners by their respective, bird-specific World Health Organization toxic equivalence factors [11]. Total $TEQ_{WHO\text{-}Avian}$ concentrations were calculated as the sum of detectable individual non-*ortho*-substituted and mono-*ortho*-substituted PCB congeners (PCBs 77, 81, 105, 118, 126, 156,

157, 167, and 169). When at least one of the coplanar congeners (except for PCB 169) could not be quantified because of interfering compounds, the sample was removed from statistical analyses. Congener 169 was not regularly detected in the samples. For congeners that occurred at concentrations less than the limit of quantification, a proxy value of half the limit of quantification was assigned to report a conservative estimate of exposure. The true value of the congeners below the limit of quantification lies somewhere between zero and the detection limit, which was determined based on a congener-specific basis. For the present study, all but eight samples had at least one congener with a proxy value assigned. This method had minimal effects on samples taken from TB, but because more congeners were below the limit of quantification at the reference area, FC, the effect was greater [16]. Some mono-*ortho*-substituted congeners coeluted with other congeners, so to report the most conservative estimate, the total concentration of the coelution group was considered to belong to its respective mono-*ortho*-substituted congener.

Statistical analyses

For statistical analyses, each nest box was treated as a separate experimental unit, and values were reported on a per-box basis. All nesting attempts were treated as separate and individual observations. Normality was assessed with the Kolmogorov-Smirnov one-sample test with Lilliefors transformation, and homogeneity of variance was verified by the F test. All concentration data were log-transformed before analyses. All parametric data were analyzed by Student's t test or two-way analysis of variance (ANOVA), and nonparametric data were analyzed by Mann–Whitney U or Kruskal–Wallis tests. The criterion for significance used in all tests was $p < 0.05$.

Biomagnification and accumulation rates

Biomagnification factors and accumulation ratios were calculated for total PCBs and $TEQs_{WHO\text{-}Avian}$ as the lipid-normalized concentration in the upper trophic level divided by the lipid-normalized concentration in the lower trophic level. Accumulation was calculated based on live-sampled adults and nestlings and fresh sampled eggs.

Accumulation rates were calculated to determine the mass of PCBs gained by the nestlings over the nestling period through the diet. The mass of PCBs in the egg was subtracted from the total mass in the nestlings. The accumulation rate was calculated as the difference in total mass of PCBs and $TEQs_{WHO\text{-}Avian}$ between nestlings and eggs divided by the nestlings' days of life [17].

Assessment of risk

The risk of potential effects on passerine health because of exposure to PCBs existing in Kalamazoo River floodplain soils was estimated by comparing concentrations of PCBs to established TRVs for eggs, nestlings, and adults of the house wren and eastern bluebird [18]. Risk was quantified as hazard quotients (HQs), which were calculated as the concentration of PCBs in the tissue divided by the TRV.

Toxicity reference values were derived from the results of studies reported in the literature. Studies were chosen for terrestrial passerine species based on several criteria. The studies judged to be the most suitable for TRV calculation used wildlife species chronically exposed over sensitive life stages, evaluated endpoints ecologically relevant to PCB exposure, had minimal co-contamination, and were multiyear studies.

Few studies are available for the species used in the present study except for an examination of American robin exposure and effects at the Housatonic River (NY, USA), which found no correlation between reproduction and PCB concentrations [19]. American robin eggs at the Housatonic site contained a mean PCB concentration of 84 mg/kg wet weight. From this field study, it can be concluded that the threshold for effects of PCBs on robins is greater than 84 mg/kg wet weight. Studies of European starlings (*Sturnus vulgaris*) also were considered for derivation of an appropriate TRV. European starlings exhibited a decrease in parental attentiveness and increased mortality at two PCB sites compared to a reference site when Aroclor 1254 concentrations in tissues (mean $\pm$ SD) were 13.6 $\pm$ 2.9 mg PCB/kg wet weight in eggs, 5.9 $\pm$ 0.8 mg PCB/kg wet weight in nestlings, and 15.9 $\pm$ 5.3 mg PCB/kg wet weight in adults [20]. The study of starlings did not report reduced hatching success, and similar embryo mortality was observed at another study site contaminated with metals but with relatively low PCB concentrations. It has been suggested that adult incubation anomalies or co-contamination by dioxin may lead to embryo mortality, so these egg concentrations are an unacceptable basis for a TRV for the Kalamazoo River [20]. Effects found in adult starlings also may be affected by the fitness of nestlings, which may reduce feeding calls and, therefore, affect parental care. The starling study fulfilled several of the given criteria by taking place over several years, by measuring ecologically relevant endpoints, and by measuring concentrations at critical life stages in a terrestrial wild avian species. The confounding factors in the study by Halbrook et al. [20], and the fact that Henning et al. [19] did not observe abnormalities in reproduction at much greater concentrations, suggests that the figure reported by Henning et al. [19] is a more appropriate choice for the TRV based on the no-observed-adverse-effect level for PCBs of 84 mg/kg.

The TRV selected as a threshold for effects based on TEQ concentrations was that suggested by the U.S. Environmental Protection Agency [21] (http://www.epa.gov/hudson/reports.htm). That study investigated TEQ concentrations in tree swallows at the Hudson River (NY, USA) over a two-year period, was conducted on wild passerine birds, evaluated relevant ecological endpoints during critical life stages, and co-contamination was considered to be inconsequential based on the study. Tree swallows exhibited abnormal plumage, decreased hatching success, and increased abandonment during one year, but these effects were not observed the following year. The inconsistency of effects over both years suggests that the TEQ value of 13,000 ng/kg from that study should not be used as a lowest-observed-adverse-effect level but could be appropriate for a no-observed-adverse-effect level. The greatest concentration of TEQs in the year without effects was 13,000 ng/kg wet weight. No lowest-observed-adverse-effect level could be established for TEQs.

RESULTS

Gross morphology and abnormalities

No gross physiological abnormalities were observed in 180 eastern bluebird nestlings and 362 house wren nestlings, or in approximately 75 eastern bluebird adult pairs and 108 house wrens adult pairs, but some egg abnormalities were present in house wrens of the 305 eastern bluebird eggs and 607 house wren eggs evaluated. These abnormalities include desiccated

Table 1. Mean concentrations of total polychlorinated biphenyls (PCBs) and lipid content of eastern bluebirds and house wrens at the Fort Custer reference area and the Trowbridge Impoundment on the Kalamazoo River (MI, USA) Area of Concern

	Fort Custer			Trowbridge		
	n	% Lipid	PCB (mg/kg)	*n*	% Lipid	PCB (mg/kg)
Eastern bluebird						
Egg						
2001	2	6.0 (2.4)[a]	0.25 (0.077)	2	8.2 (3.1)	11 (2.9)
2002	7	8.4 (2.7)	0.13 (0.076)	3	15 (15)	11 (3.9)[b]
2003	5	8.7 (3.1)	0.19 (0.13)	2	5.9 (0.40)	1.8 (0.32)[b]
Total	14	8.2 (2.8)	0.17 (0.10)	7	11 (10)	8.3 (5.1)[c]
Nestling						
2001	6	5.7 (0.84)	0.011 (0.003)	1	4.8 (NA)	0.41 (NA)
2002	5	5.0 (0.42)	0.010 (0.004)	3	4.6 (1.2)	1.4 (2.1)[b]
2003	6	4.0 (1.2)	0.013 (0.009)	2	5.0 (1.3)	1.8 (0.83)[b]
Total	17	4.9 (1.1)	0.011 (0.006)	6	4.8 (0.96)	1.3 (1.4)[d]
House wren						
Egg						
2001	8	19 (17)	0.14 (0.15)	6	9.3 (5.4)	4.5 (2.0)[b]
2002	6	7.1 (4.0)	0.10 (0.053)	8	11 (6.2)	6.8 (5.6)[b]
Total	14	14 (14)	0.12 (0.12)	14	10 (5.7)	5.8 (4.4)[d]
Nestling						
2001	7	4.5 (1.8)	0.029 (0.024)	5	5.1 (1.4)	0.8 (0.79)[b]
2002	6	6.3 (2.6)	0.010 (0.005)	11	6.9 (2.1)	0.71 (0.62)[b]
2003	NA[e]	NA	NA	1	2.4 (NA)	1.1 (NA)
Total	13	5.3 (2.3)	0.020 (0.020)	17	6.1 (2.2)	0.77 (0.64)[f]
Adult						
2002	5	5.5 (1.1)	0.087 (0.029)	6	5.2 (1.3)	3.6 (2.3)[b]
2003	3	5.3 (0.86)	0.045 (0.022)	3	5.6 (2.1)	2.5 (1.6)[b]
Total	8	5.4 (0.93)	0.072 (0.033)	9	5.4 (1.5)	3.2 (2.1)[d]

[a] Values in parentheses represent the SD.
[b] Mean concentration was significantly greater than at the Fort Custer reference site (Student's *t* test or Kruskal-Wallis; $p < 0.05$).
[c] Mean concentration was significantly greater than at the Fort Custer reference site, but interactions between location and year exist two-way analysis of variance [ANOVA]; location, $p = 0.000$; year, $p = 0.030$; location × year, $p = 0.020$; $n = 21$).
[d] Mean concentration was significantly greater than at the Fort Custer reference site (two-way ANOVA; $p < 0.000$).
[e] NA = not available.
[f] Mean concentration was significantly greater than at the Fort Custer reference site, but year was a significant cofactor (two-way ANOVA; location, $p = 0.000$; year, $p = 0.029$; location × year, $p = 0.662$; $n = 30$).

contents, irregular shape, and abnormal texture. A proportion of house wren nests contained eggs with shells that appeared to be thinned (4% of all nests, 12% of TB nests), but the thickness of the shells was not measured.

Total PCB concentrations in tissue

Polychlorinated biphenyl concentrations in eggs, nestlings, and adults (house wrens only) from TB were significantly greater than those at the reference location (FC; two-way ANOVA: $p < 0.001$) for both eastern bluebirds and house wrens (Table 1). Year was a significant cofactor for eastern bluebird eggs (two-way ANOVA: year, $p = 0.030$, $n = 21$) and house wren nestlings (two-way ANOVA: year, $p = 0.029$, $n = 30$), and the interaction between location and year was significant for eastern bluebird eggs (two-way ANOVA: location × year, $p = 0.020$, $n = 21$). For all tissues analyzed, PCB concentrations differed by 39- to 118-fold. The trend in concentration differences between sites was the same when concentrations were lipid-normalized. In general, eastern bluebirds at TB had the greatest concentrations of PCBs in eggs and nestlings (Table 1), but the greatest tissue concentration in the study was measured in a house wren egg from TB in 2002 (PCB concentration, 26 mg/kg wet wt).

Dichlorodiphenyltrichloroethane metabolites were detected in all egg samples from a random subset analyzed for DDT concentrations. Concentrations of *p,p′*-dichlorodiphenyldichloroethylene (DDE) comprised 95 to 98% of the total sum of all DDT metabolites in eastern bluebirds and house wrens. Total DDT is defined as the sum of all isomers measured. Concentrations in eastern bluebirds were sevenfold greater than those in house wrens at FC and three-fold greater than those in house wrens at TB. Concentrations of total DDT (mean ± SD) contained in eastern bluebird eggs were 2.1 ± 1.1 mg/kg wet weight at TB and 1.5 ± 1.8 mg/kg wet weight at FC, but differences between sites were not statistically significant (Student's *t* test: $p = 0.317$). House wren eggs at TB contained DDT at a concentration of 0.66 ± 0.87 mg/kg wet weight, and eggs at FC contained DDT at a concentration of 0.20 ± 0.21 mg/kg wet weight. Again, however, differences between locations were not statistically significant (Student's *t* test: $p = 0.135$). The only statistically significant difference was for concentrations of *p,p′*-dichlorodiphenyldichloroethane in eggs

Table 2. Mean concentrations of dioxin equivalents (TEQs) and relative potency in eastern bluebird and house wren tissues sampled from the Fort Custer reference area and the former Trowbridge Impoundment area (both, MI, USA) contaminated with polychlorinated biphenyls (PCBs)

	Fort Custer			Trowbridge		
	n	TEQ (ng/kg)	Relative potency (ng TEQ/kg PCB)	*n*	TEQ (ng/kg)	Relative potency (ng TEQ/kg PCB)
Eastern bluebird						
Egg						
2001	2	10 (10)[a]	37 (29)	2	70 (4.8)	7.4 (0.63)
2002	NA[b]	NA	NA	1	220 (NA)	25 (NA)
2003	1	2.2 (NA)	47 (NA)	2	15 (10)	8.5 (3.3)
Total	3	7.6 (8.6)	40 (21)	5	77 (82)[c]	11 (8.0)
Nestling						
2001	6	1.5 (1.1)	130 (99.0)	1	3.2 (NA)	7.8 (NA)
2002	5	1.3 (0.34)	150 (71)	3	7.3 (4.9)[c]	25 (18)[d]
2003	6	1.2 (0.20)	120 (45)	2	6.3 (4.4)[c]	3.3 (0.91)
Total	17	1.3 (0.64)	130 (71)	6	6.3 (4.0)[c]	15 (16)
House wren						
Egg						
2001	4	8.0 (4.7)	300 (280)	5	160 (54)[c]	40 (16)[d]
2002	4	9.2 (3.6)	150 (130)	6	530 (380)[c]	62 (20)
Total	8	8.6 (3.9)	220 (220)	11	360 (330)[e]	52 (21)[f]
Nestling						
2001	7	1.7 (1.2)	74 (53)	5	73 (63)[c]	94 (35)[g]
2002	6	1.1 (0.43)	130 (66)	11	61 (43)[c]	97 (42)[d]
2003	NA	NA	NA	1	41 (NA)	38 (NA)
Total	13	1.4 (0.96)	100 (64)	17	63 (47)[f]	93 (40)[d]
Adult						
2002	5	5.2 (2.6)	66 (43)	6	120 (40)[c]	43 (30)
2003	3	10 (8.7)	260 (180)	3	91 (91)[c]	47 (38)
Total	8	7.1 (5.7)	140 (140)	9	110 (57)[f]	45 (30)[h]

[a] Values in parentheses represent the SD.
[b] NA = not available.
[c] Mean concentration was significantly greater than at the Fort Custer reference site (Student's *t* test; $p < 0.05$).
[d] Mean relative potency was significantly lesser than at the Fort Custer reference site (Student's *t* test; $p < 0.05$).
[e] Mean concentration was significantly greater than at the Fort Custer reference site, but year was a significant cofactor (two-way analysis of variance [ANOVA]; location, $p = 0.000$; year, $p = 0.020$; location × year, $p = 0.101$; $n = 19$).
[f] Mean concentration was significantly greater than at the Fort Custer reference site (two-way ANOVA; $p < 0.000$).
[g] Mean relative potency was significantly greater than at the Fort Custer reference site (Student's *t* test; $p < 0.05$).
[h] Mean relative potency was significantly lesser than at the Fort Custer reference site (two-way ANOVA; $p < 0.000$).

of house wrens between sites (Student's *t* test: $p = 0.021$), but sample sizes were small ($n = 10$). Addled eggs of eastern bluebirds at FC contained 2.6-fold greater concentrations of lipid-normalized total DDT than fresh eggs contained, but addled eggs of eastern bluebirds at TB had lipid-normalized total DDT concentrations 82% those in fresh eggs from TB. Addled eggs of house wrens had lipid-normalized concentrations 7.4- and 5.7-fold greater than those in fresh eggs at FC ($n = 5$) and TB ($n = 5$), respectively. Samples sizes were insufficient to perform statistical tests comparing addled and fresh eggs.

Dioxin equivalents ($TEQs_{WHO\text{-}Avian}$)

For all tissue matrices in both species, $TEQ_{WHO\text{-}Avian}$ concentrations were significantly greater at TB than at FC (Student's *t* test or two-way ANOVA: $p < 0.001$) (Table 2). In the tissues of eastern bluebirds, $TEQ_{WHO\text{-}Avian}$ concentrations were 5- to 10-fold greater at TB than at FC, whereas $TEQ_{WHO\text{-}Avian}$ concentrations in the tissues of house wrens were 15- to 47-fold greater at TB compared to FC. An addled egg of a house wren contained the greatest $TEQ_{WHO\text{-}Avian}$ concentration for all tissues in the study (1,800 ng/kg wet wt).

The relative contributions of non-*ortho*-substituted and mono-*ortho*-substituted congeners to total $TEQs_{WHO\text{-}Avian}$ were evaluated at each site. Congener 169 was not detected in 67 to 69% of all samples for eastern bluebirds and house wrens, and in the samples in which PCB 169 was detected, it represented less than 1% of the total $TEQ_{WHO\text{-}Avian}$ concentration. House wren eggs, nestlings, and adults from TB were the only matrix in which PCBs 77, 81, and 126 were all detected in the majority of samples. Congeners 77, 81, and 126 comprised the greatest proportion of the total $TEQ_{WHO\text{-}Avian}$ concentration for all species (Fig. 2). In house wrens at FC, PCB 126 comprised the greatest portion of the total $TEQ_{WHO\text{-}Avian}$ concentration, but at TB, PCB 77 made the greatest contribution to the $TEQ_{WHO\text{-}Avian}$ concentration. Contributions of every congener to the total $TEQ_{WHO\text{-}Avian}$ concentration were significantly

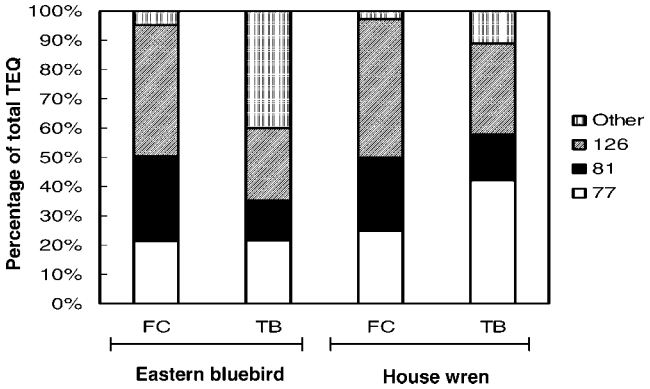


Fig. 2. Contribution of individual non-*ortho*-substituted and mono-*ortho*-substituted congeners to dioxin equivalents (TEQs) in the tissues of passerine species at the Fort Custer State Recreation Area (FC) and the Trowbridge Impoundment (TB), Michigan, USA.

different between FC and TB for house wrens (Student's *t* test: $p < 0.05$). In eastern bluebirds, PCBs 81 and 126 comprised significantly different proportions of the $TEQ_{WHO\text{-}Avian}$ concentration between sites (Student's *t* test: $p = 0.001$), but PCB 77 was similar between locations (Student's *t* test: $p > 0.05$). Congener 118 was frequently detected (83–100% of samples from both locations and all matrices and species).

Biomagnification

Ratios of concentrations between life stages of avian species for lipid-normalized and wet-weight total PCBs and $TEQs_{WHO\text{-}Avian}$ were less than 1.0 for house wren eggs to nestlings and greater than 1.0 for house wren nestlings to adults at FC and TB. Eastern bluebird egg to nestling ratios (<1.0) were less than those in house wrens (Table 3). Ratios of biomagnification between egg and nestling life stages for eastern bluebirds and house wrens were greater at FC than at TB when comparing $TEQs_{WHO\text{-}Avian}$.

Daily accumulation rates were calculated based on total PCBs and $TEQs_{WHO\text{-}Avian}$. Total PCB daily accumulation rates on a wet-weight basis for house wren nests were 0.0068 μg/d at FC during 2001 and 0.32 and 0.40 μg/d at TB during 2001 and 2002, respectively. Daily accumulation rates based on $TEQs_{WHO\text{-}Avian}$ on a wet-weight basis were 1.9 pg/d at FC during 2001 and 26 and 16 pg/d at TB during 2001 and 2002, respectively. Accumulation rates on a wet-weight basis for eastern bluebirds at FC were 0.0053 and 0.054 μg/d for 2002 and 2003, respectively. Total $TEQ_{WHO\text{-}Avian}$ accumulation rates on a wet-weight basis for eastern bluebirds were 2.1 and 12 pg/d at FC during 2002 and 2003, respectively.

Relative potency (ng TEQ/kg PCB) of PCB mixtures in samples were calculated by dividing the $TEQ_{WHO\text{-}Avian}$ concentration by the total PCB concentration for each sample (Table 3). The resulting relative potency is an indicator of the overall effect of environmental weathering and differential bioaccumulation on the toxicity of the PCB mixture [22]. Relative potencies generally were greater and more variable at FC than at TB for both species, because concentrations of PCBs generally were low and $TEQ_{WHO\text{-}Avian}$ concentrations were driven by the detection limit of the analytical method. Ratios of relative potencies among different sample types reflect changes in the potency of the PCB mixture. At TB, the potency ratio from egg to nestling was 1.3 and 1.8 for eastern bluebird and house wren, respectively, and the potency ratio at FC for the same life stages of these species was 3.3 and 0.45, respectively. Ratios for nestling to adult were 0.48 at TB and 1.4 at FC for house wrens.

DISCUSSION

DDT concentrations

Little is known about the differences in species sensitivity between other birds and eastern bluebirds or house wrens. A DDE concentration of 2.0 mg/kg in eggs has been suggested as a threshold for effects in bald eagles (*Haliaeetus leucocephalus*) [23]. In another passerine bird, the tree swallow, total DDT residue levels were 1.3 mg/kg in the attended eggs and 2.8 mg/kg in unattended eggs [24]. Although the value associated with unattended eggs may represent a threshold for effects on behavior, the likely DDE threshold for effects on reproduction of passerine birds is near 8 or 9 mg/kg [24]. No eggs of either species at the Kalamazoo River contained DDE concentrations greater than the threshold for reproductive effects, and mean concentrations were fourfold less than this same threshold. Concentrations of DDE and DDT in the eggs of eastern bluebirds were near the threshold for effects on behavior, but the eggs of house wrens had total DDT concentrations threefold less than that threshold. The co-contaminant, DDT, exists at many locations, including the Kalamazoo River Area of Concern, which makes it difficult to attribute abnormal reproduction or behavior to the presence of one contaminant versus another. At both Kalamazoo River locations, DDT is detected, but the presence of DDT is not expected to be causing reproductive impairment in the species examined.

Total PCBs

Limited data regarding passerine birds exposed to PCBs through terrestrial diets are available for comparison with the results of the present study. Tissue concentrations were similar between species at FC and other species at other relatively

Table 3. Ratios of accumulation between life stages of the eastern bluebird and house wren based on the mean lipid-normalized and wet-weight[a] total polychlorinated biphenyls (PCBs) and dioxin equivalents (TEQs) in eggs and live-sampled nestlings at the Fort Custer State Recreation Area (FC) and the Trowbridge Impoundment (TB; both MI, USA)

	Accumulation ratio (Total PCBs)				Accumulation ratio (TEQs)			
	Eastern bluebird		House wren		Eastern bluebird		House wren	
	FC	TB	FC	TB	FC	TB	FC	TB
Egg to nestling	0.11 (0.060)	0.20 (0.62)	0.79 (0.23)	0.22 (0.17)	0.31 (0.13)	0.0094 (0.48)	0.50 (0.25)	0.34 (0.24)
Nestling to adult	NA[b]	NA	3.2 (3.4)	4.4 (4.2)	NA	NA	4.3 (4.8)	1.9 (1.7)
Adult to egg	NA	NA	0.40 (1.3)	1.0 (1.4)	NA	NA	0.47 (0.82)	1.6 (2.5)

[a] Wet-weight accumulation ratios are given in parentheses.
[b] NA = not available.

Table 4. Bioaccumulation factors for wet-weight concentrations of total polychlorinated biphenyls (PCBs) and dioxin equivalents (TEQs) from the diet (average potential daily dose) [10] to bird tissues at the Fort Custer State recreation area (FC) and the Trowbridge Impoundment (TB; both MI, USA)

	Bioaccumulation factors (total PCB)				Bioaccumulation factors (TEQ)			
	Eastern bluebird		House wren		Eastern bluebird		House wren	
	FC	TB	FC	TB	FC	TB	FC	TB
Diet to egg	10	16	6.4	45	2.8	1.1	8.9	13
Diet to nestling	0.64	2.5	0.91	5.5	0.48	0.09	0.56	2.0
Diet to adult	NA[a]	NA	3.3	23	NA	NA	2.8	3.5

[a] NA = not available.

uncontaminated sites in the region [19,25]. Total concentrations of PCBs in house wren and eastern bluebird eggs and nestlings at TB were less than concentrations contained in American robin eggs and nestlings at the Housatonic River [19], but concentrations of PCBs at TB were greater than concentrations in red-winged blackbird (*Agelaius phoeniceus*) eggs at locations in the Great Lakes–St. Lawrence River Basin (ON, Canada) with the exception of one site [25]. Although few comparisons to other studies can be made for these specific species, the fact remains that total PCB concentrations were significantly greater at TB than at FC in all matrices examined. Concentrations in the tissues corresponded to greater concentrations of total PCBs in the soil at TB compared to the soil at FC [26], which suggests that concentrations in the tissues of the species examined reflected local contamination in the soil.

Tissue concentrations, expressed on a wet-weight basis, generally were greater in eastern bluebirds than in house wrens. When concentrations in all matrices were lipid-normalized, concentrations in house wrens and eastern bluebirds were more similar, but eastern bluebirds still contained greater mean total concentrations of PCBs. Differences in concentrations of PCBs between house wrens and eastern bluebirds likely were indicative of on-site dietary exposure. For eggs and nestlings at TB, eastern bluebirds contained total PCB concentrations 1.7-fold greater than those of house wrens. When site-specific average potential daily doses were calculated for TB, exposure in the diet of eastern bluebirds was found to be 3.7-fold greater than that of house wrens [10]. At FC, the present study measured greater daily accumulation rates for the nestlings of house wrens compared to eastern bluebirds, which suggests that they obtain greater concentrations from the diet at FC. Indeed, dietary exposure for house wrens was greater than that for eastern bluebirds at FC. Further study is needed, but general trends in concentration tissue gradients, described as daily accumulation rates, appear to reflect daily dietary exposure.

Dioxin equivalent (TEQ) concentrations

One measure of the toxicity of PCBs is based on toxic equivalency factors, which compare the toxicity of individual PCB congeners to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin [14]. The greatest $TEQ_{WHO\text{-}Avian}$ values were consistently measured in adults and eggs of house wrens from TB. This was opposite of what was measured for total PCB concentrations for which eastern bluebirds contained consistently greater concentrations in their tissues than house wrens. Mono-*ortho*-substituted and non-*ortho*-substituted (coplanar) congeners, which comprise $TEQ_{WHO\text{-}Avian}$ concentrations, apparently accumulate between life stages to a greater degree in house wrens than in eastern bluebirds. House wrens had $TEQ_{WHO\text{-}Avian}$ concentrations in eggs 5.7-fold greater than those in eastern bluebird eggs, and house wren nestlings contained $TEQ_{WHO\text{-}Avian}$ concentrations 10-fold greater than those in eastern bluebird nestlings. Bioaccumulation factors from the diet to tissues (calculated from data reported by Neigh et al. [10]) also suggest that accumulation of both $TEQs_{WHO\text{-}Avian}$ and total PCBs were greater in house wrens than in eastern bluebirds, especially at TB (Table 4). Unlike the relationship between total PCB concentrations and dietary exposure, $TEQs_{WHO\text{-}Avian}$ for dietary exposures do not correspond with tissue concentrations or accumulation rates. Eastern bluebirds were found to have greater accumulation rates and dietary exposure to $TEQs_{WHO\text{-}Avian}$ compared to house wrens at both TB and FC [10].

For both species, PCBs 81 and 126 contributed more to the total TEQs at FC than at TB. However, these congeners were detected in less than 30% of the samples from FC, so the detection limit of each congener contributed greatly to the analyses, being that half the detection limit was used to calculate contributions in the remaining samples. At TB, PCB 77 was the greatest contributor in most matrices. The contribution of PCB 77 to total $TEQs_{WHO\text{-}Avian}$ increased from eggs to nestlings of house wrens, but it decreased from eggs to nestlings of eastern bluebirds. Congener 126 followed an opposite trend by contributing more to the total $TEQs_{WHO\text{-}Avian}$ between eggs and nestlings of eastern bluebirds and less between eggs and nestlings of house wrens. Adult concentrations did not follow any discernible trend. Adults likely are exposed to a variety of contamination sources over a lifetime, whereas patterns in nestlings more closely reflect local sources of contamination [25,27].

Biomagnification

Ratios of accumulation were similar for terrestrial species and tree swallows from the Kalamazoo River [3]. Ratios ranging from approximately 2.0 to 5.0 between nestlings and adults suggest that birds are accumulating PCBs from their diet, but ratios less than 1.0 between eggs and nestlings suggest that the concentration or the rate of consumption is not great enough in nestlings to overcome growth dilution. Tree swallows from the Great Lakes-St. Lawrence River Basin also followed a similar trend, with eggs having greater concentrations than nestlings [25]. The greater accumulation ratio at FC compared to TB between eggs and nestlings for eastern bluebirds and house wrens may be a result of variation around the limits of detection for chemical analysis because of the small sample mass of many eggs.

Daily accumulation rates were less in house wrens at the Kalamazoo River than in other bird species at locations contaminated with PCBs. House wrens at TB had accumulation rates more than 10-fold less than the accumulation rates for

tree swallows at the same location [3] but from 10- to 240-fold less than those at locations on the Housatonic River [28]. Likewise, dietary exposure was estimated to be six- to eight-fold greater for concentrations of PCBs in the diet of the tree swallow compared to that of the house wren [10]. House wrens and eastern bluebirds also had accumulation rates that were less than those in black-crowned night heron (*Nycticorax nycticorax*) and Forster's tern (*Sterna forsteri*) from Green Bay (WI, USA) [17,29].

Relative potency and potency ratios calculated for birds were intended to describe the increase or decrease in toxicity of the PCB mixture at the study locations. Potency ratios greater than 1.0 were observed for most comparisons, which suggests that the mixture of congeners in the food chain become more potent at increasingly greater trophic levels through the bioaccumulation of aryl hydrocarbon receptor–active congeners [30]. Relative potencies at FC were influenced by several samples with low concentrations of PCBs or concentrations of $TEQs_{WHO\text{-}Avian}$ driven by the detection limit of the analytical method. These scenarios led to greater relative potencies in FC samples compared to TB samples, but the ratio of the relative potencies between trophic levels at each location (potency ratio) was similar. Ubiquitous congeners, such as PCBs 118 and 156, appear to bioaccumulate regardless of local sources of contamination, which contributes to the overall $TEQ_{WHO\text{-}Avian}$ concentrations at the reference location. The presence of these ubiquitous congeners in the diet adds to the increased potency of the internal mixture as nestlings and adults feed. Congener 126, which has the greatest toxic equivalency factors for PCBs in avian species [11], contributes the greatest relative percentage to the total $TEQ_{WHO\text{-}Avian}$ concentrations in samples from FC. The contribution of this congener also increases with trophic level, which suggests that PCB 126 is not easily metabolized and bioaccumulates through the food web. These factors led to the increase in relative potency between trophic levels at FC. At TB, PCBs and 126 contribute large portions to the total $TEQ_{WHO\text{-}Avian}$ because of the presence of these congeners in the original mixture released at the site [1].

Assessment of risk based on multiple lines of evidence

Multiple lines of evidence were used to assess the risk of PCB exposure to terrestrial birds inhabiting the former impoundments at the Kalamazoo River Superfund site. Exposure assessments of PCB concentrations in eggs, nestlings, and adults are described here. A number of reproductive parameters and dietary exposures, calculated as daily dietary dose, also were assessed for both eastern bluebirds and house wrens [10,12]. Potential adverse effects based on exposure data were determined by calculating HQs derived from literature-based TRVs. All HQs at FC for total PCBs and $TEQs_{WHO\text{-}Avian}$ were less than 0.002 for all tissues of house wrens and eastern bluebirds. Similarly, HQs at TB based on the mean and upper 95% confidence limit were less than 0.16 for all species and tissue types. In addition, no individual tissue concentration of PCBs exceeded any of the threshold values for effects.

To assist in the evaluation of risk, dietary exposures, which have been described in detail elsewhere [10], were examined concurrently as another way of estimating exposure and subsequent risk potential [18]. Because little toxicity data were available for terrestrial passerine birds, dietary exposure studies reported risk based on a range of HQ values. Concurrent studies suggest little risk to terrestrial passerines exposed to PCBs in the diet [10]. Given this, the only exposure calculations resulting in HQs greater than 1.0 were $TEQs_{WHO\text{-}Avian}$ in the diet of eastern bluebirds and house wrens in the exposed area (TB) based on the most conservative TRVs. Hazard quotient values, based on more appropriate TRVs calculated from field measurements of PCBs in wild passerine birds [21], were less than the threshold for effects based on the mean dietary exposure [10]. It is expected that actual risk lies somewhere within the range of calculated HQs, but it likely lies closer to the hazard quotient based on the field measurements of wild passerine birds. The presence of PCBs in tissues at concentrations less than a threshold for effects suggests that the HQ estimate based on dietary exposure of wild passerine birds likely is a better estimate of risk.

The final line of evidence involving reproductive health suggested some reproductive impairment that likely was attributable to other factors [12]. Depressed reproduction in house wrens and eastern bluebirds was observed in some, but not all, nesting attempts and in some, but not all, parameters evaluated. Sample sizes were small, especially for eastern bluebirds, and a 40% decrease in reproductive success at TB during 2001 could be attributed to a single female. Conversely, fledging success, which is the most important measure of reproductive success, was significantly greater in the PCB-contaminated area for house wrens. Similar field studies found no significant effect on reproductive performance of passerine birds at concentrations found in birds of the Kalamazoo River, or at even greater concentrations [28,31]. Likewise, studies of reproductive success conducted at the Kalamazoo River did not find statistically significant decreases in reproductive health of tree swallows at the contaminated TB location relative to the FC reference location [13]. Another study examined dioxin exposure to eastern bluebird eggs, and no effects were observed at toxic equivalent concentrations greater than those contained in bluebird eggs at the Kalamazoo River [32]. Without observing consistency across all nesting attempts and indices, and because of the contribution of a few adults to the reproductive measurements at TB, it cannot be concluded that the observed responses were caused by exposure to PCBs but, rather, were caused by other exogenous factors, such as co-contaminants, habitat suitability, and prey availability.

The results of the lines of evidence for exposure and effects in the present study suggest that concentrations of PCBs to which house wrens and eastern bluebirds are exposed are less than the threshold for effects. Few studies have directly investigated the sensitivity of these species to PCBs, so uncertainties exist regarding the applicability of TRVs derived from other species relative to the susceptibility of the terrestrial species in the present study to the effects of PCBs. Based on the lines of evidence, there does not appear to be substantial risk of adverse effects for terrestrial passerine birds exposed to PCBs originating from the floodplain soils of the Kalamazoo River.

Acknowledgement—The authors would like to extend a special thanks to the students and technicians at the Michigan State University Aquatic Toxicology Laboratory, including M. MacCarroll, B. Joldersma, C. Joldersma, C. Park, M. Kramer, and K. Smyth. The present study was conducted in cooperation with the Kalamazoo Nature Center, the staff of which was instrumental in field collection. Funding was provided by the Kalamazoo River Study Group.

REFERENCES

1. CDM Federal Programs Corporation. 2002. Baseline ecological risk assessment, Allied Paper/Portage Creek Kalamazoo River

Superfund site. Final/Revised Report. Michigan Department of Environmental Quality, Lansing, MI, USA.
2. Millsap SD, Blankenship AL, Bradley PW, Jones PD, Kay D, Neigh AM, Park C, Strause KD, Zwiernik MJ, Giesy JP. 2004. Comparison of risk assessment methodologies for exposure of mink to PCBs on the Kalamazoo River, Michigan. *Environ Sci Technol* 38:6451–6459.
3. Neigh AM, Zwiernik MJ, Bradley PW, Kay DP, Park CS, Jones PD, Newsted JL, Blankenship AL, Giesy JP. 2006. Tree swallow (*Tachycineta bicolor*) exposure to polychlorinated biphenyls at the Kalamazoo River Superfund Site, Michigan, USA. *Environ Toxicol Chem* 25:428–437.
4. Blasland, Bouck, and Lee. 1994. Allied Paper/Portage Creek Kalamazoo River Superfund site remedial investigation/feasibility study. Technical memorandum 10: Sediment characterization, geostatistical pilot study, Volume 1 of 3. Draft. Syracuse, NY, USA.
5. U.S. Environmental Protection Agency. 2002. Removal assessment report for Allied Paper—Kalamazoo River site Otsego/Plainwell, Michigan. TDD 0103-002. Washington, DC.
6. Blasland, Bouck, and Lee. 1994. Allied Paper/Portage Creek Kalamazoo River Superfund site remedial investigation/feasibility study. Technical memorandum 2: Results of phase I TBSA soil sampling. Syracuse, NY, USA.
7. Blasland, Bouck, and Lee. 1994. Allied Paper/Portage Creek Kalamazoo River Superfund site remedial investigation/feasibility study. Technical memorandum 3: Results of the floodplain soils investigation, Volume 1 of 2. Syracuse, NY, USA.
8. Blasland, Bouck, and Lee. 1994. Allied Paper/Portage Creek Kalamazoo River Superfund site remedial investigation/feasibility study. Technical memorandum 12: Former impoundment sediment geochronological plot study, Volume 1 of 4. Syracuse, NY, USA.
9. Pinkowski BC. 1978. Feeding of nestling and fledgling eastern bluebirds. *Wilson Bull* 90:84–98.
10. Neigh AM, Zwiernik MJ, Blankenship AL, Bradley PW, Kay DP, MacCarroll MA, Park CS, Jones PD, Millsap SD, Newsted JL, Giesy JP. 2006. Multiple lines of evidence assessment of PCBs in the diets of passerine birds at the Kalamazoo River Superfund Site, Michigan. *Hum Ecol Risk Assess* 12 (in press).
11. Van den Berg M, Birnbaum L, Bosveld ATC, Brunström B, Cook P, Feeley M, Giesy JP, Hanberg A, Hasegawa R, Kennedy SW, Kubiak T, Larsen JC, van Leeuwen FXR, Liem AKD, Nolt C, Peterson RE, Poellinger L, Safe S, Schrenk D, Tillitt D, Tysklind M, Younes M, Waern F, Zacharewski T. 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environ Health Perspect* 106:775–792.
12. Neigh AM, Zwiernik MJ, Joldersma CA, Blankenship AL, Strause KD, Millsap SD, Newsted JL, Giesy JP. 2006. Reproductive success of passerines exposed to PCBs through the terrestrial food web of the Kalamazoo River. *Ecotoxicol Environ Saf* (in press).
13. Neigh AM, Zwiernik MJ, MacCarroll MA, Newsted JL, Blankenship AL, Jones PD, Kay DP, Giesy JP. 2006. Productivity of tree swallows (*Tachycineta bicolor*) exposed to PCBs at the Kalamazoo River Superfund Site. *J Toxicol Environ Health Part A* 69 (in press).
14. Giesy JP, Kannan K. 1998. Dioxin-like and nondioxin-like toxic effects of polychlorinated biphenyls: Implications for risk assessment. *Crit Rev Toxicol* 28:511–569.
15. Nakata H, Kannan K, Jing L, Thomas N, Tanabe S, Giesy JP. 1998. Accumulation pattern of organochlorine pesticides and polychlorinated biphenyls in southern sea otters (*Enhydra lutris nereis*) found stranded along coastal California, USA. *Environ Pollut* 103:45–53.
16. Blankenship AL, Zwiernik MJ, Coady KK, Kay DP, Newsted JL, Strause K, Park C, Bradley PW, Neigh AM, Millsap SD, Jones PD, Giesy JP. 2005. Differential accumulation of PCB congeners in the terrestrial food web at the Kalamazoo River, Michigan, USA. *Environ Sci Technol* 39:5954–5963.
17. Custer TW, Custer CM. 1995. Transfer and accumulation of organochlorines from black-crowned night-heron eggs to chicks. *Environ Toxicol Chem* 14:533–536.
18. Fairbrother A. 2003. Lines of evidence in wildlife risk assessments. *Hum Ecol Risk Assess* 9:1475–1491.
19. Henning MH, Robinson SK, McKay KJ, Sullivan JP, Bruckert H. 2003. Productivity of American robins exposed to polychlorinated biphenyls, Housatonic River, Massachusetts, USA. *Environ Toxicol Chem* 22:2783–2788.
20. Halbrook R, Woolf A, Arenal C. 1998. European starling (*Sturnus vulgaris*): Avian model and monitor of contaminant and remedial effects at Crab Orchard National Wildlife Refuge. RR-E84, 1 58. Illinois Waste Management and Research Center, Champaign, IL, USA.
21. U.S. Environmental Protection Agency. 2000. Baseline ecological risk assessment, Hudson River PCBs Reassessment. Revised Phase 2 Report. Washington, DC.
22. Froese KL, Verbrugge DA, Ankley GT, Niemi GJ, Larsen CP, Giesy JP. 1998. Bioaccumulation of polychlorinated biphenyls from sediments to aquatic insects and tree swallow eggs and nestlings in Saginaw Bay, MI. *Environ Toxicol Chem* 17:484–492.
23. Elliot JE, Harris ML. 2002. An ecotoxicological assessment of chlorinated hydrocarbon effects on bald eagle populations. *Rev Toxicol* 4:1–60.
24. DeWesse LR, Cohen RR, Stafford CJ. 1985. Organochlorine residues and eggshell measurements for tree swallows *Tachycineta bicolor* in Colorado. *Bull Environ Contam Toxicol* 35:767–775.
25. Bishop CA, Koster MD, Chek AA, Hussell DJT, Jock K. 1995. Chlorinated hydrocarbons and mercury in sediments, red-winged blackbirds (*Agelaius phoeniceus*) and tree swallows (*Tachycineta bicolor*) from wetlands in the Great Lakes–St. Lawrence River Basin. *Environ Toxicol Chem* 14:491–501.
26. CDM Federal Programs Corporation. 2003. Baseline ecological risk assessment, Allied Paper/Portage Creek, Kalamazoo River Superfund Site. Final Report. Michigan Department of Environmental Quality, Lansing, MI, USA.
27. Secord AL, McCarty JP, Echols KR, Meadows JC, Gale RW, Tillitt DE. 1999. Polychlorinated biphenyls and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents in tree swallows from the Upper Hudson River, New York State, USA. *Environ Toxicol Chem* 18:2519–2525.
28. Custer CM, Custer TW, Dummer PM, Munney KL. 2003. Exposure and effects of chemical contaminants on tree swallows nesting along the Housatonic River, Berkshire County, Massachusetts, USA, 1998–2000. *Environ Toxicol Chem* 22:1605–1621.
29. Ankley GT, Niemi GJ, Lodge KB, Harris HJ, Beaver DL, Tillitt DE, Schwartz TR, Giesy JP, Jones PD, Hagley C. 1993. Uptake of planar polychlorinated biphenyls and 2,3,7,8-substituted polychlorinated dibenzofurans and dibenzo-*p*-dioxins by birds nesting in the lower Fox River and Green Bay, Wisconsin, USA. *Arch Environ Contam Toxicol* 24:332–344.
30. Trowbridge AG, Swackhamer DL. 2002. Preferential biomagnification of aryl hydrocarbon hydroxylase-inducing polychlorinated biphenyl congeners in the Lake Michigan, USA, lower food web. *Environ Toxicol Chem* 21:334–341.
31. Secord AL, McCarty JP. 1997. Polychlorinated biphenyl contamination of tree swallows in the upper Hudson River valley, New York. Effects on breeding biology and implications for other bird species. U.S. Fish and Wildlife Service, Cortland, NY.
32. Thiel DA, Martin SG, Duncan JW, Lemke MJ, Lance WR, Peterson RE. 1988. Evaluation of the effects of dioxin-contaminated sludge on wild birds. *Proceedings*, Technical Association of the Pulp and Paper Industry Environmental Conference, Norcross, GA, USA, pp 487–506.

Available online at www.sciencedirect.com
ScienceDirect
Ecotoxicology and Environmental Safety 66 (2007) 107–118
Ecotoxicology and Environmental Safety
www.elsevier.com/locate/ecoenv

Reproductive success of passerines exposed to polychlorinated biphenyls through the terrestrial food web of the Kalamazoo River ☆

Arianne M. Neigh[a,*], Matthew J. Zwiernik[a], Carrie A. Joldersma[b], Alan L. Blankenship[a,b], Karl D. Strause[a], Stephanie D. Millsap[a], John L. Newsted[b], John P. Giesy[a,b,c]

[a]*Zoology Department, Center for Integrative Toxicology, National Food Safety and Toxicology Center, Michigan State University, East Lansing, MI 48824, USA*
[b]*ENTRIX, Inc., Okemos, MI 48864, USA*
[c]*Biology and Chemistry Department, City University of Hong Kong, Kowloon, Hong Kong, SAR, China*

Received 14 January 2005; received in revised form 24 June 2005; accepted 10 October 2005
Available online 15 December 2005

Abstract

The eastern bluebird (*Sialia sialis*) and the house wren (*Troglodytes aedon*) were identified as ecological receptors of concern due to exposure and potential effects stemming from polychlorinated biphenyl (PCB) contamination in floodplain soils of the Kalamazoo River Superfund Site, Michigan, USA. Measures of population health were compared at a contaminated and a less-contaminated reference location. During this 3-year study, productivity of bluebirds was significantly less at the downstream location than at the reference location. Hatching success, clutch size, and predicted brood size were significantly less in early clutches of house wrens at the more contaminated location than at the upstream reference location, but fledging success was significantly greater at the contaminated location. Studies concurrent to the study presented here reported that concentrations of PCBs in the tissues and diets of the passerine birds were less than the predicted threshold for adverse effects. The results of our study, taken along with the measured exposure data, suggest that other factors in addition to PCB exposure such as habitat, prey availability, small sample size, and cocontaminants were likely causes of the differences that were observed at the two locations.
© 2005 Elsevier Inc. All rights reserved.

Keywords: Polychlorinated biphenyls; Productivity; Birds; Eastern bluebird; House wren; Eggs; Hatching success

1. Introduction

The Kalamazoo River was designated a Superfund site in 1990 due to the presence of wastes contaminated with polychlorinated biphenyls (PCBs) that were released during the carbonless copy paper recycling process (MDEQ, 2003). The Kalamazoo River Area of Concern (KRAOC) supports a diverse terrestrial ecosystem along its course, including 205 ha of formerly impounded contaminated sediment exposed when three dams were partially dismantled to their sill levels. While the effects of PCB contamination on various species with aquatic-based diets is well documented (Kubiak et al., 1989; Ludwig et al., 1993; Yamashita et al., 1993; Giesy et al., 1994; Millsap et al., 2004), few studies have evaluated PCB contamination in wildlife exposed through terrestrial food webs associated with riverine ecosystems. The presence of PCBs in soil and sediment at the site requires identification of effects in terrestrial and aquatic endpoints during the risk assessment process. This study focused on the effects of PCB contamination in two passerine birds with predomi-

☆Funding for this study was provided by the Kalamazoo River Study Group. The authors assure that all portions of this study involving animal subjects were conducted in accordance with university, state, and federal animal welfare practices. Sampling and salvage permits were obtained prior to start of sample collection. Michigan State University All-University Committee on Animal Use and Care provided oversight on the care and welfare of all birds used in this study.
*Corresponding author. Fax: +1 (517) 381 1435.
E-mail address: neighari@msu.edu (A.M. Neigh).

0147-6513/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.ecoenv.2005.10.004

nantly terrestrial diets, while risk to passerines dependent on the aquatic food web is presented elsewhere (Neigh et al., 2006a).

There is a well-established history for the use of passerines as biosentinals of contamination by scientists at the US Environmental Protection Agency (USEPA) (Ankley et al., 1993), Environment Canada (Bishop et al., 1995), the Canadian Wildlife Service (Elliott and Harris, 2002), and the US Fish and Wildlife Service (USFWS) (McCarty and Secord, 1999), but studies frequently focused on a receptor of the aquatic food web, the tree swallow. Presently, there is no such favored passerine receptor that exists for terrestrial systems, although in terrestrial risk assessments, depending on habitat type and species applicability to site-specific monitoring criteria, a number of passerine species have been utilized. These species include the European starling (*Sturnus vulgaris*) (Halbrook et al., 1998), American robin (*Turdus migratorius*) (Henning et al., 1997, 2002), red-winged blackbird (*Agelaius phoeniceus*) (Bishop et al., 1995), western bluebird (*Sialia mexicana*) (Fair and Myers, 2002), ash-throated flycatcher (*Myiarchus cinerascens*) (Fair and Myers, 2002), American redstart (*Setophaga ruticilla*), barn swallow (*Hirundo rustica*), eastern phoebe (*Sayornis phoebe*), rose-breasted grosbeak (*Pheucticus ludovicanus*), wood thrush (*Hylocichla mustelina*), and yellow warbler (*Dendroica petechia*) (Henning et al., 1997). To our knowledge, eastern bluebirds (*Sialia sialis*) have been used infrequently as a reproductive monitor of exposure (Bishop et al., 1995), and the house wren (*Troglodytes aedon*) is a novel monitor of terrestrial PCB exposure. In addition, there is a lack of field and laboratory toxicity studies on these species even though they are fairly ubiquitous, abundant, and easily captured contaminant receptors. There are also a large number of studies in the current literature investigating breeding, behavior, and other aspects of biology at relatively uncontaminated ecosystems from which to establish baseline values for comparison to contaminated locals. These two species are often listed as species of concern at sites contaminated with PCBs, so, to fill the data gap in the current literature, this part reports empirical information on the responses of these species to the potential effects of PCBs under field conditions.

The house wren and eastern bluebird were selected as monitors of PCB exposure at the Kalamazoo River Superfund site based on a number of criteria but primarily because they were named as resident species of concern due to their exposure to contaminated soils through the diet (MDEQ, 2003). They were not chosen as surrogate species or sensitive sentinels for other species but rather were studied to determine the potential for exposure of these species and to determine ecologically relevant reproductive parameters. Eastern bluebirds and house wrens frequently used nest boxes at the Kalamazoo River locations, so sample sizes were expected to be sufficient to demonstrate population health. It was also thought that the exposure of this species to PCBs would coincide with local sources of contamination in the soil due to their diet of terrestrial insects (Pinkowski, 1978), and thus be useful to assess potential reproductive impairment by PCBs. The eastern bluebird was also identified as a species of concern due to plant-derived sources of PCB exposure in its diet (MDEQ, 2003). Here, we describe productivity and growth of house wrens and eastern bluebirds located within the Kalamazoo River Superfund site and an upstream reference site.

2. Materials and methods

2.1. Site details

During 2000, nest boxes were established at two locations along the Kalamazoo River's 100-year floodplain. The Fort Custer State Recreation Area (FC) is located upstream of the sources of contamination and therefore was chosen to serve as a background location due to the low background levels of contamination existing at the site (Anonymous, 1997). Situated 67 km downstream, the target area was located at the former Trowbridge Impoundment (TB) within the KRAOC, which was designated a Superfund site by the USEPA in 1986 (Fig. 1). The former TB was formed when the Trowbridge Dam was removed to the sill, which exposed 132 ha of former depositional lake bottom sediments.

Previous studies quantified concentrations of total PCBs, dichlorodiphenyltrichloroethane (DDT) isomers, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TEQs) (Neigh et al., 2006b) based on World Health Organization toxic equivalence factors ($TEF_{WHO\text{-}Avian}$) (van den Berg et al., 1998) in the diet, eggs, nestlings, and adults of eastern bluebirds and house wrens. This study was designed as a comparative study between two different locations; therefore, concentrations in eggs were not converted to fresh weight values. Eggs were sampled at similar times and stored for similar periods of time, so moisture content was expected to be similar in sampled eggs at the two sites. In all tissues from both eastern bluebirds and house wrens, mean concentrations of total PCBs and TEQs were significantly greater at the KRAOC than they were at the reference location, FC (Table 1). Among all matrices, concentrations of total PCBs at TB were 39- to 122-fold greater than concentrations at FC, while concentrations of TEQs at TB were 5- to 47-fold greater than concentrations at FC. Although not significantly greater, concentrations of DDT isomers in eastern bluebird eggs were 1.4-fold greater at TB than at FC, and concentrations of DDT isomers in house wren eggs were 3.3-fold greater at TB than at FC. Concentrations of total PCBs moderately bioaccumulated from lower trophic level species to upper trophic level predators, but concentrations in biota were less than those in soils. The decrease in the concentrations of total PCBs from soil to biota was accompanied by a decrease in relative potency (mg TEQ/kg PCB) of the PCB mixture, which was attributed to environmental weathering (Blankenship et al., 2005).

2.2. Productivity observations

Throughout the 2001–2003 nesting seasons, nest boxes were monitored for occupancy by eastern bluebirds and house wrens at FC ($n = 64$) and TB ($n = 68$). Boxes were monitored daily to determine the date of clutch initiation and day of hatch. Reproductive success was determined based on nest observations. Hatching success was described as the percentage of eggs hatched in each nest. Fledging success was described as the percentage of nestlings fledged per nestling hatched, and productivity was described as the percentage of nestlings fledged per egg laid. Egg mass and length was recorded within 24 h of laying. In an effort to minimize the accidental breakage of eggs in the field due to measurement, egg width and therefore egg volume were not investigated. Day of hatch was also confirmed by assessing physical development of the nestling (Pinkowski, 1975). Each nestling was examined for gross external morphological abnormalities. Weight of each nestling was recorded on days 3, 9, and 12

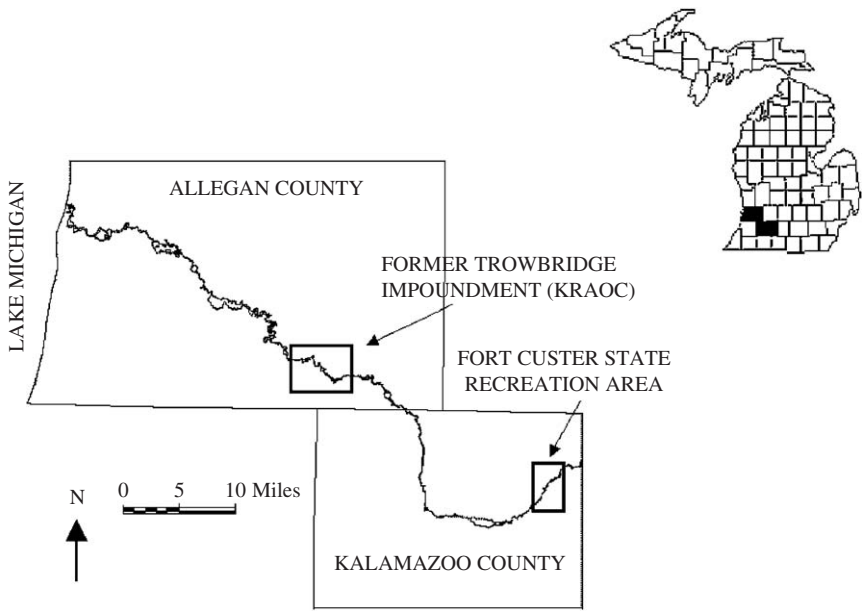


Fig. 1. Map of the Kalamazoo River Area of Concern (KRAOC) and reference site. The inset shows the location of the two counties (black) in Michigan where reproductive studies were conducted (Neigh et al., 2006a). The boxes designate the boundaries of the upstream reference location (Fort Custer State Recreation Area) and the Trowbridge Impoundment, located within the KRAOC.

Table 1
Mean (±SD) concentrations (wet weight) of total PCBs, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TEQs), and DDT in the tissues of terrestrial passerine species at the Fort Custer State Recreation Area (FC) and at the Trowbridge Impoundment (TB) (Neigh et al., 2006b)

	Total PCBs (mg PCB/kg)				TEQs[a] (μg TEQ/kg)				DDT[b] (mg DDT/kg)			
	n	FC	*n*	TB	*n*	FC	*n*	TB	*n*	FC	*n*	TB
Eastern bluebird												
Egg	14	0.17 (0.10)	7	8.3 (5.1)[c]	3	7.6 (8.6)	5	77 (82)[c]	5	1.5 (1.8)	5	2.1 (1.1)
Nestling	17	0.011 (0.006)	6	1.3 (1.4)[c]	17	1.3 (0.64)	6	6.3 (4.0)[c]	5	NA	5	NA
House wren												
Egg	14	0.12 (0.12)	14	6.3 (6.0)[c]	8	8.6 (3.9)	11	400 (450)[c]	5	0.66 (0.87)	5	0.20 (0.21)
Nestling	13	0.020 (0.020)	17	0.77 (0.64)[c]	13	1.4 (0.96)	17	63 (47)[c]		NA		NA
Adult	8	0.072 (0.033)	9	3.2 (2.1)[c]	8	7.1 (5.7)	9	110 (57)[c]		NA		NA

[a]TEQs based on World Health Organization Toxic Equivalence Factors (van den Berg et al., 1998).
[b]DDT includes *o*, *p*′ and *p*, *p*′ isomers of DDT, DDD, and DDE.
[c]Mean is significantly greater than that at the FC reference area (Students *t* test, $P<0.05$).

(hatch day = day 0) for eastern bluebirds and on days 3, 6, and 9 for house wrens. When chicks were sampled for contaminant analysis at approximately day 9 for house wrens and day 12 for eastern bluebirds, each individual was weighed and the length of the entire body (tip of beak to longest rectrix), tarsus (tibiotarsal joint to hind toe base), and unflattened wing chord were recorded. Measurements were taken during 2001 and 2002 for house wrens and from 2001 to 2003 for eastern bluebirds.

2.3. Statistical analysis

For statistical analyses (SYSTAT, Evanston, IL, USA), each nest box was treated as a separate experimental unit and values were reported on a per box basis. All nesting attempts were treated as separate and individual observations. Normality was assessed with Kolmogorov–Smirnov's one-sample test with Lilliefors transformation, and homogeneity of variance was verified by *F* test. All parametric data were analyzed by one-way analysis of variance (ANOVA), and non parametric data were analyzed by Mann–Whitney *U* or Kruskal–Wallis tests. The criterion for significance used in all tests was $P<0.05$. Statistical comparisons were made between means and distributions at the two locations. In addition, the relationships between concentrations of PCBs, measured as both total PCBs and TEQs, and specific endpoints were examined by both Spearman and the Pearson correlations.

3. Results

3.1. Reproductive success

A total of 34 house wren and 18 eastern bluebird nests were completed at the TB study site and 71 house wren and 57 eastern bluebird nests were completed at the FC site over the study period (Table 2). For the entire study, 32% of eastern bluebird nests failed and 24% of house wren

Table 2
Nest fate and percentage of initiated nests of eastern bluebirds and house wrens at the Fort Custer State Recreation Area (FC) reference site and at the Trowbridge (TB) contaminated site

	2001		2002		2003	
	FC	TB	FC	TB	FC	TB
Eastern bluebird						
Successful[a]	14 (93%)	2 (40%)	15 (63%)	2 (25%)	14 (78%)	4 (80%)
Predated[b]	1 (7%)	0 (0%)	6 (25%)	4 (50%)	1 (6.5%)	1 (20%)
Abandoned[c]	0 (0%)	1 (20%)	2 (8%)	2 (25%)	2 (11%)	0 (0%)
Unknown	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Other	0 (0%)	2 (40%)	1 (4%)	0 (0%)	1 (6.5%)	0 (0%)
Total	15	5	24	8	18	5
House wren						
Successful[a]	17 (89.5%)	8 (73%)	38 (73%)	17 (74%)		
Predated[b]	1 (5.25%)	0 (0%)	6 (11%)	2 (9%)		
Abandoned[c]	0 (0%)	2 (18%)	0 (0%)	3 (13%)		
Unknown	0 (0%)	1 (9%)	5 (10%)	0 (0%)		
Other	1 (5.25%)	0 (0%)	3 (6%)	1 (4%)		
Total	19	11	52	23		

[a]Successful nests fledged at least one nestling.
[b]Predated nests included those nests with evidence of predation such as disturbed nesting material or broken eggs.
[c]Nests were considered abandoned when eggs were cold for at least 7 days and adults were not present.

nests failed. The failure of the majority of eastern bluebird nests was attributed to predation, which comprised over 50% of the nest failures at both FC and TB. Abandonment accounted for 29% of the nest failures at FC and 30% at TB. Failure of house wren nests was attributed to a greater proportion of abandoned nests at TB (56% of all failures), while no nest failures were attributed to abandonment at FC. Predation accounted for 44% of house wren nest failures at FC but only 22% at TB.

Both house wrens and eastern bluebirds can produce two broods in a season. Birds were not banded at either location, and therefore it was not possible to definitively determine when a nesting pair established subsequent nests. In all years, there were 7 and 12 suspected house wren second broods at TB and FC, respectively, and 3 and 15 suspected eastern bluebird second broods at TB and FC, respectively. Nests were still considered to be independent samples because second broods could not be verified since adults were not banded. To limit the effect of second nestings, reproductive parameters were evaluated for the entire nesting season and based on dates of clutch initiation, where nests from early in the season were grouped together and nests from late in the season were grouped together. Nesting attempts were categorized as early or late nests to account for potential differences in reproductive parameters between the first and the second nestings. For example, the productivity of second nesting attempts in eastern bluebirds was about 21% (Pinkowski, 1979). The median dates of initiation for house wrens in 2001 were May 20 and June 24 for early and late nests, respectively, and May 28 and July 1 during 2002, respectively. The median date of nest initiation for eastern bluebirds was between April 21 and 25 for early nests and between June 9 and 22 for late nests during the study. There was little correlation (Spearman, $r^2 < 0.10$) between date of nesting and reproductive success in either house wrens or eastern bluebirds.

All measures of reproductive success, including hatching success, fledging success, and productivity, were adjusted for fresh egg sampling, except clutch size, by eliminating the egg collected for residue analyses from the reproductive analyses because the subsequent potential fate of the eggs collected for residue analyses could not be predicted at the time of sampling. Nests were observed for activity until the date of fledging, so it was assumed that live sampled nestlings would have fledged successfully. Therefore, the number of nestlings sampled did not affect the outcome of statistical analyses, but the number of fresh eggs sampled could have affected the observed brood size and number of fledglings. A predicted value based on other parameters was used to evaluate brood size and the number of fledglings. The brood size for each nest in which an egg was sampled was calculated as the clutch size multiplied by the hatching success. The predicted number of fledglings for each nest in which an egg was sampled was calculated as the clutch size multiplied by the productivity. All other interactions attributed to the collection of eggs or nestlings were considered to be negligible as compared to exposure to PCBs. The study design, which requires at least one egg or nestling to be sampled from each nest for residue analysis, may limit the compatibility of this study to the published literature, but the main focus of the study was aimed at comparing reproductive success of an unexposed versus an exposed population.

Owing to relatively small sample sizes within a year, measurements of reproductive health in eastern bluebirds

were combined among all years. When the results for all 3 years were combined, a statistically significant difference between FC and TB in productivity (Mann–Whitney *U*) and nearly statistically significant differences ($P<0.1$) in hatching success (Mann–Whitney *U*) and fledging success (Mann–Whitney *U*) were observed (Table 3). Statistically significant differences were detected in the date of clutch initiation, so, to eliminate interactions between date of initiation and reproductive success, all nests were separated into one of two groups, which were designated "early" or "late" nests. Reproductive success was not statistically different in late nests, but the clutch size (Mann–Whitney *U*) and fledging success (Mann–Whitney *U*) were statistically greater at FC than at TB in early nests.

Reproductive parameters for house wrens were also not statistically different between years, so all years were combined. When all years were combined, the only statistically significant difference between locations was fledging success, which was greater at TB than at FC (Mann–Whitney *U*) (Table 3). Although the date of clutch initiation for house wrens was not significantly different between locations, clutches were also separated into early and late nests. In early clutches, clutch size (Mann–Whitney *U*), hatching success (Mann–Whitney *U*), and predicted brood size (Mann–Whitney *U*) were statistically greater for nests at FC than at TB.

3.2. Egg measurements

No statistically significant differences in egg parameters among years were detected, so the results from all 3 years were combined. The mean weights of eggs of neither species were statistically different between locations (Table 4). The mean length of house wren eggs was not statistically different between sites, but eggs were significantly longer at FC than at TB for eastern bluebird eggs (Student's *t* test). Egg parameters were evaluated for interaction with each other and with date of initiation based on ANCOVA. No interactions between any parameters existed for house wren, but a statistically significant correlation between weight and length did exist for eastern bluebirds (ANCOVA), which changed the significance of the comparison of mean egg length ($P<0.05$ to $P>0.05$).

3.3. Growth measurements and growth curves

Differences in growth parameters between years could not be evaluated due to small sample sizes, so all years were combined. Growth parameters in 8- and 9-day-old house wrens were statistically similar, so ages were combined. Eastern bluebird growth measurements consisted of nestlings at day 12. Correlations between growth parameters within locations were first taken into account when growth

Table 3
Eastern bluebird and house wren nest productivity measurements (mean ± SD) at a reference site (Fort Custer) and at a PCB-contaminated target site (Trowbridge) on the Kalamazoo River for early and late clutches and all clutches combined

	Early clutches				Late clutches				All clutches			
	Fort Custer		Trowbridge		Fort Custer		Trowbridge		Fort Custer		Trowbridge	
	n	Mean (±1 SD)	*n*	Mean (±1 SD)	*n*	Mean (±1 SD)	*n*	Mean (±1 SD)	*n*	Mean (±1 SD)	*n*	Mean (±1 SD)
Eastern bluebird												
Hatching success[a]	25	0.82 (0.27)	10	0.61 (0.44)	24	0.75 (0.34)	4	0.54 (0.42)	49	0.79 (0.31)	14	0.59 (0.42)
Fledging success[b]	24	0.96 (0.20)	6	0.75[c] (0.42)	21	0.95 (0.22)	3	1.0 (0.0)	45	0.96 (0.21)	9	0.83 (0.35)
Productivity[d]	25	0.79 (0.32)	9	0.44 (0.47)	24	0.73 (0.38)	4	0.54 (0.42)	49	0.76 (0.34)	13	0.47[c] (0.44)
Clutch size	26	4.7 (0.47)	11	3.5[c] (1.4)	31	3.6 (0.93)	7	3.7 (1.7)	57	4.2 (1.0)	18	3.6 (1.5)
Predicted brood size	24	4.1 (1.2)	7	3.5 (1.2)	21	3.6 (0.93)	3	2.9 (2.0)	45	3.8 (1.1)	10	3.3 (1.4)
Predicted number of fledglings	23	4.1 (1.2)	5	3.1 (1.5)	20	3.6 (0.94)	3	2.9 (2.0)	43	3.9 (1.1)	8	3.0 (1.6)
House wren												
Hatching success[a]	32	0.83 (0.24)	15	0.58[c] (0.39)	28	0.78 (0.25)	16	0.71 (0.44)	60	0.81 (0.25)	31	0.64 (0.41)
Fledging success[b]	31	0.90 (0.26)	13	1.0 (0.0)	28	0.94 (0.21)	12	1.0 (0.0)	59	0.92 (0.24)	25	1.0[c] (0.0)
Productivity[d]	32	0.75 (0.30)	15	0.58 (0.39)	28	0.73 (0.30)	16	0.71 (0.44)	60	0.74 (0.30)	31	0.64 (0.41)
Clutch size	38	6.2 (0.93)	15	5.6[c] (1.2)	33	5.3 (1.2)	21	5.2 (1.5)	71	5.7 (1.1)	36	5.4 (1.4)
Predicted brood size	31	5.5 (1.5)	13	3.9[c] (2.2)	28	4.4 (1.6)	12	5.4 (1.3)	59	5.0 (1.6)	25	4.6 (2.0)
Predicted number of fledglings	29	5.2 (1.4)	13	3.9 (2.2)	27	4.2 (1.6)	12	5.4 (1.3)	56	4.8 (1.6)	25	4.6 (2.0)

[a]Hatching success is calculated as the number of eggs hatched per egg laid.
[b]Fledging success is calculated as the number of fledglings per nestling hatched.
[c]Mean of the Trowbridge population is statistically different from Fort Custer population ($P<0.05$).
[d]Productivity is calculated as the number of fledglings per egg laid.

Table 4
Mean (±1 SD)[a] reproductive endpoints for eastern bluebird and house wren eggs and nestlings reared at the Fort Custer State Recreation Area and at the Trowbridge Impoundment along the Kalamazoo River Area of Concern

	Fort Custer		Trowbridge	
	Eastern bluebird	House wren	Eastern bluebird	House wren
Eggs				
n	36	74	11	37
Egg weight (g)	3.22 (0.67)	1.46 (0.01)	3.01 (0.12)	1.51 (0.09)
n	34	74	11	37
Egg length (mm)	21.4 (1.04)	16.9 (0.08)	20.6 (0.28)[b]	16.8 (0.16)
Nestlings[c]				
n	12	22	3	12
Body weight (g)	27.79 (2.04)	9.43 (0.81)	29.17 (2.03)	9.32 (1.39)
Body length (mm)	92.3 (6.2)	64.9 (6.2)	98.1 (14.6)	61.5 (4.2)
Tarsal length (mm)	19.3 (1.8)	16.8 (2.7)	19.7 (1.4)	16.2 (2.4)
Wing chord (mm)	49.9 (8.5)	23.1 (4.2)	47.9 (4.9)	24.4 (4.6)

[a]Mean values were calculated based on the mean measurements per nest for all nests.
[b]Statistically different from Fort Custer population (Students *t* test, $P<0.05$).
[c]Eastern bluebird nestling measurements were taken at day 12 and house wren nestling measurements at day 8 or 9.

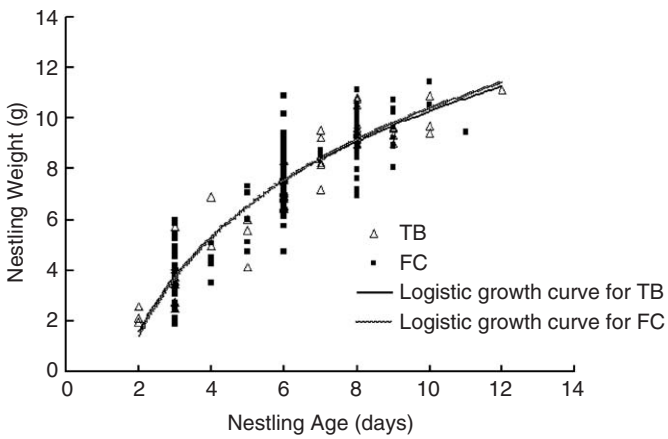


Fig. 2. House wren nestling growth curves based on mean nestling weights for each nest box at the Trowbridge (TB) target area and the Fort Custer (FC) reference site. The equation for the line of best fit at FC is $y = 5.563\,\mathrm{Ln}(x) - 2.4223$, $r^2 = 0.836$. The line of best fit for TB is $y = 5.378\,\mathrm{Ln}(x) - 2.1186$, $r^2 = 0.896$.

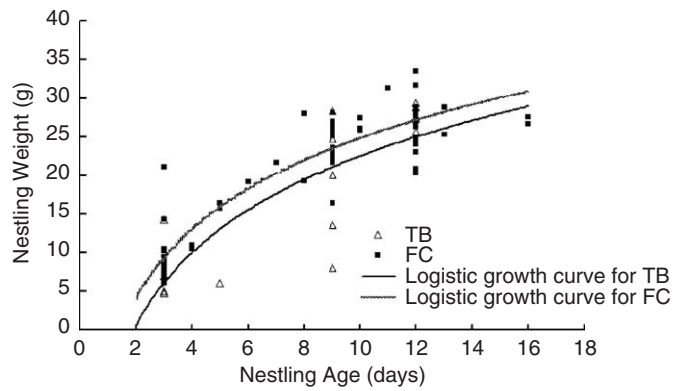


Fig. 3. Eastern bluebird nestling growth curve based on mean nestling weights in each active nest box at the Trowbridge (TB) target site and the Fort Custer (FC) reference site. The line of best fit for the curve is described by the equation $y = 13.668\,\mathrm{Ln}(x) - 8.8892$, $r^2 = 0.647$ at TB and $y = 12.891\,\mathrm{Ln}(x) - 4.7993$, $r^2 = 0.861$.

was evaluated between locations. There were no significant correlations present for eastern bluebirds, but there were several potential correlations for house wrens. The correlation between body length, wing chord length, and tarsal length drove the difference in mean body length between sites to a nearly statistically significant value (ANCOVA, $P<0.1$), but no growth parameters for either species were statistically different between sites (Table 4).

Growth of nestlings was compared between sites based on curves generated from nestling mass gained per day as an indicator of stress on the species (Zach and Mayoh, 1982). A logistic growth curve was fitted to nestling masses at each site. Growth curves, based on average nestling mass for each box at each nestling day, were strikingly similar for both species at both sites when all years were combined. Growth curves of house wrens, compared between sites, overlapped over the entire nesting period (Fig. 2). Growth curves for eastern bluebirds followed a similar trajectory, but the curve was shifted toward greater mean nestling weights at FC (Fig. 3).

Growth curves, based on average nestling mass gain over the nestling period for the two sites, were compared statistically. Nestling mass between nestling days 3 and 10 for eastern bluebirds and between nestling days 2 and 8 for house wrens was log-transformed and the mass gained per day of life was compared between sites. House wren mass gain per day was significantly different between years (ANOVA), but that of eastern bluebirds was not. House wren mass gain was significantly less at TB than at FC during 2002 (Students *t* test) but not during 2001. Eastern

bluebird mass gain per day was not different between sites in 2001 or 2003, but in 2002 it was significantly different (Kruskal–Wallis).

4. Discussion

4.1. Reproductive success

Eastern bluebirds made fewer nesting attempts than house wrens at TB and FC, but confounding factors such as vegetation structure and age of the nest trail may contribute to this difference (Munro and Rounds, 1985). The bluebird box trail at FC has been established for over 30 years, which may cause an increase in the number of returning adults and nestlings to the site.

Abandonment rates for tree swallows have been reported to be greater at PCB-contaminated sites (5.95–29.5 mg PCB/kg, mean concentrations in eggs, wet weight (ww)) compared to a less-contaminated habitat (0.103 mg PCB/kg, mean concentration in eggs, ww) (McCarty and Secord, 1999). Few data for the bluebird or the house wren are available for abandonment rates at PCB-contaminated sites. Abandonment rates of eastern bluebirds and house wrens at the Kalamazoo River were between 0% and 25%, which was near the range (4.5–23.7%) reported for uncontaminated populations of bluebirds (Rustad, 1972). Abandonment rates of eastern bluebirds may be partly attributed to interactions with house wrens as indicated by observations of house wrens preventing eastern bluebirds from entering the nest or returning with prey items for the nestlings and frequent squabbling between the two species at nest locations. House wrens are known to interfere with the nesting of other species (Finch, 1990) and, unlike other studies of this type, house wren nesting was not discouraged. Abandoned nests had no physical damage to the structure of the nest, as is the case in 85% of house wren predations (Belles-Isles and Picman, 1986), so abandoned nests may have been improperly labeled as abandoned rather than associated with house wren interference. Abandonment was not observed for house wrens at FC, but some nests were abandoned at TB. Again, interactions among house wrens may have caused abandonment without damage to the nest, where in some cases as much as 89% of unsuccessful nesting attempts have been linked to conspecific interference (Finch, 1990).

In an effort to further characterize interactions between eastern bluebird and house wren populations, the correspondence of house wren presence and nesting failure was investigated for eastern bluebird nests. A simple nearest-neighbor-type analysis was conducted to determine whether eastern bluebird failed more often when the nearest nest box was occupied by a house wren or whether a house wren nested in the box immediately following predation of abandonment of the eastern bluebird nest (Table 5). For eastern bluebird nests, 78% of nest predation and 40% of nest abandonments were associated with house wren presence, whereas only 21% of successful nests were associated with house wren presence. These values suggest that there is an association between presence of house wrens in the temporal and proximal vicinity of eastern bluebird nests and success of eastern bluebird nests.

The date of clutch initiation did not affect the reproductive success of either house wrens or eastern bluebirds. Some studies have reported that second clutches were not as likely to be successful as first clutches, with only 21% of second nests producing fledglings (Pinkowski, 1979). At the Kalamazoo River, there was no reduction in nest success in the clutches classified as "late" nests, but which nests were in fact second nests and which nests were late first nest attempts could not be determined. Other studies also did not find a correlation between Julian date and reproductive parameters (Fair and Myers, 2002).

PCBs may affect reproductive capacity by decreasing hatching success, possibly by causing infertility of eggs (McCarty and Secord, 1999). Hatching success in early clutches of house wrens was significantly less at TB than at FC (Mann–Whitney U, $P = 0.045$), which was also observed in tree swallows from the Hudson River where hatching success was 64–79% at contaminated sites (11.7–42.1 mg PCB/kg, mean concentrations in eggs, ww) and 93% at a reference location (0.103 mg PCB/kg, mean concentrations in eggs, ww) (Secord and McCarty, 1997). Conversely, hatching success (87.4%) was not impaired at the Housatonic River for several species of passerines exposed to concentrations of PCBs in floodplain soils (mean concentrations in soils = 12 mg PCB/kg, dry weight, (dw)) (Henning et al., 1997), similar to those at TB (11 mg PCB/kg, dw). Of the nonviable eggs at the Hudson River, 60% were infertile, while the remaining nonviable egg mortality was attributed to the failure of the embryo to develop and the death of the embryo (McCarty and Secord, 1999). Hatching success of both the bluebird and the house wren at the Kalamazoo River are most strongly correlated with productivity (Spearman, $r^2 = 0.78$). Productivity was significantly less in the eastern bluebird at TB, but this value is dependent on a single female in 2001 that twice attempted to produce a brood without success but did not abandon the eggs. This one female alone accounted for a 40% decrease in reproductive success at TB because only five nests were established by bluebirds during that year. If this one individual was removed from the data set, the hatching success at TB for all nests would increase by 10% and the productivity would increase by 9%. Productivity for all nests was no longer significantly different between locations when the nest was removed from the analyses, but all other statistical comparisons were similar to those made previously. Based on clutch size, hatching success, and productivity, reproductive performance was apparently depressed at TB compared to FC, but measured concentrations of PCBs in the tissue and diet of both species were below the threshold concentrations for effects (Neigh et al., 2006b, c). Little risk of reproductive effects was predicted from concentrations of PCBs in tissues and diet. Thus, the lesser reproductive success observed in these

Table 5
Correspondence of eastern bluebird nest fate[a] to proximal and temporal presence of house wrens

	House wren		Other bird species		Empty nest	
	Nearest neighbor	Later nest	Nearest neighbor	Later nest	Nearest neighbor	Later nest
Eastern bluebird						
Successful (33)	5 (15%)	2 (6%)	16 (49%)	9 (27%)	12 (36%)	22 (67%)
Predated (9)	3 (33%)	4 (45%)	1 (11%)	3 (33%)	5 (56%)	2 (22%)
Abandoned (5)	1 (20%)	1 (20%)	1 (20%)	3 (60%)	3 (60%)	1 (20%)
Other (3)	0 (0%)	1 (33%)	1 (33%)	1 (33%)	2 (67%)	1 (33%)
Total (50)	9 (18%)	8 (16%)	19 (38%)	16 (32%)	22 (44%)	26 (52%)

[a]Two eastern bluebird nests known to be predated by raccoons were removed from the analysis.

Table 6
Power ($1-\beta$) to detect a decrease in reproductive success at Trowbridge compared to that at Fort Custer and the sample size (n) needed at each location to detect a 20% reduction in reproductive health

	Eastern bluebird						House wren					
	Separate clutches				All clutches		Separate clutches				All clutches	
	Early clutches		Late clutches				Early clutches		Late clutches			
	$1-\beta$	n	$1-\beta$	n	$1-\beta$	n	$1-\beta$	n	$1-\beta$	n	$1-\beta$	n
Hatching success	0.29	63	0.16	80	0.38	68	0.63	46	0.09	67	0.56	56
Fledging success	0.22	37	0.18	9	0.19	29	0.57	14	0.33	8	0.73	11
Productivity	0.54	81	0.14	92	0.60	84	0.32	67	0.05	83	0.22	74
Clutch size	0.79	15	0.05	45	0.36	28	0.41	10	0.06	20	0.20	15
Predicted brood size	0.21	26	0.09	60	0.19	33	0.67	35	0.55	35	0.14	42
Predicted number of fledglings	0.29	35	0.09	59	0.33	39	0.50	41	0.70	38	0.07	45

birds was most likely the result of other factors present in the environment. The habitat at the more contaminated location was also deemed to be poor habitat due to frequent flooding and the presence of a dense cover of chord grass.

Statistical power to detect differences in reproductive measurements at the different locations was generally less than an acceptable limit for both species ($1-\beta<0.80$) (Table 6). Sample sizes greater than those observed at the Kalamazoo River would have been needed to detect a 20% decrease in reproductive performance at TB compared to FC. Unlike laboratory studies where sample sizes can be adjusted based on statistical power analyses to allow differences of a particular magnitude, it is not possible to increase the number of nests in field studies beyond the carrying capacity of the site. At the Kalamazoo River, nest boxes were placed to maximize occupancy and not affect survivorship. Therefore, the limiting factor for the number of nest boxes was the size of the study area, which was beyond the control of the investigators. Although some uncertainty may be associated with measures of reproductive performance due to small sample sizes, there is inherently less uncertainty in the evaluation of reproductive performance and associated exposure levels in the Kalamazoo River populations of eastern bluebirds and house wrens that were actually measured than to uncertainties associated with predicting responses in these receptors of concern based on distantly related surrogate laboratory species.

The fledging success and growth of nestlings, which may be related to nestling survival, has been associated with proximity to contamination (Fair et al., 2003). Fledging success and growth over the entire study was not impaired in house wrens at TB compared to FC. In eastern bluebird nests, fledging success was less at TB in early nests but not for all clutches combined, and growth over the entire study was not different. Although growth for both species was different in 1 year but not over the entire study, the recruitment of the population, described as the predicted number of fledglings, was not different. The impact of one year's reduction in growth to the sustainability of the population is thought to be minimal. Some differences in fledging success and growth of nestlings were observed in the house wren and eastern bluebird, but these differences were not prevalent throughout the length of the study. Due to the inconsistency of results, nestling survival, which was predicted in this study as fledging success and growth, could not be linked to exposure to PCBs.

No data on reproductive measurements in the eastern bluebird or house wren at other PCB-contaminated sites are available to compare. All measures of reproductive success for eastern bluebirds at the Kalamazoo River, except productivity at TB, were similar to those for uncontaminated populations (Pinkowski, 1979). There is little information on the reproductive success of unmanipulated house wren populations, but one population had productivity similar to those of the Kalamazoo River house wren populations (Finch, 1989). Other studies with greater tissue concentrations of PCBs in terrestrial species did not indicate reproductive effects (Bishop et al., 1995; Henning et al., 2002). The observations made in our study, coupled with those made by others at both PCB-contaminated and uncontaminated locations, indicate that it was unlikely that the concentrations of PCBs measured in birds at TB were causing any population-level effects on survival of nestlings.

4.2. Egg measurements

It has been suggested that eggs containing PCBs may exhibit differential dimensions (Fair and Myers, 2002) or mass compared to those of uncontaminated individuals (Fernie et al., 2000). PCBs may contribute to the smaller volume of eggs in ash-throated flycatchers at contaminated sites (Fair and Myers, 2002), but this difference was attributed to interaction with the width parameter in the calculation of egg volume (Pinkowski, 1975). Egg length but not width was evaluated in eggs at the Kalamazoo River, so differences in egg length may not be predictive of PCB exposure. As for measurements of egg weight, the yolks of eggs from PCB-dosed females (34.1 mg PCB/kg, ww in eggs) were found to be greater in mass than that of controls, with greater mass associated with greater lipid content (Fernie et al., 2000). When comparing fresh eggs, the lipid content in house wren eggs from TB was not significantly greater than that of eggs from FC. Sample sizes of fresh eggs of eastern bluebirds were insufficient for analysis. Natural variation in unexposed populations of eastern bluebirds has been described as an egg size gradient ranging from observably smaller eggs (mean length $\times$ mean width, 19.0×15.3 mm) to comparatively larger eggs (22.4×17.7 mm). The natural variation inherent in egg size limits the discernment of PCB-induced effects on egg size in this population, but it does not appear that exposure to PCBs had any significant effects on the sizes of eggs.

4.3. Growth measurements

Growth curves generated for each species were similar between sites. Logistic curves gave the best overall fit to the growth data of the Kalamazoo River, which is similar to the results for other locations (Zach and Mayoh, 1982). There are no published growth curves for house wren nestlings, but the relative similarity of curves for birds at TB and FC, over the length of the study, suggests that growth was not affected by exposure to PCBs. Growth curves for eastern bluebirds at the KRAOC were compared to those for an uncontaminated population (Pinkowski, 1975), and the logistic curve at TB was similar to that at the uncontaminated populations until day 8. From days 8 to 10, the rate of weight increase in Kalamazoo populations decreased relative to that at the uncontaminated site, but weights coincided again by day 12. TB nestling growth during 2001 had the greatest mean nestling mass at each day but the least during 2002. There is no apparent reason for the variation among years, but the inconsistent results among years may be associated with small sample sizes at the site. Nestlings at TB had a curve similar to those at FC, but the mass of nestlings at each day was less. When mass gain for each box over the nestling period of all years was examined, there was no statistically significant difference between TB and FC. The results of a study conducted at another location suggest that availability of insects accounts for 18–51% of the variation, while unexplained variation (error) accounted for a similar proportion (Quinney et al., 1986). Potential variations in error at the Kalamazoo River locations may be associated with variation between individuals, egg quality, or measurement error. Based on visual examination of growth curves and statistical analysis of nestling growth expressed as mass gain per day, no consistent differences in nestling growth were identified when evaluating nests over the entire study period. Although some significant differences between sites existed when years were evaluated separately, the differences were found in only 1 of the 3 years of the study. Thus, it can be concluded that the exposure to PCBs at TB did not affect the rates of growth of either bluebirds or house wrens.

4.4. Correlations between total PCB and TEQ concentration and measurement endpoints

Concentration–effect relationships between the two measures of exposure (PCB or TEQ) and the measures of reproductive performance based on individual nests were examined by correlation analyses—Pearson product-moment correlations and general linear regression models (Table 7). There were no significant correlations ($P<0.05$) between total concentrations of total PCBs or TEQs and any of the measures of reproductive success for house wrens. There were some significant and negative correlations between concentrations of PCBs and clutch size, hatching success, fledging success and productivity for eastern bluebirds, but only fledging success was correlated with TEQ concentrations even though concentrations of TEQ and PCBs were positively and significantly correlated ($r^2 = 0.86$, $P<0.01$, $n = 25$).

The trends in correlations were not consistent. Of the four significant negative correlations between concentrations of PCBs, only one, fledging success, was correlated with concentrations of TEQ. For eastern bluebirds, concentrations of TEQs generally described $<30\%$ of the

Table 7
Pearson product-moment correlations (r^2) with probabilities of exceeding this value by chance alone (P) between concentrations of PCBs or TEQ and each of the measurement endpoints for the eastern bluebird and house wren

Species	Exposure	Endpoint	r^2	$P>$
Eastern bluebird	Total PCB	**Clutch size**	**(−) 0.62**	**<0.01**
		Mean egg weight	(−) 0.48	0.06
		Hatching success	**(−) 0.28**	**0.03**
		Fledging success	**(−) 0.93**	**<0.01**
		Productivity	**(−) 0.33**	**0.02**
	TEQ	Clutch size	(−) 0.09	0.52
		Mean egg weight	(−) 0.27	0.29
		Hatching success	(+) 0.01	0.82
		Fledging success	**(+) 0.91**	**0.01**
		Productivity	(−) <0.01	0.94
House wren	Total PCB	Clutch size	(+) 0.01	0.62
		Mean egg weight	(+) 0.01	0.65
		Hatching success	(−) 0.13	0.10
		Fledging success	(+) 0.02	0.54
		Productivity	(−) 0.07	0.25
	TEQ	Clutch size	(+) 0.04	0.50
		Mean egg weight	(+) <0.01	0.93
		Hatching success	(−) 0.08	0.32
		Fledging success	(+) 0.03	0.62
		Productivity	(−) 0.04	0.50

Directions of correlations are shown in parentheses. Significant correlations are shown in bold-face.

variability in reproduction, but concentrations of TEQs described 91% of the fledging success and total PCBs explained 28–93% of variability in reproductive parameters. It was determined, after examining the residuals and the scattergram of the data, that these correlations were spurious and were artifacts of the data structure. Although these values seem to show a significant correlation, the sample sizes were small and, because of the difference between concentrations of PCBs at the two locations, a regression based on these values effectively becomes a two-point curve driven by two clusters of data points, which is unreliable for describing relationships. There were no consistent patterns in the relationships between the two measures of exposure and the measures of reproductive performance for any of the species studied. In general, the proportion of the total variance explained by either measure of exposure, total PCBs or TEQs was small.

5. Conclusions

Measures of growth and overall productivity were used to evaluate whether adverse population-level effects were occurring in two terrestrial avian species exposed to PCBs at the Kalamazoo River Superfund site. While the values of some measures of reproductive success of house wrens were significantly less at TB than at FC in some years, overall fledging success, which is a predictor of population-level effects, was greater at TB than at FC. Also during 1 year, growth of each species was less at TB than at FC, but when evaluated over the entire study period, growth of both species was not significantly different between TB and FC. Other studies also examined reproductive effects in terrestrial species due to PCB exposure and indicated no observed effects at concentrations of 18.6 mg PCB/kg, ww (Bishop et al., 1995) to 83.6 mg PCB/kg, ww (Henning et al., 2002) in eggs, which is greater than those contained in eggs at TB (6.3 mg PCB/kg, ww in house wren eggs and 8.3 mg PCB/kg, ww in eastern bluebird eggs). Based on the results of the study reported here and the results of other studies investigating the effects of PCBs on terrestrial passerine birds, it can be concluded that it was unlikely that exposure to PCBs at the Kalamazoo River Superfund site was having adverse effects.

Colocated studies were conducted in conjunction with the present study to quantify the concentration of PCBs in the tissues and diets of Kalamazoo birds and to compare various approaches to estimating risk (Neigh et al., 2006b, c). Concentrations of PCBs were significantly greater in tissues of the eastern bluebird and house wren at TB, which was the more contaminated location, than in tissues of birds at FC, which was the upstream reference area (Neigh et al., 2006b). However, concentrations of PCBs in eggs and nestlings did not exceed a threshold above which effects were expected. Concurrent studies also investigated dietary exposure and determined that total PCBs in the diet of terrestrial species were less than the threshold concentration to cause adverse effects on reproduction (Neigh et al., 2006c). Concentrations of TEQs in the diet were in a range that, based on the most conservative threshold determined from laboratory studies with other species, could cause some adverse effects. However, it was determined that the threshold was not exceeded when toxicity reference values based on a more appropriate field-derived threshold were applied in the risk assessment. The assessment of risk for concentrations in tissues and dietary exposure depend heavily on the threshold of effects and, since there are no studies specifically dealing with the species examined here, there are uncertainties associated with the choice of a threshold concentration, especially with dietary studies.

Even based on an approach to evaluate risk that includes three lines of evidence (tissue concentrations, dietary exposure, and reproduction), it was difficult to definitively determine whether PCBs are eliciting effects on birds at the Kalamazoo River. The results of the studies presented here suggest some reproductive impairment of eastern bluebirds and house wrens at some locations of the Kalamazoo River, but other factors in addition to PCBs, such as cocontaminants, habitat suitability, and prey availability, are likely causes for the effects. Colocated tissue studies at the Kalamazoo River locations (Neigh et al., 2006b) suggested that eastern bluebird eggs contained concentrations of DDT and its isomers near the threshold of effects for raptor species (Elliott and Harris, 2002), and the shells

of house wren eggs at the contaminated location were apparently thinned, which is a known effect of dichlorodiphenyldichloroethylene exposure (Lincer, 1975). Reproductive effects at the Kalamazoo River can not be decisively linked to exposure to PCBs and, with the exception of the potentially conservative estimate of risk based on exposure to non-*ortho* and mono-*ortho*-substituted PCBs, the tissue and dietary lines of evidence arrive at similar conclusions of no unacceptable risk. Based on three lines of evidence, the authors do not believe that terrestrial passerine species are exposed to concentrations of PCBs in contaminated areas of the Kalamazoo River that may contribute to the present or future risk of reproductive dysfunction.

Acknowledgments

The authors extend a special thanks to the students and technicians at the Michigan State University Aquatic Toxicology Laboratory. Monica MacCarroll, Breton Joldersma, Ryan Holem, Cyrus Park, Michael Kramer, and Karen Smyth were all of great help over the past 5 years. This study was conducted in cooperation with the Kalamazoo Nature Center whose staff was instrumental in field collection. The Kalamazoo River Study Group provided funding for this study.

References

Anonymous, 1997. An Assessment of Wildlife and Organochlorine Contaminants from the Upjohn Pond and Two Control Areas near Kalamazoo, Michigan. Final Report of an Ecological Risk Assessment for Hazardous Waste Amendment Peritting at Pharmacia & Upjohn Manufacturing Facility. 12-1-1997. SERE Group, Ltd., Kingsville, Ontario, Canada.

Ankley, G.T., Niemi, G.J., Lodge, K.B., Harris, H.J., Beaver, D.L., Tillitt, D.E., Schwartz, T.R., Giesy, J.P., Jones, P.D., Hagley, C., 1993. Uptake of planar polychlorinated biphenyls and 2,3,7,8-substituted polychlorinated dibenzofurans and dibenzo-*p*-dioxins by birds nesting in the lower Fox River and Green Bay, Wisconsin, USA. Arch. Environ. Contam. Toxicol. 24, 332–344.

Belles-Isles, J., Picman, J., 1986. House wren nest-destroying behavior. Condor 88, 190–193.

Bishop, C.A., Koster, M.D., Chek, A.A., Hussell, D.J.T., Jock, K., 1995. Chlorinated hydrocarbons and mercury in sediments, red-winged blackbirds (*Agelaius phoeniceus*) and tree swallows (*Tachycineta bicolor*) from wetlands in the Great Lakes-St. Lawrence River Basin. Enivron. Toxicol. Chem. 14, 491–501.

Blankenship, A.L., Zwiernik, M.J., Coady, K.K., Kay, D.P., Newsted, J.L., Strause, K., Park, C., Bradley, P.W., Neigh, A.M., Millsap, S.D., Jones, P.D., Giesy, J.P., 2005. Differential accumulation of PCB congeners in the terrestrial food web at the Kalamazoo River, Michigan, USA. Environ. Sci. Technol. 39, 5954–5963.

Elliott, J.E., Harris, M.L., 2002. An ecotoxicological assessment of chlorinated hydrocarbon effects on bald eagle populations. Rev. Toxicol. 4, 1–60.

Fair, J.M., Myers, O.B., 2002. Early reproductive success of western bluebirds and ash-throated flycatchers: a landscape-contaminant perspective. Environ. Pollut. 118, 321–330.

Fair, J.M., Myers, O.B., Ricklefs, R.E., 2003. Immune and growth response of western bluebirds and ash-throated flycatchers to soil contaminants. Ecol. Appl. 13, 1817–1829.

Fernie, K.J., Bortolotti, G.R., Smits, J.E., Wilson, J., Drouillard, K.G., Bird, D.M., 2000. Changes in egg composition of American kestrels exposed to dietary polychlorinated biphenyls. J. Toxicol. Environ. Health Part A 60, 291–303.

Finch, D.M., 1989. Relationships of surrounding riparian habitat to nest-box use and reproductive outcome in house wrens. Condor 91, 848–859.

Finch, D.M., 1990. Effects of predation and competitor interference on nesting success of house wrens and tree swallows. Condor 92, 674–687.

Giesy, J.P., Ludwig, J.P., Tillitt, D.E., 1994. Deformities in birds of the Great Lakes region: assigning causality. Environ. Sci. Technol. 28, 128A–135A.

Halbrook, R., Woolf, A., Arenal, C., 1998. European Starling (*Sturnus vulgaris*): Avian Model and Monitor of Contaminant and Remedial Effects at Crab Orchard National Wildlife Refuge. RR-E84, 1-58. Illinois Waste Management and Research Center, Champaign, IL.

Henning, M.H., Ebert, E.S., Keenan, R.E., Martin, S.G., Duncan, J.W., 1997. Assessment of effects of PCB-contaminated floodplain soils on reproductive success of insectivorous songbirds. Chemosphere 34, 1121–1137.

Henning, M., Robinson, S., Jenkins, K., 2002. Robin productivity in the Housatonic Watershed. April 2002 Report, Arcadis G&M, Incorporated Report for General Electric Company, Portland, Maine, USA.

Kubiak, T.J., Harris, H.J., Smith, L.M., Schwartz, T.R., Stalling, D.L., Trick, J.A., Sileo, L., Docherty, D.E., Erdman, T.C., 1989. Micro-contaminants and reproductive impairments of the Forster's tern on Green Bay, Lake Michigan-1983. Arch. Environ. Contam. Toxicol. 18, 706–727.

Lincer, J.L., 1975. DDE- induced eggshell thinning in the American kestrel: a comparison of the field situation and laboratory results. J. Appl. Ecol. 12, 781–793.

Ludwig, J.P., Auman, H.J., Kurita, H., Ludwig, M.E., Campbell, L.M., Giesy, J.P., Tillitt, D.E., Jones, P.D., Yamashita, N., Tanabe, S., Tatasukawa, R., 1993. Caspian tern reproduction in the Saginaw Bay ecosystem following a 100-year flood event. J. Great Lakes Res. 19, 96–108.

McCarty, J.P., Secord, A.L., 1999. Reproductive ecology of tree swallows (*Tachycineta bicolor*) with high levels of polychlorinated biphenyl contamination. Environ. Toxicol. Chem. 18, 1433–1439.

MDEQ, 2003. Final (Revised) Baseline Ecological Risk Assessment, Allied Paper, Inc., Portage Creek, Kalamazoo River Superfund Site. Report April 2003. 1-6-12. Prepared by Camp, Dresser, and McKee for Michigan Department of Environmental Quality. Remediation and Development Division, Lansing, MI.

Millsap, S.D., Blankenship, A.L., Bradley, P.W., Jones, P.D., Kay, D., Neigh, A.M., Park, C., Strause, K.D., Zwiernik, M.J., Giesy, J.P., 2004. Comparison of risk assessment methodologies for exposure of mink to PCBs on the Kalamazoo River, Michigan. Environ. Sci. Technol. 38, 6451–6459.

Munro, H.L., Rounds, R.C., 1985. Selection of artificial nest sites by five sympatric passerines. J. Wildlife Manage. 49, 264–276.

Neigh, A.M., Zwiernik, M.J., Bradley, P.W., Kay, D.P., Park, C.S., Jones, P.D., Newsted, J.L., Blankenship, A.L., Giesy, J.P., 2006a. Tree Swallow (*Tachycineta bicolor*) exposure to polychlorinated biphenyls at the Kalamazoo River Superfund Site. Environ. Toxicol. Chem., (25)2, in press.

Neigh, A.M., Zwiernik, M.J., Bradley, P.W., Kay, D.P., Jones, P.D., Holem, R.R., Blankenship, A.L., Strause, K.D., Newsted, J.L., Giesy, J.P., 2006b. Accumulation of polychlorinated biphenyls (PCBs) from floodplain soils by passerine birds. Environ. Toxicol. Chem., (25)2, in press.

Neigh, A.M., Zwiernik, M.J., Blankenship, A.L., Bradley, P.W., Kay, D.P., MacCarroll, M.A., Park, C.S., Jones, P.D., Millsap, S.D., Newsted J.L., Giesy J.P., 2006c. Multiple lines of evidence assessment of PCBs in the diets of passerine birds at the Kalamazoo River Superfund Site, Michigan. Human Ecol. Risk Assess., 12(3), in press.

Pinkowski, B.C., 1975. Growth and development of eastern bluebirds. Bird-Banding 46, 273–289.

Pinkowski, B.C., 1978. Feeding of nestling and fledgling eastern bluebirds. Wilson Bull. 90, 84–98.

Pinkowski, B.C., 1979. Annual productivity and its measurement in a multi-brooded passerine, the eastern bluebird. Auk 96, 562–572.

Quinney, T.E., Hussell, D.J.T., Ankney, C.D., 1986. Sources of variation in growth of tree swallows. Auk 103, 389–400.

Rustad, O.Q., 1972. An eastern bluebird nesting study in south central Minnesota. Loon 44, 80–84.

Secord, A.L., McCarty, J.P., 1997. Polychlorinated Biphenyl Contamination of Tree Swallows in the Upper Hudson River Valley, New York. US Fish and Wildlife Service, New York Field Office, Cortland, NY, pp. 1–50.

van den Berg, M., Birnbaum, L., Bosveld, A.T.C., Brunström, B., Cook, P., Feeley, M., Giesy, J.P., Hanberg, A., Hasegawa, R., Kennedy, S.W., Kubiak, T., Larsen, J.C., van Leeuwen, F.X.R., Liem, A.K.D., Nolt, C., Peterson, R.E., Poellinger, L., Safe, S., Schrenk, D., Tillitt, D., Tysklind, M., Younes, M., Waern, F., Zacharewski, T., 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ. Health Perspect. 106, 775–792.

Yamashita, N., Tanabe, S., Ludwig, J.P., Kurita, H., Ludwig, M.E., Tatsukawa, R., 1993. Embryonic abnormalities and organochlorine contamination in double-crested cormorants (*Phalacrocorax auritus*) and caspian terns (*Hydroprogne caspia*) from the upper Great Lakes in 1988. Environ. Pollut. 79, 163–173.

Zach, R., Mayoh, K.R., 1982. Weight and feather growth of nestling tree swallows. Can. J. Zool. 60, 1080–1090.

SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 26, No. 7, pp. 1386–1398, 2007
© 2007 SETAC
Printed in the USA
0730-7268/07 $12.00 + .00

Environmental Toxicology

RISK ASSESSMENT OF GREAT HORNED OWLS (*BUBO VIRGINIANUS*) EXPOSED TO POLYCHLORINATED BIPHENYLS AND DDT ALONG THE KALAMAZOO RIVER, MICHIGAN, USA

KARL D. STRAUSE,† MATTHEW J. ZWIERNIK,*† SOOK HYEON IM,‡ PATRICK W. BRADLEY,† PAMELA P. MOSELEY,† DENISE P. KAY,§ CYRUS S. PARK,† PAUL D. JONES,† ALAN L. BLANKENSHIP,†§ JOHN L. NEWSTED,†§ and JOHN P. GIESY†‖#

†Zoology Department, Center for Integrative Toxicology, National Food Safety and Toxicology Center, Michigan State University, East Lansing, Michigan 48823, USA

‡DK Science Research & Development Center, DK B/D, 27-4 Gongduck-dong, Mapo-gu, Seoul, Korea

§ENTRIX, Okemos, Michigan 48823, USA

‖Department of Biomedical Veterinary Sciences and Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5B3, Canada

#Biology and Chemistry Department, City University of Hong Kong, Kowloon, Hong Kong, Special Administrative Region, China

(*Received* 25 *July* 2006; *Accepted* 19 *December* 2006)

Abstract—The great horned owl (GHO; *Bubo virginianus*) was used in a multiple lines of evidence study of polychlorinated biphenyls (PCBs) and *p,p′*-dichlorodiphenyltrichloroethane (DDT) exposures at the Kalamazoo River Superfund Site (KRSS), Kalamazoo, Michigan, USA. The study examined risks from total PCBs, including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{\text{World Health Organization [WHO]-Avian Toxicity Equivalency Factor [TEF]}}$), and total DDTs (sum of DDT, dichlorodiphenyldichloroethylene [DDE], and dichlorodiphenyldichloroethane [DDD]; ΣDDT) by measuring concentrations in eggs and nestling blood plasma in two regions of the KRSS (upper, lower) and an upstream reference area (RA). An ecological risk assessment compared concentrations of the contaminants of concern (COCs) in eggs or plasma to toxicity reference values. Productivity and relative abundance measures for KRSS GHOs were compared with other GHO populations. Egg shell thickness was measured to assess effects of *p,p′*-DDE. The concentrations of PCBs in eggs were as great as 4.7×10^2 and 4.0×10^4 ng PCB/g, wet weight at the RA and combined KRSS sites, respectively. Egg $TEQ_{\text{WHO-Avian}}$ calculated from aryl hydrocarbon receptor–active PCB congeners and WHO TEFs ranged to 8.0 and 1.9×10^2 pg $TEQ_{\text{WHO-Avian}}$/g, (wet wt) at the RA and combined KRSS, respectively. Egg ΣDDT concentrations were as great as 4.2×10^2 and 5.0×10^3 ng ΣDDT/g (wet wt) at the RA and combined KRSS, respectively. Hazard quotients (HQs) for the upper 95% confidence interval (UCI) (geometric mean) and least observable adverse effect concentration (LOAEC) for COCs in eggs were ≤1.0 for all sites. Hazard quotient values based on the no observable adverse effect concentration (NOAEC) 95% UCI in eggs were ≤1.0, except at the LKRSS (PCB HQ = 3.1; $TEQ_{\text{WHO-Avian}}$ HQ = 1.3). Productivity and relative abundance measures indicated no population level effects in the UKRSS.

Keywords—Raptors Bioaccumulation Terrestrial food chain 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin equivalents

INTRODUCTION

Due to the presence of elevated polychlorinated biphenyl (PCB) concentrations in fish, sediments, and floodplain soils, a portion of the lower Kalamazoo River was placed on the Superfund National Priorities List in August 1990 [1]. Polychlorinated biphenyls were used in the production of carbonless copy paper and paper inks for approximately 15 years [2]. During this period, recycling of paper, including some carbonless copy paper, resulted in releases of PCBs to the Kalamazoo River. The Kalamazoo River Superfund Site (KRSS) includes 123 km of river extending from the city of Kalamazoo, Michigan, USA, to Lake Michigan at Saugatuck, Michigan, USA. The primary contaminants of concern (COCs) are PCBs, including total 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{\text{World Health Organization [WHO]-Avian Toxicity Equivalency Factor [TEF]}}$) from non-*ortho* (coplanar) and mono-*ortho* PCB congeners. However, other persistent polyhalogenated aromatic hydrocarbons such as *p,p′*-dichlorodiphenyltrichloroethane (DDT) and its metabolites dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD) (hereafter, ΣDDT) are also present. Each of these COCs has been linked to adverse reproductive effects in numerous mammals [3,4] and birds [5,6]. In addition to concerns about exposure through the aquatic food web, potential exposures of terrestrial-based receptors may also occur through the riparian floodplain soils that were former sediments in impoundments in the river. In 1986, three dams on the KRSS were partially dismantled, which exposed over 205 ha of PCB-contaminated former sediments, which now are floodplain soils. Concentrations of PCBs in surficial floodplain soils (0–25 cm) are generally greater than those of surficial sediments and range from <1 ng PCB/g (dry wt) to 8.5×10^4 ng PCB/g (dry wt) with a mean concentration of approximately 1.1×10^4 ng PCB/g (dry wt) [7–9].

The great horned owl (GHO; *Bubo virginianus*) was selected as a surrogate species to estimate risk to raptors in the terrestrial food chain. Raptors have long been used as environmental monitors [10,11]. Their sensitivity to the toxic effects of the types of COCs found at the KRSS, and their position at the top of the terrestrial food chain increases their potential for exposure to bioaccumulative contaminants. Great horned owls are highly territorial, year-round residents of the floodplain. Great horned owls also offer broad applicability as

* To whom correspondence may be addressed (zwiernik@msu.edu).

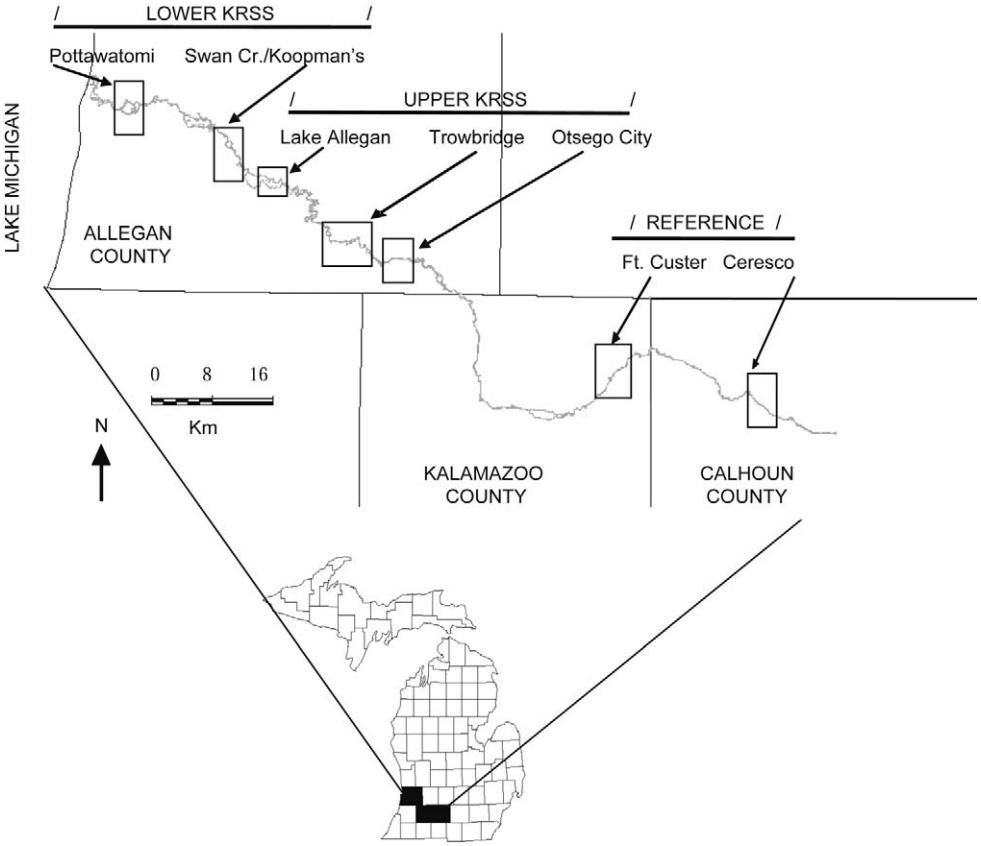


Fig. 1. Kalamazoo River superfund site (KRSS), Kalamazoo, Michigan, USA, great horned owl (*Bubo virginianus*) study sites.

environmental monitors due to their longevity (up to 28 years in the wild) and stable rates of reproduction (normally one or two fledglings per year) [12]. The propensity of GHOs to use artificial nesting platforms also allows for better experimental control compared to wildlife studies that focus exclusively on natural nests. Great horned owl nestlings are sedentary and rely solely on prey collected by adults from areas proximal to the nest. Both eggs and nestlings are easily accessed, and GHO nestling exposure has been directly related to local contaminant concentrations (R.A. Frank, 1997, Master's thesis, University of Wisconsin, Madison, WI, USA). As a result, information on the GHO allowed site-specific estimation of the risk posed by PCBs, total $TEQ_{WHO\text{-}Avian}$, and ΣDDT to terrestrial raptors at the KRSS.

The 5-year study used multiple lines of evidence to assess the potential effects of PCBs and ΣDDT on resident GHO populations in support of a baseline ecological risk assessment [13]. The specific objectives of this study were to measure concentrations of total PCBs, $TEQ_{WHO\text{-}Avian}$, and ΣDDT in eggs and blood plasma of GHO nestlings; conduct a site-specific risk assessment based on measured concentrations of these residues; evaluate whether egg shell thinning was occurring at this site from historical sources of DDT and its metabolites; and determine the relative abundance, site use, and productivity of GHO at the KRSS, relative to a reference location upstream of the KRSS PCB sources.

MATERIALS AND METHODS

Study sites

Study sites within the KRSS were chosen to provide maximum exposure of resident GHO to PCBs during normal foraging activities associated with nesting and rearing of offspring. Study sites included three segments of the Kalamazoo River between the cities of Marshall and Saugatuck, Michigan, USA, a distance of approximately 190 km by river (Fig. 1 and Table 1). The upstream reference location represented current regional background exposures in the watershed where PCB concentrations in river sediments and floodplain soils were less than those in the KRSS (less than 180 ng PCB/g [dry wt]). The reference location included two areas upstream of the KRSS, on the Ceresco reservoir (CR) and Fort Custer State Recreation Area (FC). The upper KRSS (UKRSS) was located closest to known point sources of PCBs, and included the three formerly impounded areas (Otsego, Plainwell, and Trowbridge) and two additional sites at existing impoundments created by the Otsego City Dam and Calkins Dam (Lake Allegan). The lower KRSS (LKRSS) included areas located downstream of Calkins Dam, which is the first in-stream dam inland from Lake Michigan, and extended to Lake Michigan at Saugatuck. This stretch of river is characterized by a free-flowing channel and frequently inundated wetland forest and marsh habitat. The FC and Trowbridge (TB) areas on the river were the sites of additional investigations that were used to make direct comparisons between GHO responses on a ''high potential exposure'' (TB) versus background ''no elevated exposure'' basis.

Artificial nesting platforms were placed within study sites based on surveys of the GHO population and habitat. Locations of actively defended GHO territories were determined using call–response and nest location surveys [14]. Nest trees were selected on the basis of a qualitative habitat inventory. Nest platforms were placed to provide for a maximum exposure

Table 1. Kalamazoo River great horned owl (*Bubo virginianus*) study sites (Kalamazoo River superfund site [KRSS], Kalamazoo, MI, USA) physical and chemical characterization

Study site	Habitat[a]	Length[b] (km)	Area of former sediments[c] (ha)	Mean surficial PCBs[d] (ng/g, dry wt)
Reference area				
Ceresco	UH, SS	11.5	NA[e]	170
Ft. Custer	FH	5.6	NA	9
Upper KRSS				
Plainwell	WM	2.5	24	15,000
Otsego City	M, FH	2.7	NA	1,138
Otsego	WM	3.0	31	12,000
Trowbridge	SS, WM, FH	7.6	132	15,000
Lake Allegan	M, FH	13.7	NA	NS[f]
Lower KRSS				
Koopman's Marsh	M, FH	2.1	NA	545
Swan Creek Highbanks	M	3.0	NA	396
Pottawatomi Marsh	M, WM	4.3	NA	567

[a] UH = upland hardwoods; SS = scrub/shrub wetlands; FH = deciduous forested wetlands; WM = emergent wetlands, seasonally flooded wet meadow; M = emergent wetlands, semipermanently flooded marsh.
[b] Run of river.
[c] Formerly impounded floodplain.
[d] Arithmetic mean polychlorinated biphenyl (PCB) concentration in 0- to 15-cm depth.
[e] NA = not applicable.
[f] NS = Not sampled.

due to foraging in the most expansive areas of the contaminated floodplain. The numbers of nest sites (including both artificial and natural nests) monitored at each site were as follows: reference area (RA)-26, UKRSS-22, LKRSS-6.

Field sampling

Specific details and rationale for the GHO study design and detailed descriptions of the field methods and sampling techniques employed are provided elsewhere [15]. A brief discussion of the sampling methods for each phase of the sample collection and analyses activities is provided below.

Fresh or addled egg sampling

Fresh eggs were collected as soon as possible following confirmed initiation of incubation. Addled eggs [16] were collected when blood was sampled from nestlings 4 to 6 weeks post-hatch or in instances where nest abandonment had occurred. Eggs were labeled, transported back to the laboratory, and stored at 4°C until processed. Length, width, whole-egg weight, and whole-egg water volume were measured. Egg contents were removed, weighed, and saved for subsequent residue analyses. Eggshells were rinsed, air-dried, and eggshell thickness measured (to the nearest 0.01 mm) at two to eight places by use of a Starrett Model 1010M micrometer (L.S. Starrett, Athol, MA, USA). Dry shell weight was measured and normalized to egg volume to calculate a Ratcliffe Index value [17]. All concentrations of residues in eggs were corrected for moisture loss [18].

Nestling blood plasma collections

Blood samples were taken using previously described methods [19] when nestlings were approximately 4 to 6 weeks of age and had attained a minimum body weight of 0.75 kg. A sample of 5 to 7 ml was withdrawn from the brachial vein with a 25-gauge hypodermic needle and syringe and sterile technique. Blood was transferred to a heparinized Vacutainer™ (BD, Franklin Lakes, NJ, USA) and labeled. Vacutainers containing whole blood were centrifuged at 1,200 rpm for 10 min within 48 h of field sampling. Plasma (supernatant) was transferred to a new Vacutainer appropriately labeled and stored upright at −20°C until measurement of PCBs and ΣDDT. The nestlings were banded with U.S. Fish and Wildlife Service leg bands and total body weight, bill depth, and length of the culman, foot pad, and eighth primary feather measured following standard techniques [20] (data not presented) after which the birds were returned to the nest unharmed.

Predicted egg concentrations

Concentrations of total PCBs in nestling plasma were used to calculate predicted concentrations in eggs. Great horned owl nesting territories in the KRSS and RA were closely monitored to allow fresh egg and nestling plasma samples to be collected from within the same nest or nesting territory [15]. Polychlorinated biphenyl concentrations of the colocated samples were compared using regression methods to develop a relationship from which concentrations in eggs could be predicted from those in blood plasma [21]. For comparative purposes, predicted egg concentrations also are discussed in the risk assessment.

TEQ computation

Concentrations of $TEQ_{WHO\text{-}Avian}$ in bird tissues were calculated by summing the products of concentrations of individual non-*ortho* and mono-*ortho* PCB congeners (77, 81, 105, 118, 126, 156, 157, 167, 169) and their respective bird-specific WHO TEFs [22]. Polychlorinated-dibenzo-*p*-dioxins and polychlorinated-dibenzo-furans were not measured and were not included in TEQ computation. Whenever a congener was not detected, a proxy value equal to one-half the limit of quantification was multiplied by the toxic equivalence factor to calculate the congener-specific TEQs. Co-eluting congeners were evaluated separately. Polychlorinated biphenyl congener 105 frequently co-eluted with congener 132, congener 156 frequently co-eluted with 171 and 202, congener 157 co-eluted with congener 200, and congener 167 co-eluted with congener 128. In order to report the maximum $TEQ_{WHO\text{-}Avian}$, the entire

concentration of the co-elution groups was assigned to the mono-*ortho* congener. Overall contributions to total $TEQ_{WHO\text{-}Avian}$ from congeners 105, 156, 157 and 167 ranged from 11 to 13%, 5 to 19%, 1 to 2%, and 1 to 2%, respectively.

Relative abundance and site use (vocalization surveys)

Vocalization surveys consisted of an active method in which GHO hoots were broadcast to provoke responses (call–response method) from adult and juvenile owls [14,15]. Great horned owl relative abundance was monitored over three years (2000–2002) at the FC (reference) and TB (UKRSS) locations. Abundance and site use investigations were not conducted at the LKRSS. Hoot call–response surveys were conducted from late April through early January (up to two surveys per location per month) to determine the relative abundance and site use characteristics for juvenile, nonterritorial individuals (foraging adults) and territorial nesting pairs of owls. Surveys were completed under dry, windless conditions during crepuscular hours, beginning approximately 60 min prior to sunrise or approximately 30 min after sunset. Calls were broadcast at 0.5-km intervals within the river corridor at predetermined locations using a global positioning system to locate the exact coordinates.

Productivity monitoring

Active nests at the FC and TB locations were monitored to confirm fledgling success either visually and/or audibly during vocalization surveys (based on begging call responses of juveniles to broadcasts of adult GHO hoot calls). Productivity measurements were not conducted in the LKRSS.

Chemical analysis—Extraction and clean-up

Total concentrations of PCBs (congener-specific analysis) and ΣDDT were determined using U.S. Environmental Protection Agency method 3540 (SW846), Soxhlet extraction, as described elsewhere [23]. Measured quantities of plasma and egg were homogenized with anhydrous sodium sulfate (EM Science, Gibbstown, NJ, USA) using a mortar and pestle. All samples, blanks, and matrix spikes included PCB 30 and PCB 204 as surrogate standards (AccuStandard, New Haven, CT, USA). Extraction blanks were included with each set of samples. Quality assurance and quality control sets composed of similar tissues were included with each group of 20 samples. Concentrations of PCBs, including di-*ortho*- and mono-*ortho*-substituted congeners (coplanar) were determined by gas chromatography (PerkinElmer AutoSystem, Waltham, MA, USA, and Hewlett Packard, Palo Alto, CA, USA, 5890 series II) equipped with a ^{63}Ni electron capture detector. Concentrations of non-*ortho*-substituted PCB congeners and ΣDDT were determined by gas chromatograph mass selective detector (Hewlett Packard 5890 series II gas chromatograph interfaced to a HP 5972 series detector). Concentrations of the COCs were reported on a volumetric (plasma) and mass (egg) wet weight (wet wt) basis. A solution containing 100 individual PCB congeners was used as a standard. Individual PCB congeners were identified by comparing sample peak retention times to those of the known standard, and congener concentrations were determined by comparing the peak area to that of the appropriate peak in the standard mixture. Coplanar PCB congeners and ΣDDT were detected by selected ion monitoring of the two most abundant ions of the molecular cluster. The limit of quantification for di-*ortho*- and mono-*ortho*-substituted PCB congeners was conservatively estimated (minimum surface to noise ratio of 10.0) to be 1.0 ng PCB/g (wet wt), using an extraction mass of 20 g, a 25 pg/μl standard congener mix, and 1-μl injection volume. For coplanar PCB congeners and ΣDDT analytes, method detection limits varied among samples. This was achieved using sample-specific extraction mass and a minimum surface to noise ratio of 3.0 to maintain the method detection limit for all samples at <0.1 ng/g (wet wt). Either TurboChrom (PerkinElmer) or GC Chemstation software (Agilent Technologies, Wilmington, DE, USA) was used to identify and integrate the peaks. Total concentrations of PCBs were calculated as the sum of all resolved PCB congeners.

Table 2. Toxicity reference values (TRVs) for total polychlorinated biphenyls (PCBs), 2,3,7,8- tetrachlorodibenzo-*p*-dioxin equivalents (TEQs), and total dichlorodiphenyltrichloroethane (ΣDDT) concentrations in great horned owl (*Bubo virginianus*) eggs. Reference number is located next to each value

	Tissue based TRV	Response endpoint[a]	Reference
Total PCBs (ng/g, wet wt)			
NOAEC[b]	7,000	EST, CS, EV, FS	[24]
LOAEC[c]	21,000	EST, CS, EV, FS	[24]
Total TEQs (pg/g, wet wt)			
NOAEC	135	EI, EV	[26–28]
LOAEC	400	EI	[26]
Total DDT (ng/g, wet wt)			
NOAEC	3,600	EST, FS	[29,30]
LOAEC	12,000	EST, EV, FS	[31,32]

[a] EST = egg shell thickness; CS = clutch size; EV = egg viability; FS = fledgling success; EI = enzyme induction.
[b] NOAEC = no observable adverse effect concentration.
[c] LOAEC = lowest observable adverse effect concentration.

Toxicity reference values

In the present study, tissue-based toxicity reference values (TRVs) were used to evaluate the potential for adverse effects due to PCBs, $TEQ_{WHO\text{-}Avian}$, and ΣDDT at each study site. Ideally, TRVs are derived from chronic toxicity studies in which a dose–response relationship has been observed for ecologically relevant endpoints in the species of concern, or a closely related species (e.g., other raptor species). Chronic studies must include sensitive lifestages to evaluate potential developmental and reproductive effects, and there must be minimal impact from cocontaminants on the measured effects. Toxicity reference values used in this assessment were based on values reported in the literature for no observable adverse effect concentrations (NOAECs) and lowest observable adverse effect concentrations (LOAECs) for total PCBs, $TEQ_{WHO\text{-}Avian}$, and ΣDDT in eggs of owls or similar raptor species (eagles, ospreys).

For PCBs in GHO eggs, TRVs based on the NOAEC and LOAEC were determined to be 7×10^3 and 21×10^3 ng PCB/g egg (wet wt). These TRV values are based on a feeding study with screech owls (*Otus asio*) exposed via diet to Aroclor 1248 in which no effects were seen at mean egg concentrations of 7.0×10^3 ng/g (wet wt) and a maximum concentration of 1.8×10^4 ng/g (wet wt) [24] (Table 2). Since a LOAEC was not determined in that study, the LOAEC was estimated by multiplying the NOAEC by an uncertainty factor of 3 [25].

No relevant studies on effects of $TEQ_{WHO\text{-}Avian}$ in the eggs of owl species were available from which to derive a TRV.

Thus, a tissue-based NOAEC for $TEQ_{WHO\text{-}Avian}$ in GHO eggs was estimated to be greater than 1.4×10^2 pg $TEQ_{WHO\text{-}Avian}$/g egg (wet wt) from the no observable effect concentration observed in bald eagle (*Haliaeetus leucocephalus*) chicks (presented on an egg basis) [26] with additional supporting evidence from studies of osprey (*Pandion heliaetus*) egg exposures [27,28]. A LOAEC concentration of 4.0×10^2 pg $TEQ_{WHO\text{-}Avian}$/g egg (wet wt), based on CYP1A induction, was also adopted from the lowest observable effect concentration determined in the same eagle study [26] (Table 2). It should be noted that no adverse effects on developmental or any other ecologically relevant endpoints were observed at these concentrations. Thus, these TRVs would be expected to be conservative and protective of GHOs.

A TRV based on the NOAEC for ΣDDT in GHO eggs was estimated to be greater than 3.6×10^3 ng ΣDDT/g egg (wet wt). The selection of this value as a conservative estimate of the TRV is supported by the analyses of effects presented by Wiemeyer et al. [29] for bald eagles and reinforced by Elliott and Harris [30], who identified 6.0×10^3 ng DDE/g egg (wet wt) as a LOAEC threshold for bald eagles. A LOAEC concentration of 1.2×10^4 ng ΣDDT/g egg (wet wt) was selected from the study of effects in the barn owl (*Tyto alba*) [31] and is supported by recommendations for bald eagles [32] (Table 2).

Risk assessment

Potential risk was assessed by calculation of hazard quotients (HQs) by dividing concentrations of PCBs, total $TEQ_{WHO\text{-}Avian}$, and ΣDDT measured in GHO eggs by tissue-based (egg) NOAEC and LOAEC TRVs identified for these chemicals (Table 2). Concentrations of total PCBs, total $TEQ_{WHO\text{-}Avian}$, and ΣDDT in eggs were considered to be the most sensitive measures of exposure with which to assess the potential effects of these COCs. When compared to the selected TRVs, this measure of exposure was considered to be a conservative estimate of risk at all life stages [5]. The HQs were calculated by dividing concentrations of each COC in egg (using both the lower and upper 95% confidence interval [CI] of the geometric mean) by the egg-based TRV. The shell-thinning effects of DDE were evaluated by comparing current measurements of eggshell thickness and shell weight (Ratcliffe Index) to the pre-1947 benchmark values reported for GHOs [33].

Statistical analyses

Data sets for each of the variables were analyzed for normality by use of the Kolmogorov–Smirnov, one-sample test with Lilliefors transformation, and for homogeneity of variance by Levene's test. Concentrations of COCs were generally log-normally distributed, and therefore all concentrations were log-transformed to more closely approximate the normal distribution. Variables that satisfied assumptions of normality and homogeneity (log-transformed values for ΣDDT in plasma, $TEQ_{WHO\text{-}Avian}$ in eggs, shell thickness, and Ratcliffe Index) were analyzed using parametric methods, including one-factor analysis of variance (ANOVA) with Tukey's honestly significant difference (multiple comparisons) and *t* test for simple pairwise comparisons. When parameters did not satisfy either or both assumptions of normality and homogeneity (log-transformed values for PCBs in eggs and plasma and ΣDDT in eggs), nonparametric statistical methods were used, including Kruskal–Wallis ANOVA and Median Test (multiple comparisons) and the Mann–Whitney *U* test. Associations between parameters were made with Pearson Product Correlations. Results of the vocalization survey expressed as relative abundance or site use were made with the chi-square test (χ^2). Tests for normality, homogeneity of variance and treatment effects (spatial trends) were completed using the Statistica (Version 6.1) statistical package (Statsoft, Tulsa, OK, USA). The criterion for significance used in all tests was $p < 0.05$.

For eggs and plasma, the experimental unit for concentrations of PCBs, ΣDDT, $TEQ_{WHO\text{-}Avian}$, and egg measurements (e.g., shell thickness, Ratcliffe index) was the nest. Where multiple samples were analyzed from the same clutch of eggs or brood of nestlings, analytical results for the associated samples were reported as the arithmetic mean for each nest.

RESULTS

Between 2000 and 2004, a total of 54 nesting sites (48 artificial and six natural) were sited and/or identified, and monitored for GHO occupancy with samples of eggs and/or blood plasma collected from some of these sites (Table 3). Total PCB and ΣDDT concentrations were measured in a total of 24 eggs and 16 nestling blood plasma samples that were collected from 25 active nests. Dioxin equivalent concentrations ($TEQ_{WHO\text{-}Avian}$) were calculated only for eggs. After consolidating multiple egg collections, egg sample sizes for each COC were: total PCBs, $n = 17$; $TEQ_{WHO\text{-}Avian}$, $n = 15$; ΣDDT, $n = 17$. Relative abundance and site use estimates at FC and TB are based on the completion of 46 successful call–response surveys. Productivity measurements for FC and TB are based on observations of seven active nests that produced a total of seven fledglings.

Total PCB concentrations

Geometric mean concentrations of total PCBs in eggs of GHOs inhabiting the Kalamazoo River floodplain were progressively greater downstream than upstream. The least PCB concentrations were measured in samples from the upstream RA and the greatest concentrations occurred in eggs from the LKRSS (Table 4, Fig. 2). Geometric mean egg PCB concentrations at the RA and UKRSS sites were not significantly different from each other (Kruskal–Wallis, $p = 0.157$), but the geometric mean concentrations at both of these sites were significantly less than concentrations at the LKRSS (Kruskal–Wallis, $p < 0.05$). Concentrations of PCBs in blood plasma exhibited the same spatial distributions as eggs. The results of statistical testing reflect the limited sample sizes for RA ($n = 3$) and LKRSS ($n = 2$) plasma samples. Geometric mean concentrations of PCBs in blood plasma at both the RA and LKRSS sites were not significantly different from each other (Kruskal–Wallis, $p = 1.36$), and the geometric mean concentrations at both of these sites were significantly less than concentrations at the UKRSS (Kruskal–Wallis, $p < 0.01$) (Table 4).

Temporal trends in PCB concentrations in eggs or plasma were examined to identify potential confounding influences on GHO exposure to PCBs at the site. No trends were evident in PCB concentrations of eggs or plasma between 2000 and 2004 at any of the study sites where samples were collected in at least three of the five years of sampling (Kruskal–Wallis, $p > 0.38$).

Predicted egg concentrations

A total of 14 paired GHO nestling plasma and egg samples were obtained in this study. Log-normalized GHO PCB data

Table 3. Numbers of active great horned owl (*Bubo virginianus*) nests and samples collected by year (2000–2004)

	Sample site[a]			
	Reference area		Kalamazoo River Superfund Site (KRSS)	
	Ceresco	Ft. Custer	Upper KRSS	Lower KRSS
2000				
Active nests	0	0	1	2
Plasma	0	0	1	0
Eggs	0	0	0	3
Sampling scope	NS	RA, P, NP	RA, P, NP	E
2001				
Active nests	1	1	2	1
Plasma	0	1	4	0
Eggs	1	0	0	2
Sampling scope	E	RA, P, NP, E	RA, P, NP, E	E
2002				
Active nests	2	0	4	2
Plasma	1	0	3	2
Eggs	2	0	1	5
Sampling scope	E, NP	RA, P, NP, E	RA, P, NP, E	E, NP
2003				
Active nests	1	1	2	1
Plasma	1	0	1	2
Eggs	1	1	3	0
Sampling scope	E, NP	E, NP	E, NP	E, NP
2004				
Active nests	0	0	2	2
Plasma	0	0	0	0
Eggs	0	0	3	2
Sampling scope	E, NP	E, NP	E, NP	E, NP

[a] Kalamazoo River Superfund Site, Kalamazoo, Michigan, USA. NS = not sampled; E = egg; NP = nestling plasma; RA = relative abundance; P = productivity.

are plotted in Figure 3. The formula describing the egg-to-plasma relationship (conversion factor equation) for PCBs in GHOs is expressed on a log-basis as log (PCB_{egg} μg/g) = 1.647[log (PCB_{plasma} ng/ml)] − 2.578 ($r^2 = 0.666$, $p < 0.001$). Predicted mean concentrations of PCBs in eggs (μg PCB/g egg, wet wt) and ranges are given along with corresponding values measured in eggs for each sample site (Table 5). Predicted and measured geometric mean concentrations of PCBs in eggs from the RA and UKRSS were not significantly different (Mann–Whitney U test, $p = 0.65$ [RA], $p > 0.9$ [UKRSS]). The predicted geometric mean PCB concentration in eggs from the LKRSS was approximately one-third that measured in eggs. However, the difference between the predicted and measured concentrations was not statistically significant (Mann–Whitney U test, $p = 0.24$).

$TEQ_{WHO\text{-}Avian}$ concentrations

Dioxin equivalent concentrations ($TEQ_{WHO\text{-}Avian}$) were calculated solely from GHO egg samples since minimum achievable method detection limits for individual coplanar PCB congeners in GHO plasma were limited by sample volume. Concentrations of $TEQ_{WHO\text{-}Avian}$ in GHO eggs at the RA and both KRSS sites were significantly correlated with concentrations of total PCBs ($r = 0.89$, $p < 0.001$). Concentrations of $TEQ_{WHO\text{-}Avian}$ were greater downstream than upstream with the greatest concentrations calculated for eggs from LKRSS. Geometric mean concentrations of $TEQ_{WHO\text{-}Avian}$ were significantly different among all three sites (ANOVA with Tukey's, $p < 0.005$) (Table 4, Fig. 2). All four non-*ortho*-substituted PCBs (International Union of Pure and Applied Chemistry [IUPAC] congener numbers 77, 81, 126, 169) and five of the eight mono-*ortho*-substituted PCBs (IUPAC numbers 105, 118, 156, 157, 167) were regularly detected in egg samples from the three study sites. Mono-*ortho*-substituted congeners 114, 123, and 189 were not detected. Together, the non-*ortho*-substituted PCB congeners contributed 73.4%, 67.4%, and 54.2% of total $TEQ_{WHO\text{-}Avian}$ at the RA, UKRSS, and LKRSS, respectively (Fig. 4). At least one of the non-*ortho*-substituted congeners monitored in the study was not present at concentrations greater than the detection limit in 80% of the samples from the RA site, 60% of the samples from the UKRSS, and 20% of the samples from the LKRSS. The rank order of the frequency of detection for both non-*ortho*- and mono-*ortho*-substituted PCBs in eggs was: RA, detected in 100% of samples (105, 118, 167, 169), 80% of samples (126, 157), 60% of samples (156), 40% of samples (77, 81); UKRSS, detected in 100% of samples (118, 126, 167, 169), 80% of samples (77, 105, 156, 157), 40% of samples (81); LKRSS, detected in 100% of samples (77, 105, 118, 126, 156, 157, 167, 169), 80% of samples (81).

Polychlorinated biphenyl congeners 81 and 126 have the greatest $TEF_{WHO\text{-}Avian}$ values relative to other congeners. Congeners 81 and 126 were detected in 53% and 93%, respectively, of all egg samples. Together they comprised 63.1% of the total concentration of $TEQ_{WHO\text{-}Avian}$ in eggs from the RA location, 61.8% at the UKRSS, and 42.1% at the LKRSS.

Relative potency

The relative contributions of non-*ortho*- and mono-*ortho*-substituted congeners can be evaluated by standardizing the

Table 4. Geometric mean, wet weight (range), total polychlorinated biphenyls (PCBs), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalent ($TEQ_{WHO\text{-}Avian}$) concentrations, relative potency, and lipid concentrations in great horned owl (*Bubo virginianus*) eggs and plasma (total PCBs only) from the Kalamazoo River Superfund Site (KRSS), Kalamazoo, Michigan, USA

	Plasma PCB			Egg PCB[b]			$TEQ_{WHO\text{-}Avian}$[c]			Relative potency[cd]			Lipid[e]		
Study site[a]	ng/ml	Range	*n*	ng/g	Range	*n*	pg/g	Range	*n*	μg/g	Range	*n*	%	Range	*n*
Reference	14	7.9–25.9	3	258	165–474	5	3.19	1.52–8.37	5	12.40	7.78–17.65	5	6.31	5.74–7.3	5
Upper KRSS	46	25.2–80.4	6	1,441	530–4,408	5	14.38	7.11–28.67	5	9.98	5.18–24.29	5	6.25	3.78–8.51	5
Lower KRSS	68	31.1–147	2	7,897	1,305–39,722	7	137	85–192	5	13.17	4.85–46.7	5	6.52	5.26–8.01	7

[a] Reference sample site includes samples from the Ceresco and Ft. Custer locations, Kalamazoo, Michigan, USA. Upper KRSS sample site includes samples from the Trowbridge, Otsego City Dam, and Lake Allegan impoundments. Lower KRSS sample site includes samples from Koopman's Marsh, Swan Creek Highbanks, and Pottawatomi Marsh.
[b] PCB concentrations include fresh eggs and addled eggs.
[c] TEQ, relative potency, and lipid concentrations include fresh and addled eggs.
[d] Relative potency = TEQ(pg/g)/PCB(μg/g).

$TEQ_{WHO\text{-}Avian}$ to the total PCB concentration to obtain a relative potency value [34]. Relative potency values can be used to assess the degree of weathering and to evaluate exposure and bioaccumulation between trophic levels of an impacted food web and resulting changes in toxic potency of the weathered mixture. Geometric mean relative potency values for $TEQ_{WHO\text{-}Avian}$ and total PCBs in GHO eggs are similar among the KRSS and RA sites. The greatest geometric mean concentration, (1.3×10^1 μg/g, wet wt) was observed for eggs collected at LKRSS (Table 4).

ΣDDT concentrations and eggshell measurements

Total DDT was detected in all egg and plasma samples analyzed. *p,p′*-Dichlorodiphenyldichloroethylene occurred at the greatest concentration of the measured DDT analytes and contributed roughly 98% and 95% to the ΣDDT in all egg and plasma samples, respectively. Total DDT concentrations in eggs were greater from the KRSS than were those from the RA location, and concentrations in the UKRSS and LKRSS were approximately equal. Geometric mean concentrations of ΣDDT were significantly different between the RA site and both UKRSS and LKRSS (Kruskal–Wallis, $p < 0.03$). Geometric mean ΣDDT concentrations in eggs were not significantly different between UKRSS and LKRSS (Kruskal–Wallis, $p = 0.95$) (Table 6 and Fig. 5). The spatial distribution of ΣDDT concentrations in blood plasma was similar to that of concentrations in eggs, but there was no statistically significant difference among the three sites (ANOVA with Tukey's $p = 0.22$). Egg shell thickness and Ratcliffe Index measurements displayed similar trends among the RA site and KRSS (Table 6 and Fig. 5). The mean Ratcliffe Index at LKRSS was slightly less than values observed at the UKRSS and RA site. However, this difference was not statistically significant and neither egg shell thickness, nor the Ratcliffe Index were significantly different among the three sampling locations. (ANOVA with Tukey's, $p = 0.27$, $p = 0.44$, respectively). Additionally, eggshell thickness and Ratcliffe Index were not significantly correlated with ΣDDT concentrations in eggs ($r = 0.35$, $p = 0.17$ and $r = 0.04$, $p = 0.8$ respectively).

Relative abundance and site use

Rates of hoot call responses for individual, pairs, and juvenile birds did not vary by time of survey (AM vs PM) or season (Table 7). Significant differences in the distribution and frequency of responses of individual ($\chi^2 = 16.79$, $df = 2$, $p = 0.001$; $\chi^2 = 16.6$, $df = 1$, $p = 0.001$) and the frequency of responses of paired owls ($\chi^2 = 7.0$, $df = 1$, $p = 0.01$) were observed between FC and TB, with TB having a greater relative abundance of both resident classes. Juvenile response frequencies were also significantly greater ($\chi^2 = 7.57$, $df = 1$, $p = 0.01$) at TB.

Productivity

Over the period of 2000 to 2002, there was no discernible difference in productivity (fledglings/active nest) between the nest sites in the upstream (FC) and downstream (TB) study areas where fledgling success was monitored (active nests $n = 1$, FC and $n = 6$, TB; no statistical testing performed due to the small sample size at FC). For the 3-year study period, the arithmetic mean rate of productivity was 1.0 successful fledglings per active nest at both locations (Table 7). There were more active nests (six vs one) and fledglings at TB (six vs one) than FC. At TB there were two nests that each produced

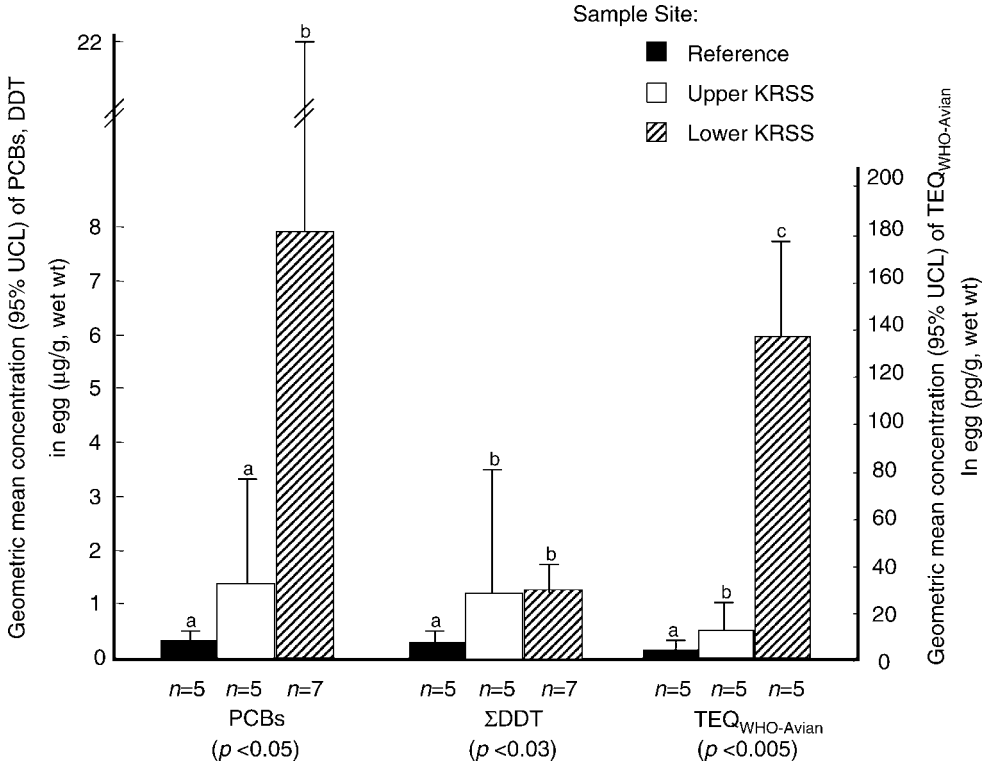


Fig. 2. Geometric mean (wet wt) total polychlorinated biphenyls (PCBs), total dichlorodiphenyltrichloroethane (ΣDDT), and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$) in great horned owl (*Bubo virginianus*) eggs collected from the Kalamazoo River superfund site (KRSS), Kalamazoo, Michigan, USA, error bars show the 95% upper confidence interval (UCI).

two fledglings, and also two failed nesting attempts (both nests abandoned during incubation). The observed number of active nests at TB compared to FC was very similar to the results of the call–response surveys. Measures of relative abundance and site use obtained with the call–response surveys also indicated that there was a greater number of actively defended territories (or resident owls) in the floodplain at TB.

Risk assessment

Measured 95% UCI (geometric mean) concentrations of PCBs in eggs collected from the UKRSS did not exceed the egg-based NOAEC TRV. The maximum HQ_{NOAEC} in the UKRSS was <1.0 (HQ = 0.5). The PCB concentrations in eggs from LKRSS included the four greatest individual PCB concentrations out of 12 eggs for the entire KRSS. In the LKRSS, the 95% UCI (geometric mean) concentration of PCBs in eggs, resulted in HQs of 1.0 and 3.1 when compared to the LOAEC and NOAEC, respectively (Fig. 6).

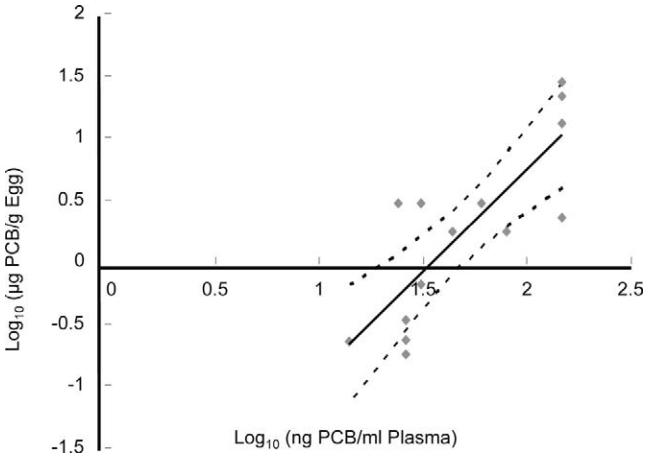


Fig. 3. Egg to plasma polychlorinated biphenyl (PCB) relationship for Kalamazoo River superfund site (KRSS), Kalamazoo, Michigan, USA, great horned owls (*Bubo virginianus*), including the 95% confidence interval on the line of best fit.

Hazard quotient values for $TEQ_{WHO\text{-}Avian}$, based on both the LOAEC and NOAEC, were <1.0 for all individual egg samples from the RA and UKRSS locations. The greatest HQ_{NOAEC} was 0.2 for the UKRSS 95% UCI (geometric mean). Dioxin equivalent concentrations ($TEQ_{WHO\text{-}Avian}$) in the LKRSS, which included the five greatest concentrations out of 10 eggs in the KRSS, resulted in HQs of 0.5 and 1.3, respectively, when the 95% UCI (geometric mean) concentration was compared with the LOAEC and NOAEC (Fig. 6).

Hazard quotient values for the 95% UCI (geometric mean) ΣDDT concentrations, based on both the NOAEC and LOAEC were ≤1.0 for all three sites with a maximum HQ_{NOAEC} value of 1.0 at the UKRSS (Fig. 6). Geometric mean eggshell thickness values at the RA and UKRSS were not significantly different and ≤1% below the pre-1947 benchmark for GHO, but mean thickness at the LKRSS was 4% less than the pre-1947 value. Values for the Ratcliffe Index were 6% less at the RA location than the pre-1947 values while values were 4% and 7% less at UKRSS and LKRSS, respectively.

Hazard quotient values for predicted 95% UCI (geometric mean) concentrations of total PCBs in eggs at all three sites are less than values based on measured concentrations in eggs (Table 5). Use of the predicted concentrations of PCBs in eggs at LKRSS resulted in a geometric mean HQ_{NOAEC} of 0.37 compared to a value of HQ_{NOAEC} of 1.3 based on the comparable measured geometric mean concentration in eggs.

Table 5. Predicted egg (from plasma)[a] and measured egg total polychnorinated biphenyl (PCB) concentrations (ng/g) and calculated geometric mean no-observable-adverse effect concentration (NOAEC) hazard quotients (HQs) in resident great horned owls (*Bubo virginianus*) from the Kalamazoo River Superfund Site (KRSS), Kalamazoo, Michigan, USA

	Reference		Upper KRSS		Lower KRSS	
	Predicted egg $n = 3$	Measured egg $n = 5$	Predicted egg $n = 6$	Measured egg $n = 5$	Predicted egg $n = 2$	Measured egg $n = 7$
Range	80–562	165–474	558–3,630	530–4,408	760–10,299	1,305–39,722
Geometric mean	209	258	1,462	1,441	2,798	7,897
Arithmetic mean	282	277	1,760	1,978	5,530	14,867
Standard deviation (SD)	250	123	1,116	1,628	6,745	14,527
Arithmetic mean ± 1 SD	32–532	154–400	644–2,876	350–3,606	0–12,275	340–29,394
NOAEC HQ (geometric mean)	0.03	0.04	0.21	0.21	0.40	1.13

[a] Predicted egg concentrations calculated using the great horned owl plasma to egg conversion factor [21]: log (egg, μg/g) = 1.674[log (plasma, ng/ml)] − 2.578.

DISCUSSION

A behavioral attribute that favors use of GHOs in ecological studies is their preference to use nests built by other bird species. To our knowledge, the present study is the first to successfully incorporate this behavior into a study designed to induce GHOs to occupy areas of maximum exposure potential, and provide for conservative and worst-case exposure assessment evaluations of the terrestrial food web in a site-specific baseline ecological risk assessment. Previously, Strigiformes have been used to determine ambient or baseline environmental conditions of avian exposure to DDE and other chlorinated hydrocarbon COCs [35]. A second class of investigations focused on local and acute poisoning episodes stemming from use of the acetylcholinesterase-inhibiting organophosphate and carbamate pesticides [36].

Comparison of total PCB concentrations to other locations

Few studies have measured concentrations of PCBs in eggs of wild GHO ([37]; B.G. Rosenberg, 1990, Master's thesis, University of Manitoba, Winnipeg, MB, Canada), and in some instances the analytical results (e.g., PCB quantification on an Aroclor-basis) are not directly comparable to PCB concentrations generated from congener-specific analyses. Surveys of healthy GHO populations in Ohio, USA [37] and Saskatchewan, Canada (B.G. Rosenberg, 1990, Master's thesis) found arithmetic mean PCB concentrations of 3.1×10^3 ng/g (wet wt) and 3.3×10^3 ng/g (wet wt), in eggs, respectively. These concentrations are greater than the arithmetic mean PCB concentration observed in GHO eggs from the UKRSS (2.0×10^3 ng/g egg, wet wt). Although these studies were limited in scope, the results support the conclusions of the risk assessment which suggest that GHOs in the UKRSS are unlikely to be affected by exposure to PCB.

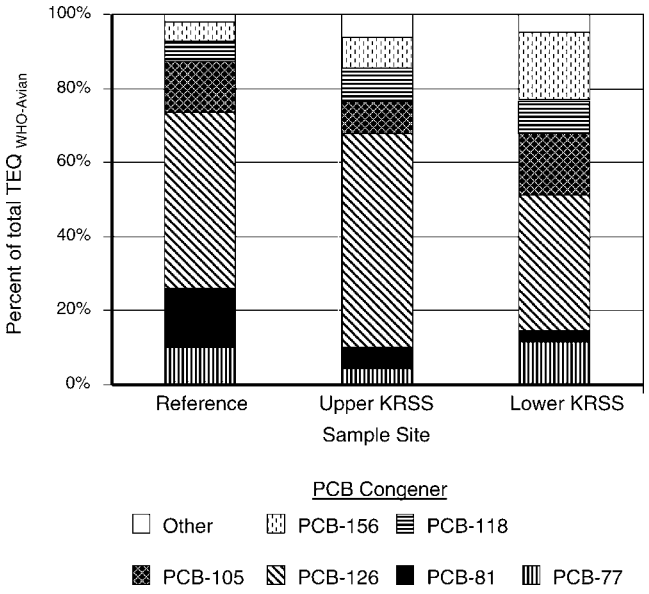


Fig. 4. Percent contribution of polychlorinated biphenyl (PCB) coplanar and mono-*ortho*-substituted congeners to total 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{\text{World Health Organization [WHO]-Avian Toxicity Equivalency Factor [TEF]}}$) in great horned owl (*Bubo virginianus*) egg samples at the Kalamazoo River superfund site (KRSS), Kalamazoo, Michigan, USA.

PCB congener profiles

The relative concentrations of PCB congeners used to calculate $TEQ_{\text{WHO-Avian}}$ in KRSS GHO eggs were similar to those observed in eggs of barn owls [38] and eagles [39,40] in North America and Europe. Similarities among the patterns include the predominance of PCB126 as the maximum detected concentration expressed on a wet weight basis (ratio of PCBs 126: 77 ~2:1 or greater) among coplanar congeners, and as the greatest relative contributor to total $TEQ_{\text{WHO-Avian}}$. This is consistent with observations that PCB 77 and 81 are more susceptible to metabolism than PCB 126 and 169 [41]. Among mono-*ortho*-substituted congeners, PCB 118 occurred at the greatest concentrations and PCBs 105 and 156 contributed the greatest relative proportion to $TEQ_{\text{WHO-Avian.}}$

ΣDDT in eggshell measurements

The ΣDDT concentrations in GHO eggs from all regions of the KRSS were within the range of ΣDDT concentrations reported for investigations of healthy GHO populations associated with nonpoint source exposures to ΣDDT ([37,42]; B.G. Rosenberg, 1990, Master's thesis). The fact that the relatively small concentrations of ΣDDT measured in GHO eggs from the KRSS were similar to those measured in eggs from other healthy GHO populations indicates that ΣDDT concentrations in KRSS GHO eggs were not having an adverse effect on GHO in the KRSS. The spatial distributions of ΣDDT concentrations observed in this study indicate that there was relatively little historical use of this pesticide in the RA. Greater concentrations of ΣDDT in the UKRSS and LKRSS may be related to historical agricultural use since both of these sites receive significant inflow from tributaries with drainage basins that contain agricultural development, including fruit production. Additionally for the LKRSS, the greater concentrations

Table 6. Geometric mean, wet weight (range), total dichlorodiphenyltrichloroethane (ΣDDT)[a] concentrations, eggshell thickness, and Ratcliffe Index values for great horned owl (*Bubo virginianus*) eggs and plasma (ΣDDT only) from the Kalamazoo River Superfund Site (KRSS), Kalamazoo, Michigan, USA

Study site[b]	Plasma ΣDDT (ng/ml)	*n*	Egg ΣDDT (ng/g)	*n*	Eggshell thickness (mm)	*n*	Ratcliffe Index No.	*n*
Reference area	47 (14–168)	3	314 (246–417)	5	0.376 (0.346–0.393)	5	1.9 (1.81–1.96)	5
Upper KRSS	107 (60–169)	6	1,269 (306–4,987)	5	0.377 (0.363–0.412)	5	1.93 (1.89–2)	5
Lower KRSS	94 (59–152)	2	1,305 (618–2,013)	7	0.363 (0.347–0.384)	7	1.88 (1.76–1.99)	7

[a] Total DDT concentrations include dichlorodiphenyltrichloroethane (DDT) and metabolites dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD).

[b] Reference area sample sites include the Ceresco and Ft. Custer locations, Kalamazoo, Michigan, USA. Upper KRSS sample sites include the Trowbridge, Otsego City Dam, and Lake Allegan impoundments. Lower KRSS sample sites include Koopman's Marsh, Swan Creek Highbanks, and Pottawatomi Marsh.

of ΣDDT bioavailability may be associated with exposures in Great Lakes–influenced habitats where fish are known to have greater concentrations of ΣDDT [43].

Toxicity reference values

Few studies meet all of our criteria for deriving a TRV for PCBs in GHOs. While birds, especially raptors, are generally considered to be some of the most exposed and sensitive animals to the effects of chlorinated hydrocarbons, there is a wide range of sensitivities to PCB and other aryl hydrocarbon receptor–active chemicals among species [44,45]. Application of laboratory or field-derived TRVs among dissimilar avian orders, such as Galliformes and Strigiformes, introduces uncertainty and associated conservative bias that can result in protective but unrealistic TRV values. Additionally, values for thresholds of effect, based on the results of acute studies are of little use when trying to establish TRVs for chronic effects in wildlife. Cocontaminants in test diets or from field studies can substantially confound the toxicity results relative to a single chemical or class of chemicals. In particular, assignment of causality can be problematic when elevated levels of cocontaminants are present. Similarly, complex mixtures such as PCBs, which are subject to environmental weathering, are dynamically changing in relative congener composition and toxic potency depending on the environment to which they are exposed. Quantifying the toxicity of neat mixtures or even weathered mixtures from different systems may not reflect the actual toxicity of the mixture of concern. To address any one of these uncertainties, risk assessors frequently apply an uncertainty factor to the published toxicological benchmark. Aside from applying an uncertainty factor of 3 to derive the PCB LOAEC from a validly determined NOAEC, application of additional uncertainty or extrapolation factors to our selected TRVs was not necessary. This is because the selected studies meet the key requirements as described above. Most specifically, the test species used were closely related wildlife species (a specific preference stated in the Great Lakes Water Quality Criteria documents) [25]. The selected studies also employed chronic exposures over sensitive life stages and measured ecologically relevant endpoints with minimal impact from cocontaminants.

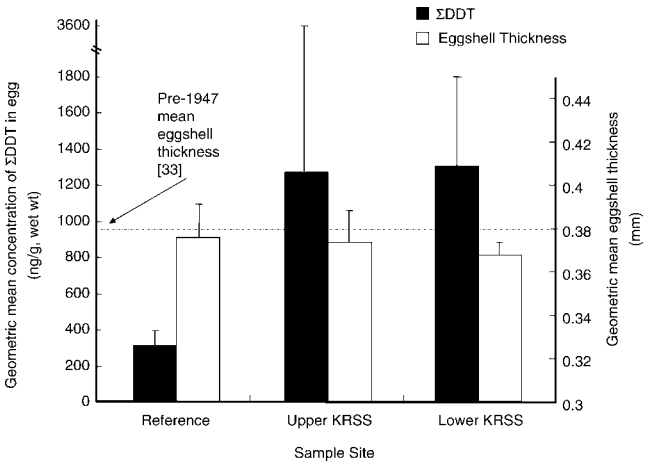


Fig. 5. Geometric mean (wet wt) total dichlorodiphenyltrichloroethane (ΣDDT) concentrations in great horned owl (*Bubo virginianus*) eggs and eggshell thickness at the Kalamazoo River superfund site (KRSS), Kalamazoo, Michigan, USA, error bars show the 95% upper confidence interval (UCI).

Risk assessment

Studies of PCB accumulation patterns in the terrestrial food web at the KRSS [46] have found considerable variation in site-specific patterns of bioavailability, bioaccumulation, and biomagnification. Site-specific exposure potentials are altered by habitat and hydrologic conditions. Site characterization studies have shown the mean PCB concentration in floodplain soils to be greatest in UKRSS and more than twice the concentration observed in floodplain soils of the LKRSS (Table 1). Our measures of PCBs in GHO eggs and GHO nestling blood plasma did not parallel these spatial patterns of PCB exposure potential. Inconsistencies of this type underscore the importance of site-specific studies at sites as large and diverse as the Kalamazoo River. The greater exposure of GHOs to PCBs at the LKRSS may be due to exposure through trophic pathways that include both terrestrial and aquatic pathways including fish from Lake Michigan. For example, diet studies of GHO in this stretch of the KRSS may show that a large portion of the diet is composed of Anseriform (waterfowl) or Charadriform (gulls) prey.

In the present study, use of either total concentrations of PCBs or $TEQ_{WHO\text{-}Avian}$ as measures of exposure in GHO eggs resulted in similar estimates of risk. A review of the 95% UCI (geometric mean) concentration ranges of HQ for total PCBs and total $TEQ_{WHO\text{-}Avian}$ indicates a high degree of concordance between these two measures of exposure. Ranges of HQ_{NOAEC} and HQ_{LOAEC} based on either total PCBs or $TEQ_{WHO\text{-}Avian}$ almost completely overlap and HQ_{LOAEC} were consistently ≤ 1.0 at each of the three study sites (Fig. 6).

Concentrations of ΣDDT in eggs of GHO at the KRSS were correlated with neither eggshell thickness (mm) nor Ratcliffe Index. Changes in values relative to the pre-1947 values for mean eggshell thickness (maximum −4%) and Ratcliffe Index

Table 7. Relative abundance and productivity of resident great horned owls (*Bubo virginianus*) at Ft. Custer (reference area) and Trowbridge (upper KRSS[a]) from 2000 to 2002

	2000		2001		2002		All years (2000–2002)	
	Ft. Custer	Trowbridge	Ft. Custer	Trowbridge	Ft. Custer	Trowbridge	Ft. Custer	Trowbridge
Relative abundance[b]	CS = 4[c]	CS = 7	CS = 9	CS = 7	CS = 11	CS = 8	CS = 24	CS = 22
Adults				Mean response rate[d]				
Total[e]	2.5	2.57	0.89	2.71	0.55	3	1.31	2.76
Foraging[f]	1.5	1.43	0.67	1.86	0.37	1.63	0.85	1.64
Paired[g]	1	1.14	0.22	0.86	0.18	1.38	0.47	1.13
Juveniles				Response frequency no. (%)[h]				
Fledgling[i]	0 (0)	0 (0)	1 (11)	7 (100)	0 (0)	1 (12)	1 (04)	8 (36)
Productivity[j]	n = 0[k]	n = 1	n = 1	n = 2	n = 0	n = 3	n = 1	n = 6
Fledglings	0	1	1	4	0	1	1	6
Fledglings/nest	0	1	1	2	0	0.3	1	1

[a] Kalamazoo River Superfund Site, Kalamazoo, Michigan, USA.
[b] Relative abundance estimates derived from hoot call/response surveys completed at dawn and dusk.
[c] CS = number of complete surveys.
[d] Mean response rate is averaged across number of completed surveys for each year. All years mean is the mean of the means.
[e] Includes discrete responses from both individual and paired (male + female) owls.
[f] Includes responses from unpaired individuals only.
[g] Includes responses from paired (male + female) owls only.
[h] Average response frequency of fledgling owls (No. = number [percent] of completed surveys with at least one fledgling begging call response) expressed on a yearly basis, and averaged over all years.
[i] A measure of current and successful breeding activity.
[j] The total number of successful fledglings from all active nests (no. fledglings/no. active nests) per year within each sampling area, and averaged over all years/sum total of all active nests.
[k] n = number of active nests.

(maximum −7%) in the KRSS were not close to threshold values of 15 to 20% associated with adverse effects on successful raptor reproduction and population maintenance [17,33].

The results of the hazard assessment suggest that GHO populations residing in the RA and the UKRSS are not at risk for effects induced by PCBs, $TEQ_{WHO\text{-}Avian}$, or ΣDDT in floodplain soil. This conclusion is consistent with measurements of fledgling productivity. At the LKRSS, the HQ_{NOAEC} values for eggs are slightly greater than 1.0, with values as great as 3.1 and 1.3 for 95% UCI (geometric mean) concentrations of total PCBs and $TEQ_{WHO\text{-}Avian}$, respectively. These HQ_{NOAEC} values indicate that exposure of GHO to PCBs in this reach of the Kalamazoo River were near the threshold for effects. It is important to note that the true effect level for individuals lies somewhere between the NOAEC and LOAEC and, even conservatively, population effects have not been expected at a HQ of 10.0. The HQ_{NOAEC} values near 1.0 are not likely to be associated with adverse reproductive effects in individual resident GHOs in the LKRSS.

Multiple lines of evidence and assessment of population-level effects

This assessment employed a multiple lines of evidence approach to minimize uncertainty in assessment endpoints and to provide the best available information for remedial decision-making for later stages of site clean-up efforts. Included in the tissue-based ''top-down'' HQ approach [13], on which we report here, the potential effects of PCBs and ΣDDT on GHO productivity and relative abundance and site use were monitored in the FC and TB areas of the RA and UKRSS sites.

Mean productivity rates were similar among locations where exposures to PCBs were much different, with 1.0 successful fledglings per active nest at the two locations where reproductive success was monitored. The mean rate of 1.0 fledgling per active nest observed at both locations is consistent with productivity measures for healthy midwestern [47] GHO populations residing in varied upland habitats. Measures of site use indicate TB populations, as determined by territory-holding nesting pairs, were near the carrying capacity (roughly one pair per 1,600 ha and a total of three pairs in the TB floodplain) [12]. Furthermore, nest acceptance rates and nest fidelity of actively breeding GHOs across all nesting seasons included in the study were consistent with previous studies of artificial nest acceptance and habitat usage by Strigiformes in midwestern forests [11,47,48]. For unknown reasons, the observed number of active nests at FC (1.0 active pair in any study year) was less than the carrying capacity of this study location. Adult mortality may have been a prime factor in the low density of active nesting pairs at FC, as we are aware of four confirmed adult deaths (three owl–car collisions, one owl–train collision) during the study period from 2000 to 2004. Unfortunately, we were not able to identify the sex of these dead owls. Owl population health data support the conclusion that TB populations are not suffering adverse effects to population maintenance.

The top-down assessment of potential hazards to resident GHO populations completed in this investigation has employed intensive sampling effort of maximum exposure, state of the art analytical techniques, multiple methods to estimate exposure to PCBs, an assessment of potentially confounding chemical stressors, valid statistical methods, and conservative benchmarks of toxicity. The results of these studies suggest that current concentrations of PCBs, expressed either as total PCB concentrations or as $TEQ_{WHO\text{-}Avian}$ are not sufficient to pose a significant risk to GHO populations in the UKRSS where large areas of former sediments are now exposed as floodplain soils. The data also indicate that risk in the LKRSS, while greater, is unlikely to be sufficient to cause adverse population-level effects.

Results from the present study concur with earlier investigations at the KRSS that indicated GHOs would effectively

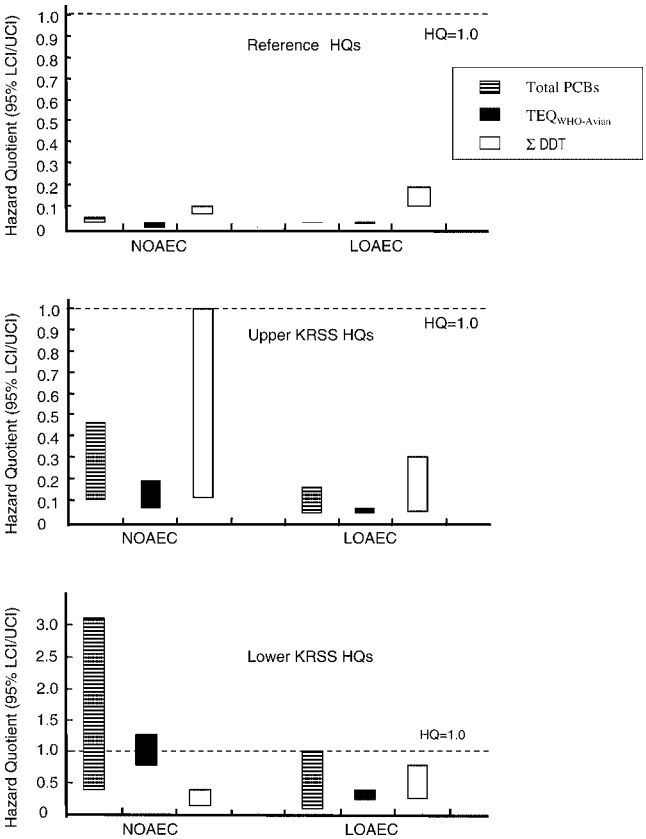


Fig. 6. Hazard quotients (HQ) for the effects of total polychlorinated biphenyls (PCBs), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{\text{World Health Organization [WHO]-Avian Toxicity Equivalency Factor [TEF]}}$), and total dichlorodiphenyltrichloroethane (ΣDDT) for great horned owl (*Bubo virginianus*) eggs at the Kalamazoo River superfund site (KRSS), Kalamazoo, Michigan, USA, based on the no observable adverse effect concentration (NOAEC) and the lowest observable adverse effect concentration (LOAEC). Each box encompasses the 95% upper and lower confidence interval (UCI/LCI) about the geometric mean concentration.

integrate exposures from primary environmental media through multiple trophic levels [46]. The present study confirms that GHOs are a useful sentinel species for site-specific baseline ecological risk assessments employing a multiple lines of evidence approach that includes using top-down methodology to combine measured residues of COCs in tissues and counts of population and productivity. The present study's successful use of GHO provides a workable model that can be applied to other large sites with extensive areas of contaminated soils that require ecological investigations of risk or long-term monitoring for potentially impacted terrestrial communities. In instances where elevated levels of contaminants cause concern for potential environmental effects, measures of owl chemical exposure, productivity, and abundance can serve as an index of overall ecosystem health.

Acknowledgement—The authors extend special thanks to three indispensable project volunteers, Donna Pieracini, Simon Hollamby, and Heather Simmons, who provided technical support and logistic assistance. We also thank the students and technicians at the MSU Aquatic Toxicology Laboratory, including Michael Kramer, Ryan Holem, Scott Kissman, and Theresa Gratton. We would like to recognize aid provided by U.S. Fish and Wildlife Service personnel Lisa Williams and Dave Best, U.S. Fish and Wildlife Service contractor Charles Mehne, and staff of the MSU Kellogg Biological Station. We also thank Karen Smyth and Katie Coady. Funding for this study was provided by the Kalamazoo River Study Group. This study was conducted under U.S. Fish and Wildlife Service permit MB775945-O. This manuscript is dedicated to the memory of William Claflin, whose passion for birds and love of the field contributed greatly to the success of this study.

REFERENCES

1. Blasland, Bouck, Lee. 1993. Allied Paper, Inc./Portage Creek/Kalamazoo River Superfund Site description of the current situation. Syracuse, NY, USA.
2. U.S. Environmental Protection Agency. 1976. PCBs in the United States industrial use and environmental distribution: Task I. EPA 560/6-76/005. Washington, DC.
3. Bursian SJ, Sharma C, Aulerich RJ, Yamini B, Mitchell RR, Orazio CE, Moore DRJ, Svirsky S, Tillitt DE. 2006. Dietary exposure of mink (*Mustela vison*) to fish from the Housatonic River, Berkshire County, Massachusetts, USA: Effects on reproduction, kit growth, and survival. *Environ Toxicol Chem* 25: 1533–1540.
4. Grant DL, Moodie CA, Phillips WEJ. 1974. Toxicodynamics of Aroclor 1251 in the male rat. *Environ Physiol Biochem* 4:214–225.
5. Giesy JP, Ludwig JP, Tillitt DE. 1994. Deformities in birds of the Great Lakes region: Assigning causality. *Environ Sci Technol* 28: 128A–135A.
6. McLane MAR, Hall LC. 1972. DDE thins screech owl eggshells. *Bull Environ Contam Toxicol* 8:65–68.
7. Blasland, Bouck, Lee. 1994. Allied Paper, Inc./Portage Creek/Kalamazoo River Superfund Site remedial investigation/feasibility study. Technical memorandum 2: Results of phase I TBSA soil sampling. Syracuse, NY, USA.
8. Blasland, Bouck, Lee. 1994. Allied Paper, Inc./Portage Creek/Kalamazoo River Superfund Site remedial investigation/feasibility study. Technical memorandum 3: Results of the floodplain soils investigation, volume 1 of 2. Syracuse, NY, USA.
9. Blasland, Bouck, Lee. 1994. Allied Paper, Inc./Portage Creek/Kalamazoo River Superfund Site remedial investigation/feasibility study. Draft technical memorandum 12: Former impoundment sediment geochronological plot study, volume 1–4. Syracuse, NY, USA.
10. International Joint Commission. 1991. Report of the ecological committee's biological effects subcommittee to the Great Lakes science advisory board. *Proceedings*, Expert Consultation Meeting on Bald Eagles, International Joint Commission, Windsor, Ontario, Canada, February 12–13, 1990, p 33.
11. Council on Environmental Quality. 1972. Development of a continuing program to provide indicators and indices of wildlife and the natural environment. Final Report, April 15, 1972. Council on Environmental Quality, Ecology Program, Smithsonian Institution, Washington, DC, USA.
12. Houston CS, Smith DG, Rohner C. 1998. Great Horned Owl (*Bubo virginianus*). In Poole A, Gill F, eds, *The Birds of North America*, No 372. The Birds of North America, Philadelphia, PA, USA, pp 1–28.
13. Fairbrother A. 2003. Lines of evidence in wildlife risk assessments. *Journal of Human and Ecological Risk Assessment* 9: 1475–1491.
14. Brenner FJ, Karwoski D. 1985. Vocalizations as an index of abundance for the great horned owl (*Bubo virginianus*). *Proc PA Acad Sci* 59:129–130.
15. Strause KD, Zwiernik MJ, Kay DP, Park CS, Blankenship AL, Giesy JP. 2007. Utilizing the great horned owl in site-specific assessments of environmental risk and natural resource damage. *Journal of Human and Ecological Risk Assessment* (in press).
16. Houston CS, Crawford RD, Oliphant LW. 1993. Unhatched eggs in raptor nests in Saskatchewan. In Redig PT, Cooper LW, Remple JL, Hunter DB, Hahn T, eds, *Raptor Biomedicine.* University of Minnesota Press, Minneapolis, MN, USA, pp 49–53.
17. Ratcliffe DA. 1968. Changes attributable to pesticides in egg breakage frequency and eggshell thickness in some British birds. *J Appl Ecol* 7:67–105.
18. Stickel J, Wiemeyer SN, Blus LJ. 1973. Pesticide residues in eggs of wild birds: Adjustment for loss of moisture and lipid. *Bull Environ Contam Toxicol* 9:193–196.
19. Mauro L. 1987. Raptor physiology—Hematology and blood chemistry. In Pendleton BG, Millsap BA, Kline KW, Bird DA, eds, *Raptor Management Techniques Manual.* National Wildlife

Federation Scientific and Technical Series No. 10. National Wildlife Federation, Washington, DC, USA.
20. Bortolotti GR. 1984. Criteria for determining age and sex of nestling bald eagles. *J Field Ornithol* 55:467–481.
21. Strause KD, Zwiernik M, Im SH, Newsted JL, Kay DP, Bradley PW, Williams L, Blankenship AL, Giesy JP. 2007. Plasma to egg conversion factor for evaluating polychlorinated biphenyl and dichlorodiphenyldichloroethylene exposures in great horned owls and bald eagles. *Environ Toxicol Chem* (this issue).
22. Van den Berg M, Birnbaum L, Bosveld AT, Brunstrom B, Cook P, Freeley M, Giesy JP, Hanberg A, Hasegawa R, Kennedy SW, Kubiak T, Larsen JC, Rolaf van Leeuwen FX, Djien Liem AK, Nolt C, Peterson RE, Poellinger L, Safe S, Schrenk D, Tillett D, Tysklind M, Younes M, Waern F, Zacharewski T. 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environ Health Perspect* 106:775–792.
23. Neigh AM, Zwiernik MJ, Bradley PW, Kay DP, Park CS, Jones PD, Newsted JL, Blankenship AL, Giesy JP. 2006. Tree swallow (*Tachycineta bicolor*) exposure to polychlorinated biphenyls at the Kalamazoo River Superfund Site, Michigan, USA. *Environ Toxicol Chem* 25:428–437.
24. McLane MAR, Hughes DL. 1980. Reproductive success of screech owls fed Aroclor 1248. *Arch Environ Contam Toxicol* 9:661–665.
25. U.S. Environmental Protection Agency. 1995. Great Lakes water quality initiative criteria documents for the protection of wildlife: DDT, mercury, 2,3,7,8-TCDD, PCBs. EPA 820-B-95-0083. Washington, DC.
26. Elliott JE, Norstrom RJ, Lorenzen A, Hart LE, Philibert H, Kennedy SW, Stegeman JJ, Bellward GD, Cheng KM. 1996. Biological effects of polychlorinated dibenzo-*p*-dioxins, dibenxofurans, and biphenyls in bald eagle (*Haliaeetus leucocephalus*) chicks. *Environ Toxicol Chem* 15:782–793.
27. Elliott JE, Wilson LK, Henny CJ, Trudeau S, Leighton FA, Kennedy SW, Cheng KM. 2000. Biological effects of chlorinated hydrocarbons in osprey chicks. *Environ Toxicol Chem* 20:866–879.
28. Woodford JE, Karasov WH, Meyer MW, Chambers L. 1998. Impact of 2,3,7,8-TCDD exposure on survival, growth and behavior of ospreys breeding in Wisconsin, USA. *Environ Toxicol Chem* 17:1323–1331.
29. Wiemeyer SN, Bunck CM, Stafford CJ. 1993. Environmental contaminants in bald eagle eggs—1980–84—and further interpretations of relationships to productivity and shell thickness. *Arch Environ Contam Toxicol* 24:213–227.
30. Elliott JE, Harris ML. 2002. An ecotoxicological assessment of chlorinated hydrocarbon effects on bald eagle populations. *Rev Toxicol* 4:1–60.
31. Mendenhall VM, Klaas EE, McLane MAR. 1983. Breeding success of barn owls (*Tyto alba*) fed low levels of DDE and dieldrin. *Arch Environ Contam Toxicol* 12:235–240.
32. Nisbet ICT, Risebrough RW. 1994. Relationship of DDE to productivity of bald eagles *Haliaeetus leucocephalus* in California and Arizona, USA. In Mayburg B-U, Chancellor RD, eds, *Raptor Conservation Today*, World Working Group on Birds of Prey. Pica Press, Berlin, Germany, pp 771–773.
33. Anderson BW, Hickey JJ. 1972. Eggshell changes in certain North American birds. *Proceedings*, International Ornithological Congress, The Hague, The Netherlands, August 30–September 5, 1970. 15:514–540.
34. Froese KL, Verbrugge DA, Ankley GT, Nieme GJ, Larsen CP, Giesy JP. 1998. Bioaccumulation of polychlorinated biphenyls from sediments to aquatic insects and tree swallow eggs and nestlings in Saginaw Bay, Michigan, USA. *Environ Toxicol Chem* 17:484–492.
35. Sheffield SR. 1997. Owls as biomonitors of environmental contamination. General Technical Report NC-190. U.S. Forest Service, North Central Research Station, St. Paul, MN.
36. Blus LJ. 1996. Effects of pesticides on owls in North America. *J Raptor Res* 30:198–206.
37. Springer MA. 1980. Pesticide levels, egg and eggshell parameters of great horned owl. *Ohio J Sci* 80:184–187.
38. Wiesmuller T, Schlatterer B, Wuntke B, Schneider R. 1999. PCDDs/PCDFs, coplanar PCBs and PCBs in barn owl eggs from different areas in the State of Brandenburg, Germany. *Bull Environ Contam Toxicol* 63:15–24.
39. Helander B, Olsson A, Bignert A, Asplund L, Litzen K. 2002. The role of DDE, PCB, coplanar PCB and eggshell parameters for reproduction in the white-tailed sea eagle (*Haliaeetus albicilla*) in Sweden. *Ambio* 31:386–403.
40. Eliott JE, Norstrom RJ, Smith GE. 1996. Patterns, trends, and toxicological significance of chlorinated hydrocarbon and mercury contaminants in bald eagle eggs from the Pacific coast of Canada, 1990–1994. *Arch Environ Contam Toxicol* 31:354–367.
41. Smith LM, Schwartz TR, Feltz K, Kubiak TJ. 1990. Determination and occurrence of AHH-active polychlorinated biphenyls, 2,3,7,8-tetrachloro-*p*-dioxin and 2,3,7,8-tetrachlorodibenzofuran in Lake Michigan sediment and biota. The question of their relative toxicological significance. *Chemosphere* 90:1063–1085.
42. Seidensticker JC IV, Reynolds HV III. 1971. The nesting, reproductive performance, and chlorinated hydrocarbon residues in the red-tailed hawk and great horned owl in south-central Montana. *Wilson Bull* 83:408–418.
43. Giesy JP, Verbrugge DA, Othoudt RA, Bowerman WW, Mora MA, Jones PD, Newsted JL, Vandervoort C, Heaton SN, Aulerich RJ, Bursian SJ, Ludwig JP, Ludwig M, Dawson GA, Kubiak TJ, Best DA, Tillitt DE. 1994. Contaminants in fishes from Great Lakes-influenced sections and above dams of three Michigan rivers. I: Concentrations of organic chlorine insecticides, polychlorinated biphenyls, dioxin equivalents, and mercury. *Arch Environ Contam Toxicol* 27:202–212.
44. Gasiewicz TA, Elferink CJ, Henry EC. 1991. Characterization of multiple forms of the Ah receptor: Recognition of a dioxin-responsive enhancer involves heteromer formation. *Biochemistry* 30:2909–2916.
45. Karchner SI, Franks DG, Kennedy SW, Hahn ME. 2006. The molecular basis for differential dioxin sensitivity in birds: Role of the aryl hydrocarbon receptor. *Proc Natl Acad Sci USA* 103: 6252–6257.
46. Blankenship AL, Zwiernik MJ, Coady KK, Kay DP, Newsted JL, Strause K, Park C, Bradley PW, Neigh AM, Millsap SD, Jones PD, Giesy JP. 2005. Differential accumulation of PCB congeners in the terrestrial food web of the Kalamazoo River superfund site, Michigan, USA. *Environ Sci Technol* 39:5954–5963.
47. Holt JB. 1996. A banding study of Cincinnati area great horned owls. *J Raptor Res* 30:194–197.
48. Bohm RT. 1985. Use of artificial nests by great gray owls, great horned owls, and red-tailed hawks in northeastern Minnesota. *Loon* 57:150–152.

SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 26, No. 7, pp. 1399–1409, 2007
© 2007 SETAC
Printed in the USA
0730-7268/07 $12.00 + .00

PLASMA TO EGG CONVERSION FACTOR FOR EVALUATING POLYCHLORINATED BIPHENYL AND DDT EXPOSURES IN GREAT HORNED OWLS AND BALD EAGLES

KARL D. STRAUSE,† MATTHEW J. ZWIERNIK,*† SOOK HYEON IM,‡ JOHN L. NEWSTED,†§ DENISE P. KAY,§ PATRICK W. BRADLEY,† ALAN L. BLANKENSHIP,†§ LISA L. WILLIAMS,‖ and JOHN P. GIESY#††

†Zoology Department, Center for Integrative Toxicology, National Food Safety and Toxicology Center, Michigan State University, East Lansing, Michigan 48824, USA

‡DK Science Research & Development Center, DK B/D, 27-4 Gongduck-dong, Mapogu, Seoul, Korea

§ENTRIX Okemos, Michigan 48823, USA

‖U.S. Fish and Wildlife Service East Lansing Field Office, 2651 Coolidge Road, East Lansing, Michigan 48823

#Biology and Chemistry Department, City University of Hong Kong, Kowloon, Hong Kong, Special Administrative Region, China

††Department of Biomedical Veterinary Sciences and Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5B3, Canada

(*Received* 25 *July* 2006; *Accepted* 19 *December* 2006)

Abstract—The benefits of nondestructive sampling techniques, such as plasma sampling, to directly measure contaminant exposure levels in at-risk or protected raptor populations are many. However, such assays are generally inconsistent with the most certain source of toxicity reference values, which are based on feeding studies and quantified as dietary or ''in ovo'' (egg-based) concentrations. An accurate conversion factor to translate nondestructive plasma-based contaminant concentrations to comparable egg-based concentrations will prove valuable to risk assessors investigating the potential effects of chemical exposures to raptors. We used databases describing the concentrations of total polychlorinated biphenyls (PCBs) in great horned owls (GHO; *Bubo virginianus*) and total PCBs and *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE) in bald eagles (*Haliaeetus leucocephalus*) from the Great Lakes region (Michigan, Wisconsin, USA) to develop a relationship to predict concentrations of PCBs and DDE in eggs. To develop a robust predictive relationship, all of the source data included concentrations of both total PCBs and/or DDE for nestling blood plasma and egg samples collected from within discrete active nesting territories and, in most instances, the same nest. The key characteristics (slope and elevation) of each relationship were tested for differences related to species and geographic region. Predicted variability of relationships were examined and compared to variability associated with natural systems. The results of statistical testing indicate that applying the conversion factors between species (GHO to bald eagle) and among geographic regions yields predicted egg concentrations that are not statistically dissimilar and are within the natural variability observed for residue concentrations among eggs of raptors within species and region.

Keywords—Raptors Polychlorinated biphenyls Plasma Egg Nondestructive sampling

INTRODUCTION

Because raptors are at the top of the food chain, they are maximally exposed to many persistent and bioaccumulative residues [1]. This, combined with the fact that they are susceptible to the toxic effects of many contaminants of concern, means that raptors can be used as effective and sensitive biological monitors for contaminant exposures and assessment of environmental effects [2] (www.ncrs.fs.fed.us/epubs/owl/SHEFFIE.PDF). Raptors also are often used as environmental sentinels for monitoring of contaminants [3] or as primary or surrogate receptor species in ecological risk assessments [4] (www.dnr.state.wi.us/ORG/water/wm/foxriver/documents/ra/Final%20BLRA%20Section%201.pdf). Raptors are particularly useful, because they are often territorial and long lived, reproducing in the same territory over long periods of time. Thus, extensive databases of historical contaminant exposures are often available.

Historically, contaminant monitoring programs utilizing raptors have primarily used eggs because of the several advantages of using them for assessing contaminant exposure and effects. These include ease of collection and the fact that the proximal exposure of the developing embryo to the chemicals gives a direct measure of one of the most sensitive endpoints, embryo lethality [5]. Eggs are relatively easy to transport and store, and egg samples from wild bird populations are available independent of egg fertility. In addition, since lipophilic compounds tend to accumulate in lipids of eggs, quantification of residues is facilitated. Furthermore, controlled laboratory studies, including feeding and egg-injection studies, offer direct comparisons of concentrations of residues in eggs with effects such as survival, eggshell integrity, and developmental deformities [6–9]. Egg-based contaminant exposure measurements have also been correlated with temporal and spatial effects. Nevertheless, egg sampling has some serious limitations when used in site-specific and long-term investigations of potential ecological risk. These include the destructive nature of the sample, a high level of nest disturbance that significantly increases the frequency of nest abandonment, high levels of uncertainty for assigning spatial origin to the observed exposure concentration, and narrow temporal limits to the ''window'' of monitored exposure ([10,11]; R. Frank, 1997, Master's thesis, University of Wisconsin, Madison, WI, USA). Egg sampling efforts also may be limited by the gender-restricted nature of the sample.

When the disadvantages of determining egg-based exposure data outweigh the advantages, residues are measured in blood plasma [12–14]. This approach also has several advantages.

* To whom correspondence may be addressed (zwiernik@msu.edu).

These include the ability to collect blood without destroying the individual, the ability to collect samples from the same individual over time, and the ability to collect samples from nestlings [14]. Because nestlings are sedentary and most residues in their blood are accumulated from food; nestling blood plasma is an integrated measure of concentrations of residues in the area proximal to the nest site, much more so than are concentrations of residues in eggs or adult plasma samples [11,15]. For most raptor species, collection of blood plasma from nestlings reduces the risk of injury to the bird and minimizes abandonment or nest relocation by adult birds. Also, blood samples need not be gender or age specific. Use of blood plasma has been further advanced by development of more sensitive methods of residue analyses that has lessened the mass of analyte required for quantification.

Limitations of plasma contaminant data are primarily in interpreting the effects of residues in blood plasma. There is relatively little information relating the concentrations of specific residues in blood plasma of nestling raptors to adverse outcomes, while there is more information on the effects of concentrations of residues in eggs on effects on both individuals and populations. Long-term monitoring of residues in eggs, blood, and raptor populations has demonstrated that trends in concentrations of residues are similar for eggs and blood plasma [15]. Thus, the use of blood plasma for monitoring populations for adverse effects would be facilitated by predicting concentrations of residues in blood to concentrations in eggs.

We used synoptic sampling of blood plasma from nestling great horned owls (GHO; *Bubo virginianus*) and bald eagles (*Haliaeetus leucocephalus*) from the Great Lakes region to develop a relationship to predict concentrations of polychlorinated biphenyls (PCBs) and *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE) in eggs. We compared these relationships to those previously published for bald eagles from other regional subpopulations and assessed the variability of predicting total PCB concentrations in eggs from those in blood plasma.

MATERIALS AND METHODS

Collection of great horned owl blood plasma and eggs

Blood plasma from nestlings and fresh or addled eggs of GHO were collected from the Kalamazoo River Superfund Site (KRSS). Collections were made between April 2000 and April 2004 along a 190-km stretch of the river's floodplain between the cities of Marshall and Saugatuck, Michigan, USA (Fig. 1). Collections were made from both naturally occurring nests and artificial nesting platforms. This location represented a gradient of concentrations of both PCBs and ΣDDT (dichlorodiphenyltrichloroethane [DDT] and its metabolites DDE and dichlorodiphenyldichloroethane [DDD]) that ranged from local ''background concentrations'' to relatively great concentrations of PCBs and ΣDDT [16]. Matched egg and nestling plasma samples were collected from nests of the same mated pair occupying the same nest in the same reproductive year. In other instances, matched egg and nestling plasma were collected from the same nest over a period of two or more years. In cases where nesting pairs selected a new nest site, samples were collected from alternate nests within the same territory over two or more consecutive years. To encourage renesting after collection of eggs, fresh eggs were collected as soon as possible following confirmation of incubation. Addled eggs were obtained when nests were abandoned.

Eggs were labeled, transported back to the laboratory, and stored at 4°C until processing. Length, width, and whole-egg weight and water volume were measured prior to removal of contents. Egg contents were stored in solvent-rinsed glass jars at −20°C until measurement of PCBs and ΣDDT.

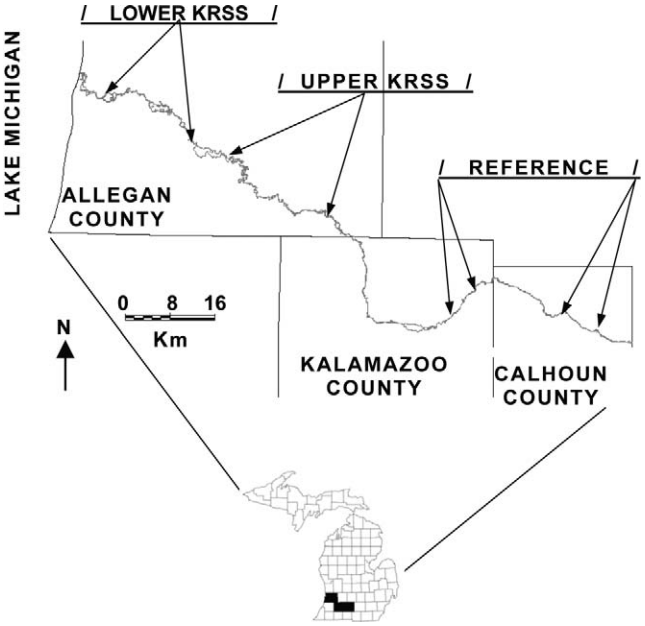


Fig. 1. Map of area of the Kalamazoo River superfund site (KRSS), indicating the location in southern Michigan, USA, as well as the three reaches across which a gradient of polychlorinated biphenyl (PCB) concentrations was observed.

Nestling blood samples were collected when nestlings were approximately 4 to 6 weeks of age and had attained a minimum body weight of 0.75 kg. A sample of 5 to 7 ml was withdrawn from the brachial vein with a heparinized disposable syringe (25-gauge hypodermic needle) and sterile technique. Blood was transferred to a heparinized Vacutainer™ (BD, Franklin Lakes, NJ, USA) tubes and labeled. Vacutainers containing whole blood were centrifuged at 1,200 rpm for 10 min within 48 h of field sampling. Plasma (supernatant) was transferred to a new Vacutainer appropriately labeled and stored upright at −20°C until measurement of PCBs and ΣDDT.

Whole egg homogenates and nestling plasma samples were processed and analyzed for congener-specific total PCBs and ΣDDT using methods described previously [17]. All chemical concentrations in eggs were corrected for moisture loss [18].

Collection of bald eagle blood plasma and eggs

The values used to develop the egg to plasma relationships for bald eagles in the Great Lakes region were compiled from studies conducted by several state and federal agencies as well as public and private research institutes, the majority of which were completed between 1996 and 2002. With a few exceptions, most egg samples included in this database originated from the U.S. Fish and Wildlife Service (USFWS) environmental contaminants program using addled egg collection [19]. Most measurements of residues in blood plasma were from the Michigan wildlife contaminant trend monitoring program administered by the Michigan Department of Environmental Quality (MDEQ), Office of Surface Water Quality [20–23] (web links for these references are located at the following web links, respectively: (1) www.deq.state.mi.us/documents/deq-wd-swqas-wildlifetrendreport02.pdf, (2) www.deq.state.mi.us/documents/deq-wd-swqas-wildlifetrendreport.pdf, (3) www.

deq.state.mi.us/documents/deq-wd-swqas-2000eaglereport.pdf, and (4) www.deq.state.mi.us/documents/deq-wd-swq-gleas-99baldeaglereport.pdf). Additional data were also used ([17,24,25]; W. Bowerman, 1991, Master's thesis, Northern Michigan University, Marquette, MI, USA).

All studies reported concentrations of total PCB, and/or *p,p′*-DDE for blood plasma of nestlings and/or egg samples collected from discrete active eagle nesting territories. From these reports, concentrations of individually paired blood plasma and egg samples were assembled according to the following three general guidelines: First, plasma samples collected from 1996 forward were paired with egg samples collected within a 5-year window of sampling for the two media (e.g., egg [1997] paired with plasma [2001]); second, samples collected prior to 1996 were paired only for the same or two consecutive collection years; third, for either grouping, a third or fourth sample was included in instances where two consecutive collections of plasma or egg were made (e.g., egg [1986–1987], plasma [1987–1988]), in which case the geometric mean concentration of the two combined samples was used. The selection of a 5-year maximum window for pairing the most recent samples was based on the trend monitoring data for PCB and *p,p′*-DDE concentrations in eggs that were not significantly different within subpopulations, between years from 1996 onward [19].

Sample collection and processing for these studies are consistent with the methods described for Kalamazoo River GHO, but methods of chemical analyses varied to some degree. The USFWS analyses of *p,p′*-DDE and total PCBs in addled eggs were completed by the USFWS Patuxent Analytical Control Facility (Shepherdstown, MD, USA) using gas-liquid chromatography. Nominal lower limits of detection were 10 ng/g, (wet wt) for DDE and 50 ng/g for total PCBs. Egg concentrations were corrected for moisture loss [19]. The MDEQ analyses of *p,p′*-DDE and total PCBs (sum of 20 PCB congeners) in nestling plasma were completed at the Clemson Institute of Environmental Toxicology (Clemson, SC, USA) using capillary gas chromatography with electron capture device following U.S. Environmental Protection Agency approved methods [23]. All reported results were confirmed by dual column analyses. Quantification levels for both compounds were 2 ng/g [20–23]. For use in this assessment, the MDEQ PCB plasma concentrations for the 20 quantified PCB congeners were converted to a total PCB equivalent using the relationship: total PCBs = 4.57(sum 20 PCB congeners, ng/g, wet wt) + 0.98 [24]. Analyses of *p,p′*-DDE and total PCBs in nestling plasma for the Green Bay and Fox River samples [25] were completed at Michigan State University and included the use of gas chromatography with electron capture detection and confirmation with mass spectrometry. Detection limits were 2.5 ng/g for DDE and 5 ng/g for total PCBs. Analytical methods for additional egg and plasma samples from Green Bay and Fox River [26] are provided by the authors.

Statistical analyses

Sample sets were analyzed for normality by the Kolmogorov–Smirnov, one-sample test with Lilliefors transformation. Concentration data were log-normally distributed and after log-transformation satisfied assumptions of normality. To evaluate the plasma to egg relationship for each PCB and *p,p′*-DDE database, a Pearson product-moment correlation analysis was performed on the log-transformed values. Paired blood plasma and egg concentrations of residues were plotted as a function of the blood plasma values and the line of best fit for each sample set was derived through simple regression and residuals analyses. Normality and correlation analyses were completed using the Statistica (Ver 6.1) statistical package (Statsoft, Tulsa, OK, USA). Regression residuals were calculated using Excel (Microsoft® Windows PE, 2002; Microsoft, Redmond, WA, USA).

To assess the robustness of the relationships developed for the GHO, predictions were compared with measured values for bald eagles at other locations available in the literature. Tests for homogeneity of regression coefficients and elevation used analysis of covariance methods (ANCOVA) [27]. Multiple comparisons among elevations were made by use of Tukey's honestly significant difference (HSD) for unequal sample size [27]. The criterion for significance used in all tests was $p < 0.05$. Comparisons of conversion factor predictive variability were made by calculating the relative percent difference (RPD).

To test the similarity between egg to nestling plasma relationships between bald eagles of the Pacific Coast versus bald eagles of the Great Lakes, an ANCOVA was used to test for equality of the population regression coefficients (slope) and elevation. Tests of elevation may be considered to be the same as asking whether the two population y intercepts are different. However, one must be cautious of misleading interpretations from such a characterization if discussion of the y intercepts would require a risky extrapolation of the regression lines far below the range of x for which data were obtained [27,28]. The use of elevations instead of y intercepts also assures comparison of relationships over only the range of plasma or egg concentrations measured in each study and eliminates the potentially confounding effects that analytical detection limits may contribute to a test of y intercepts. If either test identified a statistically significant difference within the pool of data being evaluated, additional pair-wise comparisons were completed using Tukey's HSD to identify which of the population slopes or elevations differed from one another.

RESULTS

Great horned owls

The fidelity of GHO to established home territories and preferred nesting sites resulted in multiple instances of renesting that provided samples of both nestling blood plasma and eggs for the same breeding pair in the same nesting territory. A total of 14 paired GHO nestling blood plasma and egg samples were assembled from a total of 16 blood plasma and 17 egg samples. This included four nests where the samples of blood plasma and eggs were collected in the same nest during the same year and three nests where the paired samples were collected from the same nest but during different years. In seven instances, samples were collected from two different nests within an active nesting territory but during different years.

Relationships between concentrations of both total PCBs and ΣDDT were investigated. Total PCB concentrations for these 14 data points (Table 1) exhibited a gradient among the three reaches of the KRSS and there was a significant positive correlation ($r = 0.766$, $p = 0.001$) between log-normalized nestling blood plasma and egg PCB concentrations (Fig. 2). The narrow range of ΣDDT concentrations detected in KRSS GHO plasma and egg did not exhibit such a gradient and concentrations of ΣDDT in eggs were negatively correlated

Table 1. Great Lakes (Kalamazoo River) great horned owl plasma to egg polychlorinated biphenyl (PCB) conversion factor sample pairing

PCB sample location[a] (sample year: plasma/egg)	Plasma (ng PCB/ml)	Egg (μg PCB/g) (wet wt)
Lower KRSS (2002/2000)	147	12.2
Lower KRSS (2002/2001)	147	19.8
Lower KRSS (2002/2002)	147	25.7
Lower KRSS (2002/2004)	147	2.09
Upper KRSS (2002/2003)	80.4	1.61
Upper KRSS (2000/2002)	60.2	2.74
Upper KRSS (2001/2003)	43.7	1.61
Upper KRSS (2003/2003)	31.3	0.61
Lower KRSS (2003/2004)	31.0	2.78
Reference (2003/2001)	25.9	0.31
Reference (2003/2002)	25.9	0.17
Reference (2003/2003)	25.9	0.22
Upper KRSS (2001/2002)	24.1	2.74
Reference (2002/2002)	14.0	0.21

[a] Kalamazoo River superfund site, Kalamazoo, Michigan, USA.

with those in blood plasma ($r = -0.735$, $p = 0.003$). Thus, a ΣDDT egg to blood plasma predictive relationship was not developed for GHO.

Great Lakes bald eagles

A total of 30 (total PCBs) and 31 (*p,p′*-DDE) paired nestling plasma and egg samples were assembled from the available individual samples from the Great Lakes region (Table 2). Pairings originate from a single nesting territory and are combined following the pairing guidelines discussed previously. Great Lakes bald eagles exhibited significant positive correlations between total PCB and *p,p′*-DDE concentrations in nestling blood plasma and egg samples (PCBs: $r = 0.789$, $p < 0.001$; *p,p′*-DDE: $r = 0.569$, $p = 0.001$). Log-normalized PCB and *p,p′*-DDE concentrations were plotted along with the line of best fit and 95% confidence interval for the regression line (Figs. 3 and 4).

Confirmation of concept

To test if the generality of relationships for the prediction of concentrations of residues in eggs from those in blood plasma of nestlings are consistent between owls and eagles, and among geographically distinct subpopulations, ANCOVA for slope and elevation were conducted. The relationship between residue concentrations in nestling blood plasma and egg has not been previously investigated for GHO, but Elliott and Norstrom [13] and Elliot and Harris [29] have examined the distribution of PCBs and *p,p′*-DDE in bald eagle nestling plasma and egg samples from the Pacific Coast of Canada and the United States. We have presented a comparison of the key characteristics of each of the four PCB databases (one GHO, three bald eagle) and three bald eagle *p,p′*-DDE databases for which nestling plasma and egg contaminant relationships are now available (Tables 3 and 4). A more robust egg to blood plasma relationship is obtained by using individual samples from the same nest or a much larger number of summary mean concentrations [29].

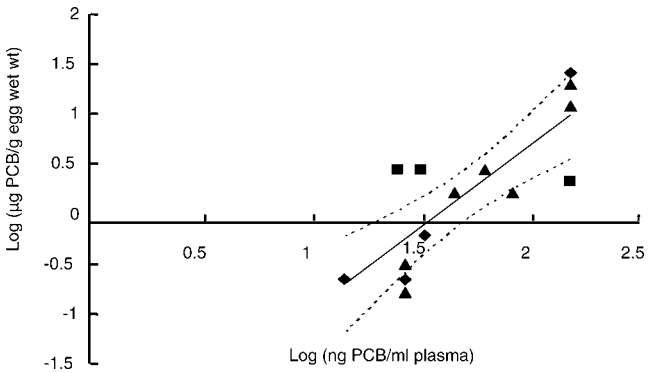


Fig. 2. Concentration of total polychlorinated biphenyls (PCBs) in eggs of great horned owls as a function of PCBs in blood plasma of nestling great horned owls along the Kalamazoo River, Michigan, USA. ◆ = same nest/same year; ■ = same nest/different year; ▲ = same territory/different year. Regression line with 95% confidence intervals of the predicted line: [$\log_{10}$(μg PCB_{egg}/g, wet wt) = 1.647[$\log_{10}$(ng PCB_{plasma}/ml)] − 2.578 ($p < 0.001$, $r^2 = 0.666$).

There were no significant differences between the slopes of regression lines for concentrations of PCB in eggs and nestling blood plasma (ANCOVA, $p > 0.05$) for the three bald eagle groups. A graphical representation of the PCB and *p,p′*-DDE egg to nestling blood plasma relationships between the four PCB sample groups and three *p,p′*-DDE sample groups is provided (Figs. 5 and 6). There were some statistically significant differences among elevations for these three groups (ANCOVA, $p < 0.003$). The elevation of the Great Lakes bald eagle group was significantly different from the two Pacific Coast groups (Tukey's test, $p < 0.03$). These findings indicate that the three population samples are all estimates of the common population regression coefficient and are approximately parallel, but with differing elevations and differing values for a predicted *y* (egg concentration). The slope of the relationship for the GHO from the Kalamazoo River was not different from that of the bald eagles (ANCOVA, $p < 0.05$), but the elevation was significantly different (Tukey's test, $p < 0.001$). The elevation of the relationship for GHO was significantly different from that of the two Pacific Coast bald eagle groups (Tukey's test, $p < 0.03$) but not significantly different (Tukey's test, $p < 0.05$) than the Great Lakes bald eagle group. Taken together, the results of the ANCOVA and Tukey's tests of differences in elevation for the PCB egg to plasma relationship indicate that the observed differences among the four groups is unlikely to be related to differences between species. For the *p,p′*-DDE data set, there were no statistically significant differences among either slopes or elevations of the relationship between concentrations in eggs and nestling blood plasma for the three bald eagle groups (ANCOVA, $p > 0.05$). This indicates that the three sample groups could have been drawn at random as subpopulations from the same population.

Egg predictions

To examine the predictive variability of results obtained from the various relationships, measured nestling blood plasma concentrations from the Great Lakes bald eagle database were used in each of the four PCB conversion factor equations and three DDE conversion factor equations to predict concentrations of PCBs and DDE in eggs. The predicted PCB–DDE egg concentration was then compared to the measured egg PCB–DDE concentration comprising the plasma–egg pair from the same Great Lakes bald eagle database. This approach allowed predictions made from each relationship to be compared to measured PCB–DDE concentrations in eggs. Differences between the predicted and measured egg PCB–DDE concentrations were assessed by calculating the RPD between the two values: RPD = [|measured_{egg} − predicted_{egg}|/(measured_{egg} + predicted_{egg})/2]·100. The analysis was conducted with three

Table 2. Great Lakes (Michigan, Wisconsin, USA) bald eagle plasma to egg polychlorinated biphenyl (PCB) and *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE) conversion factor sample pairing

PCB sample location (sample year: plasma/egg)	Plasma (ng PCB/ml)	Egg (μg PCB/g) (wet wt)	Plasma (ng DDE/ml)	Egg (μg DDE/g) (wet wt)	*p,p′*-DDE sample location (sample year: plasma/egg)
Peshtigo River (1992/1992)[ab]	901	66.6	361	14.7	Peshtigo River (1992/1992)[ab]
10 Mile Creek (1999/1997)[acd]	866	8.0	83.0	2.90	10 Mile Creek (1999/1997)[ac]
10 Mile Creek (1999/1999)[acd]	866	9.0	83.0	2.60	10 Mile Creek (1999/1999)[ac]
North Lake (1999–2000/1999)[aed]	856	3.30	114	5.20	North Lake (1999–2000/1999)[af]
Ottawa Marsh (2000/2000)[g]	773	42.2	27.0	8.10	Ottawa Marsh (2000/2000)[ae]
Skull/Stoney Island (1999/1996)[acd]	394	12.0	15.0	4.90	Skull/Stoney Island (1999/1996)[ac]
Swan Creek (1999/1996)[acd]	367	41.2	14.0	8.19	Swan Creek (1999/1996)[ac]
Boutlier Lake (1987/1986)[ah]	319	55.3	235	29.9	Boutlier Lake (1987/1986)[ah]
Dinsmoore (2002/1999)[aid]	281	6.16	39.0	4.0	Dinsmoore (2001/1999)[ai]
Pere Marquette River (2000–2001/2002)[g]	235	23.9	31.0	14.9	Pere Marquette River (2000/2002)[eg]
Pere Marquette River (2000–2001/1998)[ag]	235	34.0	31.0	0.29	Pere Marquette River (2000/1998)[ae]
Upper Peninsula Vulcan (2002/1999)[aid]	206	13.0	5.0	15.0	Upper Peninsula Vulcan (2002/1999)[ai]
Oxbox Lake (2000/2000)[aed]	176	3.40	4.0	0.72	Oxbox Lake (2000/2000)[ae]
Badwater Lake (2002/2000)[aid]	175	1.50	7.0	0.30	Badwater Lake (2002/2000)[ai]
Big Charity Island (1999/1996)[acd]	162	18.9	9.0	3.74	Big Charity Island (1999/1996)[ac]
Upper Peninsula Vulcan (1999/1999)[acd]	146	13.0	5.0	15.0	Upper Peninsula Vulcan (1999/1999)[ac]
Copper Peak (2000/1996)[ade]	106	4.30	14.0	1.30	Copper Peak (2000/1996)[ae]
Badwater Lake (1999–2000/2000)[ade]	52.0	1.50	4.0	0.30	Badwater Lake (1999–2000/2000)[ae]
Huron Bay (1999–2000/2000)[ade]	45.0	1.45	5.0	0.91	Huron Bay (1999–2000/2000)[ae]
Peavy Pond East (1999–2000/2000)[ade]	41.0	2.20	12.0	1.30	Peavy Pond East (1999–2000/2000)[ae]
Iron Lake (2002/2000)[adi]	34.0	0.65	9.0	0.62	Iron Lake (2002/2000)[ai]
Fortune Lake Island (2000/2000)[ade]	28.0	0.47	10.0	0.51	Fortune Lake Island (2000/2000)[ae]
Paint Lake (2002/2000)[adi]	23.0	0.48	18.0	0.26	Paint Lake (2002/2000)[ai]
Rock Lake/Carney Outlet (2002/1999)[adi]	22.0	1.10	10.0	0.10	Rock Lake/Carney Outlet (2002/1999)[ai]
Boney Falls (2000–2001/1999)[adef]	16.0	1.90	3.0	0.60	Boney Falls (2000–2001/1999)[aef]
Boney Falls (2000–2001/2000)[adef]	16.0	2.20	3.0	0.93	Boney Falls (2000–2001/2000)[aef]
Buck/Armstrong Lake (2001/1997)[adf]	14.0	0.55	5.0	0.62	Iron Lake (1999–2000/2000)[ae]
Fox River (1991–1992/1990)[j]	195	36.0	4.0	0.38	Thousand Isle Lake (1999–2000/2000)[ae]
Big Creek (2000/2001)[g]	43.0	0.98	3.0	0.62	Black Creek Flooding (1999/1997)[ac]
Otter Lake (2000–2001/2000)[adef]	13.0	0.49	3.0	0.10	Michigamme River (1999/1998)[ac]
			3.0	0.44	Blue Lakes (1999/1997)[ac]

[a] Egg concentrations from United States Fish and Wildlife Service [19].
[b] Plasma and egg concentrations from Dykstra et al. [25].
[c] Plasma concentrations from Michigan Department of Environmental Quality [23].
[d] PCB plasma concentrations for 20 PCB congeners are converted to a total PCB equivalent using the relationship: total PCBs = 4.57(sum 20 PCB congeners, ng/g) + 0.98 [26].
[e] Plasma concentrations from Michigan Department of Environmental Quality [22].
[f] Plasma concentrations from Michigan Department of Environmental Quality [21].
[g] Plasma and/or egg concentrations from Strause [17].
[h] Plasma concentrations from Bowerman, 1991 (Master's thesis, Northern Michigan University, Marquette, MI, USA).
[i] Plasma concentrations from Michigan Department of Environmental Quality [20].
[j] Plasma and egg concentrations from Dykstra and Meyer [24].

randomly selected subsamples from the Great Lakes eagle database. Using a random number generator, three sets of 10 paired plasma–egg samples were selected from the 30 PCB samples and 31 DDE samples comprising the Great Lakes bald eagle database.

A second and more restrictive evaluation included calculating the mean predicted versus measured egg RPD for a subset of the Great Lakes eagle plasma–egg samples with a restricted range of plasma concentrations that matches or falls within the range of plasma concentrations used to establish each specific egg to plasma relationship.

Predictive variability was assessed in each of the three randomly selected subsets and the single restricted subset. Results of the RPD analysis are expressed as mean RPD. (Tables 5 and 6). For the randomly selected subsets and PCB relationships, predicted egg PCB concentrations from the Great Lakes eagle database produced the lowest range of mean RPD (73–78%), which would be expected since this relationship was derived from the same plasma and egg samples used in the comparison. Using the GHO conversion factor, mean PCB RPD values ranged from 74 to 97%. Mean PCB RPD values for the two Pacific Coast eagle conversion factors had the widest ranges from 73 to 97% and 70 to 100%. Predictive variability for the restricted PCB subsets shows that the GHO conversion factor produced the lowest mean RPD (55%) among the four PCB conversion factors. For *p,p′*-DDE, the predicted egg concentrations from the Great Lakes eagle database again produced the lowest range of mean RPD (52–81%) for the randomly selected subsets and the restricted sample (77%), as expected. Mean DDE RPD ranges for the two Pacific Coast eagle conversion factors included 89 to 93% and 95 to 97%, and restricted mean RPDs were 99% and 105%.

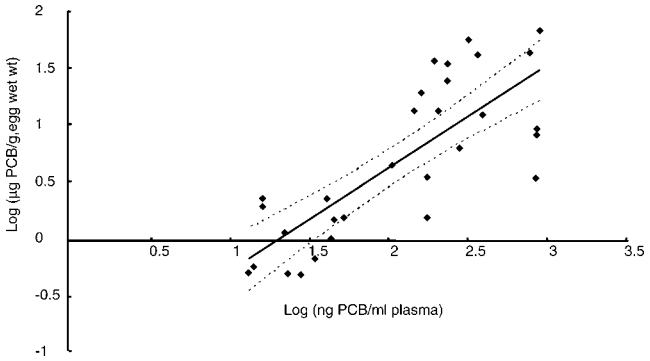


Fig. 3. Concentration of total polychlorinated biphenyls (PCBs) in eggs of bald eagles as a function of PCBs in blood plasma of nestling bald eagles in the Great Lakes region (Michigan, Wisconsin, USA). Regression line with 95% confidence intervals of the predicted line: $\log_{10}$ (µg PCB_{egg}/g, wet wt) = 0.905[$\log_{10}$ (ng PCB_{plasma}/ml)] − 1.193 ($p < 0.001$, $r^2 = 0.623$).

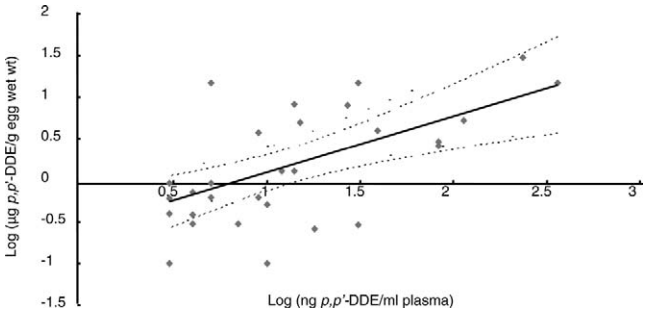


Fig. 4. Concentration of total *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE) in eggs of bald eagles as a function of *p,p′*-DDE in blood plasma of nestling bald eagles in the Great Lakes region (Michigan, Wisconsin, USA). Regression line with 95% confidence intervals of the predicted line: $\log_{10}$ (µg *p,p′*-DDE_{egg}/g, wet wt) = 0.676[$\log_{10}$ (ng *p,p′*-DDE_{plasma}/ml)] − 0.578 ($p < 0.001$, $r^2 = 0.324$).

DISCUSSION

Because nestlings can be captured and handled more easily than adults, it is less likely to cause harm to individuals or the population [13,14]. The use of blood plasma from nestlings eliminates the potentially confounding influence of adult exposures during migrations or on their wintering grounds or in instances where resident, nonmigratory species or individuals shift or greatly expand winter foraging territories [30]. Because most of the residues in the blood plasma of nestlings is accumulated from the area proximate to the nest, these results can be more easily compared with the results of diet studies to identify significant contributing sources of environmental contaminants in the food web [31]. Also, because the embryo and nestling are the most sensitive life stages for population-level effects of chlorinated hydrocarbon contaminants, field monitoring of nestling plasma combined with laboratory feeding and egg injection studies often provides the best opportunities to integrate laboratory and field studies in efforts to derive and verify field-based toxicity reference values for plasma-based endpoints [32,33].

Predictive accuracy of the plasma to egg conversion factor

Focused studies to specifically examine the predictive accuracy of the plasma to egg relationship are not available in the literature. Nevertheless, the relationship has been used in several studies as a tool for further interpretation of adult and nestling plasma data. The earliest efforts to predict concentrations of a residue in eggs from measurements in blood plasma was for ΣDDT concentrations in adult falcons and accipiter hawks. In wild American kestrels (*Falco sparverius*) of the Pacific Northwest, ΣDDT residues in adult female plasma closely paralleled ΣDDT residues in eggs laid by the same birds [34]. Concentrations of DDE in eggs (lipid basis) corresponded well with concentrations of DDE in adult European sparrow hawks (*Accipiter nisus*) [35]. A significant decrease in total concentrations of DDE in the bodies of females due to translocation to eggs was also observed. In both laboratory and field studies, concentrations of DDE in blood plasma of American kestrels correlated with exposure in the diet. Concentrations of ΣDDT in adult female blood plasma in populations of three accipiters (goshawk [*A. gentilis*], Cooper's hawk [*A. cooperii*], sharp-shinned hawk [*A. striatus*]) were correlated with ΣDDT concentrations in eggs [12]. Those authors also described a large decline in female kestrel plasma

Table 3. Plasma to egg conversion factors (CF) for total polychlorinated biphenyls (PCBs), Orders Strigiformes and Falconiformes

		PCB Plasma to Egg Relationships					
Species (subpopulation)	Reference	Sample pairing (*n*)	Strength[a]	Plasma sample range (ng PCB/ml)	Egg sample range (µg PCB/g) (wet wt)	Slope[b]	Intercept[b]
Great horned owl (Great Lakes)	[17]	Individual pairs[c] (14)	$r^2 = 0.666$ $p < 0.001$	14–147	0.2–25.7	1.647	−2.578
Bald eagle (Great Lakes)	[17]	Individual pairs[c] (30)	$r^2 = 0.623$ $p < 0.001$	13–901	0.47–66.6	0.905	−1.193
Bald eagle (Pacific Coast)	[13]	Geometric mean pairs[d] (6)	$r^2 = 0.869$ $p < 0.03$	14–80	2.6–12.7	0.734	−0.409
Bald eagle (Pacific Coast/Great Lakes)	[29]	Geometric mean pairs[d] (9)	$r^2 = 0.785$ $p = 0.001$	14–207	2.4–31.3	0.824	−0.583

[a] r^2 and significance values for the line of best fit. Values for CF Equations [13,29] describe the database as it is presented in each respective manuscript. All remaining values are derived from the databases and analyses included in this assessment.
[b] Regression slope and *y*-intercept values presented in Table 3 describe a log-log relationship between nestling plasma total PCB concentration (x = ng/ml) and fresh or addled whole egg total PCB concentration (y = µg/g wet wt).
[c] Individual nestling plasma and egg sample pairings representative of exposure conditions within an active raptor nesting territory.
[d] Geometric mean nestling plasma and egg sample pairings representative of exposure conditions within a regional raptor breeding area.

Table 4. Plasma to egg conversion factors (CF) for *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE), Order Falconiformes

Species (subpopulation)	Reference	*p,p′*-DDE plasma to egg relationships					
		Sample pairing (*n*)	Strength[a]	Plasma sample range (ng DDE/ml)	Egg sample range (μg DDE/g) (wet wt)	Slope[b]	Intercept[b]
Bald eagle (Great Lakes)	[17]	Individual pairs[c] (31)	$r^2 = 0.324$ $p < 0.001$	3–361	0.1–29.9	0.676	−0.578
Bald eagle (Pacific Coast)	[13]	Geometric mean pairs[d] (6)	$r^2 = 0.912$ $p < 0.01$	7–100	2.2–9.7	0.637	−0.220
Bald eagle (Pacific Coast/Great Lakes)	[29]	Geometric mean pairs[d] (9)	$r^2 = 0.918$ $p < 0.001$	3–100	1.0–9.7	0.680	−0.318

[a] r^2 and significance values for the line of best fit. Values for conversion factor equations [13,29] describe the database as it is presented in each respective manuscript. All remaining values are derived from the databases and analyses included in this assessment.
[b] Regression slope and *y*-intercept values presented in Table 4 describe a log-log relationship between nestling plasma total PCB concentration (x = ng/ml) and fresh or addled whole egg total PCB concentration (y = μg/g wet wt).
[c] Individual nestling plasma and egg sample pairings representative of exposure conditions within an active raptor nesting territory.
[d] Geometric mean nestling plasma and egg sample pairings representative of exposure conditions within a regional raptor breeding area.

ΣDDT concentrations due to egg laying or transfer to eggs, similar to that observed for sparrow hawks. Studies of ΣDDT exposure were conducted during a period when researchers were investigating the egg-thinning effects of DDT and its metabolites DDD and DDE, and the maternal transfer of contaminates to eggs was of primary interest to researchers who were deciphering the mechanisms of action for this compound. Separate adult plasma (female) to egg conversion factors for ΣDDT were developed for wild, nesting kestrels (Falconidae) and accipiter hawks (Accipitridae) [12]. The parameters describing the two relationships were not statistically different for the two species. Therefore, those authors suggested use of a pooled regression to predict egg concentrations from concentrations of DDT in adult blood plasma for these two families. Use of the pooled data set in the log-log relationship for the two species resulted in a relationship that could be used to make comparisons among species. While these relationships provide useful background information, the DDT relationships were not compared to the relationships developed in this paper because they did not meet the selection criteria in the present study.

The pooled relationship developed for kestrel and accipiter hawks in one region [12] has been used to predict concentrations of ΣDDT and PCBs in eggs from concentrations in blood plasma from wintering adult eagles in Colorado and Missouri, USA [36]. The pooled relationship was used to predict concentrations of ΣDDT in eggs from plasma and to assess the potential for DDE-caused eggshell thinning and potential impacts to reproductive success of individual eagles. The egg to adult plasma ΣDDT relationship also was used to estimate the potential hazard to reproductive success of migrating peregrine falcons exposed to DDE on their wintering grounds [37]. The same relationship was used to predict concentrations of DDE in egg from concentrations of DDE in blood plasma of adult bald eagles so that the exposure of those eagles could be compared to addled eggs collected from the same subregion [38]. In that study predicted concentrations of DDE in eggs corresponded very well with measured values (RPDs <10%). However, concentrations of DDE in blood plasma were predicted from concentrations of DDE in whole blood that had been adjusted for loss of DDE during storage and dilution. The relationship developed for kestrels and accipiter hawks also was used in two separate studies to predict concentrations of ΣDDT in eggs of several South African raptors, including the

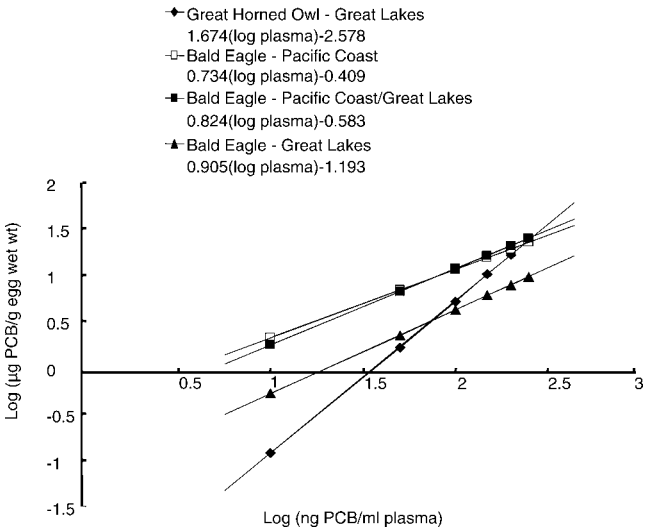


Fig. 5. Concentration of total polychlorinated biphenyls (PCBs) in eggs of great horned owls bald eagles as a function of PCBs in blood plasma of nestlings, of the same species, respectively. Regression lines are plotted and predictive equations are given in the figure legend.

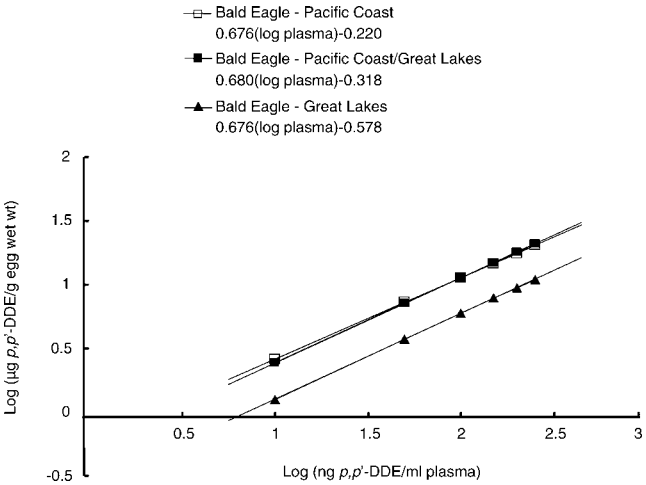


Fig. 6. Concentration of *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE) in eggs of bald eagles as a function of *p,p′*-DDE in blood plasma of nestlings, of the same species, respectively. Regression lines are plotted and predictive equations are given in the figure legend.

Table 5. Bald eagle and great horned owl (GHO) relative percent difference[a] (RPD) assessment for predicted versus measured egg polychlorinated biphenyl (PCB) concentrations across three randomly selected[b] cohorts (n = 10) and the plasma-restricted[c] cohort drawn from the Great Lakes bald eagle database[d]

Predicted vs measured PCB concentrations		Mean predicted RPD (%) from measured			
		Pacific Coast bald eagle [13]	Pacific Coast/Great Lakes bald eagle [29]	Great Lakes GHO [17]	Great Lakes bald eagle[e] [17]
Random sample no.1		100	97	93	77
Plasma range (ng PCB/ml)	16–866				
Egg range (μg PCB/g wet wt)	1–55				
Random sample no.2		92	90	74	73
Plasma range (ng PCB/ml)	16–773				
Egg range (μg PCB/g wet wt)	0.5–55				
Random sample no.3		70	73	83	78
Plasma range (ng PCB/ml)	106–866				
Egg range (μg PCB/g wet wt)	4–42				
Plasma-restricted sample		117	97	55	74
Plasma range (ng PCB/ml)		14–52 ng/ml	14–206 ng/ml	14–146 ng/ml	13–901 ng/ml
Egg range (μg PCB/g wet wt)		0.5–2.2 μg/g	0.5–362 μg/g	0.5–13 μg/g	0.5–67 μg/g
n		11	18	13	30

[a] RPD = $\{|Y_P - Y_M|/[(Y_P + Y_M)/2]\}\cdot(100)$. Y_P = predicted egg, Y_M= measured egg.
[b] Selected subsample identified using random number generator to select n = 10 plasma/egg paired samples from n = 30 (PCB) and n = 31 (DDE) possible individual paired data points in the Great Lakes bald eagle database.
[c] Plasma-restricted subsample includes all individual paired data points in the Great Lakes bald eagle database lying within the range of plasma concentrations used to derive each specific PCB and *p,p′*-DDE conversion factor databases examined in this manuscript. Sample sizes specific to the plasma-restricted subsample are noted below.
[d] See Table 2 for individually paired plasma and egg data.
[e] Great Lakes bald eagle database included for comparison purposes, illustrating effects of sample variability and site-specific exposure potentials on the intrinsic predictive accuracy of the line of best fit across the source database.

greater kestrel (*F. repicoloides*) and the lanner falcon (*F. biarmicus*), and pied kingfishers (*Ceryle rudis*). In both studies a screening-level hazard assessment was conducted by comparing the predicted concentration of ΣDDT to a toxicity reference value based on concentrations of DDE in the eggs of sensitive raptor and piscivorous species [39–42]. More recently, the USFWS and MDEQ [26] derived toxicity reference values based on concentrations of PCBs and DDE in blood plasma of nestling bald eagles using plasma to egg relationships [29] and field-based benchmarks (concentrations in eggs that were associated with reduced productivity) [43,44].

The major obstacle to assessing the predictive accuracy of the plasma to egg relationship is the absence of an extensive database containing individually matched plasma and egg con-

Table 6. Bald eagle relative percent difference[a] (RPD) assessment for predicted versus measured egg *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE) concentrations across three randomly selected cohorts[b] (n = 10) and the plasma-restricted cohort[c] drawn from the Great Lakes bald eagle database[d]

Predicted vs measured *p,p′*-DDE concentrations		Mean predicted RPD (%) from measured		
		Pacific Coast bald eagle [13]	Pacific Coast/Great Lakes bald eagle [29]	Great Lakes bald eagle[e] [17]
Random sample no.1		95	89	81
Plasma range (ng/ml)	3–39			
Egg range (μg/g wet wt)	0.1–15			
Random sample no.2		99	93	52
Plasma range (ng/ml)	3–361			
Egg range (μg/g wet wt)	0.1–15			
Random sample no.3		97	93	66
Plasma range (ng/ml)	4–361			
Egg range (μg/g wet wt)	0.3–15			
Plasma-restricted sample		105	99	77
Plasma range (ng DDE/ml)		7–83 ng/ml	3–83 ng/ml	3–361 ng/ml
Egg range (μg DDE/g wet wt)		0.1–15 μg/g	0.1–15 μg/g	0.1–30 μg/g
n		16	28	31

[a] RPD = $\{|Y_P - Y_M|/[(Y_P + Y_M)/2]\}\cdot(100)$. Y_P = predicted egg; Y_M= measured egg.
[b] Randomly selected subsample identified using random number generator to select of n = 10 plasma/egg paired samples from n = 30 (PCB) and n = 31 (DDE) possible individual paired data points in the Great Lakes bald eagle database.
[c] Plasma-restricted subsample includes all individual paired data points in the Great Lakes bald eagle database lying within the range of plasma concentrations used to derive each specific PCB and *p,p′*-DDE conversion factor databases examined in this manuscript. Sample sizes for the plasma-restricted subsample are noted below.
[d] See Table 2 for individually paired plasma and egg data.
[e] Great Lakes bald eagle database included for comparison purposes, illustrating effects of sample variability and site-specific exposure potentials on the intrinsic predictive accuracy of the line of best fit across the source database.

Table 7. Estimated polychlorinated biphenyl (PCB) concentration in egg (±95% PI)[a] from a relevant range of field-based nestling exposure or plasma concentrations

Plasma concentration (ng PCB/ml)	Predicted egg concentrations (μg PCB/g wet wt) total PCB exposure[b]							
	Individual paired samples				Geometric mean paired samples			
	Great Lakes great horned owl		Great Lakes bald eagle		Pacific Coast bald eagle		Pacific Coast/Great Lakes bald eagle	
	Slope	Intercept	Slope	Intercept	Slope	Intercept	Slope	Intercept
	1.674	−2.578	0.905	−1.193	0.734	−0.409	0.824	−0.583
10	0.12 (0.01–1.49)		0.52 (0.06–4.57)		2.11 (0.83–5.39)		1.74 (0.58–5.25)	
50	1.66 (0.17–15.8)		2.22 (0.27–17.9)		6.89 (2.98–16.0)		6.56 (2.46–17.5)	
100	5.20 (0.51–52.4)		4.15 (0.52–33.0)		*11.5 (4.28–30.8)*		11.6 (4.17–32.4)	
150	10.1 (0.92–111)		5.99 (0.75–47.7)		*15.43 (5.12–46.7)*		16.2 (5.53–47.6)	
200	*16.3 (1.36–193)*		7.77 (0.97–62.2)		*19.1 (5.76–63.4)*		20.6 (6.7–63.2)	
250	*23.5 (1.83–300)*		9.51 (1.18–76.5)		*22.4 (6.28–80.7)*		*24.7 (7.73–79.1)*	

[a] Upper and lower 95% prediction interval (PI) for predicted egg *y*. Predicted egg concentrations (±95% PIs) for plasma values lying beyond the range of data used to derive each respective conversion factor are in italics.
[b] Regression slope and *y*-intercept values describe a log-log relationship for total PCB concentrations in plasma (ng PCB/ml) and egg (μg PCB/g wet wt).

centrations from the same nest. The database assembled from the USFWS addled egg monitoring [19] and the MDEQ plasma sampling [20–23] that is presented here provided a basis for this evaluation. The predictive variability RPD results indicate that applying the conversion factor between species (GHO to bald eagle) and among geographic regions (Pacific Coast to Great Lakes) yielded predicted egg concentrations that are within the natural variability observed for residue concentrations among eggs of raptors within a species and region. Within-clutch RPD values for total organochlorine pesticide concentrations in nonmigratory sparrow hawks in Great Britain have been observed to be as great as 32%, while mean concentration (clutch means) between-clutch RPD values from the same female on the same territory are as great as 63% [45]. Relative percent difference values as great as 171% for PCBs (3-year interval) and 80% for DDE (4-year interval) have been seen for sparrow hawks from a subpopulation of females nesting within the same geographic subregion [46]. Studies of PCB and DDE concentrations in eggs sampled from the same population of eagles nesting in the vicinity of Green Bay exhibited between-clutch variability that ranged from 38% (1-year interval) to 125% (4-year interval) RPD for PCBs, and from 31% (1-year interval) to 133% (4-year interval) RPD for *p,p′*-DDE [43].

Utility and application

There is variability inherent in predicting concentrations of PCBs and *p,p′*-DDE in eggs using the egg to plasma relationships for GHO and bald eagle presented in the present assessment. The predicted values and widely bounded 95% prediction intervals for a relevant range of nestling blood plasma concentrations illustrate the homogeneity of slopes and heterogeneity of elevations among the four PCB and three DDE relationships (Tables 7 and 8). The predicted PCB and DDE egg concentrations for the Pacific Coast bald eagles show a consistent difference from the Great Lakes eagle and GHO predicted values. Even at their greatest, the observed divergences represent only a three-fold difference in the range of predicted egg concentrations that would be significant for an ecological risk assessment (e.g., at or above the toxicity reference value threshold concentration). This indicates that for ecological risk assessment applications, the use of a plasma

Table 8. Estimated *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE) concentrations (±95% PI)[a] in egg from a relevant range of field-based nestling exposure/plasma concentrations

Plasma concentration (ng DDE/ml)	Predicted egg concentrations (μg DDE/g wet wt) *p,p′*-DDE exposure[b]					
			Geometric mean paired samples			
	Individual pairs Great Lakes bald eagle		Pacific Coast bald eagle		Pacific Coast/Great Lakes bald eagle	
	Slope	Intercept	Slope	Intercept	Slope	Intercept
	0.676	−0.578	0.637	−0.220	0.668	−0.282
10	1.26 (0.08–20.0)		2.61 (1.29–5.29)		2.43 (1.34–4.41)	
50	3.72 (0.22–61.9)		7.28 (3.52–15.0)		7.13 (3.86–13.2)	
100	5.94 (0.34–105)		11.3 (5.03–24.4)		11.3 (5.85–21.9)	
150	7.81 (0.42–144)		*14.7 (6.1–35.1)*		*14.9 (7.39–29.8)*	
200	9.48 (0.50–180)		*17.6 (6.95–44.4)*		*18.0 (8.70–37.2)*	
250	11.0 (0.56–216)		*20.3 (7.68–53.4)*		*20.9 (9.86–44.2)*	

[a] Upper and lower 95% prediction interval for predicted egg *y*. Predicted egg concentrations (±95% PIs) for plasma values lying beyond the range of data used to derive each respective conversion factor are in italics.
[b] Regression slope and *y* intercept values describe a log:log relationship for *p,p′*-DDE concentrations in plasma (ng DDE/ml) and egg (μg DDE/g, wet wt).

to egg conversion factor would introduce minimal levels of uncertainty to calculations of risk.

CONCLUSION

Birds will continue to be used as indicators of environmental contamination due to their ubiquitous global distributions, high metabolic rates, and diverse foraging habits. The advantages that bird eggs provide as a medium for assessing the bioavailability of lipophilic contaminants will undoubtedly encourage the selection of egg-based sampling programs and toxicity reference values will continue to be based on concentrations of residues in eggs. To minimize effects on populations and maximize the site-specific assessment of exposures in some cases, measurement of residues in blood plasma will be more practical.

We have demonstrated that concentrations of residues in blood plasma can be used to predict concentrations of persistent and bioaccumulative compounds in eggs by use of a blood plasma to egg conversion factor. The egg to plasma relationships derived herein from individually paired great horned owl and bald eagle samples in Great Lakes populations are not statistically dissimilar from comparable egg to plasma relationships provided in the literature for Pacific Coast bald eagles. These findings also indicate that raptors express similar relationships between nestling plasma and egg concentrations across closely related avian orders and across geographically isolated subpopulations. The plasma to egg conversion factor can be used as an accurate and reliable tool to translate nondestructive plasma-based contaminant exposure measurements to comparable egg-based concentrations. These calculated egg-basis concentrations can then be used with egg-based toxicity reference values derived from population-level benchmark effects (e.g., embryo mortality, developmental deformities, fledgling productivity) to assess the health of animal communities. The plasma to egg conversion factor also offers ecological risk assessors an additional tool to aid with interpretations of dissimilar data. It is our hope that by incorporating plasma-based sampling protocols into long-term monitoring plans and site-specific hazard assessments that this method will contribute to more efficient and less disruptive studies of raptor populations in all habitats.

Acknowledgement—The authors thank the many private landowners in the Kalamazoo River floodplain, all of whom generously provided access to their properties for the great horned owl study, most notably Jerry and Anne Peterson, Jerry and Kathy Arnett, and Shirley, Brent, and Sadie Foster. Training and support received from Jim Sikarskie, DVM and Simon Hollamby of the Michigan State University College of Veterinary Medicine, Wildlife Rehabilitation Center, was essential to the success of this study. Additional aid was provided by Dave Best of the USFWS and Nina Consolatti of the Michigan State University Kellogg Biological Station. We thank Charles Mehne and Karen Smyth. Funding for this study was provided by the Kalamazoo River Study Group.

REFERENCES

1. Giesy JP, Bowerman WW, Mora MA, Verbrugge DA, Othoudt RA, Newsted JL, Summer CL, Aulerich RJ, Bursian SJ, Ludwig JP, Dawson GA, Kubiak TJ, Best DA, Tillitt DE. 1995. Contaminants in fishes from Great Lakes-influenced sections and above dams of three Michigan rivers: Implications for health of bald eagles. *Arch Environ Contam Toxicol* 29:309–321.
2. Sheffield SR. 1997. Owls as biomonitors of environmental contamination. NC-190. General Technical Report. U.S. Forest Service, North Central Research Station, St. Paul, MN.
3. International Joint Commission. 1991. Report of the ecological committee's biological effects subcommittee to the Great Lakes science advisory board. *Proceedings*, Expert Consultation Meeting on Bald Eagles, Windsor, Ontario, Canada, February 12–13, 1990, p 33.
4. RETEC Group. 2002. Final baseline human health and ecological risk assessment. Lower Fox River and Green Bay, Wisconsin Remedial Investigation and Feasibility Study. December. Wisconsin Department of Natural Resources, Madison, WI, USA.
5. Fox GA. 2001. Wildlife as sentinels of human health effects in the Great Lakes—St. Lawrence basin. *Environ Health Perspect* 109:853–861.
6. Hoffman DJ, Melancon MJ, Klein PN, Eisemann JD, Spann JW. 1998. Comparative developmental toxicity of planar polychlorinated biphenyl congeners in chickens, American kestrels, and common terns. *Environ Toxicol Chem* 17:747–757.
7. Summer CL, Giesy JP, Bursian SJ, Render JA, Kubiak TJ, Jones PD, Verbrugge DA, Aulerich RJ. 1996. Effects induced by feeding organochlorine-contaminated carp from Saginaw Bay, Lake Huron to laying white leghorn hens. I. Effects on health of adult hens, egg production, and fertility. *J Toxicol Environ Health* 39: 389–407.
8. Summer CL, Giesy JP, Bursian SJ, Render JA, Kubiak TJ, Jones PD, Verbrugge DA, Aulerich RJ. 1996. Effects induced by feeding organochlorine-contaminated carp from Saginaw Bay, Lake Huron to laying white leghorn hens. II. Embryonic and teratogenic effects. *J Toxicol Environ Health* 39:407–438.
9. McLane MAR, Hall LC. 1972. DDE thins screech owl eggshells. *Bull Environ Contam Toxicol* 8:65–68.
10. Martin PA, DeSolla SR, Ewins P. 2003. Chlorinated hydrocarbon contamination in osprey eggs and nestlings from the Canadian Great Lakes Basin, 1991–1995. *Ecotoxicology* 12:209–224.
11. Olsson A, Ceder K, Bergman A, Helander B. 2000. Nestling blood of the white-tailed sea eagle (*Haliaeetus albicilla*) as an indicator of territorial exposure to organohalogen compounds—An evaluation. *Environ Sci Technol* 34:2733–2740.
12. Henny CJ, Meeker DL. 1981. An evaluation of blood plasma for monitoring DDE in birds of prey. *Environ Pollut Ser A Ecol Biol* 25:291–304.
13. Elliott JE, Norstrom RJ. 1998. Chlorinated hydrocarbon contaminants and productivity of bald eagle populations on the Pacific Coast of Canada. *Environ Toxicol Chem* 17:1142–1153.
14. Bowerman WW. 1993. Regulation of bald eagle (*Haliaeetus leucocephalus*) productivity in the Great Lakes Basin: An ecological and toxicological approach. PhD thesis. Michigan State University, East Lansing, MI, USA.
15. Donaldson GM, Shutt JL, Hunter P. 1999. Organochlorine contamination in bald eagle eggs and nestlings from the Canadian Great Lakes. *Arch Environ Contam Toxicol* 36:70–80.
16. Blankenship AL, Zwiernik MZ, Coady KK, Kay DP, Newsted JL, Strause K, Park C, Bradley PW, Neigh AM, Millsap SD, Jones PD, Giesy JP. 2005. Differential accumulation of polychlorinated biphenyl congeners in the terrestrial food web of the Kalamazoo River superfund site, Michigan. *Environ Sci Technol* 39:5954–5963.
17. Strause KD. 2006. Exposure of great horned owls (*Bubo virginianus*) and bald eagles (*Haliaeetus leucocephalus*) to PCBs and DDTs at the Kalamazoo River superfund site, Michigan, USA. PhD thesis. Michigan State University, East Lansing, MI, USA.
18. Stickel J, Weimeyer SN, Blus LJ. 1973. Pesticide residues in eggs of wild birds: Adjustment for loss of moisture and lipid. *Bull Environ Contam Toxicol* 9:193–196.
19. Best DA. 2002. Monitoring of bald eagle exposure to organochlorine compounds in the Great Lakes via addled eggs, 1986–2000. Environmental Contaminants Program, U.S. Fish and Wildlife Service, East Lansing Field Office, MI.
20. Roe A, Birrenkoff A, Bowerman W, Bush D, Sikarskie J. 2004. Michigan wildlife trend monitoring: Year 2002 annual report: Nestling bald eagles. MI/DEQ/WD-04/024. Michigan Department of Environmental Quality, Lansing, MI, USA.
21. Roe A, Birrenkoff A, Bowerman W, Bush D, Sikarskie J. 2004. Michigan wildlife trend monitoring: Year 2001 annual report: Nestling bald eagles. MI/DEQ/WD-04/012. Michigan Department of Environmental Quality, Lansing, MI, USA.
22. Roe A, Bowerman W, Bush D, Sikarskie J. 2003. Michigan wildlife trend monitoring: Year 2000 annual report: Nestling bald eagles. MI/DEQ/WD-03/088. Michigan Department of Environmental Quality, Lansing, MI, USA.
23. Summer CL, Bush D, Bowerman W. 2002. Michigan wildlife

trend monitoring: Year 1999 annual report: Nestling bald eagles. MI/DEQ/WD-02/023. Michigan Department of Environmental Quality, Lansing, MI, USA.
24. Dykstra CR, Meyer MW. 1996. Effects of contaminants on reproduction of bald eagles on Green Bay, Lake Michigan, Wisconsin. Wisconsin Department of Natural Resources, Rhinelander, WI, USA, and U.S. Fish and Wildlife Service, Green Bay, WI.
25. Dykstra CR, Meyer MW, Stromborg KL, Warnke DK, Bowerman WW, Best DA. 2001. Association of low reproductive rates and high contaminant levels in bald eagles on Green Bay, Lake Michigan. *J Gt Lakes Res* 27:239–251.
26. Stratus Consulting. 2005. Stage I Assessment Report, Volume I. Injury assessment: Kalamazoo river environment. Final Report. Michigan Department of Environmental Quality, Michigan Attorney General, U.S. Fish and Wildlife Service, and National Oceanic and Atmospheric Administration, Ann Arbor, MI.
27. Zar JH. 1999. *Biostatistical Analysis*, 4th ed. Prentice Hall, Upper Saddle River, NJ, USA.
28. Blus LJ. 1984. DDE in birds' eggs: Comparison of two methods for estimating critical levels. *Wilson Bull* 96:268–276.
29. Elliott JE, Harris ML. 2002. An ecotoxicological assessment of chlorinated hydrocarbon effects on bald eagle populations. *Rev Toxicol* 4:1–60.
30. Henny CJ, Riddle KE, Hulse CS. 1988. Organochlorine pollutants in plasma of spring migrant peregrine falcons from coastal Texas, 1984. In Cade TJ, Enderson JH, Thelander CG, White CM, eds, *Peregrine Falcon Populations: Their Management and Recovery.* The Peregrine Fund, San Francisco, CA, USA, pp 423–427.
31. Dykstra CR, Meyer MW, Warneke DK, Karasov WH, Andersen DE, Bowerman WW, Giesy JP. 1998. Low reproductive rate of Lake Superior bald eagles: Low food delivery rates or environmental contaminants? *J Gt Lakes Res* 24:32–44.
32. Elliott JE, Wilson LK, Henny CJ, Trudeau SF, Leighton FA, Kennedy SW, Cheng KM. 2001. Assessment of biological effects of chlorinated hydrocarbons in osprey chicks. *Environ Toxicol Chem* 20:866–879.
33. Hoffman DJ, Melancon MJ, Klein PN, Rice CP, Eisemann JD, Hines RK, Spann JW, Pendleton GW. 1996. Developmental toxicity of PCB 126 (3,3′,4,4′,4-pentachlorobiphenyl) in nestling American kestrels (*Falco sparverius*). *Fundam Appl Toxicol* 34: 188–200.
34. Henny CJ. 1977. Birds of prey, DDT, and tussock moths in Pacific Northeast. *Trans N Am Wildl Nat Resour Conf* 42:397–411.
35. Bogan JA, Newton I. 1977. Redistribution of DDE in sparrowhawks during starvation. *Bull Environ Contam Toxicol* 18:317–321.
36. Henny CJ, Griffin CR, Stahlecker DW, Harmata AR, Cromartie E. 1981. Low DDT residues in plasma of bald eagles (*Haliaeetus leucocephalus*) wintering in Colorado and Missouri. *Can Field-Nat* 95:249–252.
37. Henny CJ, Ward FP, Riddle KE, Prouty RM. 1982. Migratory peregrine falcons, (*Falco peregrinus*) accumulate pesticides in Latin America during winter. *Can Field-Nat* 96:333–337.
38. Frenzel RW. 1985. Environmental contaminants and ecology of bald eagles in southcentral Oregon. PhD thesis. Oregon State University, Corvallis, OR, USA.
39. Newton I. 1979. *Population Ecology of Raptors.* Buteo Books, Vermillion, SD, USA.
40. Fasola M, Vecchio I, Caccialanza G, Gandini C, Kitsos M. 1987. Trends of organochlorine residues in eggs of birds from Italy, 1977 to 1985. *Environ Pollut* 33:25–36.
41. Evans SW, Bouwman H. 2000. The geographic variation and potential risk of DDT in the blood of pied kingfishers from northern KwaZulu-Natal, South Africa. *Ostrich* 71:351–354.
42. Smith I, Bouwman H. 2000. Levels of organochlorine pesticides in raptors from the North-West province, South Africa. *Ostrich* 71:36–39.
43. Stratus Consulting. 1999. Injuries to Avian Resources, Lower Fox River/Green Bay Natural Resource Damage Assessment. Final Report. U.S. Fish and Wildlife Service, Green Bay, WI.
44. Wiemeyer SN, Bunck CM, Stafford CJ. 1993. Environmental contaminants in bald eagle eggs—1980–1984—and further interpretations of relationships to productivity and shell thickness. *Arch Environ Contam Toxicol* 24:213–227.
45. Newton I, Bogan J. 1978. The role of different organo-chlorine compounds in the breeding of British sparrowhawks. *J Appl Ecol* 15:105–116.
46. Newton I, Bogan JA, Rothery P. 1986. Trends and effects of organochlorine compounds in sparrowhawk eggs. *J Appl Ecol* 23: 461–478.

Integrated Environmental Assessment and Management — Volume 4, Number 1—pp. 24–40
© 2008 SETAC

Original Research

Risk Assessment Methodologies for Exposure of Great Horned Owls (*Bubo virginianus*) to PCBs on the Kalamazoo River, Michigan

Karl D Strause,† Matthew J Zwiernik,† John L Newsted,†‡ Arianne M Neigh,† Stephanie D Millsap,† Cyrus S Park,† Pamela P Moseley,† Denise P Kay,‡ Patrick W Bradley,† Paul D Jones,† Alan L Blankenship,†‡ James G Sikarskie,§ and John P Giesy†||#*

†Zoology Department, Center for Integrative Toxicology, National Food Safety and Toxicology Center, Michigan State University, East Lansing, Michigan 48824, USA
‡ENTRIX, Okemos, Michigan 48823, USA
§College of Veterinary Medicine, Center for Integrative Toxicology, Wildlife Rehabilitation Center, Michigan State University, East Lansing, Michigan 48824, USA
||Department of Biomedical Veterinary Sciences and Toxicology Center, University of Saskatchewan, Saskatoon, Saskatchewan S7K 5B3, Canada
#Biology and Chemistry Department, City University of Hong Kong, Kowloon, Hong Kong, SAR, China

(Received 27 February 2007; Accepted 13 September 2008)

ABSTRACT

Dietary exposures of great horned owls (GHO; *Bubo virginianus*) to polychlorinated biphenyls (PCBs) in the terrestrial food web at the Kalamazoo River, Michigan, USA, were examined. Average potential daily doses (APDD) in GHO diets were 7- to 10-fold and 3-fold greater at the more contaminated location versus a reference location for site-specific exposures quantified as total PCBs and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$), respectively. Wetland/aquatic prey contributed significantly to PCB exposure and APDD. Estimates of risk based on comparison of modeled dietary intake (e.g., APDD) to toxicity reference values (TRVs), using a hazard quotient (HQ) methodology, varied between diet composition methods (mass basis vs numeric basis). Mass-basis compositions yielded greater HQs at all sites. Potential risks associated with dietary exposures ("bottom-up" risk assessment methodology) were less than (HQ $<$ 1) benchmarks for effects,. This result is consistent with risk estimates based on concentrations in tissues ("top-down" risk assessment methodology), and indicated PCBs posed no significant risk to terrestrial raptor species. Colocated and concurrent studies that evaluated GHO reproductive performance (nestling productivity) and relative abundance were consistent with results of the risk assessment. Measures of risk based on HQs were consistent with direct measures of ecologically relevant endpoints (reproductive fitness). Uncertainty in risk estimates is contributed during the selection of TRVs for effects in GHO based on $TEQ_{WHO\text{-}Avian}$ because of the absence of species-specific, dose-response thresholds. This evaluation indicated that a multiple-lines-of-evidence approach provided the best estimate of risk.

Keywords: Raptors Dietary exposure Bioaccumulation 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin

INTRODUCTION

The great horned owl (GHO; *Bubo virginianus*) is a useful sentinel species for site-specific baseline ecological risk assessments at sites with large contiguous areas of contaminated environmental media. Their sensitivity to the toxic effects of some organic contaminants, such as organochlorine, organophosphate, and carbamate pesticides and their relatively great exposure as apex predators makes them valuable as a surrogate species for estimating risk to raptors in the terrestrial food chain (Sheffield 1997). Great horned owls have been used successfully in site-specific estimates of risk posed by polychlorinated biphenyls (PCBs) at the Kalamazoo River Superfund Site (KRSS) in Kalamazoo and Allegan Counties, Michigan, USA (Strause et al. 2007). This previous study utilized a "top-down" or "tissue-based" approach in which exposure was determined by measuring concentrations of PCBs in eggs and blood plasma of nestlings. The potential for risks from exposure to PCBs were assessed in a multiple-lines-of-evidence ecological risk assessment that included both comparing the measured concentrations of PCBs to toxicity reference values (TRVs) as well as concurrent measures of productivity and abundance in a "weight of evidence" to identify cause and effect linkages between the chemical stressor and any observed suboptimal population or community structure at the site (Fairbrother 2003).

A 2nd method for assessing potential risk to wildlife uses a predictive approach in which the exposure is inferred by measuring concentrations of the chemicals of concern (COCs), such as PCBs, in matrices other than the receptor species of interest. This predictive approach is often referred to as the "bottom-up" or "dietary-based" approach. In this method the exposure-response is predicted by use of food chain modeling, based on site-specific measures of concentrations of the COCs in wildlife food items or sediments or soils. In some cases, the actual dietary items can be identified via diet studies and quantified via forage base and food item sampling programs, while in other situations default or average values are used. Data specific to dietary composition and prey item COC concentrations are combined with receptor species' ingestion rate and body weight parameters

*To whom correspondence may be addressed: strausek@msu.edu
Published on the Web 9/13/2007.

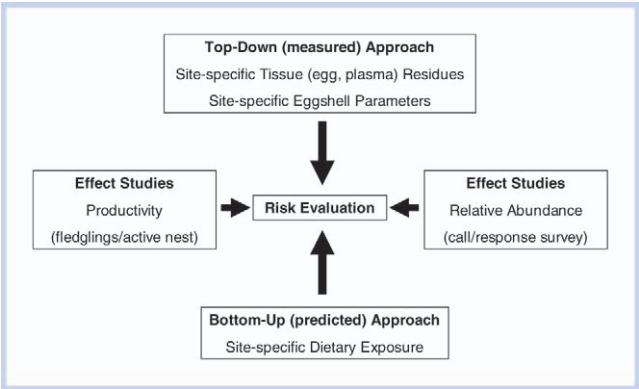


Figure 1. Multiple-lines-of-evidence used to assess risk to resident Kalamazoo River Superfund Site great horned owl (*Bubo virginianus*) populations.

to compute an average potential daily dose (APDD). This estimated daily dose is then compared to a dietary TRV to assess potential risks at the site. This study described the site-specific dietary exposure pathways to PCBs for GHOs at the KRSS. Site-specific dietary exposures (expressed as APDD) were then compared to TRVs for adverse effects determined in controlled laboratory studies to calculate hazard quotients (HQs) based on predicted exposure through diet. Additionally, the site-specific method of estimating dietary exposures was compared to a literature-derived APDD computed using diet composition values for an off-site GHO subpopulation residing in southeast Michigan (Craighead and Craighead 1956).

Due to limitations in the time and or resources available, few assessments apply both risk assessment methods simultaneously at the same location. Most assessments use the predictive method. While the 2 approaches are inherently linked, the accuracy and precision of the 2 methods are seldom compared. Therefore, the overall object of this study was to use a multiple-lines-of-evidence approach to evaluate the results of the predictive assessment approach with actual measurements of exposure (PCB concentrations in tissues, eggshell thickness) and population-level effects such as abundance or productivity at the same time at the same location (Figure 1). The 2 established methodologies of risk assessment (i.e., top-down vs bottom-up) were compared to determine how similar the predictions of risk would be based on both total PCBs, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) equivalents (TEQ) calculated from aryl hydrocarbon receptor-active PCBs. The equivalents are calculated by summing the products of concentrations of individual non-*ortho* (coplanar) (PCB 77, 81, 126, 169) and mono-*ortho* PCB congeners (PCB 105, 118, 156, 157, 167) and their respective World Health Organization (WHO) 2,3,7,8-TCDD toxic equivalency factors for avian receptors (Van den Berg et al. 1998). Avian-specific 2,3,7,8-TCDD toxic equivalents ($TEQ_{WHO\text{-}Avian}$) provide an estimate of potential risk from exposures to the most toxic PCB congeners.

Meeting these objectives required completion of the following:

1. Collection of GHO pellet and prey remains samples from active nest sites to identify dietary components and enumerate dietary composition;
2. Collection of representative prey item samples for the categories of prey (e.g., passerine birds, mice/voles) that contributed most significantly to GHO diet;
3. Determination of concentrations of PCBs and $TEQ_{WHO\text{-}Avian}$ based on congener-specific measurements;
4. Calculation and comparison of HQs based on total PCBs and total $TEQ_{WHO\text{-}Avian}$ between site-specific and literature-based diets;
5. Comparisons of bottom-up and top-down estimates of risk based on total PCBs and $TEQ_{WHO\text{-}Avian}$ using the HQ methodology; and
6. Incorporation of both the top-down and bottom-up approaches into a multiple-lines-of-evidence assessment that includes concurrent investigation of GHO relative abundance and reproductive productivity at each study site.

Additionally, information on the PCB/$TEQ_{WHO\text{-}Avian}$ concentrations between prey categories and food web sources (e.g., terrestrial vs. aquatic) was also assessed.

METHODS AND MATERIALS

Study sites

The KRSS includes 123 km of river extending from the city of Kalamazoo to Lake Michigan at Saugatuck, Michigan, USA. The primary COCs are PCBs, including total $TEQ_{WHO\text{-}Avian}$ from coplanar and mono-*ortho* PCB congeners. Polychlorinated biphenyls were used in the production of carbonless copy paper and paper inks for approximately 15 y (USEPA 1976). During this period, recycling of paper, including some carbonless copy paper resulted in releases of PCBs to the Kalamazoo River. The Kalamazoo River was placed on the Superfund National Priorities List in August 1990 due to the presence of elevated PCB concentrations in fish, sediments, and floodplain soils (BBL 1993).

Two sites within the KRSS were chosen for the GHO dietary study (Figure 2). These included the Fort Custer State Recreation Area (FC) and the former Trowbridge Impoundment (TB). Characterizations of these sites have been provided in earlier assessments of GHO exposure at the KRSS (Strause et al. 2007). The FC site is a reference area located approximately 7 km upstream of Morrow Pond Dam (the upstream limit of the KRSS) and 40 km upstream of TB. The FC site contains floodplain habitat similar to that present at TB and represents "current" regional background exposures in the watershed. The TB site is located in the Upper KRSS downstream of the point sources in the KRSS and it is 1 of 3 former impoundments in the Upper KRSS where removal of an in-stream dam to sill level has exposed former river sediments that are now heavily vegetated floodplain soils and riparian wetland habitat. The former TB impoundment includes the greatest area of contaminated soils (132 ha) and the greatest mean PCB soil concentrations (1.5×10^4 ng/g dry weight [dw]) in the river floodplain. The FC and TB sites were selected to make direct comparisons between GHO responses on a "high potential exposure" versus background "no elevated exposure" basis.

The GHO populations studied at each site were restricted to mated pairs occupying natural nests and artificial nesting platforms within the 100-y floodplain. The propensity for GHOs to use artificial nesting platforms allowed for better experimental control compared to wildlife studies that rely exclusively on natural nests. Nest platforms were placed throughout the FC and TB sites, and at TB the artificial platforms were placed to provide for a "worst-case exposure"

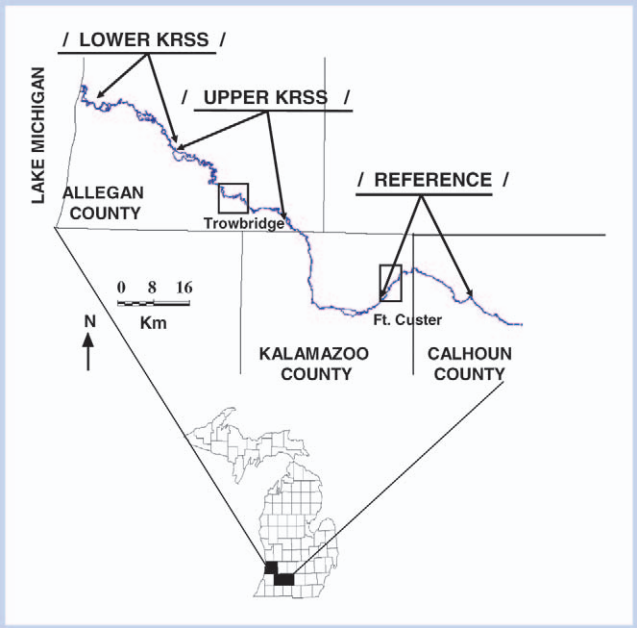


Figure 2. Kalamazoo River great horned owl (*Bubo virginianus*) study sites including the Reference sampling location (Ft. Custer), the Upper Kalamazoo River Superfund Site (Trowbridge), and Lower Kalamazoo River Superfund Site (LKRSS) sampling locations.

by maximizing GHO foraging in the most expansive areas of the contaminated floodplain.

Sample collection

The studies of GHO exposure to PCBs at the KRSS were part of a broader study to investigate PCB congener bioaccumulation and dynamics in the terrestrial and aquatic food webs of the Kalamazoo River floodplain that included representative samples from all tropic levels in resident terrestrial and aquatic communities (Millsap et al. 2004; Blankenship et al. 2005; Kay et al. 2005; Neigh, Zwiernik, Blankenship, et al. 2006; Neigh, Zwiernik, Bradley, Kay, Jones, et al. 2006; Neigh, Zwiernik, Bradley, Kay, Park, et al. 2006). All sample collections were completed within the 100-y floodplain. Representative taxa included raptors (owls and eagles), passerine birds, aquatic and terrestrial mammals, fish, terrestrial and aquatic invertebrates, plants and colocated soil, and sediment samples (Blankenship et al. 2005; Kay et al. 2005). The principal components of the GHO food chain in the KRSS floodplain are likely to include terrestrial mammals and terrestrial passerine or aquatic birds, although limited numbers of aquatic invertebrates also may be eaten (Figure 3).

Pellets and prey remains

Site-specific studies of GHO diet were completed only at active nest sites. Pellets and prey remains were collected to determine the principal prey items that comprised the diet of nestlings. Diet investigations were undertaken in conjunction with other GHO study objectives that included collection of blood plasma from nestling GHOs and monitoring of productivity (Strause et al. 2007). To minimize nest disturbances and to avoid bias this required that collection of pellets and prey remains were coordinated with these other investigations. Pellet and prey remains were collected from the nest, the base of the nest tree and beneath adult perch trees during the nestling blood sampling event. Additional samples were collected from the base of the nest tree and beneath feeding perches after the nestling GHOs fledged from the nest (2–3 weeks after blood was collected), and on 10-d intervals thereafter until no more samples could be collected. A final sample was collected from the nest and associated feeding perches during a "post-fledge" nest climb to clean and maintain the artificial nesting platform. Post-fledge climbs occurred between 4 and 10 weeks after the young had fledged. During each collection event, pellets and prey remains were systematically and completely removed from each location and packaged in a plastic jar as a composite sample representative of the specific collection site (e.g., nest, nest tree base, feeding/roosting perch). Samples were labeled to identify the nesting pair, dated and transported to the laboratory where they were exposed to naphthalene while drying to eliminate invertebrate scavengers. Prior to processing, pellet samples were sterilized by autoclave.

During each field season, all pellets and prey remains collected at an active nest were processed into 2 separate composite samples. Each composite sample was constructed around collections made during the 2 separate nest climbs completed at each active nest (nestling banding/plasma sample collection; post-fledge nest climb). Each composite included all collections from the ground that were completed concurrent with the nest climb and prey remains/pellet collections from the nest. Ground collections made subsequent to the banding event were combined into the 2nd (post-fledge nest climb) composite sample. This compositing scheme provided for reconciling the presence of large prey and bird remains among the ground and nest collections thus reducing the chance of overestimating the frequency of occurrence of large prey species because of their tendency to be represented in more than 1 pellet or prey sample (Marti 1974). The 2 distinct composite samples were processed independently and the prey items identified in each were summed to arrive at a final count for each nesting event.

Prey item identification/dietary composition

Relative proportions of prey items in the site-specific diet were determined by examining unconsumed prey remains (bones, fur, and feathers of animals too large to consume whole) as well as the skeletal remains in regurgitated pellets (Errington 1932, 1938; Hayward et al. 1993). All identifiable remains were sorted and quantified as to the minimum number of individuals from each taxon necessary to account for the assemblage of remains present in any given composite of samples. For mammalian prey items too large for owls to swallow whole (~100 g) and avian prey, the remains of the same prey item were frequently present in multiple samples. When this occurred, the items from within each discrete sampling event were examined together to reconcile the frequency of occurrence of larger prey and birds. Multiple prey item identification keys were utilized for comparative identification of mammalian and avian remains including owl pellet identification keys (Carolina Biological Supply, Burlington, NC, USA) and the vertebrate skeletal collection from the Michigan State University (MSU) museum. Avian remains (feathers) were identified with the aid of MSU Kellogg Biological Station bird sanctuary personnel. Prey items were identified to the best practical taxonomic classification and grouped by species/family and order into 7 prey categories relating to food web (aquatic vs terrestrial) and trophic level (primary vs secondary consumers) position.

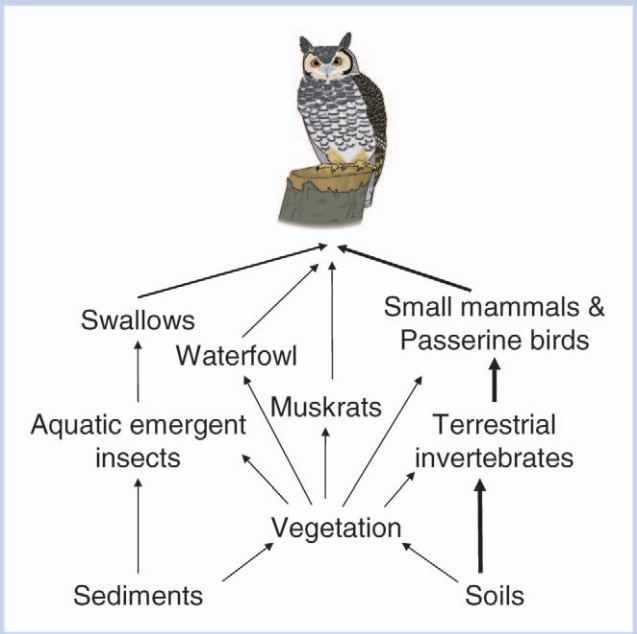


Figure 3. Great horned owl (*Bubo virginianus*) food chain and exposure pathways at the Kalamazoo River Superfund Site (KRSS).

These categories included: Passerine (terrestrial avian), waterfowl (aquatic avian), mice/vole (terrestrial primary consumers, small mammal), shrew (terrestrial secondary consumers, small mammal), muskrat (aquatic primary consumers, medium-size mammal), rabbit/squirrel (terrestrial primary consumers, medium-size mammal), and crayfish (detritivor/aquatic primary consumer, invertebrate).

The estimated dietary composition was based on the frequency of occurrence of all identifiable prey items and compiled on the basis of percent composition on a numeric basis (% number) and percent composition on a biomass basis (% biomass; Wink et al. 1987). Percent biomass was calculated by multiplying each identified prey item by the mean adult weight (male + female) for the particular species or family (Dunning 1984; Baker 1983). The small number of individual prey items that could not be positively identified to family or order was limited to unidentifiable parts of terrestrial birds and medium-size mammals. For biomass calculations, these items were assigned a mass value equal to the average mass computed for the representative species identified for that category at each respective nest site.

Prey collections for chemical analyses

Prey species represented in site-specific and literature-based GHO diets were collected from the FC and TB study sites and analyzed for total PCBs. Analyses of pellet and prey remains samples collected from FC and TB identified 6 general categories of GHO prey. These included passerine birds, waterfowl, mice/voles, shrews, muskrats, and rabbit/squirrel. A 7th category, crayfish, is represented in the literature-based diet and included in the diet analysis. Field sampling and processing methods for representative individuals from each of the 7 prey categories are described below.

Passerine birds collected from the FC and TB sites included the tree swallow (*Tachycineta bicolor*), house wren (*Troglodytes aedon*), and American robin (*Turdus migratorius*). A single European starling (*Sturnus vulgaris*) also was collected at TB. All live birds were collected at the end of the nesting period. Adult wrens and swallows were captured with mist nets or a trap-door mechanism. Additionally, dead individuals found at nest boxes were salvaged for analyses (Neigh, Zwiernik, Bradley, Kay, Jones, et al. 2006; Neigh, Zwiernik, Bradley, Kay, Park, et al. 2006). Adult robins were collected using pellet guns (MSU Aquatic Toxicology Laboratory, unpublished data). The starling (carcass) was recovered beneath an active GHO nest. Birds were promptly euthanized by cervical dislocation and carcasses were placed in solvent-rinsed sample jars and frozen at −20 °C. For chemical sample analysis, feathers, beaks, wings, legs, and stomach contents were removed and the whole body was homogenized in a solvent-rinsed grinder.

Waterfowl species sampled included merganser (*Mergus* spp.), mallard (*Anas platyrhynchos*), wood duck (*Aix sponsa*), and blue-winged teal (*Anas discors*). Waterfowl sampling was not included in the MSU Kalamazoo River food web investigations. Waterfowl samples used in this GHO diet exposure study were collected in August 1985 by the US Fish and Wildlife Service. The US Fish and Wildlife Service collected adult and immature ducks from 5 locations in the KRSS. Sampling locations included Morrow Pond and the Menasha and Trowbridge impoundments in the Upper KRSS, and the Allegan State Game Area and Saugatuck Lake downstream of Allegan Dam in the Lower KRSS. For chemical analysis the feathers, beaks, and entrails were removed and the remaining carcass was homogenized in a solvent-rinsed grinder (MDNR 1987).

Small mammals collected included mice (*Peromyscus* spp., *Zapus hudsonius*), voles (*Microtus pennsylvanicus*), shrews (*Sorex cinereus*, *Blarina brevicauda*), red squirrels (*Tamiasciurus hudsonicus*), and chipmunks (*Tamias striatus*). All small mammals were trapped using pit-fall or Sherman live traps placed alternately within a 30×30 m^2 sampling grid sited in the floodplain. Two sampling grids were located at FC and 4 grids were set up at TB (see figure 1 of Blankenship et al. [2005]). Captured species were sacrificed by cervical dislocation and carcasses were placed in solvent-rinsed sample jars and frozen at −20 °C. Prior to chemical analysis, stomach contents were removed and the remaining whole body (including pelage) was homogenized in a solvent-rinsed grinder (Blankenship et al. 2005).

Muskrats (*Ondatra zibethicus*) were collected along the riverbank throughout FC and TB using body-gripping "conibear" traps. Samples were frozen at −20 °C until processing for chemical analysis. Processing of whole-body samples included removal of the pelage, a coarse grind, and further homogenization in a commercial blender (Millsap et al. 2004).

Crayfish (*Cambarus* spp. and *Orconectes* spp.) were collected along the riverbank at FC and TB by use of wire minnow traps set adjacent to the small mammal sampling grids. For chemical analyses, the whole body was homogenized in a solvent-rinsed grinder (Millsap et al. 2004).

Chemical analysis—extraction/clean-up

Concentrations of PCB congeners were determined by use of US Environmental Protection Agency (USEPA) method 3540 (SW846). The details of the soxhlet extraction and sample preparation and clean-up have been described previously (Neigh, Zwiernik, Bradley, Kay, Park, et al. 2006). Prey items were homogenized with anhydrous sodium sulfate (EM Science, Gibbstown, NJ, USA) using a mortar and

pestle. All samples, blanks, and matrix spikes included PCB 30 and PCB 204 as surrogate standards (AccuStandard, New Haven, CT, USA). Extraction blanks were included with each set of samples. Quality assurance/quality control sets composed of similar tissues were included with each group of 20 samples. Concentrations of PCBs, including di- and mono-*ortho*-substituted congeners were determined by gas chromatography (Perkin Elmer AutoSystem, Perkin Elmer, Waltham, MA, USA; and Hewlett Packard 5890 series II, Hewlett Packard, Palo Alto, CA, USA) equipped with a ^{63}Ni electron capture detector (GC-ECD). Concentrations of coplanar PCB congeners were determined by gas chromatograph mass selective detector (GC-MS; Hewlett Packard 5890 series II gas chromatograph interfaced to a HP 5972 series detector). Polychlorinated biphenyls were reported on a mass wet weight (ww) basis. A solution containing 100 individual PCB congeners was used as a standard. Individual PCB congeners were identified by comparing sample peak retention times to those of the known standard, and congener concentrations were determined by comparing the peak area to that of the appropriate peak in the standard mixture. Di-and mono-*ortho*-substituted PCB congeners were detected by selected ion monitoring of the 2 most abundant ions of the molecular cluster and the limit of quantification was conservatively estimated (minimum surface to noise ratio of 10.0) to be 1.0 ng PCB/g ww, using an extraction mass of 20 g, a 25 pg/μL standard congener mix and 1-μL injection volume. For coplanar PCB congeners, method detection limits varied among samples but were maintained at $\leq$0.1 ng/g ww for all samples using the sample-specific extraction mass and a minimum surface to noise ratio of 3.0. TurboChrom (Perkin Elmer) was used to identify and integrate the peaks. Total concentrations of PCBs were calculated as the sum of all resolved PCB congeners. Total PCB concentrations in waterfowl samples collected by the US Fish and Wildlife Service were quantified as Aroclor 1260 (MDNR 1987).

TEQ computation

Concentrations of $TEQ_{WHO\text{-}Avian}$ in prey item tissues were calculated to produce the most conservative estimate of body burden concentrations originating from coplanar and mono-*ortho* PCB congeners. Polychlorinated-dibenzo-*p*-dioxins and polychlorinated-dibenzofurans were not measured and were not included in TEQ computation. Whenever a PCB congener was not detected, a proxy value equal to one-half the limit of quantification was multiplied by the toxic equivalency factor to calculate the congener-specific TEQs. Co-eluting congeners were evaluated separately. Polychlorinated biphenyl congener 105 frequently co-eluted with congener 132, congener 156 frequently co-eluted with 171 and 202, congener 157 co-eluted with congener 200, and congener 167 co-eluted with congener 128. In order to report the maximum $TEQ_{WHO\text{-}Avian}$, the entire concentration of the co-elution groups was assigned to the mono-*ortho* congener. Among the 6 GHO prey categories analyzed at the MSU Aquatic Toxicology Laboratory (excludes waterfowl samples), the maximum combined contributions to total $TEQ_{WHO\text{-}Avian}$ of congeners 105, 156, 157, and 167 was 4.2% (passerine), 46% (mice/vole), 5% (shrew), 1.2% (muskrat), <1% (rabbit/squirrel), and <1% (crayfish), respectively.

Individual non-*ortho* and mono-*ortho* PCB congener concentrations in waterfowl samples were estimated from the quantified Aroclor 1260 total PCB concentrations by multiplying the geometric mean total PCB concentration by a congener-specific fractional composition value (Schwartz et al. 1993). The greatest observed congener-specific fractional value (percent composition basis) determined among 4 technical Aroclor mixtures (1242, 1248, 1254, 1260) was selected to account for inherent differences in Aroclor batch production processes. Bioaccumulation factors of 10 and 3 were applied to PCB congeners 126 and 169 to account for the selective enrichment (weathering, metabolism) of these 2 congeners that was measured in bald eagle (*Haliaeetus leucocephalus*) (PCB 126) and lake trout (*Salvelinus namaycush*) (PCB 169) eggs analyzed as part of the Schwartz et al. (1993) study. Using this approach, the combined contribution of congeners 105, 156, 157, and 167 to total $TEQ_{WHO\text{-}Avian}$ in waterfowl was <1%.

Toxicity reference values

In this study, TRVs were used to evaluate the potential for adverse effects due to PCBs including $TEQ_{WHO\text{-}Avian.}$ Ideally, TRVs are derived from chronic toxicity studies in which a total PCB or $TEQ_{WHO\text{-}Avian}$ dose–response relationship has been observed for ecologically relevant endpoints in the species of concern, or alternately in a wildlife species rather than a traditional laboratory species. Chronic studies should also include sensitive life stages to evaluate potential developmental and reproductive effects, and there must be minimal impact from co-contaminants on the measured effects.

Toxicity reference values used in this assessment were based on values reported in the literature for no observable adverse effect levels (NOAELs) and lowest observable adverse effect levels (LOAELs) for total PCBs and $TEQ_{WHO\text{-}Avian}$ (Table 1). The dietary PCB NOAEL for GHO was based on the controlled, laboratory study on the reproductive effects of PCBs on the screech owl (*Otus asio*; McLane and Hughes 1980). In that study, screech owls were fed a diet that contained 3 mg PCB/kg, ww. At this dose, no effects were observed on eggshell thickness, number of eggs laid, young hatched and fledged. A TRV for the GHO was estimated from the toxicity information available for the screech owl by use of allometric relationships, body weight and food consumption given by Sample et al. (1996). This resulted in a TRV for GHO, expressed as a daily dose, of 4.1×10^2 ng PCB/g bw/d. A LOAEL was not identified in that study so the LOAEL value of 1.23×10^3 ng PCBs/g body weight (bw)/d was estimated by applying a NOAEL to LOAEL uncertainty factor of 3. No additional uncertainty factors were applied to account for potential intertaxon variability, because the NOAEL is in the range determined for the chicken, the most sensitive bird species tested (Platonow and Reinhart 1973; Lillie et al. 1974).

No studies of the effects of $TEQ_{WHO\text{-}Avian}$ were available for deriving TRVs, and no studies were found in which there was a closely related test species to GHO. A subchronic laboratory study (10-week exposure period) by Nosek et al. (1992) found that intraperitoneal injections of 2,3,7,8-TCDD at concentrations of 1.0×10^3 pg TCDD/g/week (1.4×10^2 pg TCDD/g bw/d) caused a 64% decrease in fertility and a 100% increase in embryo mortality in ring-necked pheasants (*Phasianus colchicus*). This exposure concentration was used as the dietary TEQ-based LOAEL for GHO. A NOAEL was not directly available from this study and had to be derived from the limited dose data. Because effects due to the

Table 1. Toxicity reference values (TRVs) used to calculate hazard quotients for total polychlorinated biphenyls (PCBs) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$) in great horned owl (*B. virginianus*) diet and eggs[a]

	Dietary-based TRVs (ng PCBs/g bw/d) (pg $TEQ_{WHO\text{-}Avian}$/g bw/d)		Tissue-based TRVs (ng PCBs/g) (pg $TEQ_{WHO\text{-}Avian}$/g)	
	NOAEL	LOAEL	NOAEC	LOAEC
Total PCBs[b]	410	1,230	7,000	21,000
$TEQ_{WHO\text{-}Avian}$	14[c]	140[c]	135[d]	400[d]

[a] NOAEL = no observable adverse effect level; LOAEL = lowest observable adverse effect level; NOAEC = no observable adverse effect concentration; LOAEC = lowest observable adverse effect concentration; bw = body weight.
[b] McLane and Hughes (1980).
[c] Nosek et al. (1992).
[d] Elliott et al. (1996, 2000) and Woodford et al. (1998).

exposure were pronounced in the test subjects, a safety factor of 10 was applied to derive a NOAEL of 1.4×10^1 pg TEQ/g bw/d. Limitations of the study include the evaluation of TCDD exposure and not PCB-TEQ exposure, differences in species, and use of injections of 2,3,7,8-TCDD in a subchronic study verses feeding 2,3,7,8-TCDD contaminated food in a chronic exposure study.

Because co-eluting congener contributions are included in some mono-*ortho* PCB congener concentrations used in this risk assessment the PCB-based TEQs may overestimate exposure relative to 2,3,7,8-TCDD. In this instance, the use of a TRV based on 2,3,7,8-TCDD exposure is likely to yield conservative estimates of risk when applied to PCB exposure. Also, tolerance to TEQ-based exposure by birds is species specific (Woodford et al. 1998), and the TRVs derived from Nosek et al. (1992) are likely to be protective of GHO because the *Galliformes* used in the study are among the more sensitive species to the effects of 2,3,7,8-TCDD (Hoffman et al. 1998). The available information indicates that raptors, such as the American kestrel (*Falco sparverius*), osprey (*Pandion haliaetus*), and bald eagle, are more tolerant than gallinaceous species to the effects of PCBs and TEQ (Elliott et al. 1996, 1997; Hoffman et al. 1998; Woodford et al. 1998). Thus, for GHO a more closely related raptorial species such as American kestrels would be the ideal basis for TRVs. However, in the few studies in which kestrels were exposed to PCBs, there was either inadequate dose–response information or incomplete assessment of ecologically relevant endpoints.

Tissue-specific TRVs (egg-basis) for total PCBs and $TEQ_{WHO\text{-}Avian}$ used in previous assessments of GHO exposure at the site (Strause et al. 2007) are included (Table 1). The egg-based TRVs are included to aid interpretation of the bottom-up and top-down methodology comparisons completed in this study.

Average potential daily dose (APDD)/risk assessment

The amount of PCBs ingested by GHOs was calculated using the wildlife dose equation for dietary exposures (USEPA 1993). The APDDs for total PCBs and $TEQ_{WHO\text{-}Avian}$ were calculated for GHOs using the site-specific diets for GHO determined in this study, and for comparison purposes a literature-based diet for a separate population of Michigan GHOs (Craighead and Craighead 1956). All APDDs were based on diets with prey composition compiled on a biomass basis (Equation 1). Average potential daily dose calculations also included the incidental ingestion of floodplain soils that could potentially be associated with GHO foraging activity.

$$\mathrm{APDD} = \sum (C_k \times FR_k \times NIR_k) \qquad (1)$$

C_k = Geometric mean and upper 95%CL concentration of total PCBs or $TEQ_{WHO\text{-}Avian}$, ww in the *k*th prey item category of GHO diet, or alternatively floodplain soils.

FR_k = Fraction of GHO diet (based on mass) represented by the *k*th prey item category.

NIR_k = Normalized GHO ingestion rate of the *k*th prey item (g prey/g bw/d, ww).

Concentrations of PCBs and $TEQ_{WHO\text{-}Avian}$ in representative prey items collected from the KRSS were determined using the methods described previously and are presented in the following section. FR_k (mass basis) was determined for the GHO subpopulation cohorts at both FC and TB, and from a previous study (literature-based diet) of GHO populations in southeast Michigan (Craighead and Craighead 1956). A conservative assumption for the value of FR_k is that GHO at the KRSS will obtain 100% of their diet requirements from the 100-y floodplain (site use factor = 1). NIR_k (0.056 g/g bw/d) was derived from daily ingestion rates and mean body weights reported for GHO (Craighead and Craighead 1956). Additional PCB and $TEQ_{WHO\text{-}Avian}$ dietary exposure from incidental soil ingestion was calculated for TB GHOs using both the site-specific and the literature-based dietary composition and geometric mean concentrations of PCBs measured for TB soils. Incidental soil ingestion contributions to dietary exposure were not calculated for the FC GHOs because of the very low concentrations of PCBs present in FC soils. Geometric mean and upper 95% confidence level (CL) geometric mean concentrations of total PCBs in TB floodplain soils (as an added protective measure nondetects were removed from the data set prior to computing mean and upper 95% CL values) were obtained from previous investigations at the site (BBL 1994). Concentrations of total PCBs in soils were considered to be 85% bioavailable and contain 65% moisture (estimated from Studier and Sevick [1992]) to make the dry weight soil concentrations comparable to wet weight concentrations in prey. The dietary fraction of incidental soil ingestion (2%) for GHOs was based on reports in the literature (USEPA 1993). An absorbance factor of 1.0 was applied to incidental soil ingestion exposures. Dioxin equivalent concentrations in soils were not measured at the site and were estimated from the Aroclor-based soil data using the methods described previously for waterfowl (Schwartz et al. 1993).

Comparisons of potential hazard estimated for dietary exposure to PCBs, were based on HQs. Hazard quotients were calculated as the APDD (ng PCB/g bw/d or pg

$TEQ_{WHO\text{-}Avian}$/g bw/d) divided by the corresponding TRV (Equation 2).

$$HQ = \frac{\text{APDD(ng PCBs/g bw/d or pg TEQs/g bw/d)}}{\text{dietary TRV}} \quad (2)$$

Other lines of evidence from previously published studies on KRSS GHO populations were examined to minimize uncertainties in the analysis and calculation of potential risk from dietary exposure (Strause et al. 2007). These include the top-down risk assessment approach that quantified concentrations of PCBs present in GHO eggs and nestling plasma, and examined the effects of chlorinated hydrocarbons on egg viability through measurements of eggshell thickness and Ratcliffe index (Hickey and Anderson 1968; Ratcliffe 1968). A 3rd and ancillary line of evidence investigated potential effects of PCB exposures at the KRSS by monitoring productivity (fledgling success) and relative abundance between the contaminated floodplain habitat (TB) and the reference location (FC). By evaluating multiple lines of evidence together it was possible to provide the best available information for remedial decision-making at the site, especially when 2 or more lines of evidence converged on a common finding.

Statistical analysis

Both parametric and nonparametric statistics were applied depending on which assumptions were met. Concentrations of total PCBs and $TEQ_{WHO\text{-}Avian}$ in prey populations from the site were analyzed for normality by use of the Kolmogorov-Smirnov, 1-sample test with Lilliefors transformation. Concentrations of the COCs were generally log-normally distributed and therefore all data sets were log-transformed to more closely approximate the normal distribution. Data sets that were normally distributed were compared using a *t* test. If the data did not exhibit a normal distribution, then a nonparametric version of the *t* test (Mann–Whitney *U* test) was used. Associations between parameters were made with Pearson Product Correlations. Tests for normality and treatment effects (spatial trends) were completed using the Statistica (Version 6.1) statistical package (Statsoft, Tulsa, OK, USA). The criterion for significance used in all tests was $p < 0.05$. Statistical methods for comparing COC concentrations in GHO tissues (eggs, plasma), eggshell parameters (shell thickness, Ratcliffe Index) and call/response survey measurements of GHO abundance employed in the multiple-lines-of-evidence evaluation of site-specific risk to KRSS GHO populations have been described previously (Strause et al. 2007).

RESULTS

Composition of the diet of GHOs

A total of 285 discrete prey items were identified in 59 pellet and prey remains samples collected from a combined total of 7 active nests in the FC and TB study sites from 2000 to 2002. Excepting 4 post-fledge prey remains samples collected between 4 and 28 June, all samples were collected prior to 1 June in each year of the study, and, as such, the data provide a characterization of the spring or nesting season diet for KRSS GHOs. Only prey items represented by the classes *Aves* and *Mammalia* were observed. Prey from classes *Reptilia*, *Amphibia*, or *Crustacae* were not observed in the diets of GHO at the KRSS (Table 2).

Dietary compositions at FC and TB varied slightly when compiled on a class basis. Fort Custer GHOs consumed a slightly lesser proportion of birds and slightly greater proportion of mammals (birds: 15.5% numeric, 13.8% mass; mammals: 84.5% numeric, 86.2% mass) compared to TB GHOs (birds: 27.5% numeric, 24.8% mass; mammals: 72.5% numeric, 75.2% mass). Similar results are produced whether one compiles the class-level data on either a numeric or mass basis with only a slight increase in the proportion of mammalian prey when diet is compiled on a mass basis.

Within-class differences were observed between the FC and TB diets of GHO. Large differences were observed in the proportions of passerine/terrestrial birds represented in diets of GHOs at FC and TB (11% vs 25.8% on a numeric basis and 5% vs 22% on a mass basis, respectively) and in the proportion of rabbits represented in GHO diets at FC and TB (46% vs 16% on a numeric basis and 75% vs 50% on a mass basis, respectively). Within-class differences also are seen between diet compilations based on percent number versus percent biomass. On a numeric basis, small mammals (mice/voles) and shrews account for up to 33% and 51% of the GHO diet at FC and TB, respectively. These proportions decrease to 2.2% (FC) and 6.2% (TB) of the diet on a mass basis. Likewise, the combined proportion of rabbit and muskrat prey on a numeric basis increases from 51% (FC) and 21.5% (TB) to 84% (FC) and 69% (TB) on a mass basis.

A diet compilation based on mass provides the most accurate characterization of the relative importance of prey to avian predators (Marti 1987). Mass-based characterizations of KRSS GHO diet at FC and TB are compared to diet composition values for a nearby GHO subpopulation residing in southeast Michigan (Craighead and Craighead 1956; Figure 4). Class-level differences in prey composition between KRSS GHOs and the literature-based (LB) values are greatest for classes *Aves* and *Mammalia* at FC (13.8% [FC] vs 68% [LB], 86.2% [FC] vs 31.5% (LB), respectively). Crayfish (class *Crustacae*) were also present in the LB diet of GHO. Within-class prey proportions for KRSS and literature-based GHO diets were similar with passerine/terrestrial birds and rabbits/muskrats comprising the great majority of bird and mammalian prey.

Total PCB and $TEQ_{WHO\text{-}Avian}$ concentrations in prey

Concentrations of total PCBs and lipid content of 130 discrete whole-body prey item samples were determined. Prey items collected previously from the KRSS included 17 waterfowl samples that also were used to estimate GHO exposure to PCBs via the diet. Budget limitations prevented the collection and PCB/TEQ analyses of rabbit and grey/fox squirrel samples from FC and TB. Available data for chipmunk and red squirrel were used to fill this gap in the data base. Geometric mean concentrations of total PCBs in FC prey ranged from 2 ng PCBs/g ww in rabbit surrogates (i.e., chipmunk/red squirrel) to 9.6×10^1 ng PCBs/g ww in passerines (Table 3). Geometric mean concentrations of total PCBs in TB prey ranged from 5.6×10^1 ng PCBs/g ww in muskrats to 1.3×10^3 ng PCBs/g ww in passerines. Total PCB concentrations in waterfowl were 8.9×10^2 ng PCBs/g ww, and this value was used for both the FC and TB sites because of the uncertainty associated with residence and exposure of these mobile and migratory species. Polychlorinated biphenyl concentrations in prey items from TB were significantly greater (small mammals, muskrats, crayfish; *t* test $p < 0.01$;

Table 2. Great horned owl spring diet composition at the Kalamazoo River Superfund Site (site-specific) and from the literature

	Numeric basis (% occurrence)			Mass basis (% contribution)		
	Site-specific diet ($N = 285$)		Literature-based diet ($N = 260$)	Site-specific diet ($N = 285$)		Literature-based diet ($N = 260$)
Prey item	Ft. Custer (FC)	Trowbridge (TB)	Washtenaw Co. MI[a]	Ft. Custer (FC)	Trowbridge (TB)	Washtenaw Co. MI[a]
Class Aves	15.5	27.5	41.0	13.8	24.8	68.0
Passerine[b]	11.0	25.8	39.0	5.0	22.0	65.5
Waterfowl[c]	4.5	1.7	2.0	8.8	2.8	2.5
Class Mammalia	84.5	72.5	54.0	86.2	75.2	31.5
Mice/vole	31.0	49.0	41.0	2.0	6.0	2.5
Shrew	2.5	2.0	0.0	0.2	0.2	0.0
Muskrat	5.0	5.5	2.0	9.0	19.0	6.0
Rabbit[d]	46.0	16.0	11.0	75.0	50.0	23.0
Class Crustacae	0.0	0.0	5.0	0.0	0.0	0.5
Crayfish	0.0	0.0	5.0	0.0	0.0	0.5

[a] Craighead and Craighead (1956).
[b] Passerine category includes all terrestrial birds and all unidentified bird ("unknown bird") remains.
[c] Waterfowl category includes all aquatic birds.
[d] Rabbit category includes squirrels and all unidentified medium-size mammal ("unknown mammal") remains.

passerines, shrews; Mann–Whitney U test $p < 0.01$) than those from the upstream reference area at FC. Waterfowl and rabbit (surrogate) samples (TB sample size = 1) were not tested for significant differences.

Geometric mean concentrations of $TEQ_{WHO\text{-}Avian}$ in FC prey ranged from 0.52 pg TEQ/g ww in rabbit surrogates to 7.5 pg TEQ/g ww in crayfish (Table 4). Geometric mean concentrations of $TEQ_{WHO\text{-}Avian}$ in TB prey ranged from 1.3×10^1 pg TEQ/g ww in muskrats to 7.1×10^1 pg TEQ/g ww in rabbits. Using congener-specific fractional composition values (Schwartz et al. 1993) conservatively estimated $TEQ_{WHO\text{-}Avian}$ concentrations in 17 waterfowl samples were 2.4×10^2 pg TEQ/g ww. Prey items from TB contained significantly greater concentrations of TEQ than those from FC (t test $p < 0.02$) with the exception of muskrats and small mammals, which were not statistically different (Mann–Whitney U test $p = 0.26$ and $p = 0.22$, respectively). Waterfowl and rabbit (surrogate) samples (TB sample size = 1) were not tested for significant differences.

Contributions to total $TEQ_{WHO\text{-}Avian}$ from the 4 non-*ortho* and 8 mono-*ortho* PCB congeners showed that more than 90% of total TEQ was contributed by non-*ortho* congeners 77, 81, and 126 for all prey item categories at each of the 2 sampling locations excepting TB mice/voles (45% contribution; Figure 5). The same 3 coplanar congeners were among the 3 greatest contributors to concentrations of total TEQ for all prey item categories at each sampling location excepting TB mice/voles and TB rabbits (chipmunk). The 3 greatest PCB congener contributors and their combined contribution to total TEQ for each prey item category included: PCB 77 > 126 > 81 (FC passerine [95.4%], TB passerine [97%], FC and TB waterfowl [98.1% estimated], FC and TB crayfish [99%]; PCB 77 > 81 > 126 (TB muskrat [98.6%]); PCB 126 > 77 > 81 (FC muskrat [99.7%], FC rabbit [chipmunk and red squirrel] [99.1%], TB shrew [93.3%]); PCB 126 > 81 > 77 (FC shrew [99.4%], FC mice/vole [97.1%]); PCB 126 > 77 > 118 (TB rabbit [chipmunk] [97.9%]); PCB 105 > 81 > 126 (TB mice/vole [71.1%]).

Average potential daily dose

Average potential daily doses for GHOs were calculated based on geometric mean and upper 95% CL concentrations of both total PCBs and $TEQ_{WHO\text{-}Avian}$ of each prey item category for the numeric- and mass-based range of dietary composition at the FC and TB study sites (Table 5). Calculations of both total PCB and $TEQ_{WHO\text{-}Avian}$ exposures at TB included contributions from incidental soil ingestion. Based on site-specific diet and prey item COC concentrations, GHO ingestion of total PCBs were from 7- to 10-fold greater at TB than at FC, and $TEQs_{WHO\text{-}Avian}$ were 3-fold greater at TB than FC. Average potential daily doses calculated using the upper 95% CL (geometric mean) of total PCBs and $TEQ_{WHO\text{-}Avian}$ displayed a range of differences that were similar (6- to 7-fold difference and 2-fold difference, total PCBs, $TEQ_{WHO\text{-}Avian}$, respectively) to values of APDD based on the geometric mean.

Comparisons of geometric mean, mass-based ranges of APDD between the site-specific (APDD measured) and literature-based (APDD predicted) GHO dietary compositions yielded APDD values for total PCB exposures at FC that were equivalent (≤1.5-fold difference), and TB total PCB APDD values that differed by a factor of 1.6 (literature-based > site-specific APDD; Table 5). Average potential daily dose values for mean $TEQ_{WHO\text{-}Avian}$ were 1.6- to 2.3-fold greater for FC site-specific based dietary exposures and equivalent for TB based exposures. The literature-based TB APDD calculations for both total PCB and $TEQ_{WHO\text{-}Avian}$ included contributions from incidental soil ingestion consistent with the calculations for site-specific exposures at TB. Average

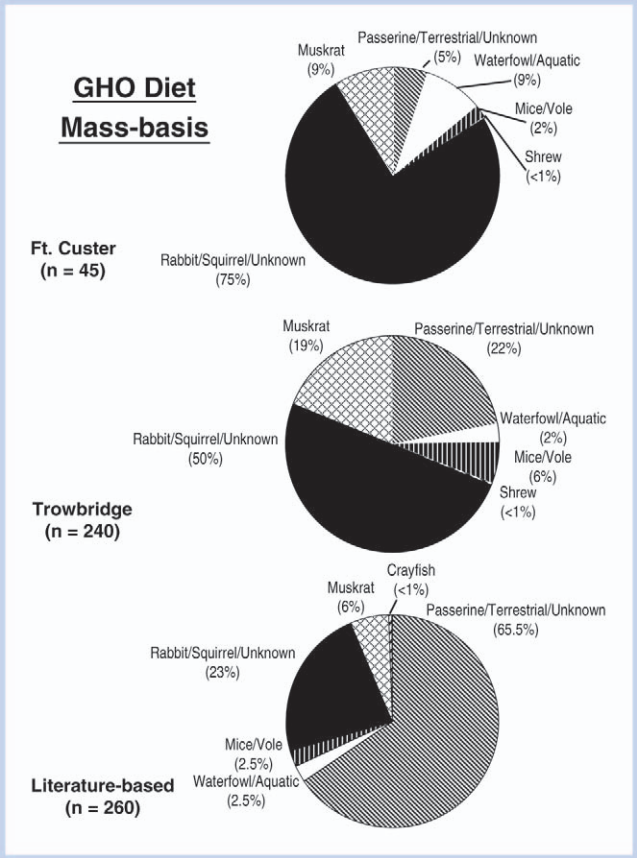


Figure 4. Site-specific great horned owl (GHO; *Bubo virginianus*) diet composition based on a biomass contribution basis for the Ft. Custer and Trowbridge sampling locations, and a literature-based (Craighead and Craighead 1956) diet composition for GHO populations in southeast Michigan (USA).

potential daily doses (site-specific vs literature-based) calculated using the upper 95% CL for total PCBs displayed a range of values similar to mean-based APDDs (FC exposures were equivalent, literature-based APDDs were 2.1-fold greater for TB exposures). Average potential daily doses calculated using the upper 95%CL concentrations of $TEQ_{WHO\text{-}Avian}$ in prey produced site-specific APDDs that were 1.5- to 2.1-fold greater than literature-based values at FC, and APDDs that were equivalent at TB.

The greatest calculated APDDs for GHOs at the KRSS originated from a mass-based dietary compilation. The 10-fold greater APDD at TB than at FC was consistent with the significant differences in total PCB and $TEQ_{WHO\text{-}Avian}$ concentrations in prey items collected from the 2 sites. The APDDs based on total PCBs and $TEQ_{WHO\text{-}Avian}$ for the site-specific and literature-based diets were less than 3-fold different for both FC and TB. The moderate differences in dietary composition observed at a class and within-class level between the 2 studies did not combine to influence APDD to a great extent.

Due to greater concentrations of COCs or greater proportions in the diet, exposures are often dependent on a few types of prey. This phenomenon was observed for GHOs at the KRSS where 2 to 4 prey item categories combine to account for more than 90% of the APDD at any given site and diet composition. At FC, mass-based APDDs for geometric mean total PCB concentrations in prey show that waterfowl (91% of APDD) and passerines (5%) drive exposures for the site-specific diet, and the same 2 prey items, albeit in an inverted ratio: waterfowl (25%) and passerines (72%), also drive the literature-based exposure (Figure 6A). At TB, passerines (45%), rabbits (43%), and soil ingestion (5%) figure predominantly in $APDD_{PCBs}$ for site-specific exposures and also literature-based exposures (passerines [62%], rabbits [26%], soil ingestion [5%]). The principal prey items responsible for APDDs calculated for $TEQ_{WHO\text{-}Avian}$ include the same prey identified for $APDD_{PCBs}$ but in some cases additional prey contribute to the 90% threshold. At FC, waterfowl (97%) and rabbits (2%) drive $APDD_{TEQ}$ for site-specific exposures, and literature-based exposures come from waterfowl (62%) and passerines (35%). At TB, rabbits (52%), passerines (18%), soil ingestion (16%), and waterfowl (10%) drive $APDD_{TEQ}$ for site-specific exposures, and literature-based exposures come from passerines (34%), rabbits (28%), waterfowl (16%), and soil ingestion (14%; Figure 6B).

Assessment of hazard

Hazard quotients were calculated for each location based on the site-specific and literature-based APDDs for total PCBs and $TEQ_{WHO\text{-}Avian}$. To conservatively estimate potential hazard to resident GHOs at the KRSS and to capture the broadest reasonable range of variability in characterizations of prey item COC concentrations and composition of the GHO diet, HQs are calculated from the range of APDD values encompassing the geometric mean and associated upper 95% CL values for each respective prey item and the dietary proportion contributed by each prey item compiled on both a numeric and mass basis. The range of HQs discussed for the NOAEL and LOAEL effect levels (HQ_{NOAEL}/HQ_{LOAEL}, respectively) will typically represent potential hazard associated with exposures to geometric mean concentrations for a numeric-based diet (low range) up to the upper 95% CL concentrations for a mass-based diet (high range). All HQs (total PCBs and $TEQ_{WHO\text{-}Avian}$) for site-specific and literature-based diets determined for both FC and TB geometric mean and upper 95% CL exposures were less than 1.0 (Table 6). The maximum FC HQ_{NOAEL} for total PCBs was 0.02 and 0.03 for the site-specific and literature-based diets, respectively. The maximum TB HQ_{NOAEL} for total PCBs was 0.15 and 0.31 for the site-specific and literature-based diets, respectively. The maximum FC HQ_{NOAEL} for $TEQ_{WHO\text{-}Avian}$ ranged from 0.27 to 0.13 for the site-specific and literature-based diets, respectively; TB $HQ_{NOAEL/TEQ}$ ranged from 0.5 to 0.73 for the site-specific and literature-based diets, respectively.

DISCUSSION

Dietary composition

The 3-y sampling program at active GHO nests for pellets and prey remains was an effective approach for characterizing site-specific exposures of nestling GHOs to the COCs at the KRSS, and to concurrently allow for nonintrusive monitoring of GHO productivity at each nest (Strause et al. 2007). Additionally, some potential biases commonly associated with pellet and prey remains sampling were also addressed in this study. Our approach capitalized on GHO preferences to use nests built by other bird species or in this instance artificial nesting platforms that were located at appropriate floodplain locations in the KRSS. In doing so, we successfully induced

Table 3. Geometric mean and upper 95% confidence level (U95% CL) concentrations of total polychlorinated biphenyls (PCBs) (ng PCBs/g wet weight) in prey items collected from 2 sites on the Kalamazoo River (MI, USA)

	Ft. Custer					Trowbridge				
	N	Range	Geometric mean	U95% CL	Lipid (%)	*N*	Range	Geometric mean	U95% CL	Lipid (%)
Passerines[a]	11	9–1,030	96	262	4.10	20	62–32,200	1,336*	3,102	4.90
Waterfowl[b]	17	130–28,000	889	1,751	3.80	17	130–28,000	889	1,751	3.80
Mice/Vole	12	2–180	13	27	3.59	20	30–548	67*	102	4.57
Shrew	16	2–18	8	10	3.66	17	25–3,150	847*	1,533	2.68
Muskrat	4	8–26	13	22	2.60	7	14–112	56*	94	2.07
Rabbit[c,d]	6	1–6	2	4	3.71	1	568	568	568	4.95
Crayfish	4	27–89	49	93	0.63	13	76–1,940	373*	597	1.62

* Trowbridge total PCB concentrations are significantly greater than concentrations at Ft. Custer ($p \leq 0.01$).
[a] Geometric mean total PCB concentrations in Ft. Custer house wren (5), tree swallow (2), and American robin (4) and Trowbridge starling (1), house wren (6), tree swallow (5), and American robin (8) used as representative of terrestrial passerine concentrations.
[b] Waterfowl samples were collected from 5 locations on the Kalamazoo River and were not divided between upstream and downstream sampling locations because of uncertain local residence status on the river.
[c] Geometric mean total PCB concentrations in Ft. Custer chipmunk (5) and red squirrel (1) used as surrogate value for rabbit concentration.
[d] Total PCB concentrations in Trowbridge chipmunk (1) used as surrogate value for rabbit concentration.

resident GHOs to occupy areas of the site having maximum exposure potential. Only one of the active nests sampled for pellets and prey remains was a natural nest, all remaining nesting activity included in the diet characterization study occurred at artificial nesting platforms. Additional advantages of GHO behavior incorporated into this study included their propensity to forage within relatively small areas because of their sedentary lifestyle and highly versatile prey capture ability (Marti 1974).

In this study, sampling of pellets and prey remains from beneath feeding perches and nest trees (ground collections) was augmented with collections from active nests during the blood sampling event and again after fledging was complete. Nest sites were the best locations for collecting avian prey remains, particularly feathers that could be positively classified as evidence of owl predation. Nest collections eliminated a significant source of uncertainty associated with feather remains collected on the ground beneath or in the general vicinity of active nests and feeding perches for which solid evidence of owl predation was mostly lacking.

Feeding studies with captive GHOs (Errington 1930; Glading et al. 1943) showed that pellets consistently reflected the food habits of adult and juvenile owls. Potential biases associated with pellet studies (under-representation of very

Table 4. Geometric mean and upper 95% confidence level (U95% CL) concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{Who\text{-}Avian}$) (pg $TEQ_{WHO\text{-}Avian}$/g wet weight) in prey items collected from 2 sites on the Kalamazoo River (MI, USA)

	Ft. Custer					Trowbridge				
	N	Range	Geometric mean	U95% CL	Lipid (%)	*N*	Range	Geometric mean	U95% CL	Lipid (%)
Passerine[a]	11	0.47–238	5.3	18	4.10	19	1.1–4,777	56*	194	4.90
Waterfowl[b]	17	36–7,678	244	725	3.80	17	36–7,678	244	725	3.80
Mice/vole	12	0.18–2.9	0.61	0.91	3.59	20	0.47–3.8	0.80	0.99	4.57
Shrew	16	0.45–49	1.28	2.18	3.66	17	4–249	47*	77	2.68
Muskrat	4	0.07–37	1.1	14	2.60	7	0.43–49	13	47	2.07
Rabbit[c,d]	6	0.13–4.0	0.52	1.51	3.71	1	71	71	71	4.95
Crayfish	4	1.5–58	7.5	33	0.63	13	3.1–374	56*	108	1.62

* Trowbridge TEQ concentrations are significantly greater than concentrations at Ft. Custer ($p \leq 0.02$).
[a] Geometric mean total $TEQ_{WHO\text{-}Avian}$ concentrations in Ft. Custer house wren (5), tree swallow (2), and American robin (4) and Trowbridge starling (1), house wren (6), tree swallow (5), and American robin (7) used as representative of terrestrial passerine concentrations.
[b] Waterfowl samples were collected from 5 locations on the Kalamazoo River and were not divided between upstream and downstream sampling locations because of uncertain local residence status on the river.
[c] Geometric mean total $TEQ_{WHO\text{-}Avian}$ concentrations in Ft. Custer chipmunk (5) and red squirrel (1) used as surrogate value for rabbit concentration.
[d] $TEQ_{WHO\text{-}Avian}$ concentrations in Trowbridge chipmunk (1) used as surrogate value for rabbit concentration.

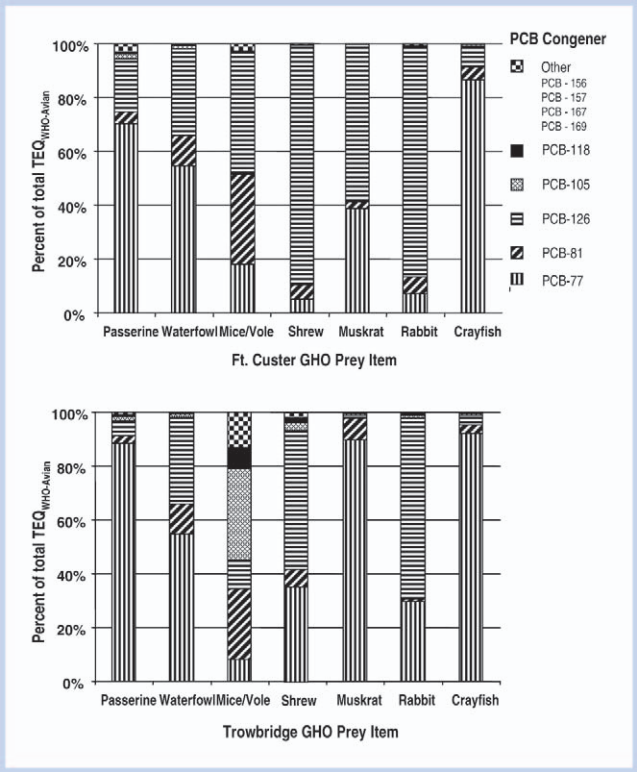


Figure 5. Percent contribution of polychlorinated biphenyl (PCB) coplanar and mono-*ortho*-substituted congeners to total 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$) in great horned owl (*Bubo virginianus*) prey at the Kalamazoo River.

small prey, prey with more easily digestible components, boneless pellets from newly hatched owlets) or prey remains collections (over-representation of larger species and avian prey) can be adequately managed through collections of both types of samples, segregation of samples among active nests, and reconciliation of all concurrently collected samples. Very small prey items (e.g., very young animals) are unlikely to carry great COC concentrations because of a very limited period of potential exposure, and even in the exception (e.g., shrews, which do frequently have great COC concentrations), their low mass will contribute negligibly to APDD at most sites. Prey with easily digestible components may still be identified in prey remains collected from the nest, and the small numbers of prey that are completely absorbed by recently hatched owlets will most likely be adequately represented in subsequent pellets.

Studies of the dietary habits of North American GHO populations show the GHO is an opportunist hunter, plastic in foraging behavior, a generalist in prey selection with the broadest diet of any North American owl (Marti and Kochert 1996). Dietary preferences depend upon habitat, season, and prey vulnerability, and GHOs are capable of expressing the most diverse prey profile of all North American raptors (Voous 1998). Contributing factors to the species' broad diet include a large body size and crepuscular/nocturnal activity range. Major determinants upon prey selection by any individual include habitat, prey abundance, and prey vulnerability (a measure that combines ephemeral and interrelated determinants of prey density/prey behavioral patterns, habitat condition, and seasonality; Houston et al. 1998).

In general, temperate North American GHO populations feed predominantly on terrestrial mammals followed by terrestrial and aquatic birds, and a minor mix of reptiles, amphibians, and arthropods (Marti and Kochert 1995; Murphy 1997). Great horned owl diets vary between physiographic regions (Wink et al. 1987) and even among individual nesting territories when land use and habitat type distributions diverge within distinct physiographic units (Marti 1974). Great horned owl diets can also show significant temporal variation when pronounced changes in prey availability occur due to natural small mammal population cycles or anthropogenic modifications to habitat or prey populations (Fitch 1947; Adamcik et al. 1978). In addition, GHO diets also may vary with seasonal changes in prey vulnerability, although this source of variation tends to be minor compared to diet alterations stemming from

Table 5. Range of average potential daily doses (APDD)[a] based on geometric mean and the upper 95% confidence level (U95% CL) of prey items for total polychlorinated biphenyls (PCBs) (ng PCBs/g body weight [bw]/d) and 2,3,7,8 tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$) (pg TEQ/g bw/d) when assuming 2 different dietary compositions for great horned owl (GHO) at the Kalamazoo River Superfund Site, Michigan, USA

	Ft. Custer		Trowbridge[b]	
	PCBs	TEQs	PCBs	TEQs
PCB-based dietary models				
Site-specific APDD (APDD measured)[c]				
Geometric mean	3–5	0.676–1.25	30–37	2.39–3.81
U95% CL	7–10	2.03–3.76	59–61	5.34–6.98
Literature-based APDD (APDD predicted)[d]				
Geometric mean	4–5	0.428–0.549	38–60	2.72–3.97
U95% CL	9–12	1.34–1.75	80–127	6.81–10.18

[a] The range of calculated APDD results from using diet estimations based on both total frequency (numeric basis) and biomass contribution (mass basis; see Table 2).
[b] Includes incidental ingestion of floodplain soils at the former Trowbridge impoundment.
[c] Based on results of field collected GHO pellets and prey remains from active nests at each Kalamazoo River study site.
[d] A study of GHO diet in Washtenaw County, Michigan, USA (Craighead and Craighead 1956).

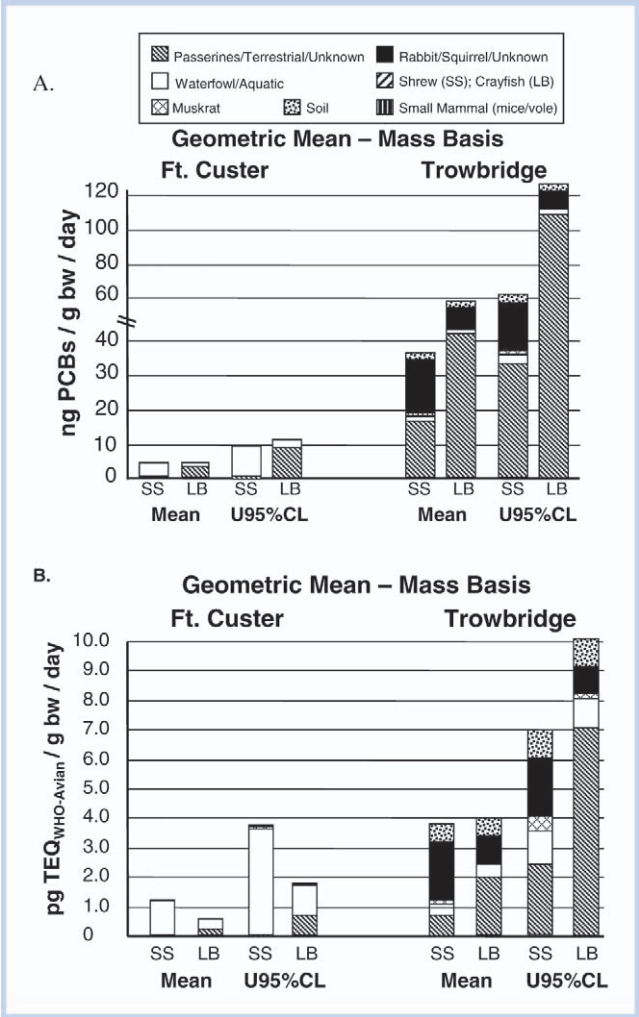


Figure 6. (**A**) Relative contribution of each principal prey component and incidental soil ingestion to total polychlorinated biphenyl (PCB) average potential daily dose (APDD; ng PCBs/g bw/d, ww) for great horned owl (*Bubo virginianus*) based on the geometric mean and upper 95% confidence level concentrations in Ft. Custer and Trowbridge prey, Trowbridge soil, and site-specific (SS) and literature-based (LB) dietary compositions (mass basis only). (**B**) Percent contribution of each principal prey component and incidental soil ingestion to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$) average potential daily dose (APDD; pg TEQ/g bw/d, ww) for great horned owl (*B. virginianus*) based on the geometric mean and upper 95% confidence level concentrations in Ft. Custer and Trowbridge prey, Trowbridge soil, and site-specific (SS) and literature-based (LB) dietary compositions (mass basis only).

differences in habitat and temporal prey availability (Errington et al. 1940; Fitch 1947; Wink et al. 1987). If present, significant seasonal variations in GHO diets may originate from changes in prey vulnerability caused by a combination of factors including vegetation changes, altered activity patterns of prey and GHOs, day length, GHO reproductive cycles, prey hibernation patterns, prey migration patterns, and prey reproductive lifecycle events (e.g., mating, dispersal of young).

Great horned owl foraging preferences are difficult to predict from surveys of prey populations in most North American temperate habitats and attempts to correlate GHO predation preferences with prey abundances have yielded mixed results (Murphy 1997). This is due in part to the fact that prey density apparently has little effect on prey vulnerability in some GHO territories (Adamcik et al. 1978; Peterson 1979). Great horned owls tend to display a density-independent dietary relationship to prey species. Changes in prey vulnerability do not necessarily correspond with changes in numerical status. Some species remain more vulnerable to GHO predation regardless of annual fluxes in abundance/density, even in periods of low population densities (Errington 1932; Peterson 1979).

Attempts to correlate GHO predation preferences to habitat types show greater success where GHOs' food habits appear to depend largely upon where the bird is situated because many birds studied seem to limit their activities to a few acres of certain favorite habitat (Errington 1932; Fitch 1947). While there is evidence to support a strong interaction between GHO foraging preferences and habitat (Rusch et al. 1972), there were still instances where some GHOs did appear to respond opportunistically to the availability of certain prey types. (Marti and Kochert 1996). However, wetlands habitats appear to be a notable exception to the habitat–prey use relationship identified for GHOs where studies of GHO use of wetland-dependent prey and proximity and extent of wetlands within nesting territories have shown almost no relationship between habitat and prey type. In these studies, GHOs sought wetland prey regardless of proximity or abundance of wetland habitats. This may stem from the fact that prey species may be more available and vulnerable due to high prey density, high prey diversity and abundance, and more favorable locations and numbers of elevated hunting perches in wetlands and wetland edge habitats (Murphy 1977; Houston 1998).

Our studies of GHO predation at the KRSS included efforts to control for spatial and temporal variability in owl diets. Great horned owl nesting platforms were specifically located in riparian floodplain habitats that were buffered from most human disturbances and situated within 100 m of the river to provide uniformity in foraging habitat, available prey populations and habitat-dependent influences on prey vulnerability. Nest trees were selected after completing a qualitative survey of nesting habitat quality so as to provide an optimal mix of cover and foraging habitat for breeding owls. Pellets and prey remains from multiple years were collected only during active nesting and brooding periods of the annual reproductive lifecycle to provide uniformity in environmental cues on GHO behavior and seasonal influences on prey availability/vulnerability.

Site-specific and literature-based diets

Because the dominant factors influencing GHO diet originate from spatial differences in habitat type and temporal alterations in prey populations, a literature-based diet selected for use in the absence a site-specific value must match the physiographic region and dominant habitat types at the site. If multiple studies of equivalent quality are available, temporal considerations can also be addressed. For instance, in the 1940s, nesting habitat of GHO populations in southeastern Michigan (Superior Township, Washtenaw County) included plant and animal community assemblages that were very similar to those present at KRSS (Craighead and Craighead 1956). When dietary composition for GHOs from KRSS was compared to the Washtenaw study, differences were observed that were principally related to differing proportions of rabbits and passerine/terrestrial birds (Table 2). Kalamazoo River owls consumed greater proportions of rabbits, and Washtenaw County GHOs consumed greater proportions of

Table 6. Hazard quotient (HQ) values based on geometric mean and the upper 95% confidence level (U95% CL) of average potential daily doses (APDD) of total polychlorinated biphenyls (PCBs) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$), when assuming 2 different dietary compositions for great horned owl (GHO) at the Kalamazoo River Superfund Site, Michigan, USA[a]

	Ft. Custer		Trowbridge	
	HQ NOAEL	HQ LOAEL	HQ NOAEL	HQ LOAEL
PCB-based dietary models				
Site specific[b]				
Geometric mean	0.01	<0.01	0.07–0.09[c]	0.02–0.03
U95% CL	0.02	0.01	0.14–0.15	0.05
Literature based[d]				
Geometric mean	0.01	<0.01	0.09–0.15	0.03–0.05
U95% CL	0.02–0.03	0.01	0.20–0.31	0.07–0.10
$TEQ_{WHO\text{-}Avian}$ based dietary models				
Site specific[b]				
Geometric mean	0.05–0.09	0.01	0.17–0.27	0.02–0.03
U95% CL	0.15–0.27	0.01–0.03	0.38–0.50	0.04–0.05
Literature based[d]				
Geometric mean	0.03–0.04	<0.01	0.19–0.28	0.02–0.03
U95% CL	0.01–0.13	0.01	0.49–0.73	0.05–0.07

[a] Toxicity reference values used to calculate HQs are provided in Table 1. NOAEL = no observable adverse effect level; LOAEL = lowest observable adverse effect level.
[b] Based on results of field collected GHO pellets and prey remains from active nests at each Kalamazoo River study site.
[c] The range of calculated APDD results from using diet estimations based on both total frequency (numeric basis) and biomass contribution (mass basis; see Table 2).
[d] A study of GHO diet in Washtenaw County, Michigan, USA (Craighead and Craighead 1956).

passerines/terrestrial birds. The greater proportion of birds in Washtenaw County GHO diets is directly attributable to a greater number of ring-necked pheasants in the diet that were at their historical peak of abundance in Michigan during the 1940s. However, their number significantly decreased by the 1980s (Luukkonen 1998) and as a result, this loss of an important dietary item was compensated for by increased GHO consumption of cottontail rabbits in the KRSS study (Springer and Kirkley 1978; Peterson 1979).

The impact of wetlands on GHO dietary composition also needs to be taken into account in that wetland habitats are well represented and in close proximity to GHO nesting territories at KRSS and the proportion of species with a direct-link (habitat based; e.g., muskrat, waterfowl, crayfish) and indirect-link (foraging based; e.g., insectivorous passerine birds, bats, weasels) to the aquatic food-web may have an important contribution to the overall diet. The combined diet composition for KRSS GHOs (FC + TB) shows that 8.8% (numeric basis) and 21% (mass basis) of GHO prey originated wholly or in part from the aquatic food-web. In comparison, aquatic prey comprised 9% of Washtenaw County. A review of GHO diet studies available in the literature showed that aquatic prey are common in diets of GHOs residing in close proximity to wetland habitat types, and in select western and upper midwestern habitats, the proportion of aquatic prey (numeric basis) in resident GHO diets can exceed 20% and 50%, respectively (Murphy 1997; Bogiatto et al. 2003).

Average potential daily dose

A conservative approach was used to calculate APDD values for GHOs such that when site-specific PCB or $TEQ_{WHO\text{-}Avian}$ concentration data were not available for a specific component of GHO diet (e.g., rabbits, grey/fox squirrels), the shortcoming was addressed by using site-specific data for red squirrels and chipmunks to represent potential COC exposures for the group. This approach incorporated a conservative estimate of potential exposure because the omnivorous diets of squirrels and chipmunks place them in a higher trophic level compared to rabbits. The conservative nature of this substitution is evident in that the total PCB and $TEQ_{WHO\text{-}Avian}$ concentrations used to calculate rabbit contributions to Trowbridge APDD were 1 to 2 orders of magnitude higher than concentrations expressed by both terrestrial and aquatic herbivorous counterparts to rabbits at the site (mice/vole, muskrat; Table 3). A similar approach was used to address the absence of site-specific data for pheasants and other galliform prey where these species were grouped with passerine prey. Passerines at the site included insectivorous representatives (e.g., tree swallows) with forage-based links to the aquatic food-web at the site. Passerines had the highest concentration of total PCBs and 2nd highest concentration of $TEQ_{WHO\text{-}Avian}$ among prey groups from the TB site (Table 3). The US Fish and Wildlife Service waterfowl database also provided an unbiased characterization of potential exposure through this aquatic pathway by including

representative concentrations from both piscivorous (merganser) and omnivorous (mallard) feeding groups. Great horned owls prey indiscriminately upon waterfowl and a variety of wading birds (Rusch et al. 1972). Piscivorous waterfowl and shorebirds have been shown to accumulate total PCB and $TEQ_{WHO\text{-}Avian}$ concentrations that are 10- to 15-fold greater than their closely related avian counterparts who are more herbivorous (Jones et al. 1993). Processing of small mammals and avian prey that included the removal of stomach contents (both prey types) and feathers, beaks, wings, and legs (avian prey) is a common practice in exposure and effects studies and is typically used to conservatively estimate soil to organism bioaccumulation factors. The method contributed to conservative measurements of the COCs in these samples because the substantial mass excluded from analyses (keratin, herbaceous forage) contained much lower contaminant concentrations compared to the remaining tissues (predominantly muscle and lipids) analyzed for the target organism. This approach is also consistent with the consumption habits of GHOs which consume very small mammals in their entirety. Larger mammals are consumed in part by ripping the flesh from the skeleton and pelt. Birds are frequently plucked of their flight feathers and other larger contour feathers during or prior to being consumed. Finally, incidental soil ingestion was also included in the site-specific APDD calculations for TB exposures to account for soil that may be associated with the pelage of small mammalian prey that tunnel through vegetation or use burrows for shelter, nesting, or food storage, and also present in avian prey that consume grit and associated soil particles as a normal course of their foraging activities (Mayoh and Zach 1986).

A conservative approach also was used to estimate $TEQ_{WHO\text{-}Avian}$ from the PCB Aroclor data for waterfowl and soils in the calculation of $APDD_{TEQ}$ for GHOs. Dioxin equivalent concentrations ($TEQ_{WHO\text{-}Avian}$) were estimated by selecting the greatest proportional contribution of each individual non-*ortho* and mono-*ortho* PCB congener across 4 technical Aroclor mixtures as the fractional composition value for each medium. The greatest potential concentrations for each congener were supplemented with additional "enrichment factor" increases to 2 bioaccumulative and toxic non-*ortho* congeners (PCB 126 and 169). As a result, the estimated $TEQ_{WHO\text{-}Avian}$ concentrations for waterfowl and soils have much greater toxicity than would be predicted from the original Aroclor mixtures. Waterfowl $TEQ_{WHO\text{-}Avian}$ concentrations were the greatest among all prey type contributions to $APDD_{TEQ}$ by a factor of 3 and 7 for the geometric mean and upper 95% CL concentrations, respectively (Table 3).

The only notable difference between estimates of APDD from a site-specific diet ($APDD_{measured}$) and literature-based diet ($APDD_{predicted}$) were greater $APDD_{predicted}$ for total PCB (geometric mean and upper 95% CL values) at TB (mass-based diet), and greater $APDD_{measured}$ for $TEQ_{WHO\text{-}Avian}$ (geometric mean and upper 95% CL) values at FC (numeric- and mass-based diets). At TB, the greater $APDD_{PCB}$ values for the literature-based diet was due to the greater proportions of pheasants and to the elevated total PCB concentrations in the passerine/terrestrial avian prey group compared to total PCB concentrations in rabbits, the predominant prey group for TB owls. The difference in predicted versus measured $APDD_{PCB}$ was not observed at FC because the large differences in the proportion of passerine/terrestrial prey (e.g., pheasant) between the site-specific and literature-based diets was mitigated by the small total PCB concentrations in the dietary items collected at FC, a larger proportion of waterfowl in the FC $APDD_{measured}$ versus literature-based $APDD_{predicted}$, and the greater waterfowl total PCB concentrations used for the $APDD_{PCB}$ calculations (Tables 2 and 3; Figure 6). At FC, the greater $APDD_{TEQ}$ for the site-specific diet originated from the overriding influence of waterfowl prey. This included a larger proportion of waterfowl in the FC $APDD_{measured}$ versus literature-based $APDD_{predicted}$, and the much greater waterfowl $TEQ_{WHO\text{-}Avian}$ concentrations used for the $APDD_{TEQ}$ calculations (Tables 2 and 4; Figure 6). This difference in $APDD_{TEQ}$ was not present between $APDD_{predicted}$ /$APDD_{measured}$ at TB. The much larger proportion of passerine/terrestrial prey (e.g., pheasant) in the literature-based diet was mitigated by greater mean $TEQ_{WHO\text{-}Avian}$ in rabbits versus passerines/terrestrial avian prey, coupled with additional TEQ contributed from muskrat prey (with greater variable TEQ concentrations) to $APDD_{measured}$ that was calculated from the upper 95% CL for $TEQ_{WHO\text{-}Avian}$ in prey populations.

Overall, calculations of $APDD_{predicted}$ /$APDD_{measured}$ in this study were primarily influenced by gaps in the site-specific data for principal prey items in both the site-specific and literature-based diets. Although $APDD_{predicted}$ and $APDD_{measured}$ values were very similar across the range of prey concentration values at FC and TB, the notable differences observed in APDD can be traced to the lack of site-specific data for pheasants and rabbits, and the lack of recent, congener-specific total PCB data for waterfowl. Because all surrogate data used to address these data gaps was chosen to insure that any potential biases contributed by these data erred in a conservative "worst-case" manner, it is reasonable to assume that if site-specific data were available for these prey, the calculated $APDD_{PCB}$/$APDD_{TEQ}$ for both diets would have decreased and the relationships between $APDD_{measured}$ and $APDD_{predicted}$ for both total PCBs and $TEQ_{WHO\text{-}Avian}$ would have changed. This exercise also illustrates that differences between $APDD_{measured}$ and $APDD_{predicted}$ may be exacerbated at sites where the contaminant distribution between proximal aquatic and terrestrial habitats is dissimilar, and prey with links to the aquatic food-web figure predominantly in site-specific GHO diets. In these instances, the unique composition of a site-specific diet that includes aquatic prey may contribute significantly to the overall assessment of exposure, therefore posing significant potential risk that may be overlooked if the hazard assessment relies upon a literature-based dietary composition that fails to identify important prey items with links to aquatic exposures.

Hazard estimates based on total PCBs and TEQs

Hazard quotients based on $TEQ_{WHO\text{-}Avian}$ were greater than those based on total PCBs. Hazard quotients calculated from NOAEL TRVs for geometric mean and upper 95% CL concentrations of $TEQ_{WHO\text{-}Avian}$ were 4- to 13-fold greater than for total PCBs at FC, and 2- to 3-fold greater at TB (Table 6; Figure 7). Hazard quotients based on total PCB concentrations are considered to be an accurate estimate of potential risk because the concentration in the diet can be compared directly to values reported in the studies from which TRVs were derived. Congener-specific analyses provided for coplanar PCB congeners to be used in a calculation of TEQ. This

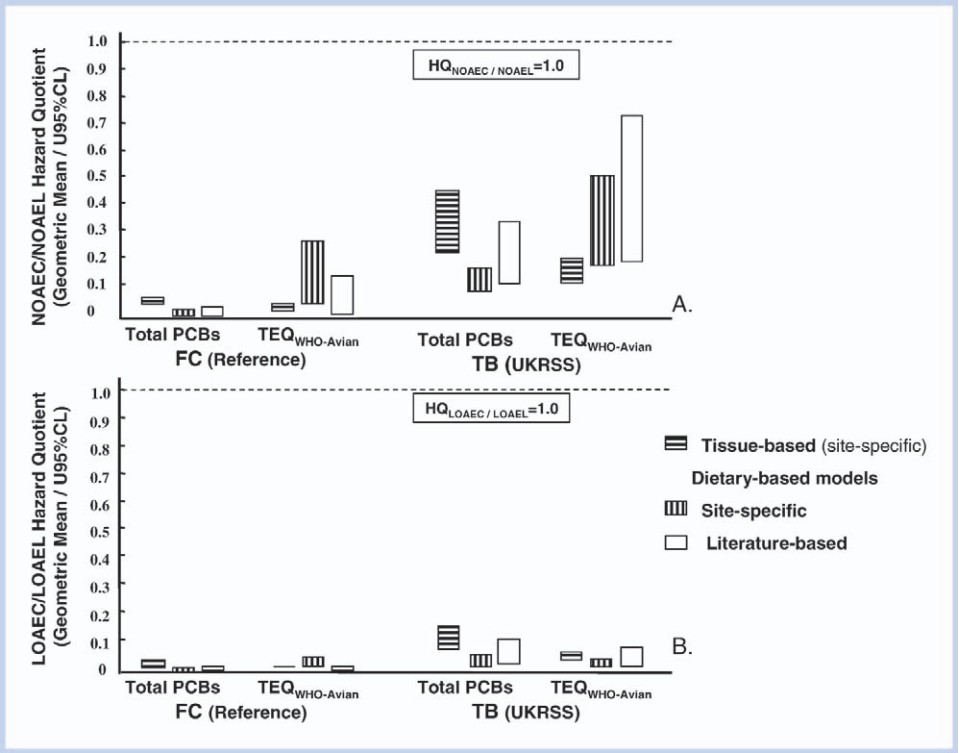


Figure 7. (**A**) Comparison of tissue-based (egg) no observable adverse effect concentration (NOAEC) and diet-based no observable adverse effect level (NOAEL) hazard quotients (HQs) at the Upper Kalamazoo River Superfund Site (Trowbridge) and Reference (Ft. Custer) locations calculated from NOAEC/NOAEL-based toxicity reference values (TRVs) for polychlorinated biphenyls (PCBs) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$). Each box encompasses the geometric mean and upper 95% confidence level concentration. Dietary HQ ranges include average potential daily dose (APDD) concentrations computed using numeric- and mass-based dietary compositions. (**B**) Comparison of tissue-based (egg) lowest observable adverse effect concentration (LOAEC) and diet-based lowest observable adverse effect level (LOAEL) Hazard Quotients (HQs) at the Upper Kalamazoo River Superfund Site (Trowbridge) and Reference (Ft. Custer) locations calculated from LOAEC/LOAEL-based TRVs for polychlorinated biphenyls (PCBs) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$). Each box encompasses the geometric mean and upper 95% confidence level concentration. Dietary HQ ranges include average potential daily dose (APDD) concentrations computed using frequency- and mass-based dietary compositions.

approach eliminated the difficulties and uncertainties involved with assessing the toxicity of environmentally weathered PCB mixtures that are quantified as Aroclors, and is generally believed to correlate better with toxicity than measures of total PCBs (Blankenship and Giesy 2002).

A number of factors contribute to the greater $TEQ_{WHO\text{-}Avian}$ HQ values calculated in this study. The absence of a well-designed TEQ feeding study for *Strigiformes* (or a more closely related raptor species) introduced uncertainties to the TRV screening process (discussed previously) and necessitated selection of a conservative TRV for HQ_{TEQ} calculations. The scientific basis for TEQ derivation and use may contribute to bias that overestimates risk when TEQs are applied to complex mixtures of PCBs. Concentrations of TEQs are calculated by multiplying each PCB congener by a class-specific (mammal, bird, fish) relative potency expressed as a toxic equivalency factor. Toxic equivalency factors are consensus values that were rounded up to be conservative estimates of potential risk (Van den Berg et al. 1998). These practices, coupled with the use of proxy values for congeners that were present at concentrations less than the method detection limit (e.g., use of one-half the method detection limit for nondetects), the summation of co-eluting congeners into a single value for some mono-*ortho* PCB congeners, and conservative estimates of congener fractional composition values for historical Aroclor PCB data are likely reasons that HQs based on $TEQ_{WHO\text{-}Avian}$ are greater than HQs estimated for total PCBs. Additionally, a recent review of tree swallow exposure studies indicated that $TEQs_{WHO\text{-}Avian}$ calculated from field-based TCDD and PCB exposures did not elicit similar endpoints of effect and may not be toxicologically equivalent (Neigh, Zwiernik, Blankenship, et al. 2006).

The multiple-lines-of-evidence approach

This study has determined that dietary exposures of resident GHO populations to total PCBs and $TEQ_{WHO\text{-}Avian}$ present in contaminated floodplain soil of the KRSS are well below the threshold for effects on reproductive success. Even when the most conservative estimates of HQ are considered in the bottom-up assessment of potential hazards at the site, all HQ values for calculated APDDs using a site-specific dietary composition are less than 0.5. Similarly, all HQ values for calculated APDDs using a literature-based dietary composition at the site are less than 0.75 (Table 6). The greater HQ value for the literature-based diet originated from overestimates of the passerine/terrestrial proportion of avian prey.

The bottom-up assessment was one component of a multiple-lines-of-evidence approach that also included a tissue-based top-down investigation of GHO exposure by investigating PCB concentrations in eggs and nestling plasma (Strause et al. 2007). Results of the tissue-based studies were consistent with the dietary findings (Figure 7). The observed total PCB/$TEQ_{WHO\text{-}Avian}$ concentrations in eggs resulted in HQs less than 1.0 for all exposures indicating that tissue-

based exposures did not pose a significant potential risk to GHO populations at the Upper KRSS. Hazard quotients calculated for tissue-based and site-specific dietary exposures show strong agreement at both the Reference and Upper KRSS study sites with less than a 3-fold difference between the ranges of $HQ_{NOAEC/NOAEL}$ and $HQ_{LOAEC/LOAEL}$ (mean and upper 95% CL concentrations) for both COCs, excepting $TEQ_{WHO\text{-}Avian}$ concentrations at FC where a 7-fold range in HQs was present.

The multiple-lines-of-evidence approach included ancillary investigations to the top-down assessment that focused on evaluating the relative abundance, site use, and productivity of resident GHOs at the Upper KRSS relative to the upstream Reference location (Figure 1). The relative abundance of territory-holding nesting pairs of GHOs in the Upper KRSS was near the carrying capacity for the available habitat area included in the study. Nest acceptance rates and nest fidelity of actively breeding Upper KRSS GHOs across all nesting seasons included in the study were consistent with previous studies of artificial nest acceptance and habitat usage by Strigiforms in midwestern forests (Holt 1996). Mean productivity rates (fledglings/active nest) were similar among locations where exposures to PCBs were much different, and were consistent with productivity measures for healthy midwestern GHO populations (Holt 1996; Strause et al. 2007). These results agree with findings for both the top-down and bottom-up approaches to evaluate chemical exposures at the site, and serve to reduce the uncertainties associated with assessment endpoints and strengthen the conclusion that potential risk to GHOs from exposures to total PCB/ $TEQs_{WHO\text{-}Avian}$ in the Upper KRSS are unlikely to be sufficient to cause adverse effects.

Results from this study suggest that it would be appropriate to estimate potential risk based on either tissue-based or dietary-based methodologies. However, if a dietary-based approach to estimate potential risk to GHOs is used, studies of site-specific diet must be completed to assure that site-specific data can be collected for principal prey items representing potential exposures for both aquatic and terrestrial food webs at any site where aquatic habitats are located in close proximity to resident GHO nesting habitats. Additionally, because budget limitations will constrain the breadth of prey item sampling and analyses at most sites, it is essential that risk assessors clearly communicate all dietary assumptions applied to the data (e.g., prey groupings, gaps in the chemical database for any prey comprising a denotive dietary proportion) and how these assumptions impact risk calculations at the site.

Acknowledgment—Sook Hyeon Im provided much-appreciated assistance in the extraction laboratory. Technical support and assistance with field sampling was received from Simon Hollamby, Heather Simmons, and Donna Pieracini. Joe Johnson of the MSU Kellogg Biological Station Bird Sanctuary aided in identifications of avian prey remains. Eva-Maria Muecke assisted with the statistical analyses. The assistance provided by the US Fish and Wildlife Service, East Lansing Field Office, notably Lisa Williams, Dave Best, and Charles Mehne and the staff of the Michigan Department of Natural Resources at Fort Custer and the Allegan State Game Area including William Kosmider, John Lerg, and Mike Baily were instrumental to the success of this study. Funding for this project was provided by the Kalamazoo River Study Group.

REFERENCES

Adamcik RS, Todd AW, Keith LB. 1978. Demographic and dietary responses of great horned owls during a snowshoe hare cycle. *Can Field-Nat* 92:156–166.

Baker RH. 1983. Michigan mammals. Detroit (MI): Michigan State University Press.

[BBL] Blasland, Bouck & Lee. 1993. Allied Paper, Inc./Portage Creek/Kalamazoo River Superfund Site description of the current situation. Syracuse (NY): BBL.

[BBL] Blasland, Bouck & Lee. 1994. Allied Paper, Inc./Portage Creek/Kalamazoo River Superfund Site remedial investigation/feasibility study. Technical memorandum 2: Results of phase I TBSA soil sampling. Syracuse (NY): BBL.

Blankenship AL, Giesy JP. 2002. Use of biomarkers of exposure and vertebrate tissue residues in the hazard characterization of PCBs at contaminated sites: Application to birds and mammals. In: Sunahara GI, Renoux AY, Thellen C, Gaulet CL, Pilon A, editors. Environmental analysis of contaminated sites. West Sussex (UK): John Wiley. p 153–180.

Blankenship AL, Zwiernik MZ, Coady KK, Kay DP, Newsted JL, Strause K, Park C, Bradley PW, Neigh AM, Millsap SD, Jones PD, Giesy JP. 2005. Differential accumulation of polychlorinated biphenyl congeners in the terrestrial food web of the Kalamazoo River superfund site, Michigan. *Environ Sci Technol* 39:5954–5963.

Bogiatto RJ, Sardella BA, Essex JJ. 2003. Food habits of great horned owls in northeastern California with notes on seasonal diet shifts. *West N Am Nat* 63:258–263.

Craighead JJ, Craighead FC. 1956. Hawks, owls and wildlife. Harrisburg (PA): Stackpole. 403 p.

Dunning JB. 1984. Body weights of 686 spp. of North American birds. Monograph 1. McKinleyville (CA): Western Bird Banding Association.

Elliott J, Kennedy SW, Lorenzen A. 1997. Comparative toxicity of polychlorinated biphenyls to Japanese quail (*Coturnix c. japonica*) and American kestrels (*Falco sparverius*). *J Toxicol Environ Health* 51:57–75.

Elliott JE, Norstrom RJ, Lorenzen A, Hart LE, Philibert H, Kennedy SW, Stegeman JJ, Bellward GD, Cheng KM. 1996. Biological effects of polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls in bald eagle (*Haliaeetus leucocephalus*) chicks. *Environ Toxicol Chem* 15:782–793.

Elliott JE, Wilson LK, Henny CJ, Trudeau S, Leighton FA, Kennedy SW, Cheng KM. 2000. Biological effects of chlorinated hydrocarbons in osprey chicks. *Environ Toxicol Chem* 20:866–879.

Errington PL. 1930. The pellet analysis method of raptor food habits study. *Condor* 32:292–296.

Errington PL. 1932. Technique of raptor food habits study. *Condor* 34:75–86.

Errington PL. 1938. The great horned owl as an indicator of vulnerability in prey populations. *J Wildl Manag* 2:190–204.

Errington PL, Hamerstrom F, Hamerstrom FN. 1940. The great horned owl and its prey in north-central United States. Ames (IA): Iowa State College Agricultural Experiment Station. Research Bulletin 277.

Fairbrother A. 2003. Lines of evidence in wildlife risk assessments. *Human and Ecological Risk Assessment* 9:1475–1491.

Fitch HS. 1947. Predation by owls in the Sierran foothills of California. *Condor* 76:45–61.

Glading B, Tillotson DF, Selleck DM. 1943. Raptor pellets as indicators of food habits. Sacramento (CA): California Fish and Game SH 11. 29:92–121.

Hayward JL, Galusha JG, Frias G. 1993. Analysis of great horned owl pellets with rhinoceros auklet remains. *Auk* 110:133–135.

Hickey JJ, Anderson BW. 1968. Chlorinated hydrocarbons and eggshell changes in raptorial and fish-eating birds. *Science* 162:271–273.

Hoffman JH, Melancon P, Klein JD, Eisemann JD, Spann JW. 1998. Comparative developmental toxicity of planar polychlorinated biphenyl congeners in chickens, American kestrels, and common terns. *Environ Toxicol Chem* 17:747–757.

Holt JB. 1996. A banding study of Cincinnati area great horned owls. *J Raptor Res* 30:194–197.

Houston CS, Smith DG, Rohner C. 1998. Great horned owl (*Bubo virginianus*). In: Poole A, Gill F, editors. The birds of North America, Philadelphia (PA): Academy of Natural Sciences of Philadelphia.

Jones PD, Ankley GT, Best DA, Crawford R, Degalen N, Giesy JP, Kubiak TJ, Ludwig JP, Newsted JL, Tillitt DE, Verbrugge DA. 1993. Biomagnification of bioassay derived 2,3,7,8-tetrachlorodibenzo-p-dioxin equivalents. *Chemosphere* 26:1203–1212.

Kay DP, Blankenship AL, Coady KK, Neigh AM, Zwiernik MJ, Millsap SD, Strause K, Park C, Bradley P, Newsted JL, Jones PD, Giesy JP. 2005. Differential accumulation of polychlorinated biphenyl congeners in the aquatic food web at the Kalamazoo River superfund site, Michigan. *Environ Sci Technol* 39:5964–5974.

Lillie RJ, Cecil HC, Bitman J, Fries GF. 1974. Differences in response of caged white leghorn layers to various polychlorinated biphenyls (PCBs) in the diet. *Poult Sci* 53:726–732.

Luukkonen DR. 1998. Abundance, distribution and winter survival of pheasants in southern Michigan. East Lansing (MI): Michigan State University.

Marti C. 1974. Feeding ecology of four sympatric owls. *Condor* 76:45–61.

Marti CD. 1987. Raptor food habits studies. In: Giron Pendleton BA, Millsap BA, Cline KW, Bird DM, editors. Raptor techniques manual. Washington DC: National Wildlife Federation. p 67–79.

Marti CD, Kochert MN. 1995. Are red-tailed hawks and great horned owls diurnal-nocturnal dietary counterparts? *Wilson Bull* 107:615–628.

Marti CD, Kochert MN. 1996. Diet and trophic characteristics of great horned owls in southwestern Idaho. *J Field Ornithol* 67:499–506.

Mayoh KR, Zach R. 1986. Grit ingestion by nestling tree swallows and house wrens. *Can J Zool* 64:2090–2093.

McLane MAR, Hughes DL. 1980. Reproductive success of screech owls fed Aroclor 1248. *Arch Environ Contam Toxicol* 9:661–665.

[MDNR] Michigan Department of Natural Resources. 1987. Kalamazoo river remedial action plan. Second draft. Lansing (MI): MDNR.

Millsap SD, Blankenship AL, Bradley PW, Jones PD, Kay DP, Neigh A, Park C, Strause KD, Zwiernik MJ, Giesy JP. 2004. comparison of risk assessment methodologies for exposure of mink to PCBs on the Kalamazoo River, MI. *Environ Sci Technol* 38:6451–6459.

Murphy RK. 1997. Importance of prairie wetlands and avian prey to breeding great horned owls (*Bubo virginianus*) in northwestern North Dakota. In: Duncan JR, Johnson DH, Nicholls TH, editors. Second international symposium, biology and conservation of owls in the northern hemisphere. Winnipeg (MB): USDA Forest Service. General technical report NC-190.

Neigh AM, Zwiernik MJ, Blankenship AL, Bradley PW, Kay DP, MacCarroll MA, Park CS, Jones PD, Millsap SD, Newsted JW, Giesy JP. 2006. Exposure and multiple lines of evidence assessment of risk for PCBs found in the diets of passerine birds at the Kalamazoo River superfund site, Michigan. *Human and Ecological Risk Assessment* 12:924–946.

Neigh AM, Zwiernik MJ, Bradley PW, Kay DP, Park CS, Jones PD, Newsted JL, Blankenship AL, Giesy JP. 2006. Tree swallow (*Tachycineta bicolor*) exposure to polychlorinated biphenyls at the Kalamazoo River superfund site, Michigan, USA. *Environ Toxicol Chem* 25:428–437.

Neigh AM, Zwiernik MJ, Bradley PW, Kay DP, Jones PD, Holem RR, Blankenship AL, Strause KD, Newsted JL, Giesy JP. 2006. Accumulation of polychlorinated biphenyls from floodplain soils by passerine birds. *Environ Toxicol Chem* 25:1503–1511.

Nosek JA, Craven SR, Sullivan JR, Hurley SS, Peterson RE. 1992. Toxicity and reproductive effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in ring-necked pheasant hens. *J Toxicol Environ Health* 35:187–198.

Peterson L. 1979. Ecology of great horned owls and red-tailed hawks in southeastern Wisconsin. Madison (WI): Wisconsin Department of Natural Resources. Technical bulletin 111.

Platonow NS, Reinhart BS. 1973. The effects of polychlorinated biphenyls (Aroclor 1254) on chicken egg production, fertility and hatchability. *Can J Comp Med Vet Sci* 37:341–346.

Ratcliffe DA. 1968. Changes attributable to pesticides in egg breakage frequency and eggshell thickness in some British birds. *J Appl Ecol* 7:67–105.

Rusch DH, Meslow EC, Doerr PD, Keith LB. 1972. Response of great horned owl populations to changing prey densities. *J Wildl Manag* 36:282–296.

Sample BE, Opresko DM, Suter GW II. 1996. Toxicological benchmarks for wildlife; 1996 Revision. Oak Ridge (TN): Oak Ridge National Laboratory, Health Sciences Division. ES/ER/TM86-R3.

Schwartz TR, Tillit DE, Feltz KP, Peterman PH. 1993. Determination of mono- and non-*O,O′*-chlorine substituted polychlorinated biphenyls in Aroclors and environmental samples. *Chemosphere* 26:1443–1460.

Sheffield SR. 1997. Owls as biomonitors of environmental contamination. St. Paul (MN): US Forest Service. General technical report NC-190.

Springer MA, Kirkley JS. 1978. Inter and intraspecific interactions between red-tailed hawks and great horned owls in central Ohio. *Ohio J Sci* 78:323–329.

Strause KD, Zwiernik MJ, Im SH, Bradley PW, Moseley PP, Kay DP, Park CS, Jones PD, Blankenship AL, Newsted JL, Giesy JP. 2007. Risk assessment of great horned owls (*Bubo virginianus*) exposed to polycholorinated biphenyls (PCBs) and DDT along the Kalamazoo River, Michigan. *Environ Toxicol Chem* 26:1386–1398.

Studier EH, Sevick SH. 1992. Live mass, water content, nitrogen and mineral levels in some insects from south-central lower Michigan. *Comp Biochem Physiol A* 103:579–595.

[USEPA] US Environmental Protection Agency. 1976. PCBs in the United States: Industrial use and environmental distribution: Task I. Washington DC: USEPA. EPA 560/6–76/005.

[USEPA] US Environmental Protection Agency. 1993. Wildlife exposure factors handbook. Washington DC: USEPA. EPA 600/R-93/187b.

Van den Berg M, Birnbaum L, Bosveld AT, Brunstrom B, Cook P, Freeley M, Giesy JP, Hanberg A, Hasegawa R, Kennedy SW, Kubiak T, Larsen JC, Rolaf van Leeuwen FX, Djien Liem AK, Nolt C, Peterson RE, Poellinger L, Safe S, Schrenk D, Tillett D, Tysklind M, Younes M, Waern F, Zacharewski T. 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environ Health Perspect* 106:775–792.

Voous KH. 1988. Owls of the northern hemisphere. Cambridge (MA): MIT Press.

Wink J, Senner SE, Goodrich LJ. 1987. Food habits of great horned owls in Pennsylvania. *Proc Pa Acad Sci* 61:133–137.

Woodford JE, Karasov WH, Meyer MW, Chambers L. 1998. Impact of 2,3,7,8-TCDD exposure on survival, growth and behavior of ospreys breeding in Wisconsin, USA. *Environ Toxicol Chem* 17:1323–1331.

Human and Ecological Risk Assessment, 13: 966–985, 2007
Copyright © Taylor & Francis Group, LLC
ISSN: 1080-7039 print / 1549-7680 online
DOI: 10.1080/10807030701506595

Site-Specific Assessments of Environmental Risk and Natural Resource Damage Based on Great Horned Owls

Matthew J. Zwiernik,[1] Karl D. Strause,[1] Denise P. Kay,[2] Cyrus S. Park,[1] Alan L. Blankenship,[1,2] and John P. Giesy[1,3]
[1]Department of Zoology, Center for Integrative Toxicology, National Food Safety and Toxicology Center, Michigan State University, East Lansing, MI, USA; [2]ENTRIX, Inc., Okemos, MI, USA; [3]Department of Veterinary Biomedical Sciences and Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, and Biology and Chemistry Department, City University of Hong Kong, Kowloon, Hong Kong, SAR, China

ABSTRACT

Selection of receptors is a key element of ecological risk and natural resource damage assessments. The great horned owl (*Bubo virginianus;* GHO) has advantages as a tertiary terrestrial receptor and integrated measure of exposure to chemical residues in a multiple-lines-of-evidence approach that includes elucidation of contaminant exposure by measured (tissue-based) and predicted (dietary) methodologies, and population-level measures of potential adverse effects (*i.e.*, productivity, abundance). Methods described herein exploited attributes of GHO behavior, including its propensity to nest in artificial nesting platforms. This approach allowed better control of experimental conditions, minimized uncertainty in assessment endpoints, and maximized data utility for testing hypotheses. During 5 years, 54 GHO nests (14 active territories) along 38 km of river floodplain were monitored at the Kalamazoo River Superfund Site (Kalamazoo/Allegan Counties, Michigan). Concentrations of polychlorinated biphenyls (PCBs) and *otho-*, *para*-substituted isomers of dichlorodiphenyltrichloroethane (DDT), including DDD/DDE (ΣDDTs), were measured in 24 eggs and 16 samples of nestling blood plasma. Dietary PCB exposure was predicted by determining site-specific dietary composition and sampling and quantifying PCB concentrations in 171 prey items collected within active GHO territories. The convergence of exposure and effects assessments improved confidence in resulting predictions of minimal risk to resident GHO populations (Hazard Quotients ≤ 1.5). Repeated GHO use of nesting platforms minimized temporal and spatial variability.

Received 11 November 2006; revised manuscript accepted 3 December 2006.
Address correspondence to Dr. Matthew Zwiernik, 224 National Food Safety and Toxicology Bld., East Lansing, MI 48848, USA. E-mail: zwiernik@msu.edu

Key Words: ERA, receptor, raptor, great horned owl, exposure assessment, multiple-lines-of-evidence.

INTRODUCTION

Raptor species have long been used as environmental monitors (IJC 1991; Sundlof *et al.* 1986; CEQ 1972) because they are sensitive to some of the more prevalent contaminants of concern (COCs), and have a high potential for exposure to those residues. Herein we describe direct, site-specific, field assessment methodologies that use the great horned owl (*Bubo virginianus;* GHO) as a sentinel or surrogate species for terrestrial-based organisms in assessing ecological hazards or natural resource damages as well as site-specific clean-up values for soils. The methodologies take advantage of useful attributes of the GHO in a multiple-lines-of-evidence approach to assess potential exposure to COCs and potential effects. Exposure was quantified both by predicting exposure through the diet and by measuring concentrations in blood plasma and eggs of GHOs. Both estimates of exposure were then compared to threshold concentrations for effects reported in the literature. Concurrent measures of GHO abundance and reproductive performance were used to assess consistency between predicted effects thresholds based on the risk assessments and observed effects in resident populations. Measurement endpoints from each line of evidence were combined in a weight of evidence approach to assess potential risks to resident GHO populations at the Kalamazoo River Superfund Site (KRSS). The methods were designed to minimize uncertainty in assessment endpoints (Fairbrother 2003), minimize the ecological impact of data collection, and maximize the utility of data in testing hypotheses.

SPECIES APPLICABILITY

Guidelines promulgated by the U.S. Environmental Protection Agency (USEPA) state that species-specific as well as site-specific factors dictate the applicability of an organism for use as a species of concern in risk assessments performed for Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA)–based ecological field studies (USEPA 1994, 1997, 1999). The ultimate goal is to select specific populations or communities for which the collected data and resulting decisions can be extrapolated across the ecosystem of interest. Both the GHO and the specific methods described herein have a broad applicability to key ecological components.

Comparisons of measurement endpoints for GHOs can be made across wide geographical regions and habitat types. The GHO is endemic throughout the temperate and sub-arctic regions of the Americas from Alaska to Argentina and has one of the largest ranges of all raptors (Houston *et al.* 1998; Burton 1984; AOU 1983). In addition, it is able to utilize more habitat types than any other American raptor species (Johnsgard 1988) while maintaining a foraging range and taxonomic dietary composition that is similar to a number of less adaptive medium and large terrestrial-based receptors (Austing and Holt 1966; Austing 1964; Craighead and Craighead 1956).

In addition to geographic applicability, a number of species-specific characteristics need to be considered when selecting organisms for study. These include intensity

(concentration) and duration (time spent on-site) of exposure, appropriateness as a surrogate species, sensitivity to some of the primary contaminants of concern at many sites, including the KRSS, ecological function, relative ease of conducting field studies with the organism, and other recognized values (USEPA 1994). The GHO is a top food web predator and year round resident throughout its range. Great horned owls are strict carnivores with large rates of ingestion, relative to their body weight (Tabaka *et al.* 1996) and have life spans known to exceed 28 yrs (Nero 1992). These attributes, as well as the fact that GHOs have no known predators, makes the GHO a useful indicator of the magnitude and bioavailable fraction of contaminants in terrestrial ecosystems.

Great horned owls are considered to be among the most sensitive animals to some of the most common environmental contaminants that occur in terrestrial environments (Hoffman 1995). Great horned owls are susceptible to environmental contaminants because of their high dose potential (*e.g.*, variety and mass of prey ingested) and inherent physiological sensitivity to chemical stressors. Dietary exposure of owls to small amounts of select contaminants such as organophosphates, organochlorines, and metals has been shown to cause lethal and sub-lethal effects including reproductive impairment or failure (Sheffield 1997). Because of these characteristics, the GHO is a useful sentinel or surrogate for other terrestrial species, or as a bio-indicator or bio-monitor for evaluating potential exposures of avian populations to contaminants (Sheffield 1997).

The nesting characteristics of GHOs provide advantages as indicators of contaminant bioavailability relative to raptors. In terms of both geographical location and habitat diversity, the GHO occupies the greatest range of nesting sites of any bird in the Americas (Baumgartner 1938). Great horned owls do not construct their own nests, but rather commandeer the nests of others, which are typically nests of squirrels, hawks, or crows. For this reason, GHOs will utilize artificial nesting platforms (Bohn 1985; Holt 1996). Great horned owls will continue to use a nest as long as it remains successful and serviceable, but will not maintain a nest. Natural nests, especially usurped nests, are rarely used for more than a single season (Frank 1997; Holt 1996). As a result, GHOs are almost always looking for a new nest within their territory and quickly move to constructed nesting platforms. The use of artificial nesting platforms obviates the need to locate and access natural nests and simplifies monitoring of GHOs. In addition, platforms allow for the dictation of foraging areas, consistency among years, and minimization of predation. Constructed platforms can be durable, placed in a wide range of locations, and maintained indefinitely. The resulting multi-year use of the same nest reduces variability in temporal and spatial exposure profiles.

Great horned owls offer advantages over other tertiary terrestrial receptors when assessing site-specific COC exposures and population health. As top predators, GHOs effectively integrate exposures to COCs from multiple trophic levels and habitats. Like most higher order terrestrial predators, GHOs are opportunistic feeders with a diet that includes a wide variety of small- and medium-sized mammals, birds, insects, amphibians, and invertebrates (Marti and Kochert 1996; Voous 1988; Marti 1974; Craighead and Craighead 1956). Exposures of GHO nestlings to residues have been shown to be directly related to local contaminant concentrations (Frank 1997) and their abundance has been shown to be directly related to available prey (Rohner

1996; Houston and Francis 1995; Rusch *et al.* 1972; Adamcik *et al.* 1978) and ultimately ecosystem health.

Concentrations of COCs in GHOs can be directly assessed through the collection of tissues, eggs, or blood. Great horned owls have relatively high rates of reproduction, a factor that offers advantages in meeting sample size requirements. Great horned owls are relatively easy to capture and handle as compared to other terrestrial-based raptors. Nestlings between 5 and 6 wks of age can be easily accessed, banded, morphological characteristics measured, blood sampled, and radio tagged (Austing and Holt 1966). Broods of pre-fledge nestlings (typically one to three individuals) are confined to the nest and rely solely on prey collected by adults from areas proximal to the nest. Parental foraging ranges of GHOs are constrained during brood rearing due to nest defense and prey transport limitations. This ensures that exposures of both adult and nestling GHOs to COCs are directly linked to the immediate area surrounding the nest site.

Exposure of GHOs through the diet can be quantified by enumerating the composition of the diet and determining the concentrations of COCs in the prey items. These two parameters can then be combined to allow calculation of a weighted average concentration of COCs in the diet and an average potential daily intake (USEPA 1993). Methods to determine site-specific dietary composition are well described and include the combined examination of prey remains and regurgitated pellets (Marti 1987; Rusch *et al.* 1972; Errington 1930). Unlike other raptors, owls prefer to swallow their smaller prey items whole. The prey enters the glandular stomach where enzymes break it down. Undigested materials, including bones and hair are regurgitated in the form of a packed pellet within 2 to 24 h after consumption. These pellets and prey remains in and around the nest (associated with adult feeding perches) can be sampled over time.

The GHO as a Key Receptor (Case Study)

The studies and results reported here were part of a large group of studies in support of an ecological risk assessment of the KRSS (Blankenship *et al.* 2005; Kay *et al.* 2005; Millsap *et al.* 2004; Neigh *et al.* 2006a,b; Strause 2007a). The KRSS was designated a Superfund site in 1990, and is comprised of nearly 100 km of the Kalamazoo River from Portage Creek in the city of Kalamazoo to the downstream terminus at Lake Michigan. The primary COCs were identified as polychlorinated biphenyls (PCBs) with some evidence of elevated exposures of raptors to dichlorodiphenyltrichloroethane (DDT) and its metabolites dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD) (hereafter, ΣDDT) (Mehne 1994).

The GHO study was designed to determine bioavailability and accumulation of the COCs from a terrestrial food web in the contaminated floodplain of the Kalamazoo River. The potential for adverse effects to resident GHO populations was estimated using a hazard quotient (HQ) approach that was validated by comparisons to the abundances and reproductive productivity of GHOs in the target study areas, as well as reference areas, and information available in the literature about these population parameters at other uncontaminated locations.

METHODS

Study Site

The study area included sections of the Kalamazoo River, both upstream and downstream of known sources of contaminants. Four contiguous study areas of ≥7 km were utilized including two target areas, Lake Allegan State Game Area and the former Trowbridge impoundment, as well as two upstream reference sites at the Fort Custer State Recreation Area and Ceresco Impoundment (Figure 1). Upstream or reference locations were selected based on habitat suitability and applicability to baseline watershed contaminant exposures, and included two areas encompassing 15 km of free flowing and impounded areas of the river. Floodplain habitats included

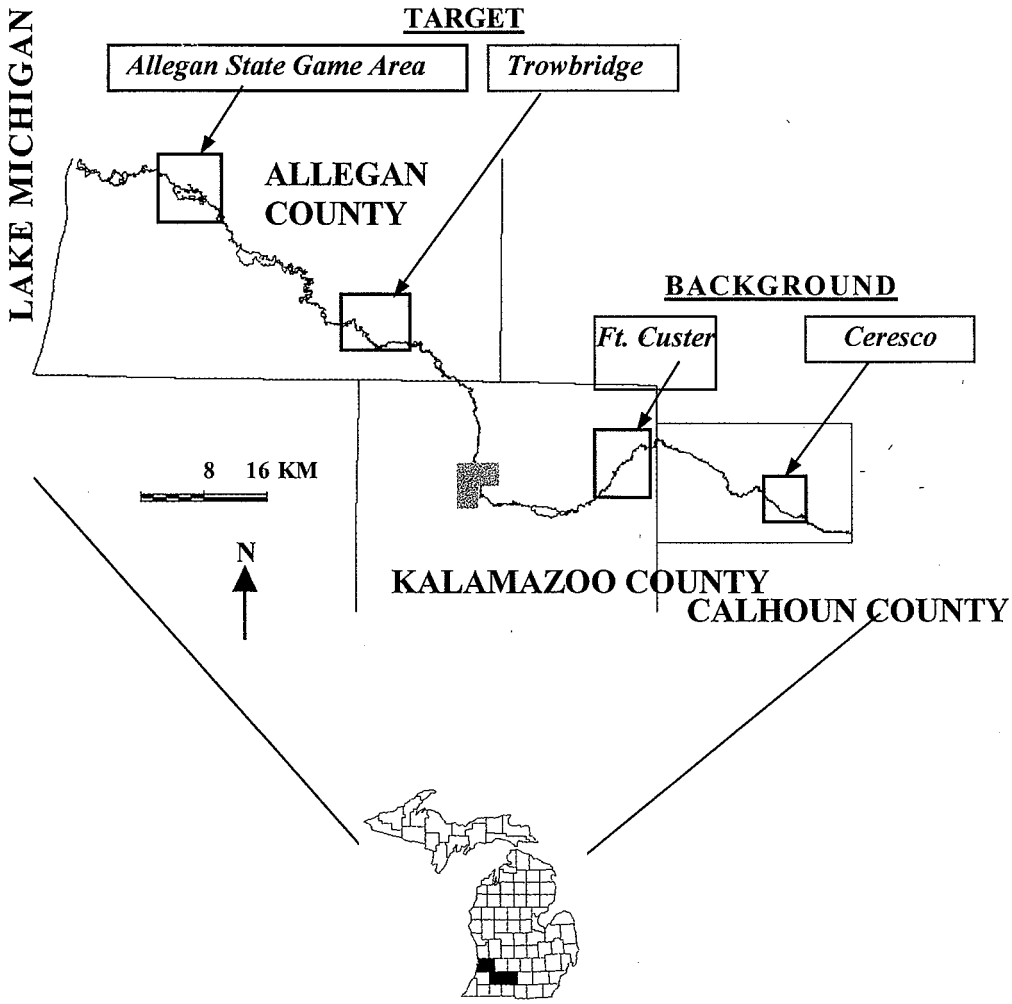


Figure 1. Map of sampling areas within Kalamazoo River floodplain. Superfund site extends 128 km from the city of Kalamazoo to its confluence with Lake Michigan. The sampling areas of Trowbridge and Allegan State Game Area lie 30 and 60 km downstream of Kalamazoo whereas the reference sampling areas of Fort Custer and Ceresco lie similar distances upstream of the start of the Superfund site, respectively.

emergent marsh, wet meadow, emergent shrub, and deciduous forested wetland. For the downstream and contaminated target areas, study locations included similar habitats of free flowing, impounded, and formally impounded sections of the river. Specific areas were selected based on a maximum potential for exposure of resident owls to the COCs from floodplain soils during foraging activities associated with nesting and subsequent rearing of offspring (Strause 2007a).

Artificial Nesting Platforms

Nest platforms were constructed, with minor modifications, as described in Henderson (1992). A 3.5′ × 3.5′ piece of 1 in "chicken wire" mesh was cut into a circle and formed into a nesting cone by making one cut from the outer edge to the center and then overlapping the two cut edges until the cone was about 18 in deep. The cut ends of the chicken wire were bent around the overlapping ends to hold the cone together and to prevent sharp ends from protruding. A 3.5′ × 3.5′ section of dark gray Tyvek® was similarly cut, folded, and placed into the wire cone. Tyvek® is strong, lightweight, and breathable and provides protection from weather, light, and moisture. A drainage hole was cut at the base of the Tyvek® cone and leaf litter was placed between the wire mesh and Tyvek®. Flexible 1/2″ and smaller stems of willow and dogwood were woven, placed at the top edge, and spiraled downward around the inside of the nest. Stems were secured to the frame with light gauge stainless steel wire (Figure 2). Once installed, one to two L of shredded wood chips were added as nesting material and to level the inside of the nest cone.

Placement of Nest Platforms

Nest platforms were placed in live trees of at least 25 cm in diameter at the base. Preferred sites included large trees on the leeward edge of shelterbelts or other areas somewhat protected from high winds. Effective nest placement was no less than 8 m from the ground and ideally at a height of 11.5 to 16.5 m. Pre-constructed nests were secured in a suitable crotch with camouflaged stainless steel adjustable pipe clamps. Because exposure to PCB contaminated floodplain soils was being evaluated, nests were located within 100 m of preferred foraging habitat and offered GHOs a combination of concealment, easy flight access, and proximity to selected foraging grounds. Ten to 15 nests were deployed per study area resulting in a density of one to three artificial nesting platforms per breeding territory. In all, a total of 54 nests were monitored including six natural nests.

Exposure Based on Measured Concentrations

The first measure of exposure included concentrations of PCBs and ΣDDT in eggs and nestling blood plasma. Egg samples were collected as soon as incubation activity was confirmed. Individual eggs were placed in pre-labeled, shock-absorbing, crush-proof transport containers placed in a pack and carried to the ground. Eggs were labeled, transported back to the laboratory and stored at 4IC until processing. Weight, volume, and eggshell thickness of eggs were determined (Stickel *et al.* 1973). The contents of the eggs were then homogenized. Blood was collected from nestlings by use of sterile technique (Henny 1997; Henny and Meeker 1981; Mauro

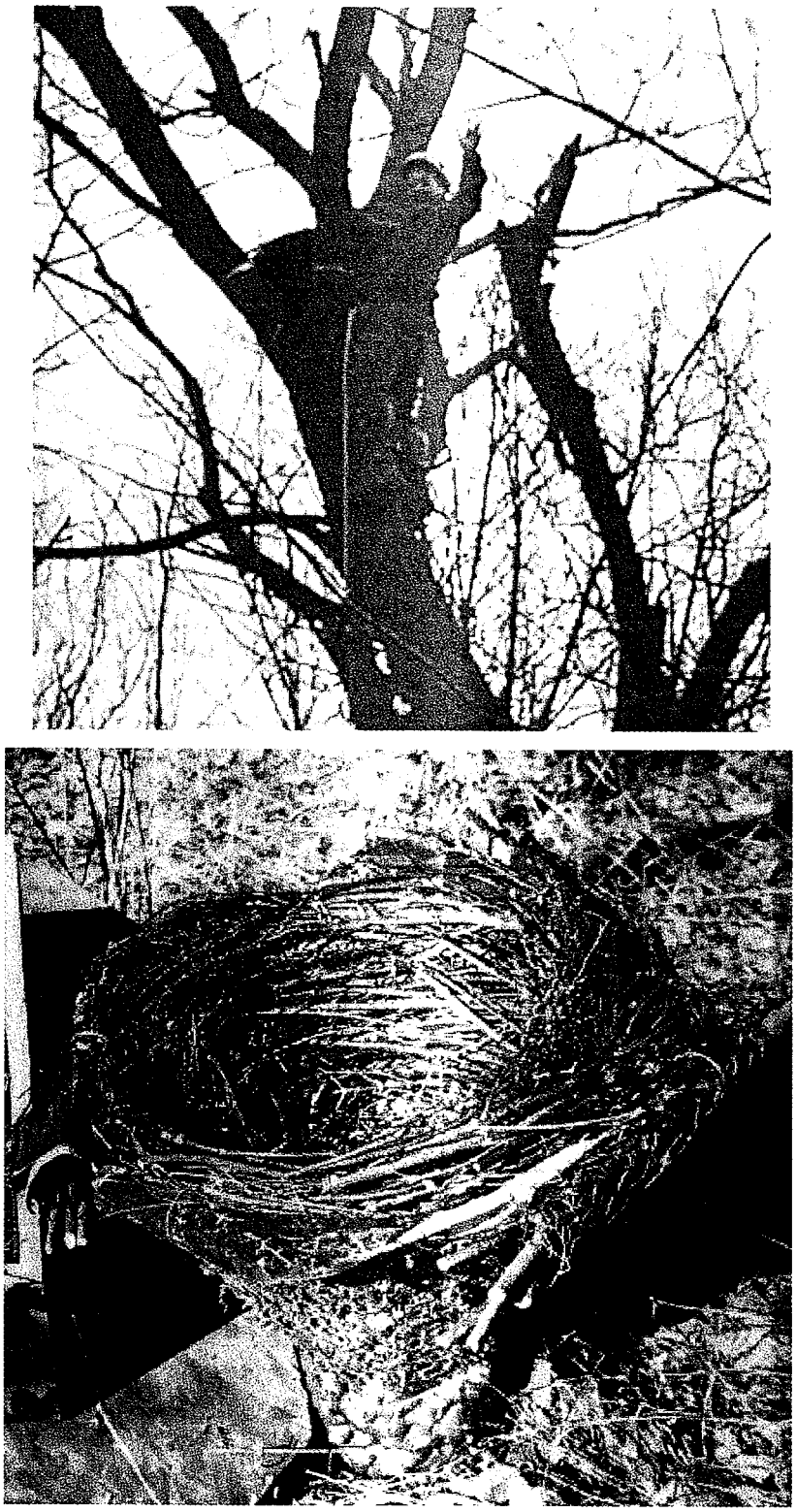


Figure 2. (Bottom) Artificial nesting platform (Top) Platform installation. *Note*: stainless steel adjustable pipe clamps are not camouflage painted for demonstration purposes.

1987) when they were 5 to 6 wk of age and weights were $\geq$0.75 kg. Owlets at this stage were relatively easy to capture, tolerated handling and could be returned unharmed to the nest. Blood was drawn with 25-gauge hypodermic needles into 10 ml syringes containing sodium heparin solution and then transferred to pre-labeled heparinized Vacutainers™ and placed on cold packs in an insulated cooler. During the collection of nestling blood samples, individual nestlings were identified by attaching leg bands (United States Fish and Wildlife Service (USFWS) #9 rivet) following standard USFWS protocols. Addled eggs and egg shell fragments also were collected at the time of banding. Vacutainers™ containing whole blood were transported to the laboratory and centrifuged at 1200 rpm for 10 min and the plasma (supernatant) was transferred into a new Vacutainer™appropriately labeled and stored upright at −20°C.

Quantification of select COCs was performed at the Michigan State University (MSU) Aquatic Toxicology Laboratory (ATL) based on project-specific data quality objectives. Total concentrations of PCBs (congener-specific analysis) and ΣDDT were determined using USEPA method 3540 (SW846), soxhlet extraction, as described elsewhere (Neigh *et al* 2006b). Briefly, concentrations of PCBs, including di- and mono-*ortho*-substituted congeners were determined by use of a gas chromatograph equipped with a ^{63}Ni electron capture detector (GC-ECD). Concentrations of non-*ortho*-substituted PCB congeners and ΣDDT were determined by gas chromatograph mass selective detector (GC-MS). The limit of quantification for di- and mono-ortho-substituted PCBs was conservatively estimated to be 1.0 ng PCB/g, ww. For coplanar PCB congeners and ΣDDT analytes, method detection limits varied among samples but were maintained for all samples at <0.1 ng/g, ww. Either TurboChrom (Perkin Elmer, Wellesley, MA, USA) or GC Chemstation software (Agilent Technologies, Wilmington, DE, USA) was used to identify and integrate the individual PCB congener peaks. Total concentrations of PCBs were calculated as the sum of all resolved PCB congeners (Kay *et al.* 2005).

Dietary Exposure

The site-specific exposure to PCBs via the diet was predicted by determining the relative proportions of prey items in the diet followed by measurement of PCBs in representative samples of those items collected from the reference and target floodplain study locations. Site-specific dietary composition for resident owls was determined from prey of actively nesting GHOs. Prey items included regurgitated pellets and any uneaten remains of prey such as bones, feathers, scales, and fur (hereafter referred to together as prey remains). All prey remains were collected from around the nest tree and beneath feeding perches prior to egg drop and incubation. Prey remains were again collected from within the nest, around the base of the nest tree, and below any associated feeding perches at time of banding and subsequently at 10-d intervals. Use of this method ensured minimal nest disturbance while ensuring that fresh prey remains were being collected. The systematic and complete removal of prey remains was done to reduce the chance of overestimating the frequency of occurrence of large prey species because of their tendency to be represented in more than one pellet or prey sample (Marti 1974). Prey remains were placed into containers and individually labeled as to collection time and relation to

nest. Prey remains collected from within the nest were limited to those items that were fully consumed. Partially consumed prey items were not collected and instead were noted as to species and size.

Relative proportions of prey items in the diet were determined by examining unconsumed prey remains as well as skeletal remains in regurgitated pellets (Hayward *et al.* 1993). Prey items were identified down to the lowest practical taxonomic classification and grouped by species, family or order. Pellet contents were quantified as to the minimum number of individuals from each taxon necessary to account for the assemblage of remains. For prey items too large to swallow whole (>100 g), individual time points and collection sites were examined together to reconcile the frequency of occurrence of larger prey species when remains of the same prey item were present in multiple samples. Multiple prey item identification keys were utilized for comparative identification including owl pellet identification keys (Carolina Biological Supply Company, Burlington, NC, USA) and the vertebrate skeletal collection from the MSU museum. Avian remains were identified with the aid of MSU Kellogg Biological Station bird sanctuary personnel. Dietary composition was based on the frequency of occurrence of all identifiable prey items and compiled on the basis of absolute (%) frequency of occurrence and relative (%) composition of biomass.

Prey Item Sampling

Once identified as a principal component of owl diet, prey species were collected from the most contaminated GHO foraging areas and a reference location. Species selection and sample sizes were determined based on sensitivity and power analyses of preliminary data and expected contribution to GHO dietary exposures. For this study, a total of 171 small mammals including meadow voles (*Microtus pennsylvanicus*), white-footed mice (*Peromyscus leucopus*), deer mice (*Peromyscus maniculatus*), meadow jumping mice (*Zapus hudsonius*), eastern chipmunks (*Tamias striatus*), short tail shrew (*Blarina brevicauda*), and masked shrews (*Sorex cinereus*) were sampled from six locations at two time points. Also sampled from these locations were arthropods, including four orders each of terrestrial and aquatic invertebrates. Larger mammals such as red squirrels (*Tamiasciurus hudsonicus*), fox squirrels (*Sciurus niger*), eastern cottontails (*Sylvilagus foridanus*), muskrats (*Ondatra zibethicus*), and mink (*Mustella vison*) as well as passerine species including the American robin (*Turdus migratorius*), house wren (*Troglodytes aedon*) and tree swallows (*Tachycineta bicolor*) were sampled opportunistically throughout the study area. Sampling techniques varied depending on target species.

Hazard Evaluation

Here we provide methodologies for site-specific assessment of the hazard of chemicals in soils to GHOs based on a multiple-lines-of-evidence approach that could enable well-informed decisions regarding potential remedial actions and determination of natural resource damages (USEPA 1997; Fairbrother 2003). Such an approach has been applied at other contaminated sites for wildlife species such as mink (Bursian *et al.* 2003) or tree swallows (Custer *et al.* 2005). However, to our knowledge, this is the first case in which field studies and multiple-lines-of-evidence have been utilized to assess potential risks of PCBs and ΣDDT to GHOs.

The multiple-lines-of-evidence included several methods of estimating exposure. Direct observations of population densities and reproductive success were made and compared to the results of the hazard assessment. Exposure of GHOs to these compounds was characterized in two ways. Concentrations of PCBs in the diet were calculated from the site-specific dietary composition and concentrations in prey items, as well as measured concentrations of PCBs and ΣDDT in eggs and blood plasma of nestling GHOs.

Each measure of exposure was compared to the threshold for a toxic effect determined from the literature and expressed as a toxicity reference value (TRV) (USEPA 1998). An ecological risk assessment (ERA) was conducted by calculating hazard quotients (HQs). Hazard quotients were determined by dividing the measured or predicted concentration in the diet, egg, or nestling blood plasma by the appropriate TRV. Toxicity reference values for the GHO hazard assessment were selected following criteria outlined by the USEPA (1995) and were derived from chronic toxicity studies in which a dose-response relationship was observed in the species of concern, or alternatively a closely related species (Strause *et al.* 2007b,c). Toxicity reference values were selected from studies that examined effects of PCBs and ΣDDT in owls and eagles, however, there were no suitable studies that used the GHO as the test species. The PCB TRVs were based on a feeding study with screech owls (*Otus asio*) that examined productivity endpoints (McLane and Hughes 1980). The ΣDDT TRVs were based on field studies that examined productivity and eggshell thinning endpoints in bald eagles (*Haliaeetus leucocephalus*) (Elliott and Harris 2002), and shell thinning endpoints in the barn owl (*Tyto alba*) (Klaas *et al.* 1978). Aside from applying an uncertainty factor of 3 to derive the PCB lowest observable adverse effect concentration from a validly determined no observable adverse effect concentration, application of additional uncertainty or extrapolation factors to our selected TRVs was not necessary. Comparisons were made between, within, and among, sites, individuals and prey species. The multiple-lines-of-evidence approach can be optimized, based on information needed, level of effort available, and site-specific criteria and characteristics.

Population Density and Reproductive Success

The final line of evidence included measurements of population health. Health of the GHO population was assessed through the evaluation of productivity including nest success, number of nestlings per nest, fledging success, and nestling age and growth measurements as well as GHO abundance. Much of the information on population dynamics was acquired in conjunction with the owl banding and nest monitoring tasks described earlier. However, an additional effort was made to evaluate GHO population health using vocalization surveys.

Vocalization Surveys

The results of vocalization surveys and triangulation were used to identify active breeding territories, locations of nests, site use, relative abundance, and confirmation of fledging success. A combination of vocalization survey methods were used including an active method in which GHO hoots were broadcast to provoke responses (Frank 1997; Brenner and Karwoski 1985), and a passive or silent survey method

during sensitive lifestage events and the periods when GHOs were most active (*e.g.*, just before and during mating) (Rohner and Doyle 1992). Relative abundance determinations were made based on the number of individuals responding on a per survey basis. Pair vocalization responses and post survey observations were evaluated and referenced to literature-based foraging areas to delineate active territories. Nestling fledge success was determined by nestling vocalizations post banding and/or subsequent visual confirmation. All positive responses and non-responses were recorded. For the positive responses, sex and age (adult or juvenile) and global positioning system coordinates of river location, and approximate azimuth values (compass readings) of response origin were recorded for the purpose of location by triangulation. Post surveys, targeted areas of 150 m radius were searched systematically by foot for signs of GHO activity (whitewash and castings) to determine roost sites and to locate nests. Active or potentially active roost sites and nest locations were recorded using a GPS receiver. To minimize disturbance to incubating birds, ground activity during the months of February and March was limited to occupancy identification of previously located nests and known nesting platforms. Nesting activity was confirmed visually by spotting scope from predetermined monitoring locations no less than 50 m from the nest or by overhead flights using fixed wing aircraft.

RESULTS

Over the course of 5 years, monitoring efforts were completed at 48 artificial and six natural nests covering approximately 14 active territories and approximately 38 km of river. Nesting activity was observed at 20 individual nests and resulted in 25 nesting attempts. Of the 20 nests utilized by GHOs, five were natural nests, including four appropriated nests of other avian species and a tree cavity nest. Artificial nesting platforms were successful in attracting GHOs to preferred study areas in the floodplain. In fact, nesting activity did not occur in natural nests in those territories for which artificial nesting platforms were in place. Reuse of artificial nesting platforms over multiple seasons allowed for the minimization of temporal and spatial variability and allowed easy access for researchers. The robust owl population was ideal for evaluating the multiple-lines-of-evidence at both the target and reference sites.

Detailed methods and results for contaminant analysis and exposure assessments are provided in separate articles (Strause *et al.* 2007b,c). The results are summarized here to illustrate the effectiveness of the methods and as an example of sample sizes that may be necessary to detect differences between the study and reference locations.

Great horned owl exposures were assessed by collecting both fresh eggs (destructive) and nestling blood plasma (non-destructive). Sample availability varied among years and locations (Table 1). A total of 40 egg and blood plasma samples were collected. Of the 24 eggs collected during the study, 5 were from the reference areas and 19 were from the target areas. Blood plasma was collected from 16 individual nestlings, this included 4 samples from the reference areas, and 12 samples from the target areas. Statistically significant differences in concentrations of total PCBs were observed among locations (reference versus target) for the predicted

Table 1. Sampling scope and blood plasma and egg summary. Description of sampling effort by year and location. Note that for years 2000–2002 the Fort Custer and Trowbridge sampling areas were monitored for productivity, thus only addled eggs were collected.

		Reference sample sites		Target sample sites	
Year		Ceresco	Fort Custer	Trowbridge Impoundments	Allegan SGA
2000	Active nests		0	1	2
	Plasma		0	1	0
	Eggs		0	0	3
	Data targeted[1]	NM	P, NP, RA	P, NP, RA	E
2001	Active nests	1	1	2	1
	Plasma	0	1	4	0
	Eggs	1	0	0	2
	Data targeted[1]	E	P, E, NP, RA	P, E, NP, RA	E
2002	Active nests	2	0	4	2
	Plasma	1	0	3	2
	Eggs	2	0	1	5
	Data targeted[1]	E, NP	P, E, NP, RA	P, E, NP, RA	E, NP
2003	Active Nests	1	1	2	1
	Plasma	1	0	1	2
	Eggs	1	1	3	0
	Data Targeted[1]	E, NP	E, NP	E, NP	E, NP
2004	Active nests	0	0	2	2
	Plasma	0	0	0	0
	Eggs	0	0	3	2
	Data targeted[1]	E, NP	E, NP	E, NP	E, NP

[1]NM = not monitored; P = productivity; E = egg sampling; NP = nestling plasma sampling; RA = relative abundance.

dietary exposure and for total PCB and ΣDDT concentrations in GHO eggs and blood plasma (Strause *et al.* 2007b, c). PCB concentrations in eggs were significantly greater at the Allegan SGA compared to the Trowbridge and reference sites ($p < 0.05$), and total DDT concentrations were significantly different among each of the Allegan SGA, Trowbridge and reference sites ($p < 0.03$).

These differences were the result of exposures to mean PCB concentrations in floodplain soil of approximately 0.17 mg PCBs/kg, dw (dry weight) in reference areas and approximately 15 mg PCBs/kg, dw in the target areas. Differences in dietary composition between the reference and target areas also were observed (Figure 3). Differences between predicted dietary exposures (average potential daily dose) were largely the result of significant differences in concentrations in the prey items (Table 2), and were not a product of differences in dietary prey item composition. Concentrations of PCBs and ΣDDT in eggs were significantly different between reference and target areas (Figure 4). Diet-based HQ values calculated from geometric mean total PCB concentrations in prey animals collected from the most contaminated areas of the KRSS floodplain were less than 1.0 at the target locations. Tissue-based

Table 2. PCB concentrations in GHO dietary items sampled from proximal foraging areas. Waterfowl were not sampled based on sensitivity analysis.

Dietary items	N	Trowbridge Mean total PCBs (± std dev) (mg/kg)[2]	N	Fort Custer Mean total PCBs (± std dev) (mg/kg)[2]
Small mammal[1]	21	*0.13 ± 0.16	18	0.021 ± 0.042
House wren adult	6	*3.57 ± 2.30	5	0.09 ± 0.032
American robin	8	*1.14 ± 1.44	4	0.091 ± 0.65
Tree swallow	5	*11.46 ± 11.90	2	1.49 ± 0.15
Shrew	17	*1.31 ± 0.94	16	0.009 ± 0.005
Muskrat	7	*0.07 ± 0.03	4	0.01 ± 0.01

[1]Includes; white-footed mouse, deer mouse, jumping mouse, meadow vole, red squirrel, and eastern chipmunk.
[2]On a wet-weight basis.
*Indicates a significant difference between sites at $p < 0.05$.

HQs calculated from the geometric mean concentrations of total PCBs and ΣDDT in eggs were ≤1.5 at all target locations (Strause *et al.* 2007b). In addition, a well-defined relationship was established for total concentrations of PCB in eggs and those in nestling blood plasma (Strause *et al.* 2007d). The statistical power of the tests were such that statistically significant differences (Type I error (α) of 0.05 and Type II error (β) < 0.20) in exposure could have been detected with as few as 4 eggs or 12 samples of nestling blood plasma per area.

Relative abundance of GHOs per river km was significantly different between the reference and target areas of the Kalamazoo River, but reproductive productivity per defended territory (number of nestlings fledged per active nest) was not significantly

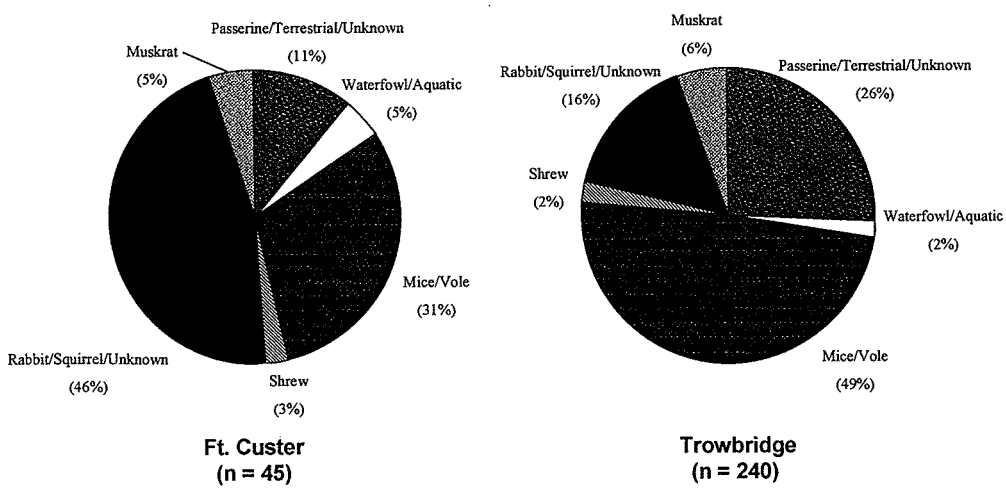


Figure 3. Dietary composition of GHOs as determined by pellet and prey remains analysis. Data presented as percent frequency of occurrence from active nests within sampling area 2000–2002.

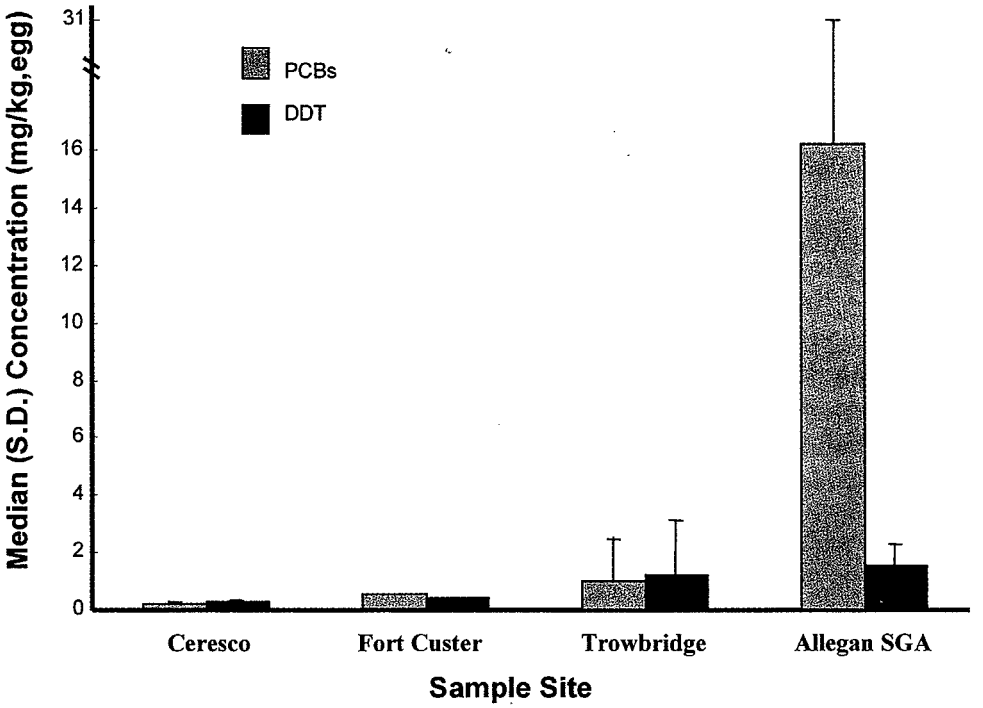


Figure 4. Concentrations of PCBs and ΣDDTs in GHO tissues (egg). Median concentrations and associated one standard deviation of samples collected at four locations. Sampling locations presented from upstream to downstream (left to right) with the two reference sites upstream of point sources (Ceresco and Fort Custer), and two target sites downstream of point sources (Trowbridge and Allegan State Game Area).

different between study sites. During the 3-year period (2000–2002) in which abundance measurements were completed at the KRSS, significant differences in the number of adult, juvenile, and paired responses of GHOs were observed, with the Trowbridge impoundment (target area) having greater numbers of each response compared to Ft. Custer (reference) (Table 3). The Trowbridge impoundment had a greater number of active nests (6 versus 1) and greater overall recruitment to floodplain populations with six successful fledglings compared to one successful fledgling at Ft. Custer, however, the mean rates of productivity for the two sites were identical at 1.0 fledgling per active nest (Table 3).

DISCUSSION

Use of the GHO as a key receptor species in ERAs is predicated on its relatively great exposure potential, broad applicability among geographic regions and ecosystems, and ease of study. Although the first two characteristics have been well documented for the GHO, its nocturnal nature and aggressive disposition may have previously dissuaded researchers from using the species in previous ERAs. For this study, the GHO proved to be a relatively easy and effective receptor species with applicability to both screening level and site-specific baseline ERAs. The single most

Table 3. Relative abundance and reproductive productivity of GHOs. Abundance based on adult, juvenile, and pair responses to great horned owl calls broadcast from predetermined locations throughout sampling areas.

	Ft. Custer	Trowbridge
Relative Abundance[1]	N[2] =24	N[2] =22
Adults	Mean Response Rate[3]	
Total[4]	1.31	2.76
Foraging[5]	0.85	1.64
Paired[6]	0.47	1.13
Juveniles	Response Frequency[7]	
	n, (%)	
Fledgling	1 (4%)	8 (36%)
Productivity		
Active Nests	1	6
Fledglings	1	6
Fledglings/Nest	1	1

[1]Derived from hoot call/response surveys completed at dawn and dusk.
[2]N = number of complete surveys.
[3]Mean response rate is averaged across N completed surveys.
[4]Includes discrete responses from both individual and paired owls.
[5]Includes responses from unpaired individuals only.
[6]Includes responses from paired (male + female) owls only.
[7]Response frequency of fledgling owls, n = number of surveys with at least one fledgling begging call response, (%)= (n)/number of surveys (N^2).

important outcome of this study was our ability to induce breeding pairs of GHOs to occupy nesting sites centrally located within areas of interest and reuse those nesting sites over multiple years. This provided for conservative and worst case exposure assessment evaluations and risk characterizations. These behavioral attributes of the GHO offered significant advantages over other top terrestrial food web receptors including all other large resident raptors.

The strategy of conducting initial surveys to identify occupied GHO territories, followed by reconnaissance of active owl territories within the areas of interest was effective for locating existing owl territories. However, successful location of optimally located natural nests (in relation to contaminated floodplain foraging habitats) was rare. Site-specific characteristics indicate that this may have been due to an absence of available nests in the floodplain of the study area because other nest building species are more limited in nesting habitat and prefer upland areas. Artificial nesting platforms were placed inside the perimeter of defended territories and centrally located within the areas of interest. The density of nesting platform placement ranged from one to three nests per territory at 500 to 1000 m intervals. Over the 5-year study period, nesting activity was identified in 81% of the territories containing nesting platforms. Nesting activity occurred in 90% of territories in which paired owls were identified. Both relative abundance and pair response were useful predictors of nesting potential. Nesting activity in natural nests was never observed

in those territories in which artificial nesting platforms were placed at least 6 mos prior to expected egg drop. Platforms were utilized preferentially even in instances where appropriate natural nests were available and/or when natural nests were utilized the year prior to artificial nest placement. Breeding and reproductive success of nesting pairs utilizing artificial nesting platforms was comparable to natural nest-based reproductive studies. Of the territories in which a platform had been placed, GHOs initiated incubation 65% of the time. In a 28-yr study, in a proximal geographical area of similar characteristics, it was found that 62% of owls in occupied territories initiated incubation. For that study, the resulting annual mean productivity expressed as the number of young/occupied territory varied moderately from 0.5 to 1.1 and the number of fledglings/successful nest was a consistent value of 1.7 (Holt 1996). In this study, reproductive productivity in both the reference and target was similar to the Holt study. The annual mean number of juveniles fledged per occupied territory ranged from 0.5 to 1.6 and the number of fledglings/successful nest was 1.4. Post-fledge survival was successfully monitored in all territories in which active surveys were systematically performed and nestlings were banded. Monitoring of survival of juveniles by their begging responses to broadcasted adult hoot calls was possible as late as 24 wk post-banding. An option for longer-term monitoring of juvenile survivorship includes the temporary attachment of a radio transmitter prior to fledging from the nest.

In this study, several methods of estimating exposure were applied. For select nests, fresh eggs were sampled shortly after identification of incubation activity, whereas other territories were monitored for productivity. Nestlings from these territories as well as nestlings from egg sampling territories (re-nesters or completed incubation of partial clutch sampling) were banded, 7 ml of blood collected, general health determined, and select morphological measurements taken. Prey remains (including pellets) were collected from active nests, the base of the nest tree, and beneath nearby feeding perches for all nests in which fledgling productivity was monitored. Pellet and prey remains analyses identified 285 individual prey items.

In order to determine which type of egg sampling would have the least effect on territorial and site-wide productivity, fresh eggs were collected using two different sampling approaches. Either the entire clutch was collected to induce re-nesting or a single egg was left to induce continued incubation of the remaining egg(s). When the total clutch sampling approach was used, two of four pairs re-nested and produced three young. For nests in which the most recently laid eggs were left for continued incubation, three of seven pairs continued incubation. Two of the nests each produced one nestling and the third nest was destroyed by severe weather. In all, 24 eggs were sampled from 12 different territories.

Over the 5-year study, the territories targeted for egg collection varied. Overall, the egg sampling effort targeted 24 territory-years in which 15 territory-years contained incubation activity. A territory-year is a level of effort term defined as any one territory monitored over one year. Thus, a single territory monitored over four years or four territories monitored over a single year both involve the same level of effort, 4 territory-years. The cumulative number of sampled eggs would have increased to 30 eggs had the entire clutch been collected for all territories targeted for fresh egg collections.

Conclusions resulting from each of the lines of evidence examined in this study were consistent between and among sites. Contaminant exposure based on both dietary- and tissue-based methodologies produced similar results of significantly different COC exposures for the downstream target versus the upstream reference area. However, the results of the hazard assessments indicated that GHO populations residing in the floodplain were not at risk for effects induced by total PCBs or ΣDDT in contaminated soils. Maximum HQ values of <1.0 (diet exposures) and <1.5 (egg exposures) indicated that exposure of GHOs to the COCs in Kalamazoo River were below or near the threshold for effects (Strause *et al.* 2007b, c). Confidence in the risk conclusions was further strengthened by site-specific measurements of productivity, abundance, and nestling growth and success. Population parameters for target area owls were not significantly different from the upstream reference areas, and were similar to those expected in a healthy environment. The mean rate of 1.0 fledgling per active nest observed at both locations was consistent with productivity measures for healthy midwestern GHO populations residing in upland habitats (Holt 1996). Additionally, measures of site-use (abundance) indicated the target area populations at Trowbridge were near the carrying capacity for undisturbed GHO habitats (Houston *et al.* 1998). This consistency across each of the multiple lines of evidence for both measurement and assessment endpoints combined with the relative certainty of each measurement, the minimal impact on the receptor and environment, and the level of effort expended, highlights the utility of the GHO as a receptor in this and possibly other ERAs and natural resource damage assessment investigations.

Here we have provided an overview of the advantages of the GHO as a site-specific surrogate species for the determination of potential risk of contaminants in terrestrial ecosystems. We have given a short overview of a case history. The space available here was limited. For that reason, neither the methods nor the results could be fully described. Detailed methods in the form of standard operating procedures are available from the authors. In addition, detailed results of the assessments are published elsewhere (Strause *et al.* 2007a, b, c).

ACKNOWLEDGMENTS

The authors thank the students and staff of the Michigan State University Aquatic Toxicology Laboratory, including Paul Jones, Dan Villeneuve, Patrick Bradley, Pam Moseley, William Claflin, Donna Pieracini, Ryan Holem, and Scott Kissman as well as personnel of the Michigan State University Veterinary Clinic, including Jim Sikarskie, Simon Hollemby, and Heather Simmons, personnel of the Michigan State University, Kellogg Biological Station, most notably Nina Consolotti, Joe Johnson, and Michael Klug. We also recognize the assistance provided by the U.S. Fish and Wildlife Service, East Lansing Field Office, most notably Lisa Williams, Dave Best, and Charles Mehne as well as the staff of the State of Michigan Department of Natural Resources at Fort Custer and the Allegan State Game Area including William Kosmider, John Lerg, and Mike Baily. We thank Ray Adams of the Kalamazoo Nature Center. Funding for this project was provided by the Kalamazoo River Study Group through an unrestricted research grant to J. P. Giesy (PI) of Michigan State University.

ANIMAL USE

All aspects of the study that involved the use of animals were conducted in the most humane way possible. Toward this end, all aspects of the study were conducted using standard operating procedures that had been approved (AUF #s 02/10-030-00; 03 /04-044-00; 08/03-105-00) by the Michigan State University's All University Committee on Animal Use and Care (AUCAUC). All of the necessary state and federal approvals and permits obtained for the project (Michigan Department of Natural Resources [MDNR] Land Use Permit #A-02-09; MDNR Scientific Collecting Permit #SC1220; USFWS Migratory Bird Scientific Collection Permit #MB081272-1) are on file at the MSU-ATL.

REFERENCES

Adamcik RS, Todd AW, and Keith LB. 1978. Demographic and dietary responses of great horned owls during a snowshoe hare cycle. The Canadian Field-Naturalist 92:156–66

American Ornithologists Union. 1983. Checklist of North American Birds, 6th ed. Washington, DC, USA

Austing GR. 1964. The World of the Red Tailed Hawk. Living World Books, JB Lippincott Co., Philadelphia, PA, USA

Austing GR and Holt JB Jr. 1966. The World of the Great Horned Owl. Living World Books, JB Lippincott Co., Philadelphia, PA, USA

Baumgartner FM. 1938. Courtship and nestling of the great horned owl. The Wilson Bulletin 50:274–85

Blankenship AL, Zwiernik MJ, Coady KK, *et al.* 2005. Differential accumulation of PCB congeners in the terrestrial food web of the Kalamazoo River superfund site, Michigan, USA. Environ Sci Technol 39:5954–63

Bohm RT. 1985. Use of artificial nests by great grey owls, great horned owls, and red-tailed hawks in northeastern Minnesota. The Loon 57:150–2

Brenner FJ and Karwoski D. 1985. Vocalizations as an index of abundance for the great horned owl (*Bubo virginianus*). Proc P Acad Sci 59:129–30

Bursian SJ, Aulerich RJ, Yamini B, *et al.* 2003. Dietary Exposure of Mink to Fish From the Housatonic River: Effects on Reproduction and Survival. Final Report. Weston Solutions, West Chester, PA, USA

Burton JA. 1984. Owls of the World. Tanager Books, Dover, NH, USA

Council on Environmental Quality. 1972. Development of a Continuing Program to Provide Indicators and Indices of Wildlife and the Natural Environment. Ecology Program, Smithsonian Institution, Final Report. Washington, DC, USA

Craighead JJ and Craighead FC Jr. 1956. Hawks, Owls and Wildlife. Stackpole Publishing Co. Harrisburg, PA, USA

Custer CM, Custer TW, Rosiu CJ, *et al.* 2005. Exposure and effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in tree swallows (*Tachycineta bicolor*) nesting along the Woonasquatucket River, Rhode Island, USA. Environ Toxicol Chem 24:93–109

Elliott JE and Harris ML. 2002. An ecotoxicological assessment of chlorinated hydrocarbon effects on bald eagle populations. Rev Toxicol 4:1–60

Errington PL. 1930. The pellet analysis method of raptor food habits study. The Condor 32:176–86

Fairbrother A. 2003. Lines of evidence in wildlife risk assessment. Hum Ecol Risk Assess 9:1475–91

Frank RA. 1997. Effects of Dieldrin on Great Horned Owls at the Rocky Mountain Arsenal National Wildlife Refuge. M.S. Thesis. University of Wisconsin, Madison, WI, USA

Hayward JL, Galusha JG, and Frias G. 1993. Analysis of great horned owl pellets with rhinoceros auklet remains. The Auk 110:133–5

Henderson CL. 1992. Woodworking for Wildlife, Homes for Birds and Mammals. Minnesota's Bookstore. St. Paul, MN, USA

Henny CJ. 1977. Birds of prey, DDT, and tussock moths in Pacific Northwest. Trans North Am Wildl Nat Resource Conf 42:397–411

Henny CJ and Meeker DL. 1981. An evaluation of blood plasma for monitoring DDE in birds of prey. Environ Pollut (Series A) 25:291–304

Hoffman DJ. 1995. Wildlife Toxicity Testing. In: Hoffman DJ, Rattner BA, Burton GA Jr., *et al.* (eds), Handbook of Toxicology, pp 47–69. Lewis Publications, Boca Raton, FL, USA

Holt JB Jr. 1996. A banding study of Cincinnati area Great Horned Owls. The Journal of Raptor Research 30:194–7

Houston CS and Francis CM. 1995. Survival of great horned owls in relation to snowshoe hare cycle. The Auk 112:44–59

Houston CS, Smith DG, and Rohner C. 1998. Great Horned Owl. In: Poole A and Gill F (eds), Birds of North America No. 372. pp 1–27. The Birds of North America Inc., Philadelphia, PA, USA

International Joint Commission. 1991. Report of the Ecological Committee's Biological Effects Subcommittee to the Great Lakes Science Advisory Board. Proceedings of the "Expert Consultation Meeting on Bald Eagles." Windsor, ON, Canada

Johnsgard PA. 1988. North American Owls Biology and Natural History. Smithsonian Institution Press, Washington, DC, USA

Kay DP, Blankenship AL, Coady KK, *et al.* 2005. Differential accumulation of polychlorinated biphenyls in the aquatic food web at the Kalamazoo River superfund site, Michigan. Environ Sci Technol. 39:5964–74

Klaas EE, Wiemeyer SN, Ohlendorf HM, *et al.* 1978. Organochlorine residues, eggshell thickness, and nest success in barn owls from the Chesapeake Bay. Estuaries 1:46–53

Marti C. 1974. Feeding ecology of four sympatric owls. The Condor 76:45–61

Marti CD. 1987. Raptor food habits studies. National Wildlife Federation, Scientific and Technical Series. 10:67–80

Marti CD and Kochert MN. 1996. Dietary and trophic characteristics of great horned owls in southwestern Idaho. Journal of Field Ornithology 67:499–506

Mauro L. 1987. Raptor physiology—hematology and blood chemistry. In: Pendleton BG, Millsap BA, Kline KW, *et al.* (eds), Raptor Management Techniques Manual. Natl Wildl Fed Sci Tech Ser No. 10, National Wildlife Federation, Washington, DC, USA

McLane MAR and Hughes DL. 1980. Reproductive success of screech owls fed Aroclor 1248. Arch Environ Contam Toxicol 9:661–5

Mehne C. 1994. Unpublished Data on Contaminant Concentrations in Bird Eggs Collected From the Kalamazoo River Basin. From Dr. C. Mehne, Kalamazoo, MI, USA

Millsap SD, Blankenship AL, Bradley PW, *et al.* 2004. Comparison of risk assessment methodologies for exposure of mink to PCBs on the Kalamazoo River, Michigan. Environ Sci Technol 38:6451–9

Neigh AM, Zwiernik MJ, Blankenship AL, *et al.* 2006a. Exposure and multiple lines of evidence assessment of risk for PCBs found in the diets of passerine birds at the Kalamazoo River Superfund Site, Michigan. Hum Ecol Risk Assess 69:395–415

Neigh AM, Zwiernik MJ, Bradley PW, *et al.* 2006b. Tree swallow (*Tachycineta bicolor*) exposures to polychlorinated biphenyls at the Kalamazoo River Superfund Site, Michigan, USA. Environ Toxicol Chem 25:428–37

Nero RW. 1992. New great horned owl longevity record. The Blue Jay 50:91–2

Rohner C. 1996. The numerical response of great horned owls to the snowshoe hare cycle: Consequences of non-territorial "floaters" on demography. J Animal Ecol 65:359–70

Rohner C and Doyle FI. 1992. Methods of locating great horned owl nests in the boreal forest. J Raptor Res 26:33–5

Rusch DH, Meslow EC, Keith LB, *et al.* 1972. Response of great horned owl populations to changing prey densities. J. Wildl Manage 36:282–96

Sheffield SR. 1997. Owls as Biomarkers of Environmental Contamination. In: Second International Symposium, Biology and Conservation of Owls of the Northern Hemisphere. In: Duncan JR, Johnson DH, and Nicholls TH (eds), Winnipeg, MB, Canada, USDA Forest Service 1997 General Technical Report NC-190

Stickel J, Wiemeyer SN, and Blus LJ. 1973. Pesticide residues in eggs of wild birds: Adjustment for loss of moisture and lipid. Bull Environ Contam Toxicol 9:193–6

Strause KD. 2007a. An Ecological Risk Assessment for Great Horned Owls (*Bubo virginianus*) and Bald Eagles (*Haliaeetus leucocephalus*) Exposed to Polychlorinated Biphenyls and total DDTs at the Kalamazoo River Superfund Site, Michigan. PhD Thesis. Michigan State University, East Lansing, MI, USA

Strause KD, Zwiernik MJ, Im SH, *et al.* 2007b. Risk assessment of great horned owls exposed to polychlorinated biphenyls (PCBs) and DDT along the Kalamazoo River, Michigan. Environ Toxicol Chem 26:1386–98.

Strause KD, Zwiernik MJ, Newsted JL, *et al.* 2007c. Comparison of risk assessment methodologies for exposure of great horned owls (*Bubo virginanus*) to PCBs on the Kalamazoo River. Integrated Environ Assess Manage (In press).

Strause KD, Zwiernik MJ, Im SH, *et al* 2007d. Plasma to egg conversion factor for evaluating PCB and DDE exposures in great horned owls and bald eagles. Environ Toxicol Chem 26:1399–1409.

Sundlof SF, Forrester DJ, Thompson NP, *et al.* 1986. Residues of chlorinated hydrocarbons in tissues of raptors in Florida. J Wildl Dis 22:71–82

Tabaka CS, Ullrey DE, Sikarskie JG, *et al.* 1996. Diet, cast composition, and energy and nutrient intake of 0red-tailed hawks (*Buteo jamaicensis*), great horned owls (*Bubo virginianus*), and turkey vultures (*Cathartes aura*). J Zoo Wildlife Med 27:187–96

USEPA (US Environmental Protection Agency). 1993. Wildlife Exposure Factors Handbook, Volume I of II. EPA-600-R-93-187a. Office of Health and Environmental Assessment, Washington, DC, USA

USEPA. 1994. ECO Update: Field Studies for Ecological Risk Assessment. EPA-540-F-94-014. Office of Solid Waste and Emergency Response, Washington, DC, USA

USEPA. 1995. Great Lakes Water Quality Initiative Criteria Documents for the Protection of Wildlife: DDT, Mercury, 2,3,7,8-TCDD, PCBs. EPA-820-B-95-0082. Washington, DC, USA

USEPA. 1997. Ecological Risk Assessment Guidance for Superfund: Process for Designing and Conducting Ecological Risk Assessments—Interim Final. EPA-540-R-97-006. Office of Solid Waste and Emergency Response, Washington, DC, USA

USEPA. 1998. Guidelines for Ecological Risk Assessment. EPA-630/R-95/002F. Risk Assessment Forum, Washington, DC, USA

USEPA. 1999. Issuance of Final Guidance: Ecological Risk Assessment and Risk Management Principles for Superfund Sites. OSWER Directive 9285.7-28 P, Washington, DC, USA

Voous KH. 1988. Owls of the Northern Hemisphere. MIT Press, Cambridge, MA, USA

Section 4

ARCADIS
Infrastructure, environment, facilities

Allied Paper, Inc./Portage Creek/Kalamazoo River Superfund Site

Supporting Document for Ecological Risk Studies Peer Review

Characteristics of the Formerly Impounded Areas

Kalamazoo River Study Group

May 2008

Allied Paper, Inc./Portage Creek/ Kalamazoo River Superfund Site

Supplemental Remedial Investigations/ Feasibility Studies

Supporting Document for Ecological Risk Studies Peer Review

Characteristics of the Formerly Impounded Areas

Kalamazoo River Study Group

May 2008

ARCADIS

Characteristics of the Formerly Impounded Areas

Allied Paper, Inc./Portage Creek/ Kalamazoo River Superfund Site

Supplemental Remedial Investigations/Feasibility Studies

Prepared for:

Kalamazoo River Study Group

Prepared by:

ARCADIS
10559 Citation Drive
Suite 100
Brighton
Michigan 48116
Tel 810.229.8594
Fax 810.229.8837

Our Ref.:

B0064524.0000.00771

Date:

May 2008

This document is intended only for the use of the individual or entity for which it was prepared and may contain information that is privileged, confidential and exempt from disclosure under applicable law. Any dissemination, distribution or copying of this document is strictly prohibited.

Table of Contents

1. Introduction 1-1

1.1 Background 1-1

1.2 History of the Formerly Impounded/Historically Inundated Areas 1-2

1.2.1 Former Plainwell, Otsego and Trowbridge Impoundments 1-2

1.2.2 Former Otsego City Impoundment 1-3

1.2.3 Plainwell No. 2 Dam Area 1-3

2. Characteristics of Exposed Sediments in Formerly Impounded Areas 2-1

2.1 Overview of Sampling Programs 2-1

2.2 Physical Soil Characteristics 2-2

2.3 PCB Distributions in Exposed Sediments 2-3

2.3.1 Areal Distribution 2-4

2.3.2 Comparison of PCB by Depth Interval among Impoundments and Distribution with Depth 2-5

2.3.3 Inclusion of USEPA Data 2-6

2.4 TBSA Data 2-8

3. Ecology and Habitat Characteristics of the Formerly Impounded Areas 3-1

3.1 Land Cover and Habitat 3-1

3.2 Plant Communities 3-3

3.2.1 Forested Wetlands 3-3

3.2.2 Emergent and Shrub-Scrub Wetlands 3-3

3.3 Wildlife 3-4

4. References 4-1

Tables

Table 1-1	Summary of Physical Characteristics for the Formerly Impounded Areas	1-2
Table 2-1	Surficial Exposed Sediment Samples Analyzed for PCBs, TOC, and Solids	
Table 2-2	Summary of Percent Solids in the Formerly Impounded Areas	2-3
Table 2-3	Summary of Percent TOC in the Formerly Impounded Areas	2-3
Table 2-4	Summary of PCB Concentrations in Depth Intervals	2-6
Table 2-5	Summary of Surficial Exposed Sediment PCB Concentrations in the Formerly Impounded Areas	2-7
Table 2-6	Summary of TBSA Data	2-8
Table 3-1	Representative Plant Species Occurring in Major Plant Community Types in the Formerly Impounded Areas of the Kalamazoo River Study Area	
Table 3-2	Wildlife Species Potentially Occurring Within the Formerly Impounded Areas of the Kalamazoo River Study Area that May Utilize Emergent Wetland Habitat	
Table 3-3	Wildlife Species Potentially Occurring Within the Formerly Impounded Areas of the Kalamazoo River Study Area that May Utilize Wetland Shrub-Scrub Habitat	
Table 3-4	Wildlife Species Potentially Occurring Within the Formerly Impounded Areas of the Kalamazoo River Study Area that May Utilize Wetland Forest Habitat	

Figures

Figure 1	Formerly Impounded Areas in the Kalamazoo River
Figure 2	Extent of Formerly Inundated Area –Plainwell No. 2 Dam
Figure 3	Extent of Exposed Sediment – Former Plainwell Impoundment
Figure 4	Extent of Exposed Sediment – Former Otsego City Impoundment
Figure 5	Extent of Exposed Sediment – Former Otsego Impoundment
Figure 6	Extent of Exposed Sediment – Former Trowbridge Impoundment
Figure 7	Remaining Dam Structures
Figure 8	Surficial Solids Results in Former Impounded Area Exposed Sediment

Figure 9 Surficial TOC Results in Former Impounded Area Exposed Sediment

Figure 10 Sample Locations – Former Plainwell Impoundment

Figure 11 Sample Locations – Former Otsego City Impoundment

Figure 12 Sample Locations – Former Otsego Impoundment

Figure 13 Sample Locations – Former Trowbridge Impoundment

Figure 14 Surficial Exposed Sediment and Surficial Floodplain Soil PCB Levels in Relation to Elevation and Former Impoundment Pool Elevation

Figure 15 Cumulative Frequency Distribution by Depth for Each Former Impoundment

Figure 16 Cumulative Frequency Distribution in Surficial Exposed Sediment

Figure 17 Cumulative Frequency Distribution in Former Plainwell Impoundment Exposed Sediment

Figure 18 Surficial PCB Results in Former Impounded Area Exposed Sediment

Figure 19 Aerial Photograph – Former Plainwell No. 2 Area

Figure 20 Aerial Photograph – Former Plainwell Impoundment

Figure 21 Aerial Photograph – Former Otsego City Impoundment

Figure 22 Aerial Photograph – Former Otsego Impoundment

Figure 23 Aerial Photograph – Downstream End of the Former Trowbridge Impoundment

Figure 24 Aerial Photograph – Middle Section of the Former Trowbridge Impoundment

Figure 25 Aerial Photograph – Upstream End of the Former Trowbridge Impoundment

Appendix A. Terrestrial Habitat of the Former Impoundments

Figure A-1 Terrestrial Habitat – Former Plainwell No. 2 Area

Figure A-2 Terrestrial Habitat – Former Plainwell Impoundment

Figure A-3 Terrestrial Habitat – Former Otsego City Impoundment

Figure A-4 Terrestrial Habitat – Former Otsego Impoundment

Figure A-5 Terrestrial Habitat – Former Trowbridge Impoundment

1. Introduction

As part of the Supplemental Remedial Investigation efforts now underway at the Allied Paper, Inc./Portage Creek/Kalamazoo River Superfund Site (Site), the Kalamazoo River Study Group (KRSG) and the U.S. Environmental Protection Agency (USEPA) have agreed to conduct a peer review of a series of ecological studies carried out on behalf of the KRSG. The studies—undertaken by a research team at Michigan State University (MSU) led by Dr. John Giesy—were designed to support assessment of potential risks to ecological receptors exposed to polychlorinated biphenyls (PCBs) in former sediments in certain areas of the Site.

This document, the *Characteristics of the Formerly Impounded Areas*, was developed to provide supporting information to the Peer Review Panel members as they consider the quality and relevance of the MSU studies as additional lines of evidence for evaluating Site-specific risk and supporting risk management decisions. The information in this report summarizes the general history, PCB distributions, ecology, and habitat in the five areas of the Site that were either formerly impounded or historically inundated.

1.1 Background

The current floodplains in the five formerly impounded areas associated with the Plainwell No. 2 Dam, Plainwell Dam, Otsego City Dam, Otsego Dam, and Trowbridge Dam (see Figure 1) contain exposed sediments that were submerged—either for prolonged periods or short intervals depending on ground elevation—when these dams were operational. As described in Section 1.2, water levels behind the dams dropped when the structures were opened and/or dismantled, significantly altering conditions along the Kalamazoo River. Sediments that were once submerged were exposed, and now comprise wide, relatively level floodplain areas that are currently covered by vegetation. Figures 2 through 6 present the extent of the formerly impounded/inundated areas associated with each of the five dams.

The impoundments created by the Allegan City and Lake Allegan Dams are not discussed in this report. Since the Allegan City and Lake Allegan Dams have been maintained over time and have not been drawn down or dismantled, they continue to impound water and there are no clearly defined areas of exposed sediments at these locations. As a result, these two areas are different from those that are the focus of this report.

Table 1-1 below presents a summary of the length of the five areas and the associated acreage of exposed sediment.

Table 1-1 Summary of Physical Characteristics for the Formerly Impounded Areas

Formerly Impounded Area	Length (miles)[1]	Area of Exposed Sediments (acres)
Plainwell No. 2	0.91	89[(2)]
Plainwell	1.9	59
Otsego City	1.7	76
Otsego	3.4	77
Trowbridge	4.7	374

Note:

1. Length is presented in river miles.
2. Estimated from topographic information only.

1.2 History of the Formerly Impounded/Historically Inundated Areas

The five dams that created the formerly impounded areas were constructed between 1856 and 1904 for hydroelectric power generation. In the mid-1960s, these dams began to be phased out as sources of hydroelectric power. Over the next several decades, the dams' gates were opened, stoplogs were removed, and in some cases the structures were partially dismantled. As a result, the water levels behind the dams in these five areas dropped and the river channel incised the sediment bed. To varying extents, some portions of the original dam structures are still in place at all five dams. Portions of the former sediments exposed by the dam drawdown now comprise part of the current floodplain, and portions of the exposed sediment areas interact with the river during high flows, depending on ground elevations. Figure 7 presents photographs of the currently remaining dam structures. A brief history of these areas is presented below. (A note on terminology: The sediments that were exposed as a result of water level dropping behind the dams are referred to as exposed sediment(s). The soils that comprised the floodplain when the dams were in operation are referred to as historic floodplain soils.)

1.2.1 Former Plainwell, Otsego and Trowbridge Impoundments

The Plainwell, Otsego and Trowbridge dams originally were constructed in 1902, 1904 and 1899 (respectively). Power generation ceased at these dams in December 1965, and the dams were deeded to a predecessor of the Michigan Department of Natural Resources (MDNR) by Consumers Power Company in September 1967. The MDNR retains ownership of the remains of these three dams. In the early 1970s, MDNR drew down these three impoundments, and then in 1987, dismantled the three dams down to their sill levels. The drawdown lowered water levels in these areas by up to 10 feet. Since the drawdowns began, grasses, woody vegetation, and trees have become established over these areas.

1.2.2 Former Otsego City Impoundment

The Otsego City Dam, also known as the Menasha Paper Company Dam, is located in the City of Otsego, near the Farmer Street Bridge. According to the United States Geologic Survey (USGS), the Otsego City Dam was built in the 1840s in an effort to create a freight business on the Kalamazoo River (Dalrymple 1972, as cited in Rheaume et al. 2002). The original dam was 5 feet high and contained a lock for the passage of barges, canoes, or rafts. Over the years, the dam was rebuilt and repaired (Rheaume et al. 2002). Available information indicates that the former Otsego City Impoundment was drawn down in mid-March 1982 when stoplogs were removed from the dam (Hayes 1982), and again in May 1991 when the dam was dismantled to its sill level. The impact on water level due to the 1982 action is not known, but the MDNR estimated that the 1991 action lowered water levels by 2 to 3 feet (Hayes 1991).

1.2.3 Plainwell No. 2 Dam Area

Unlike the dams of the four downstream formerly impounded areas, the Plainwell No. 2 Dam—which actually consists of four separate structures (two diversion structures connected by an earthen berm, a head gate, and a waste gate)—was constructed in 1856 to create and control water levels in a mill race/power canal. The diversion structures directed water from the main stem of the river into a mill race/power canal that was once used to generate water power for a flour mill, the City of Plainwell, and several other businesses. The Plainwell No. 2 Dam impounded less water than the other formerly impounded areas—the operation of the structures never created a lake-type environment and the historic floodplains upstream of this dam were inundated to a lesser extent compared to the other four formerly impounded areas. By 1979, the Plainwell Paper Company owned the dam and no power was being generated (U.S. Army Corps of Engineers [USACE] 1979).

According to the Michigan Department of Environmental Quality (MDEQ), the dam and associated structures were partially removed in the early 1980s such that there is no longer any “significant amount of water” (Hayes 1998) impounded in the area. The waste gate structure’s lift gate and stoplog guides were still present in 1980, and at that time there were no active operational procedures in place other than to permanently leave all discharge control structures adjusted so that they will permit maximum discharge capacity (USACE 1979).

2. Characteristics of Exposed Sediments in Formerly Impounded Areas

Various data have been collected over the years to characterize the exposed sediments and historic floodplain soils found within the formerly impounded areas of the Kalamazoo River. Potential exposure to PCBs in these exposed sediments and historic floodplain soils by terrestrial ecological receptors is primarily associated with the surface interval.

Since 1993, the key investigation efforts have included soil sampling by the KRSG as part of remedial investigations (RIs) in 1993, 1994 and 2000 (BBL 1994a, 1994b, 2000a), and the USEPA to support their assessment of PCB distributions in the exposed sediments and historic floodplain soils in the former Plainwell and Otsego City impoundments (USEPA 2002). Data from these sampling programs are summarized in the sections below.

In addition to PCB data, a limited amount of data exists for other constituents. As part of the 1993 Former Impoundment Exposed Sediment Investigation, one soil core from each of the former Plainwell, Otsego, and Trowbridge impoundments was analyzed for Contract Laboratory Program Target Contract List/Target Analyte List constituents. Samples analyzed include the 0- to 6-inch and 6- to 18-inch depth intervals. Those data are presented in Technical Memorandum 12 (BBL 1994b). However, PCBs have been identified as the constituent of concern for the Site and are the focus of this document.

2.1 Overview of Sampling Programs

PCB data have been collected in the formerly impounded areas with varying degrees of intensity for several different purposes. Key data sets that may contribute to understanding the PCB exposure levels for assessing terrestrial ecological risks include the following:

- **1993/1994 RI Former Impoundment Investigation.** As part of the RI, samples were collected in the former Plainwell, Otsego, and Trowbridge impoundments. Cores were collected along a series of transects that crossed the areas of exposed sediment in these three formerly impounded areas.

- **1993 RI Floodplain Soil Investigation.** As part of this investigation, exposed sediment and floodplain soil samples were collected along transects perpendicular to the river channel throughout the Site, including single transects established in the current floodplain of both the former Otsego City and Otsego impoundments, resulting in a total of four sample locations within the limits of these formerly impounded areas.

- **1993 Terrestrial Biota Sampling Area (TBSA) Soil Sampling.** Soil samples were collected from exposed sediment and the historic floodplains of the former Plainwell, Otsego, and Trowbridge impoundments as part of the 1993 RI to assess the potential exposure of terrestrial organisms to PCB for the ecological risk assessment. Multiple samples were collected from the uppermost one foot within established grids and composited for PCB analyses, resulting in two samples from the former Otsego Impoundment, eight samples from the former Plainwell Impoundment, and 24 samples from the former Trowbridge Impoundment. Sample areas covered 0.8 to 17.2 acres.

- **Focused Soil Sampling.** In 2000, at the request of the MDEQ, the KRSG collected floodplain soil samples from locations that were identified as potential depositional areas. Focused samples were collected in each of the five formerly impounded areas, but the effort was limited, and generated just one to seven samples in each area.

- **USEPA Removal Assessment Sampling.** In 2001, the USEPA conducted a two-phased floodplain soil and sediment assessment in the former Plainwell and Otsego City impoundments to better understand PCB distributions and geostatistical variability of PCB concentrations. The first phase of the investigation involved sampling on a systematic grid. In the second phase, a radial grid system was used to sample around select boring locations where elevated concentrations of PCBs were identified based on the results of the first phase.

Table 2-1 summarizes the relevant surficial exposed sediment data collected within the five formerly impounded areas. The samples that are included in the evaluations presented in this report primarily include samples from the uppermost six inches of exposed sediment. Of the 262 samples collected in the five formerly impounded areas included in Table 2-1, 257 (98%) are from the 0- to 6-inch depth increment, two are from depths shallower than 6 inches (0-2 inches and 0-5 inches), and three samples went deeper, to a maximum of eight inches. Table 2-1 does not include the results of the TBSA samples; those samples were composite samples from the upper 12 inches of exposed sediment/floodplain soil as compared to the discrete samples from the upper six inches collected during the other investigation activities. Due to inconsistencies of the TBSA sample collection with the rest of the data, the TBSA sample results are discussed separately in Section 2.4, below.

2.2 Physical Soil Characteristics

Based on the findings from the historical investigation programs, exposed sediments within the formerly impounded areas are similar with respect to type, moisture content, color, texture, and total organic content (TOC). The general consistency in physical properties in these areas is

apparent from comparing the range, median, and average percent solids data (as shown in Table 2-2, below). Comparing the three formerly impounded areas that have the most data, the average percent solids is statistically the same between the former Plainwell and Trowbridge impoundments, and only slightly lower for the former Otsego Impoundment. Percent solids data from the former Plainwell No. 2 Area and former Otsego City Impoundment are generally lower than the other former impoundments; however, considerably less data are available from these two areas. Figure 8 presents a summary of the percent solids data.

Table 2-2 Summary of Percent Solids in the Formerly Impounded Areas

Formerly Impounded Area	Number of Samples	Range of Solids (percent)	Average (percent)	Standard Deviation (percent)	95% Confidence Interval (percent)
Plainwell No. 2	3	35 to 49	43	7.0	—
Plainwell	29	33 to 71	51	9.2	48 – 55
Otsego City	2	21 to 23	22	—	—
Otsego	34	16 to 74	44	12	40 – 48
Trowbridge	71	13 to 88	53	18	49 – 57

Available TOC data from exposed sediment (summarized in Table 2-3, below) also exhibit consistency among the formerly impounded areas. The mean TOC for the individual areas ranges from 8.7 to 11 percent. Comparing the three areas that have the most available data, the average TOC concentrations in the former Plainwell, Otsego, and Trowbridge impoundments are not statistically different. Figure 9 presents a summary of the TOC data.

Table 2-3 Summary of Percent TOC in the Formerly Impounded Areas

Formerly Impounded Area	Number of Samples	Range of TOC (percent)	Average (percent)	Standard Deviation (percent)	95% Confidence Interval (percent)
Plainwell No. 2	3	7.6 to 14	11	3.0	—
Plainwell	45	1.4 to 16	9.0	4.2	7.7 – 10
Otsego City	5	3.2 to 20	10	8.4	—
Otsego	34	3.3 to 27	11	4.8	9.6 – 13
Trowbridge	71	0.23 to 39	8.7	7.0	7.0 – 10

2.3 PCB Distributions in Exposed Sediments

Among the five formerly impounded areas, the most comprehensive exposed sediment PCB data sets exist for the former Plainwell, Otsego and Trowbridge impoundments. As noted in

Section 2.1, these three former impoundments were the focus of specific RI and/or USEPA investigations aimed at determining the areal extent and distribution of PCB in the exposed sediment. Conversely, the former Otsego City Impoundment and Plainwell No. 2 Dam area have not been as extensively studied, with the majority of the data available for these areas collected during supplemental RI and/or subsequent USEPA sampling activities. As a result, much of the information regarding PCB distribution discussed in this report is based on the data from the former Plainwell, Otsego, and Trowbridge impoundments. Figures 10 through 13 present the locations of all exposed sediment and historical floodplain soil sampling points in for the former Plainwell, Otsego City, Otsego, and Trowbridge impoundments, respectively. However, as described in Section 2.1, the analyses below are restricted to those samples from exposed sediments.

2.3.1 Areal Distribution

The majority of PCBs in the current floodplains are confined to the exposed sediments within the limits of the former impoundments as demarcated by the pre-drawdown water surface elevations. Within the former Plainwell, Otsego, and Trowbridge Impoundments, the pre-drawdown water surface elevations were 712, 683, and 669 feet National Geodetic Vertical Datum 1929 (ft NGVD), respectively. These elevations provide a basis for delineating PCB occurrence in these three former impoundments, as shown in Figure 14. These boundaries define the areal extent of PCB-containing exposed sediment deposits, and are consistent with the boundaries established by discontinuities in the total solids and TOC concentration data. For the area near Plainwell Dam No. 2, an approximate impoundment water surface elevation of 724.2 ft NGVD has been estimated based on recent survey data, dam inspection reports, and historical information. Although the data are limited, the three focused samples that fall within that area are all lower than 724.2 ft NGVD, and contain PCB concentrations ranging from 7.0 to 15 milligrams per kilogram (mg/kg), with one non-detect result. For the Otsego City Impoundment, the extent of former inundation was estimated based on aerial photographs during the period of dam operation. Comparing data inside and outside of that boundary supports the same conclusion: PCBs are generally confined to historically-inundated areas, and outside of these areas PCBs are detected less frequently and at lower concentrations. For the purposes of this evaluation, only data from exposed sediments within the formerly impounded areas as delineated are included. All analyses exclude the river channel proper and any associated sediment data from within the channel.

2.3.2 Comparison of PCB by Depth Interval among Impoundments and Distribution with Depth

Among the former Plainwell, Otsego, and Trowbridge impoundments, the most systematic and comparable data sets are the PCB data from the 1993 Former Impoundment Exposed Sediment Investigation. During that investigation, exposed sediment cores were collected along transects from the river to the outer extent of the formerly impounded areas using a relatively uniform, systematic sampling approach. The sample numbers are relatively consistent among impoundments and are useful for statistical analysis and comparison of PCB concentration in these impoundments. Cumulative frequency distributions of PCB data from these three former impoundments are shown for various depth increments in Figure 15. The distributions illustrate the relative similarities of PCB concentrations in the three former impoundments as well as consistently lower PCB concentrations in sub-surface samples. The arithmetic average and median of PCB concentrations in each depth increment and each impoundment (presented in Table 2-4, below) also show a declining trend with increasing depth. The arithmetic mean of PCB concentrations (based on the 1993 data) in the exposed sediments in the former Plainwell, Otsego, and Trowbridge impoundments were not significantly different from one another at 15 mg/kg, 13 mg/kg, and 15 mg/kg, respectively. Figure 16 shows a comparison of the surficial exposed sediment PCB distributions in these three former impoundments.

Table 2-4 Summary of PCB Concentrations in Depth Intervals from 1993/1994 Samples

Depth Interval (in)	Total Number of Samples	Range of Results (mg/kg)	Average (mg/kg)	Standard Deviation (mg/kg)	Median (mg/kg)
Former Plainwell Impoundment					
0 - 6	28	0.71 - 85	15	19	6.6
6 - 18	27	0.18 - 65	14	19	3.5
18 - 30	27	0.034 - 84	8.1	18	1.5
30 - 42	21	ND - 31	3.6	6.8	1.1
42 - 54	13	ND - 13	2.0	3.6	0.32
54 - 66	7	ND - 5.3	0.91	1.9	0.083
66 - 78	1	0.38	0.38	--	0.38
Former Otsego Impoundment					
0 - 6	27	ND - 36	13	11	8.4
6 - 18	27	ND - 117	19	30	7.9
18 - 30	25	ND - 100	12	21	5.0
30 - 42	19	ND - 30	5.5	8.0	1.8
42 - 54	17	ND - 72	5.9	17	0.83
54 - 66	12	ND - 24	3.3	6.8	0.36
66 - 78	5	ND - 2.4	0.63	0.98	0.15
Former Trowbridge Impoundment					
0 - 6	64	ND - 81	15	17	9.6
6 - 18	61	ND - 77	12	17	3.0
18 - 30	46	ND - 36	4.6	7.0	1.2
30 - 42	31	ND - 10	1.2	2.3	0.43
42 - 54	18	ND - 5.6	0.63	1.3	0.17
54 - 66	7	0.029 - 2.2	0.51	0.76	0.37
66 - 78	2	0.035 - 0.048	0.042	0.0092	0.042

2.3.3 Inclusion of USEPA Data

The PCB data collected by the USEPA in 2001 affect the statistics only for the former Plainwell Impoundment; the data provide only five additional sample locations within the Otsego City Impoundment, but 118 locations within the former Plainwell Impoundment. Summary statistics for surficial exposed sediment PCB data resulting from all RI and USEPA sampling activities are provided in Table 2-5 (below).

The inclusion of the USEPA data collected in 2001 increases the average surficial exposed sediment PCB concentration from 15 mg/kg to 20 mg/kg in the former Plainwell Impoundment. However, the 20 mg/kg average in the former Plainwell Impoundment is biased by the USEPA Phase II sampling approach, which included the collection of several samples in a radial pattern in very close proximity to locations of high concentrations observed in Phase I. As a result, the average is affected by the inordinate number of higher concentration samples from the same limited area (see Figure 10). This is evident in Figure 17, which shows the cumulative frequency distributions for the RI data, the USEPA data, and all data combined.

Using the median as a measure of central tendency reduces the effect of the sampling bias in the former Plainwell Impoundment and median values from the formerly impounded areas are comparable—four of the five medians range from 7.0 mg/kg to 10 mg/kg, with the anomaly in this case being the former Otsego City Impoundment with a median PCB concentration of 1.1 mg/kg. The relatively scarce data from the former Plainwell No. 2 Dam area and the former Otsego City Impoundment prevent meaningful statistical comparisons to the other impoundments.

Table 2-5 Summary of Surficial Exposed Sediment PCB Concentrations in the Formerly Impounded Areas

Formerly Impounded Area	Frequency of Detection	Range of Detected PCB (mg/kg)	Average (mg/kg)	Standard Deviation (mg/kg)	Median (mg/kg)
Plainwell No. 2	2/3 (67%)	7.0 to 15	7.2	7.2	7.0
Plainwell(1,2)	147/147 (100%)	0.0069 to 134	20	23	10
Otsego City	5/7 (71%)	0.023 to 7.5	2.3	2.8	1.1
Otsego	33/34 (97%)	0.13 to 61	13	14	7.6
Trowbridge	65/71 (92%)	0.051 to 81	15	18	8.5

Notes:
1. Data for Plainwell include data collected by KRSG and USEPA and represent conditions prior to Time-Critical Removal Action (TCRA) activities. Some of the data used here are from areas from which soil was removed as part of the TCRA activities.
2. Data for Plainwell include data collected in a non-random sampling effort conducted by USEPA. The data presented in this table have not been corrected to account for the effect of bias of the USEPA sampling on the average.

The activities of the ongoing Time-Critical Removal Action (TCRA) at the former Plainwell Impoundment will also affect the surficial exposed sediment PCB concentration, as the areas where the highest concentrations were observed in the former impoundment are being excavated. Surficial exposed sediment PCB data within the formerly impounded areas are

summarized in Figure 18. Note that the data presented in Figure 18 for the former Plainwell Impoundment only includes PCB results from sampling locations that will remain after the TCRA removal activities are complete (i.e., all data points that fall within the planned removal areas have been excluded) to provide an estimate of the post-TCRA surficial exposed sediment PCB concentration. Considering all data, the average PCB concentration will be reduced from 20 mg/kg to an estimated 12 mg/kg after the TCRA is complete. If only the 1993 RI data are considered, the average PCB concentration in the exposed sediments in the former Plainwell impoundment is 15 mg/kg before the TCRA and declines to an estimated 5.5 mg/kg after removal is complete.

2.4 TBSA Data

The TBSA PCB data collected from the former Plainwell, Otsego, and Trowbridge impoundments in 1993 (and summarized in Table 2-6) are generally similar to the data collected in support of the RI. Average PCB concentrations in these composite samples were 7.3 mg/kg, 19 mg/kg, and 25 mg/kg for the former Plainwell, Otsego, and Trowbridge impoundments, respectively. These data were collected in areas specifically targeted to represent the habitat of mice and earthworms, and to correspond to locations where mouse and earthworm samples were collected. The TBSA data were not collected to represent the larger area or the PCB distribution of the entire former impoundments, but rather to support the ecological risk studies.

Table 2-6 Summary of TBSA Data

Former Impoundment	Total Number of Samples	Range (mg/kg)	Arithmetic Mean (mg/kg)	Standard Deviation (mg/kg)	Median (mg/kg)
Plainwell[1]	8	4.2 - 10	7.3	2.5	7.2
Otsego[2]	2	9.7 - 28	19	13	19
Trowbridge[3]	14	14 - 35	25	5.5	24

Notes:
1. Includes results from Phase I sampling at TBSA 8, TBSA 9, and TBSA 10 (3 composite samples) and Phase II sampling at TBSA 10 (5 composite samples).
2. Includes results from Phase I sampling at TBSA 6 and TBSA 7 (2 composite samples).
3. Includes results from Phase I sampling at TBSA 2 through TBSA 5 (4 composite samples) and Phase II sampling at TBSA 3 and TBSA 5 (10 composite samples).

3. Ecology and Habitat Characteristics of the Formerly Impounded Areas

A variety of land cover types are present in different proportions within each of the formerly impounded areas. These land cover types and proportions contribute to the overall ecology and habitat characteristics of each impounded area. This section provides an overview of the habitat types present in each of the formerly impounded areas and a description of the biota that has been observed or would be expected within these habitat types. For the purposes of this document, the descriptions of the biota are focused on plants, birds and mammals, and information was obtained primarily from the following sources:

- A Geographic Information System (GIS) developed by Western Michigan University's GIS Research Center, which displays land cover types as found in 1996
- Digitized National Wetlands Inventory maps, developed by the U.S. Fish and Wildlife Service (USFWS)[1]
- Aerial photographs of the formerly impounded areas
- A field survey of habitat and biota by ARCADIS staff conducted June 13-14, 2000

The 1996 land cover GIS provides the primary basis for the delineation of habitat types. Western Michigan University (WMU) developed the 1996 land cover GIS using aerials obtained from the U.S. Department of Agriculture's Natural Resource Conservation Service. The scope of the WMU project excluded the area within the municipalities of Kalamazoo and Parchment. The land cover data were then registered to the Michigan Resource Information System (MIRIS) base maps (1:24,000 scale). Arc/Info software was used to generalize, display and spatially analyze land cover types. In addition, interpretation of the 1996 land cover data was facilitated by observations made during the field survey and inspection of aerial photographs.

3.1 Land Cover and Habitat

Land cover types within the five formerly impounded areas are shown in Figures A-1 through A-5 in Appendix A, and aerial photographs of the formerly impounded areas are shown in Figures 19 through 25. The land cover and habitat descriptions provided below are general

[1] These maps were given less weight in habitat determination for the former Plainwell, Otsego and Trowbridge impoundments than the 1996 land cover maps because they were based on aerial photographs taken in 1981 prior to the dams being taken down to sill level.

descriptions, and small areas of mixed/other land cover types may be present within these general land cover areas.

Land cover types that represent the ecological habitat within these areas are primarily considered either forested, shrub-scrub, or emergent wetland. These wetland types are not continually inundated (i.e., aquatic bed wetlands), but rather are represented by at least one of the following characteristics: (1) at least periodically, the land supports predominantly hydrophytic (wetland-adapted) plant species; (2) the substrate is predominantly undrained hydric soil; or (3) the substrate is nonsoil and is saturated with water or covered by shallow water at some time during the growing season of each year (Cowardin et al. 1979). Forested wetlands are lowland wooded areas that are dominated by hydrophytic tree species. Shrub-scrub wetlands are typically dominated by shrubs or small trees and during the June 2000 field survey, areas delineated as shrub-scrub wetlands were observed to include a variety of vegetation types that were intermediate between forested and emergent wetlands. In addition to wetlands that are dominated by shrubs, this land cover type includes wetlands currently containing young trees of fast-growing species and some areas containing emergent vegetation with widely spaced trees. Emergent wetlands are dominated by hydrophytic, herbaceous vegetation with little or no woody vegetation. Additional descriptions of habitat within the formerly impounded areas and in other areas adjacent to the river can be found in Section 3.2.1 and Appendix A of the Final (Revised) Baseline Ecological Risk Assessment (CDM 2003).

The former Plainwell No. 2 Area contains primarily forested wetland areas, (Figure A-1), with a very small area identified as emergent wetland or upland successional. The former Plainwell Impoundment (Figure A-2) consists primarily of shrub-scrub wetland with a small amount of forested wetland identified in the areas farthest from the river. The former Otsego City Impoundment (Figure A-3) contains primarily emergent wetland adjacent to the river and smaller areas of shrub-scrub and forested wetland intermixed. The former Otsego Impoundment (Figure A-4) and the former Trowbridge Impoundment (Figure A-5) contain similar proportions of emergent, shrub-scrub and forested wetland with a small area of aquatic bed wetland identified on the northwest end of both impoundments. While these characterizations provide a good general overview of the habitat character in each of the formerly impounded areas, it is important to note that they are based primarily on aerial photographs and may not precisely represent current conditions either due to lack of resolution, changes in water regime or other external changes in land use. As described above, small areas of other habitat types may be present within the general categories identified here and the associated biota may vary accordingly. Thus, species associated with non-wetland habitat types may be present within smaller sub-areas of the former impoundments. The plant and

wildlife communities that have been observed or that would be expected in the general habitat types identified are discussed below.

3.2 Plant Communities

Major plant community types have been identified based on the MDNR's Allegan State Game Area Master Plan (MDNR 1993), and their extent throughout the formerly impounded areas is inferred from the land cover information discussed previously. In addition, the June 2000 field survey supported additional characterization of plant communities. Plant communities within each habitat type found in the formerly impounded areas are summarized below and in Table 3-1.

3.2.1 Forested Wetlands

Forested wetlands comprise the largest area within the former Plainwell No. 2 Area, as shown on Figure A-1, and make up smaller proportions of the remaining four formerly impounded areas. During a June 2000 field survey, dominant tree species were noted for portions of the former Plainwell No. 2 Area. The riparian plant community was strongly dominated by silver maple, a fast-growing species with brittle branches that contribute woody debris to the river. The abundance of silver maple is characteristic of an intermediate successional stage in formerly disturbed lowland hardwood forests (Hodges 1997).

3.2.2 Emergent and Shrub-Scrub Wetlands

Both emergent and shrub-scrub wetlands tend to intergrade in the formerly impounded areas. Taken together, these two habitat types comprise the majority of the area contained within the former Plainwell, Otsego, and Trowbridge impoundments as well as in the former Otsego City Impoundment (Figures A-2 through A-5). Historically, the vegetation in these reaches consisted primarily of southern floodplain forest (Brewer et al. 1984). The current extent of emergent and shrub-scrub wetlands may be due to the fluctuations in water levels associated with dam operations and drawdown of the formerly impounded areas.

Emergent wetlands in the five formerly impounded areas are generally dominated by a small number of plant species that tend to form monospecific stands, including Reed canary grass (*Phalaris arundinacea*), cattails (*Typha spp*.), purple loosestrife (*Lythra salicaria*), stinging nettle (*Urtica dioica*), and to a lesser degree green-headed coneflower (*Rudbeckia laciniata*) and giant ragweed (*Ambrosia trifida*).

The most commonly observed shrub or small tree in the formerly impounded areas was box elder, a fast-growing, short-lived pioneer species of moist areas (Preston 1989). Other commonly observed shrubs included silky dogwood (*Cornus amomum*), willow (*Salix* sp.) and elderberry (*Sambucus canadensis*). Silver maple and elm were also found in mixed emergent and shrub-scrub wetlands, particularly in the former Otsego Impoundment.

3.3 Wildlife

A wide variety of birds and mammals have been identified as using the overall terrestrial areas within the Site for foraging, nesting, and/or cover. Because the habitat within the five formerly impounded areas is generally limited to forested shrub-scrub and emergent wetlands, only a subset of these species would be expected to use the areas within these areas. The species of birds and mammals that have been observed in the formerly impounded areas and/or would be expected to occur in emergent, shrub-scrub or forested wetland habitats are listed in Tables 3-2, 3-3 and 3-4. These species are either designated wetland dependent (meaning that the species is limited to that particular habitat type) or wetland specialist (meaning that the species requires wetland habitat for all or part of its life cycle). The species listed in these tables is not considered exhaustive as many species from adjacent habitat types may use these wetland habitats opportunistically and small areas of other habitat types may be present. Rather, these tables list the species most likely to be present and or use the wetland areas to a significant degree.

A high proportion of the birds encountered along the Kalamazoo River, in general, are neotropical migrants, which breed in the U.S. or Canada and winter in Central or South America (Adams et al. 1998). Other migrants use the Site as wintering habitat, such as the snowy owl (*Nyctea scandiaca*), which migrates from northern Canada to the emergent wetlands in southwestern Michigan for the winter. Year-round avian residents are also prevalent. In general, habitat character within the formerly impounded areas varies seasonally due to differing times and degrees of inundation. Specifically, changes in vegetative cover over a season (i.e., lower lying vegetation in the early season and increasingly higher vegetation in the later season) can make an area more or less suitable for a variety of wildlife species. For example, during the early season when vegetation is lower, habitat is more suitable for ground foraging species such as the American robin. Later in the season, when the vegetation height increases, the area becomes less suitable for foraging by these ground-foraging species (Neigh et al. 2004).

Many of the mammalian species observed, such as the short-tail shrew, opossum, raccoon, and whitetail deer are adaptable to many habitats. Others, such as the river otter and beaver, depend on a specific wetland habitat type for at least part of their life cycle.

4. References

Adams, R.A., S. Allen, M.A. Evans, C. Ferguson, S. Powless, and C. Stefanich. 1998. Conservation of Bird Populations in Riparian Forests of Southwest Michigan. United States Department of the Interior, Fish and Wildlife Service.

BBL. 1994a. Technical Memorandum 3 – Results of the Floodplain Soils Investigation. Syracuse, NY, February, 1994.

BBL. 1994b. Allied Paper, Inc./Portage Creek/Kalamazoo River Superfund Site - Draft Technical Memorandum 12 – Former Impoundment Sediment and Geochronologic Dating Investigation. May 1994.

BBL. 2000a. Allied Paper, Inc./Portage Creek/Kalamazoo River Superfund Site - RI/FS Remedial Investigation Report – Phase I. October 2000.

BBL. 2000b. Allied Paper, Inc./Portage Creek/Kalamazoo River Superfund Site - Supplement to the Kalamazoo River RI/FS – Phase I. October 2000.

Brewer, L.G., T.W. Hodler and H.A. Raup. 1984. Presettlement Vegetation of Southwestern Michigan. Western Michigan University.

CDM. 2003. Final (Revised) Baseline Ecological Risk Assessment – Allied Paper, Inc./Portage Creek/Kalamazoo River Superfund Site. Prepared on behalf of the MDEQ Remediation and Redevelopment Division. April 2003.

Cowardin, L.M., V. Carter, F.C. Golet and E.T. LaRoe. 1979. Classification of Wetlands and Deepwater Habitats of the United States. Biological Services Program, Fish and Wildlife Service, U.S. Department of the Interior. FWS/OBS-79/31. December 1979.

Hayes, J., MDNR. 1982. Telephone messages re: Otsego City Dam, March 19, 1982.

Hayes, J., MDNR. 1991. Notes to the file, June 6, 1991.

Hayes, J., MDEQ. 1998. Personal Communication with D.E. Penniman, BBL re: Kalamazoo River dams. July 21, 1998.

Hodges, J.D. 1997. Development and ecology of bottomland hardwood sites. Forest Ecology and Management. 90:117-125.

MDNR. 1993. Allegan State Game Area Master Plan. October 1993.

Neigh, A.M., Zwiernik, M., Giesy, J. Kalamazoo River Area of Concern Passerine Habitat Suitability Index. (In Preparation)

Preston, R.J. 1989. North American Trees. 4th edition. Iowa State University Press, Ames, IA.

Rheaume, S.J., D.L. Hubbell, C.M. Rachol, A. Simard and L.M. Fuller. 2002. Sediment Characteristics and Configuration within the Otsego City Dam Impoundment on the Kalamazoo River, Michigan, 2001-02. USGS Report Water-Resources Investigations Report 03-4218.

USACE. 1979. National Dam Safety Program Inspection Report, Kalamazoo River Basin, Plainwell No. 2 Dam. August 1979.

USEPA. 2002. Removal Assessment Report for Allied Paper-Kalamazoo River Site Otsego/Plainwell, Michigan. TDD No. 0103-002.

ARCADIS

Tables

Kalamazoo River Study Group
Allied Paper, Inc./Portage Creek/Kalamazoo River Superfund Site
Supplemental Remedial Investigations/Feasibility Studies
Characteristics of the Formerly Impounded Areas

Table 2-1. Surficial Exposed Sediment Samples Analyzed for PCBs, TOC, and Solids

Formerly Impounded Area	Study	Total Number of Samples Analyzed for		
		PCB	TOC	Solids
Plainwell No. 2	TM3/RI (1993)	--	--	--
	TM12/RI (1993/94)	--	--	--
	RI (2000)	3	3	3
	USEPA Phase I (2001)	--	--	--
	USEPA Phase II (2001)	--	--	--
	Total	3	3	3
Plainwell	TM3/RI (1993)	--	--	--
	TM12/RI (1993/94)	28	28	28
	RI (2000)	1	1	1
	USEPA Phase I (2001)	27	16	--
	USEPA Phase II (2001)	91	--	--
	Total	147	45	29
Otsego City	TM3/RI (1993)	1	1	1
	TM12/RI (1993/94)	--	--	--
	RI (2000)	1	1	1
	USEPA Phase I (2001)	5	3	--
	USEPA Phase II (2001)	--	--	--
	Total	7	5	2
Otsego	TM3/RI (1993)	3	3	3
	TM12/RI (1993/94)	27	27	27
	RI (2000)	4	4	4
	USEPA Phase I (2001)	--	--	--
	USEPA Phase II (2001)	--	--	--
	Total	34	34	34
Trowbridge	TM3/RI (1993)	--	--	--
	TM12/RI (1993/94)	64	64	64
	RI (2000)	7	7	7
	USEPA Phase I (2001)	--	--	--
	USEPA Phase II (2001)	--	--	--
	Total	71	71	71

Notes:

RI - Remedial Investigation - 2000 Focused Soil Sampling
TM - Technical Memorandum
TM3/RI - Floodplain Soil Investigation
TM12/RI - Former Impoundment Exposed Sediment Investigation

Kalamazoo River Study Group
Allied Paper, Inc./Portage Creek/Kalamazoo River Superfund Site
Supplemental Remedial Investigations/Feasibility Studies
Characteristics of the Formerly Impounded Areas

Table 3-1. Representative Plant Species Occurring in Major Plant Community Types in the Formerly Impounded Areas of the Kalamazoo River Study Area

Plant Species[a]			
Scientific Name	**Common Name**	**Scientific Name**	**Common Name**
Forested Wetlands			
Canopy		***Understory***	
Acer negundo	boxelder	*Acer negundo*	boxelder
Acer nigrum	black maple	*Adiantum pedatum*	maidenhair fern
Acer rubrum	red maple	*Arisaema triphyllum*	jack-in-the-pulpit
Acer saccharinum	silver maple	*Asarum canadense*	wild ginger
Aesculus glabra	Ohio buckeye	*Cercis canadensis*	redbud
Carya cordiformis	bitternut hickory	*Cornus spp.*	dogwood
Catalpa speciosa	catalpa	*Impatiens capensis*	jewelweed
Celtis occidentalis	hackberry	*Lindera benzoin*	spicebush
Fraxinus nigra	black ash	*Onoclea sensibilis*	sensitive fern
Fraxinus pennsylvanica	green ash	*Parthenocissus quinquifolia*	Virginia creeper
Gleditsia triacanthos	honey locust	*Populus deltoides*	cottonwood
Gymnocladus dioicus	Kentucky coffeetree	*Rhus radicans*	poison ivy
Juglans cinerea	butternut	*Rhus typhina*	staghorn sumac
Juglans nigra	black walnut	*Salix nigra*	black willow
Morus alba	white mulberry	*Thalictrum dasycarpum*	purple meadow rue
Platanus occidentalis	American sycamore	*Urtica dioica*	stinging nettle
Populus tremuloides	quaking aspen	*Viburnum sp.*	viburnum
Quercus bicolor	swamp white oak	*Viola sp*	violet
Quercus palustris	pin oak	*Vitis riparia*	forest grape
Sassafras albidum	sassafras		
Tilia americana	basswood		
Ulmus americana	American elm		
Ulmus rubra	slippery elm		
Emergent and Shrub-Scrub Wetlands			
Shrubs and Small Trees		***Herbaceous Emergent Vegetation***	
Acer negundo	boxelder	*Alliaria officinalis*	garlic mustard
Acer saccharinum	silver maple	*Ambrosia trifida*	giant ragweed
Cornus amomum	silky dogwood	*Apocynum cannabinum*	indian hemp
Salix sp.	willow	*Conium maculatum*	poison hemlock
Sambucus canadensis	elderberry	*Galium aparine*	cleavers
Ulmus sp.	elm	*Glechoma hederacea*	gill-over-the-ground
		Hesperis matronalis	dames rocket
		Impatiens capensis	jewelweed
		Iris pseudoacorus	yellow iris
		Lythra salicaria	Purple loosestrife
		Phalaris arundinacea	Reed canary grass
		Pontederia cordata	pickerelweed
		Rudbeckia laciniata	green-headed coneflower
		Rumex verticillatus	water dock
		Sagittaria latifolia	common arrowhead
		Solanum dulcamara)	deadly nightshade
		Sparganium sp.	bur-reed
		Typha angustifolia	narrow-leaved cattail
		Typha latifolia	broad-leaved cattail
		Urtica dioica	stinging nettle

Note:
[a] Compiled from MDNR (1993), Brewer et al. (1982), Pippen (1976), and observations during a June 2000 site visit.

Kalamazoo River Study Group
Allied Paper, Inc./Portage Creek/Kalamazoo River Superfund Site
Supplemental Remedial Investigations/Feasibility Studies
Characteristics of the Formerly Impounded Areas

Table 3-2. Wildlife Species Potentially Occurring Within the Formerly Impounded Areas of the Kalamazoo River Study Area that May Utilize Emergent Wetland Habitat

Scientific Name	Common Name	Habitat Specialist[a]	Wetland Dependent[b]
Birds			
Actitis macularia	Spotted Sandpiper		X
Anas acuta	Northern Pintail		X
Anas americana	American Wigeon		X
Anas clypeata	Northern Shoveler		X
Anas discors	Blue-winged Teal		X
Anas platyrhynchos	Mallard		
Anser albifrons	Greater White-fronted Goose		X
Anthus spinoletta	American Pipit	X	X
Asio flammeus	Short-eared Owl		
Asio otus	Long-eared Owl		
Aythya affinis	Lesser Scaup		
Aythya americana	Redhead		X
Aythya collaris	Ring-necked Duck		
Aythya marila	Greater Scaup		X
Aythya valisineria	Canvasback		X
Botaurus lentiginosus	American Bittern		X
Branta bernicla	Brant		X
Branta canadensis	Canada Goose		X
Branta leucopsis	Barnacle Goose		X
Buteo lagopus	Rough-legged Hawk		
Calcarius lapponicus	Lapland Longspur		
Calidris minutilla	Least Sandpiper	X	X
Chen caerulescens	Snow Goose		X
Chen rossii	Ross' Goose		
Chlidonias niger	Black Tern	X	X
Circus cyaneus	Northern Harrier		
Cistothorus palustris	Marsh Wren	X	X
Cistothorus platensis	Sedge Wren		
Contopus borealis	Oliver-sided Flycatcher		
Cygnus columbianus	Tundra Swan		X
Cygnus olor	Mute Swan		X
Dolichonyx oryzivorus	Bobolink		
Euphagus carolinus	Rusty Blackbird		X
Euphagus cyanocephalus	Brewer's Blackbird		
Falco peregrinus	Peregrine Falcon		
Fulica americana	American Coot		X
Gallinago gallinago	Common Snipe		X
Geothlypis trichas	Common Yellowthroat		
Grus canadensis	Sandhill Crane		X
Ixobrychus exilis	Least Bittern	X	X
Melospiza georgiana	Swamp Sparrow		X
Nyctanassa violaces	Yellow-crowned Night-Heron		X
Nyctea scandiaca	Snowy Owl		
Nycticorax nycticorax	Black-crowned Night-Heron		
Oxyura jamaicensis	Ruddy Duck	X	X

Kalamazoo River Study Group
Allied Paper, Inc./Portage Creek/Kalamazoo River Superfund Site
Supplemental Remedial Investigations/Feasibility Studies
Characteristics of the Formerly Impounded Areas

Table 3-2. Wildlife Species Potentially Occurring Within the Formerly Impounded Areas of the Kalamazoo River Study Area that May Utilize Emergent Wetland Habitat

Scientific Name	Common Name	Habitat Specialist[a]	Wetland Dependent[b]
Birds (Cont'd.)			
Phalacrocorax auritus	Double-crested Cormorant		X
Pluvialis dominica	Lesser Golden Plover		
Pluvialis squatarola	Black-bellied Plover	X	X
Podiceps auritus	Horned Grebe		X
Podilymbus podiceps	Pied-billed Grebe		X
Porzana carolina	Sora		X
Progne subis	Purple Martin		X
Rallus limicola	Virginia Rail		X
Riparia riparia	Bank Swallow		
Spizella arbores	American Tree Sparrow		X
Sturnella magna	Eastern Meadowlark		
Tringa flavipes	Lesser Yellowlegs	X	X
Tringa melanoleuca	Greater Yellowlegs		X
Tringa solitaria	Solitary Sandpiper		
Xanthocephalus xanthocephalus	Yellow-headed Blackbird	X	X
Mammals			
Blarina brevicauda	Shorttail Shrew		
Condylura cristata	Starnose Mole		X
Cryptotis parva	Least Shrew		
Didelphis marsupialis	Opossum		
Eptesicus fuscus	Big Brown Bat		
Lutra canadensis	River Otter		X
Mephitis mephitis	Striped Skunk		
Mustela erminea	Ermine		
Mustela frenata	Longtail Weasel		
Mustela nivalis	Least Weasel		
Mustela vison	Mink		
Ondatra zibethicus	Muskrat		X
Sorex cinereus	Masked Shrew		
Zapus hudsonius	Meadow Jumping Mouse		

Notes:
a Compiled from MDNR (1993), Brewer et al. (1982), Pippen (1976), and observations during a June 2000 site visit.
b Oak forests are similar to Oak/Pine forests except that pines are absent.

Kalamazoo River Study Group
Allied Paper, Inc./Portage Creek/Kalamazoo River Superfund Site
Supplemental Remedial Investigations/Feasibility Studies
Characteristics of the Formerly Impounded Areas

Table 3-3. Wildlife Species Potentially Occurring Within the Formerly Impounded Areas of the Kalamazoo River Study Area that May Utilize Wetland Shrub-Scrub Habitat

Scientific Name	Common Name	Habitat Specialist[a]	Wetland Dependent[b]
Birds			
Actitis macularia	Spotted Sandpiper		X
Agelaius phoeniceus	Red-winged Blackbird		
Anas acuta	Northern Pintail		X
Anas rubripes	American Black Duck		X
Aythya collaris	Ring-necked Duck		
Botaurus lentiginosus	American Bittern		X
Butorides striatus	Green-backed Heron		X
Cardinalis cardinalis	Northern Cardinal		
Carduelis tristis	American Goldfinch		
Coccyzus americanus	Yellow-billed Cuckoo		
Coccyzus erythrophthalmus	Black-billed Cuckoo		
Colaptes auratus	Northern Flicker		
Colinus virgianius	Northern Bobwhite		
Dendroica pensylvanica	Chestnut-sided Warbler		
Dendroica petechia	Yellow Warbler		
Dumetella carolinensis	Gray Catbird		
Empidonax alnorum	Alder Flycatcher	X	X
Empidonax trailii	Willow Flycatcher		
Gallinago gallinago	Common Snipe		X
Geothlypis trichas	Common Yellowthroat		
Grus canadensis	Sandhill Crane		X
Icteria virens	Yellow-breasted Chat		
Icterus galbula	Northern Oriole		
Melospiza georgiana	Swamp Sparrow		X
Melospiza lincolnii	Lincoln's Sparrow		
Melospiza melodia	Song Sparrow		
Mergus serrator	Red-breasted Merganser		X
Nycticorax nycticorax	Black-crowned Night-Heron		
Parus atricapillus	Black-capped Chickadee		
Quiscalus quiscula	Common Grackle	X	X
Seiurus noveboracensis	Northern Waterthrush		X
Setophaga ruticilla	American Redstart		
Spizella arbores	American Tree Sparrow		X
Toxostoma rufum	Brown Thrasher		
Troglodytes aedon	House Wren		
Vermivora chrysoptera	Golden-winged Warbler		X
Vermivora peregrina	Tennessee Warbler		
Vermivora pinus	Blue-winged Warbler		
Vireo gilvus	Warbling Vireo		
Wilsonia canadensis	Canada Warbler		
Wilsonia pussila	Wilson's Warbler		

Kalamazoo River Study Group
Allied Paper, Inc./Portage Creek/Kalamazoo River Superfund Site
Supplemental Remedial Investigations/Feasibility Studies
Characteristics of the Formerly Impounded Areas

Table 3-3. Wildlife Species Potentially Occurring Within the Formerly Impounded Areas of the Kalamazoo River Study Area that May Utilize Wetland Shrub-Scrub Habitat

Scientific Name	Common Name	Habitat Specialist[a]	Wetland Dependent[b]
Mammals			
Blarina brevicauda	Shorttail Shrew		
Canis latrans	Coyote		
Castor canadensis	Beaver		X
Condylura cristata	Starnose Mole		X
Cryptotis parva	Least Shrew		
Didelphis marsupialis	Opossum		
Eptesicus fuscus	Big Brown Bat		
Lutra canadensis	River Otter		X
Mephitis mephitis	Striped Skunk		
Mustela erminea	Ermine		
Mustela frenata	Longtail Weasel		
Mustela vison	Mink		
Myotis lucifugus	Little Brown Bat		
Odocoileus virginianus	Whitetail Deer		
Ondatra zibethicus	Muskrat		
Peromyscus leucopus	White-footed Mouse		X
Procyon lotor	Raccoon		
Scalopus aquaticus	Eastern Mole		
Sorex cinereus	Masked Shrew		
Synaptomys cooperi	Southern Bog Lemming		
Vulpes vulpes	Red Fox		
Zapus hudsonius	Meadow Jumping Mouse		

Notes:
a Compiled from MDNR (1993), Brewer et al. (1982), Pippen (1976), and observations during a June 2000 site visit.
b Oak forests are similar to Oak/Pine forests except that pines are absent.

Kalamazoo River Study Group
Allied Paper, Inc./Portage Creek/Kalamazoo River Superfund Site
Supplemental Remedial Investigations/Feasibility Studies
Characteristics of the Formerly Impounded Areas

Table 3-4. Wildlife Species Potentially Occurring Within the Formerly Impounded Areas of the Kalamazoo River Study Area That May Utilize Wetland Forest Habitat

Scientific Name	Common Name	Habitat Specialist[a]	Wetland Dependent[b]
Birds			
Aegolius acadicus	Northern Saw-whet Owl		
Agelaius phoeniceus	Red-winged Blackbird		
Aix sponsa	Wood Duck		X
Anas rubripes	American Black Duck		X
Buteo lineatus	Red-shouldered Hawk	X	X
Butorides striatus	Green-backed Heron		X
Cathartes aura	Turkey Vulture		
Catharus fuscescens	Veery		
Catharus minimus	Gray-cheeked Thrush		
Certhia americana	Brown Creeper		
Coccyzus americanus	Yellow-billed Cuckoo		
Contopus borealis	Oliver-sided Flycatcher		
Dendroica cerulea	Cerulean Warbler		
Dendroica dominica	Yellow-Throated Warbler	X	X
Dendroica palmarum	Palm Warbler		
Dendroica tigrina	Cape May Warbler	X	X
Dryocopus pileatus	Pileated Woodpecker		
Empidonax flaviventris	Yellow-bellied Flycatcher	X	X
Empidonax virescens	Acadian Flycatcher		
Euphagus carolinus	Rusty Blackbird		X
Euphagus cyanocephalus	Brewer's Blackbird		
Gallinago gallinago	Common Snipe		X
Haliaeetus leucocephalus	Bald Eagle	X	X
Hylocichla mustelina	Wood Thrush		
Icterus galbula	Northern Oriole		
Larus philadelphia	Bonaparte's Gull		X
Lophodytes cucullatus	Hooded Merganser		X
Melanerpes carolinus	Red-bellied Woodpecker		
Melanerpes erythrocephalus	Red-headed Woodpecker		
Melospiza georgiana	Swamp Sparrow		X
Melospiza lincolnii	Lincoln's Sparrow		
Mergus merganser	Common Merganser		X
Mniotilta varia	Black-and-white Warbler		
Myiarchus crinitus	Great Crested Flycatcher		
Nyctanassa violaces	Yellow-crowned Night-Heron		X
Oporornis agilis	Connecticut Warbler		
Oporornis formosus	Kentucky Warbler		
Oporornis philadelphia	Mourning Warbler		
Pandion haliaetus	Osprey		X
Parula americana	Northern Parula		
Parus atricapillus	Black-capped Chickadee		
Parus bicolor	Tufted Titmouse		
Passerina cyanea	indigo Bunting		
Pheucticus Ludovicianus	Rose-breasted Grosbeak		
Picoides pubescens	Downy Woodpecker		

Kalamazoo River Study Group
Allied Paper, Inc./Portage Creek/Kalamazoo River Superfund Site
Supplemental Remedial Investigations/Feasibility Studies
Characteristics of the Formerly Impounded Areas

Table 3-4. Wildlife Species Potentially Occurring Within the Formerly Impounded Areas of the Kalamazoo River Study Area That May Utilize Wetland Forest Habitat

Scientific Name	Common Name	Habitat Specialist[a]	Wetland Dependent[b]
Birds (Cont'd.)			
Picoides villosus	Hairy Woodpecker		
Polioptila caerules	Blue-gray Gnatcatcher		
Protonotaria citrea	Prothonotary Warbler	X	X
Regulus calendula	Ruby-crowned Kinglet	X	X
Regulus satrapa	Golden-crowned Kinglet		
Sayornis phoebe	Eastern Phoebe		
Seiurus motacilla	Louisiana Waterthrush	X	X
Seiurus noveboracensis	Northern Waterthrush		X
Setophaga ruticilla	American Redstart		
Sitta canadensis	Red-breasted Nuthatch		
Sitta carolinensis	White-breasted Nuthatch		
Strix varia	Barred Owl		
Tringa solitaria	Solitary Sandpiper		
Troglodytes troglodytes	Winter Wren		
Vermivora chrysoptera	Golden-winged Warbler		X
Vermivora peregrina	Tennessee Warbler		
Vermivora ruficapilla	Nashville Warbler		
Vireo flavifrons	Yellow-Throated Vireo		
Vireo gilvus	Warbling Vireo		
Vireo olivaceus	Red-eyed Vireo		
Wilsonia canadensis	Canada Warbler		
Wilsonia citrina	Hooded Warbler		
Zonotrichia albicollis	White-throated Sparrow	X	X
Mammals			
Blarina brevicauda	Shorttail Shrew		
Canis latrans	Coyote		
Castor canadensis	Beaver		X
Condylura cristata	Starnose Mole		X
Didelphis marsupialis	Opossum		
Eptesicus fuscus	Big Brown Bat		
Glaucomys volans	Southern Flying Squirrel		
Lasionycteris noctivagans	Silver-haired Bat	X	X
Lasiurus borealis	Red Bat		
Lasiurus cinereus	Hoary Bat		
Lutra canadensis	River Otter		X
Mephitis mephitis	Striped Skunk		
Mustela erminea	Ermine		
Mustela frenata	Longtail Weasel		
Mustela vison	Mink		
Myotis keenii	Keen's Bat		
Myotis lucifugus	Little Brown Bat		
Nycticeius humeralis	Evening Bat		
Odocoileus virginianus	Whitetail Deer		
Peromyscus leucopus	White-footed Mouse		

Kalamazoo River Study Group
Allied Paper, Inc./Portage Creek/Kalamazoo River Superfund Site
Supplemental Remedial Investigations/Feasibility Studies
Characteristics of the Formerly Impounded Areas

Table 3-4. Wildlife Species Potentially Occurring Within the Formerly Impounded Areas of the Kalamazoo River Study Area That May Utilize Wetland Forest Habitat

Scientific Name	Common Name	Habitat Specialist[a]	Wetland Dependent[b]
Mammals (Cont'd.)			
Procyon lotor	Raccoon		
Scalopus aquaticus	Eastern Mole		
Sciurus carolinensis	Eastern Gray Squirrel		
Sciurus niger	Eastern Fox Squirrel		
Sorex cinereus	Masked Shrew		
Synaptomys cooperi	Southern Bog Lemming		
Tamiasciurus hudsonicus	Red Squirrel		
Urocyon cinereoargenteus	Gray Fox		
Vulpes vulpes	Red Fox		
Zapus hudsonius	Meadow Jumping Mouse		

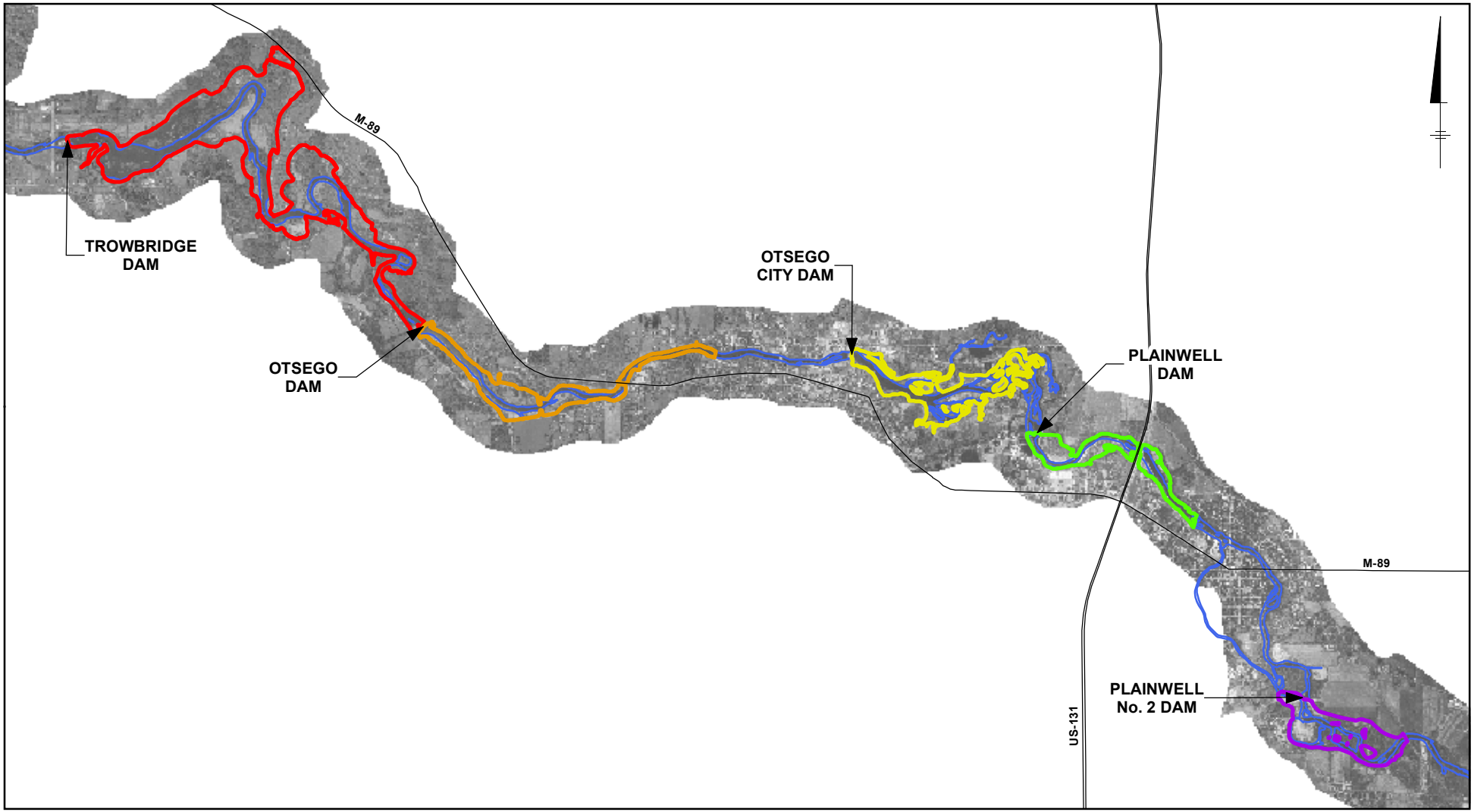
Notes:

a Compiled from MDNR (1993), Brewer et al. (1982), Pippen (1976), and observations during a June 2000 site visit.
b Oak forests are similar to Oak/Pine forests except that pines are absent.

ARCADIS

Figures

SYR-85 MTK EAL
KRSG (64524.0771)
Q:\KRSG\FormerImpoundments\EcoRiskSupportMemo_01-08-08\mxd\Former Impoundments.mxd - 5/5/2008 @ 5:37:50 PM



LEGEND:

- FORMER OTSEGO IMPOUNDMENT
- FORMER OTSEGO CITY IMPOUNDMENT
- FORMER PLAINWELL IMPOUNDMENT
- FORMER PLAINWELL No. 2 AREA
- FORMER TROWBRIDGE IMPOUNDMENT
- EXISTING SHORELINE (APPROXIMATE)

NOTES:

1. FORMER IMPOUNDED AREA BOUNDARIES ARE APPROXIMATE.
2. AERIAL IMAGE DERIVED FROM ORTHOGRAPHIC DATA BY AIR LAND SURVEYS, INC., KALAMAZOO RIVER FLOWN 4/24/99.

0 5,200 10,400 Feet

GRAPHIC SCALE

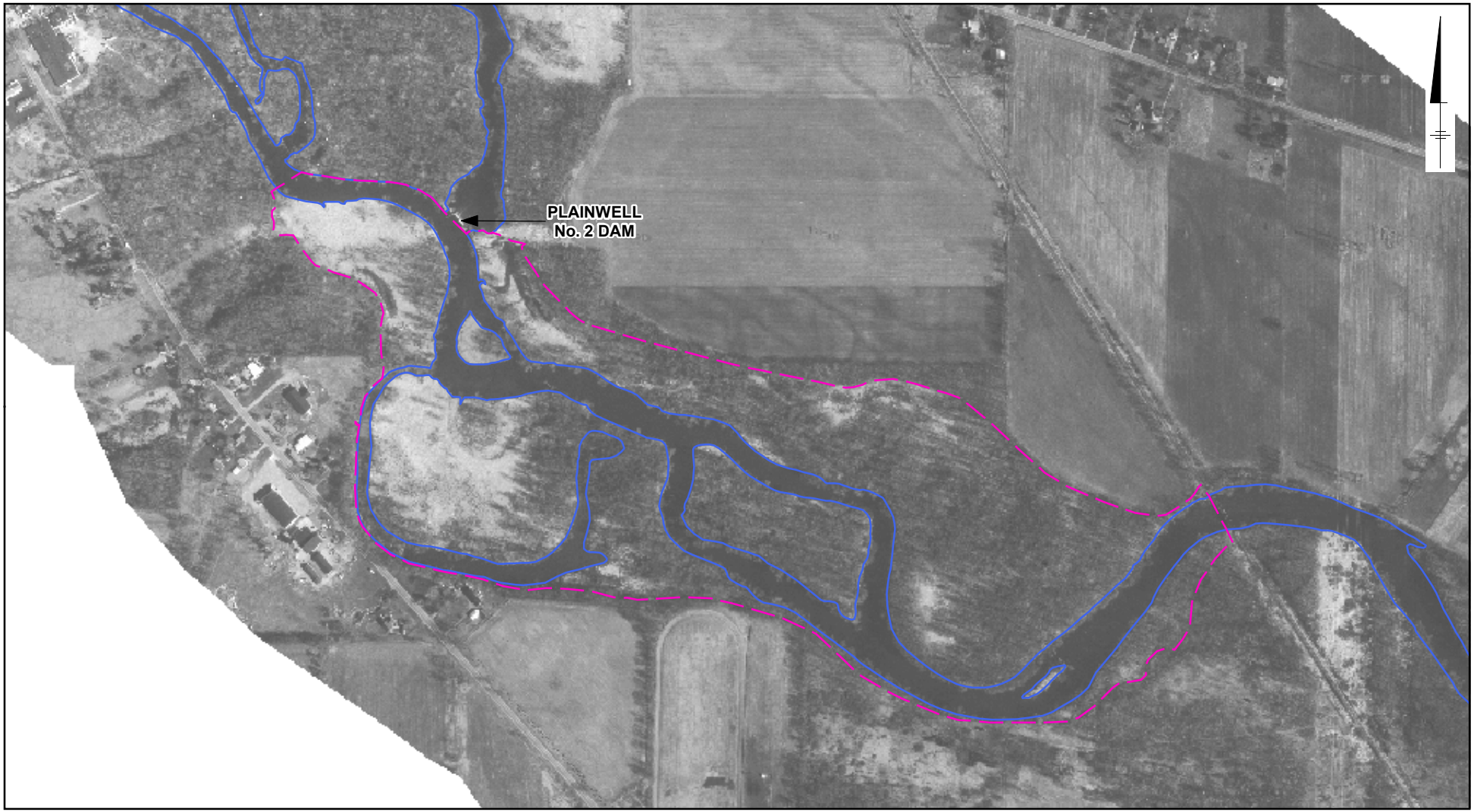
KALAMAZOO RIVER STUDY GROUP
ALLIED PAPER, INC./PORTAGE CREEK/
KALAMAZOO RIVER SUPERFUND SITE
CHARACTERISTICS OF THE FORMERLY IMPOUNDED AREAS

FORMERLY IMPOUNDED AREAS IN THE KALAMAZOO RIVER

ARCADIS

FIGURE
1

SYR-85 MTK EAL
KRSG (64524.0771)
Q:\KRSG\FormerImpoundments\EcoRiskSupportMemo_01-08-08\mxd\Exposed Sediment - Plainwell No2 Dam Impoundment.mxd - 5/5/2008 @ 5:40:13 PM



LEGEND:

- LIMITS OF FORMER INUNDATION IN THE PLAINWELL NO. 2 DAM AREA (APPROXIMATE)
- EXISTING SHORELINE (APPROXIMATE)

NOTES:

1. FORMER FLOODPLAIN BOUNDARIES ARE APPROXIMATE AND SUBJECT TO REFINEMENT.
2. AERIAL IMAGE DERIVED FROM ORTHOGRAPHIC DATA BY AIR LAND SURVEYS, INC., KALAMAZOO RIVER FLOWN 4/24/99.

0 700 1,400 Feet

GRAPHIC SCALE

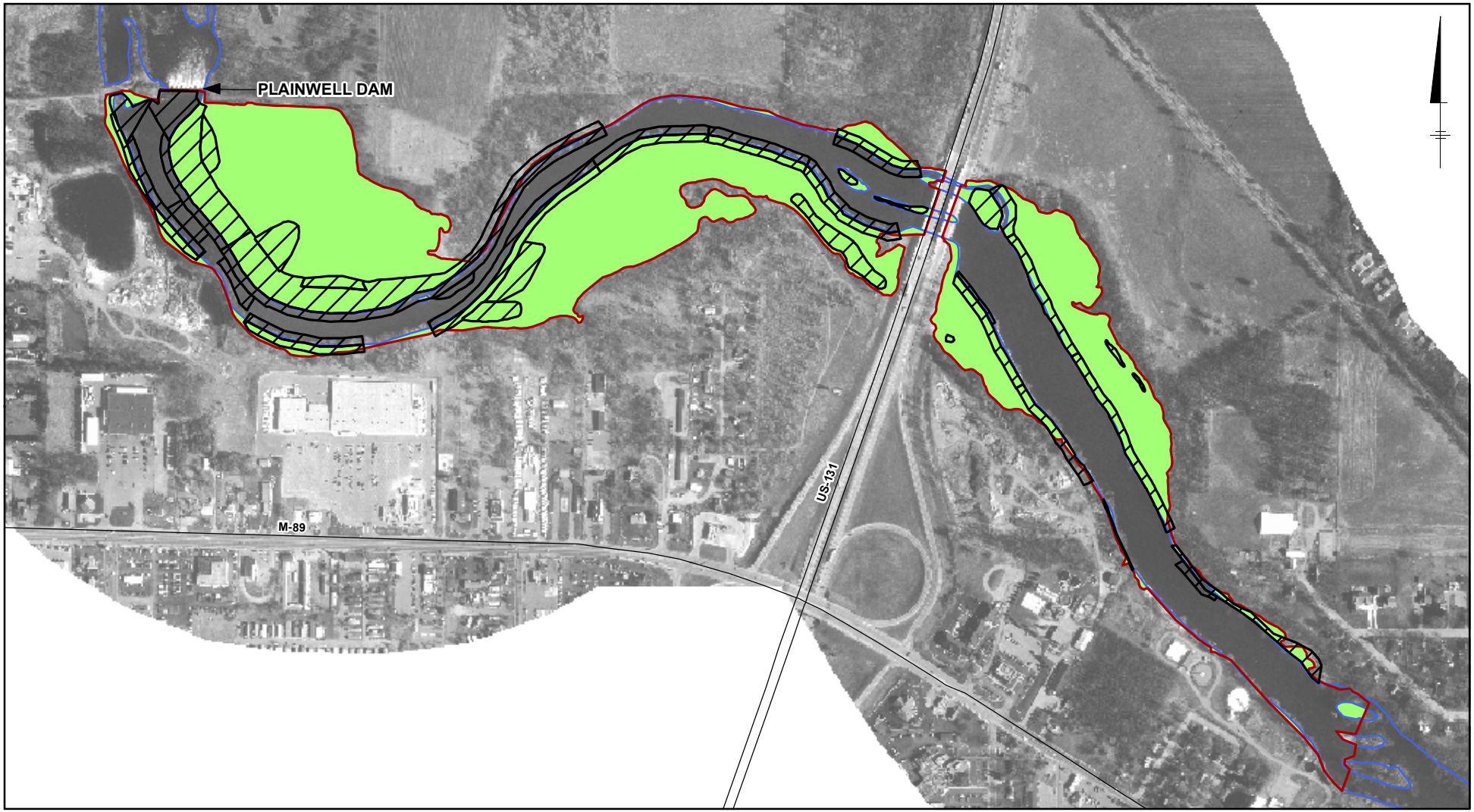
KALAMAZOO RIVER STUDY GROUP
ALLIED PAPER, INC./PORTAGE CREEK/
KALAMAZOO RIVER SUPERFUND SITE
CHARACTERISTICS OF THE FORMERLY IMPOUNDED AREAS

EXTENT OF FORMERLY INUNDATED AREA PLAINWELL NO. 2 DAM

ARCADIS

FIGURE
2

SYR-85 MTK EAL
KRSG (64524.0771)
Q:\KRSG\FormerImpoundments\EcoRiskSupportMemo_01-08-08\mxd\Exposed Sediment - Plainwell Impoundment.mxd - 5/5/2008 @ 5:40:11 PM



LEGEND:

- REMOVAL AREAS TO BE EXCAVATED IN 2007 AND 2008
- FORMER PLAINWELL IMPOUNDMENT
- EXPOSED SEDIMENT (APPROXIMATE)
- EXISTING SHORELINE (APPROXIMATE)

NOTES:

1. FORMER IMPOUNDED AREA BOUNDARIES ARE APPROXIMATE.
2. AERIAL IMAGE DERIVED FROM ORTHOGRAPHIC DATA BY AIR LAND SURVEYS, INC., KALAMAZOO RIVER FLOWN 4/24/99.

0 700 1,400 Feet

GRAPHIC SCALE

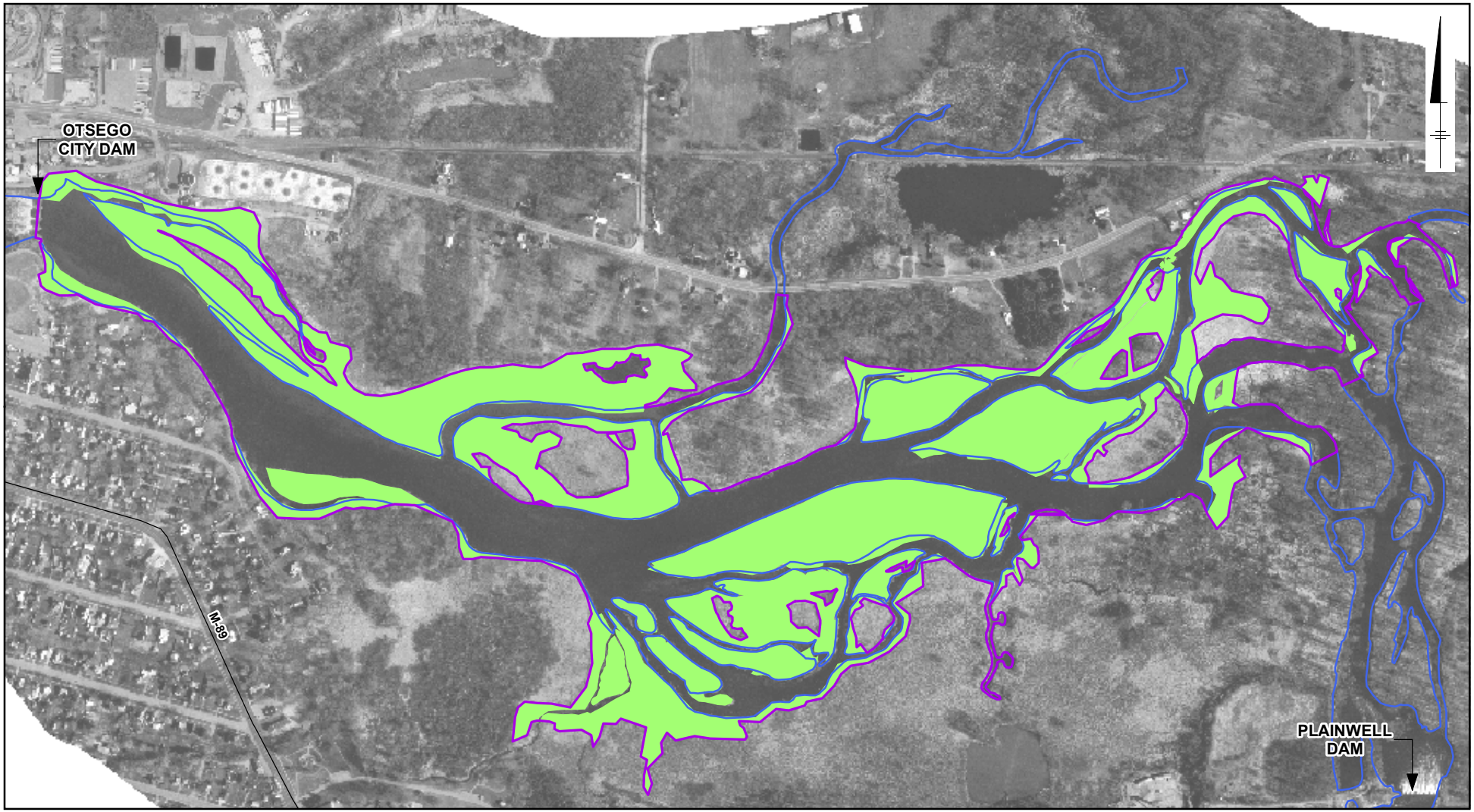
KALAMAZOO RIVER STUDY GROUP
ALLIED PAPER, INC./PORTAGE CREEK/
KALAMAZOO RIVER SUPERFUND SITE
CHARACTERISTICS OF THE FORMERLY IMPOUNDED AREAS

EXTENT OF EXPOSED SEDIMENT - FORMER PLAINWELL IMPOUNDMENT

ARCADIS

FIGURE
3

SYR-85 MTK EAL
KRSG (64524.0771)
Q:\KRSG\FormerImpoundments\EcoRiskSupportMemo_01-08-08\mxd\Exposed Sediment - Otsego City Impoundment.mxd - 5/5/2008 @ 5:57:03 PM



LEGEND:

- FORMER OTSEGO CITY IMPOUNDMENT
- EXPOSED SEDIMENT (APPROXIMATE)
- EXISTING SHORELINE (APPROXIMATE)

NOTES:

1. FORMER IMPOUNDED AREA BOUNDARIES ARE APPROXIMATE.
2. AERIAL IMAGE DERIVED FROM ORTHOGRAPHIC DATA BY AIR LAND SURVEYS, INC., KALAMAZOO RIVER FLOWN 4/24/99.

0 700 1,400 Feet

GRAPHIC SCALE

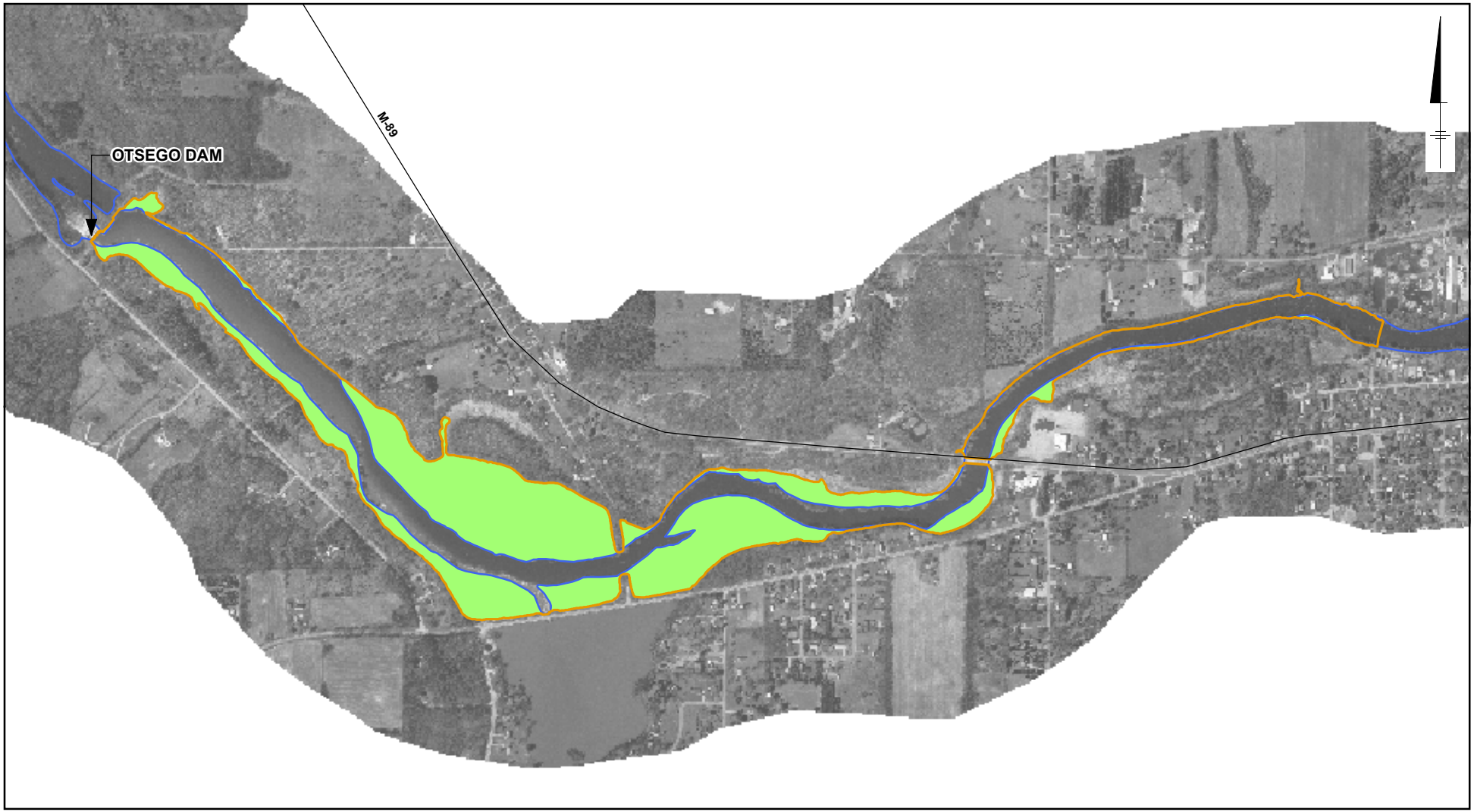
KALAMAZOO RIVER STUDY GROUP
ALLIED PAPER, INC./PORTAGE CREEK/
KALAMAZOO RIVER SUPERFUND SITE
CHARACTERISTICS OF THE FORMERLY IMPOUNDED AREAS

EXTENT OF EXPOSED SEDIMENT - FORMER OTSEGO CITY IMPOUNDMENT

ARCADIS

FIGURE
4

SYR-85 MTK AEL
KRSG (64524.0771)
Q:\KRSG\FormerImpoundments\EcoRiskSupportMemo_01-08-08\mxd\Exposed Sediment - Otsego Impoundment.mxd - 5/5/2008 @ 5:42:23 PM



LEGEND:

- FORMER OTSEGO IMPOUNDMENT
- EXPOSED SEDIMENT (APPROXIMATE)
- EXISTING SHORELINE (APPROXIMATE)

NOTES:

1. FORMER IMPOUNDED AREA BOUNDARIES ARE APPROXIMATE.
2. AERIAL IMAGE DERIVED FROM ORTHOGRAPHIC DATA BY AIR LAND SURVEYS, INC., KALAMAZOO RIVER FLOWN 4/24/99.

0 1,200 2,400 Feet

GRAPHIC SCALE

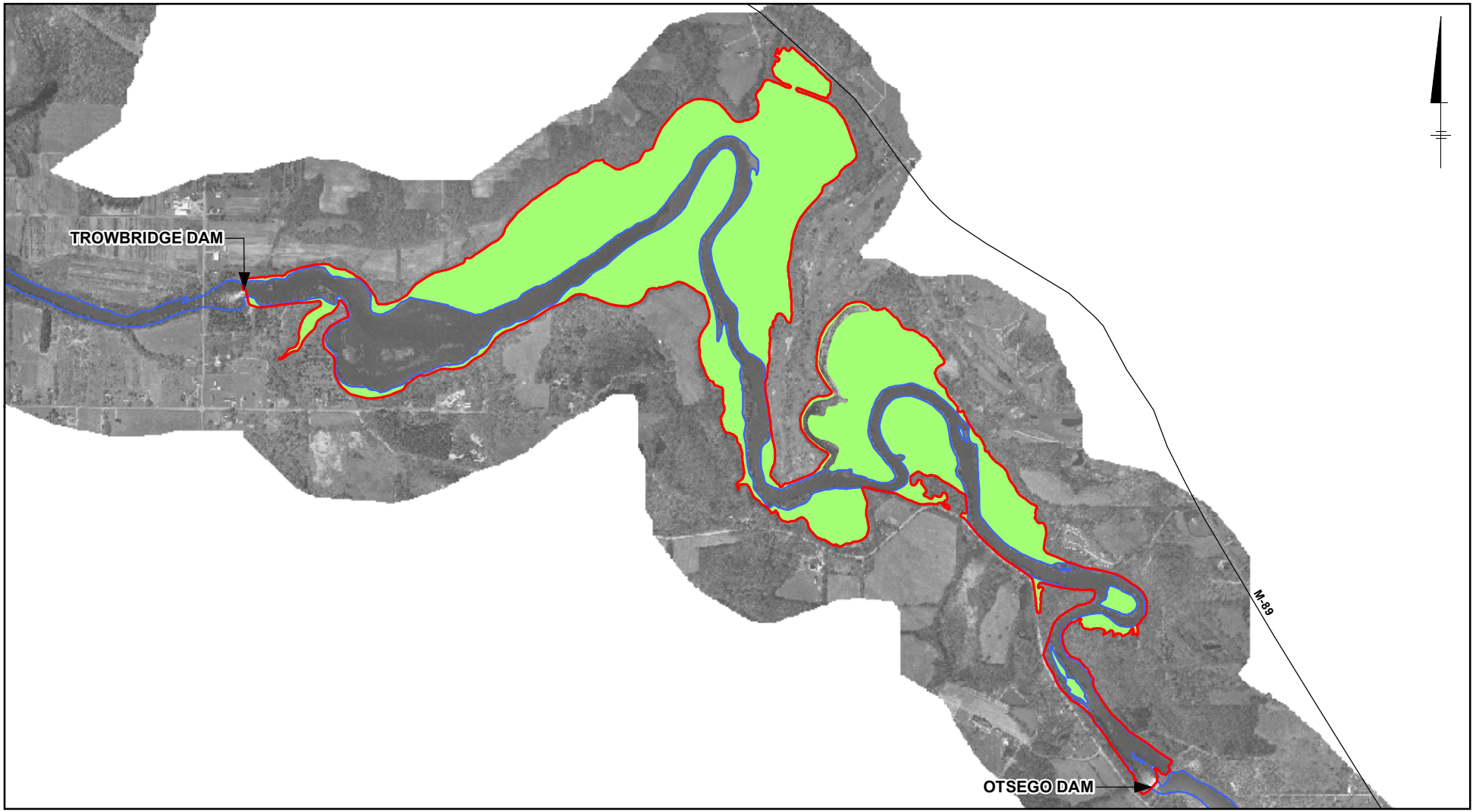
KALAMAZOO RIVER STUDY GROUP
ALLIED PAPER, INC./PORTAGE CREEK/
KALAMAZOO RIVER SUPERFUND SITE
CHARACTERISTICS OF THE FORMERLY IMPOUNDED AREAS

EXTENT OF EXPOSED SEDIMENT - FORMER OTSEGO IMPOUNDMENT

ARCADIS

FIGURE
5

SYR-85 MTK EAL
KRSG (64524.0771)
Q:\KRSG\FormerImpoundments\EcoRiskSupportMemo_01-08-08\mxd\Exposed Sediment - Trowbridge Impoundment.mxd - 5/5/2008 @ 5:42:25 PM



LEGEND:

- FORMER TROWBRIDGE IMPOUNDMENT
- EXPOSED SEDIMENT (APPROXIMATE)
- EXISTING SHORELINE (APPROXIMATE)

NOTES:

1. FORMER IMPOUNDED AREA BOUNDARIES ARE APPROXIMATE.
2. AERIAL IMAGE DERIVED FROM ORTHOGRAPHIC DATA BY AIR LAND SURVEYS, INC., KALAMAZOO RIVER FLOWN 4/24/99.

0 2,000 4,000 Feet

GRAPHIC SCALE

KALAMAZOO RIVER STUDY GROUP
ALLIED PAPER, INC./PORTAGE CREEK/
KALAMAZOO RIVER SUPERFUND SITE
CHARACTERISTICS OF THE FORMERLY IMPOUNDED AREAS

EXTENT OF EXPOSED SEDIMENT - FORMER TROWBRIDGE IMPOUNDMENT

ARCADIS

FIGURE
6

Plainwell No. 2 Dam (Right Diversion Structure)

Plainwell No. 2 Dam (Left Diversion Structure)

Plainwell Dam (View to Downstream)

Otsego City Dam (View to Upstream)

Otsego Dam (View to Upstream)

Trowbridge Dam (View to Upstream)

Note:

The Plainwell Dam picture shows construction activities related to the time critical removal action underway in October 2007.

KALAMAZOO RIVER STUDY GROUP
ALLIED PAPER, INC./PORTAGE CREEK/
KALAMAZOO RIVER SUPERFUND SITE

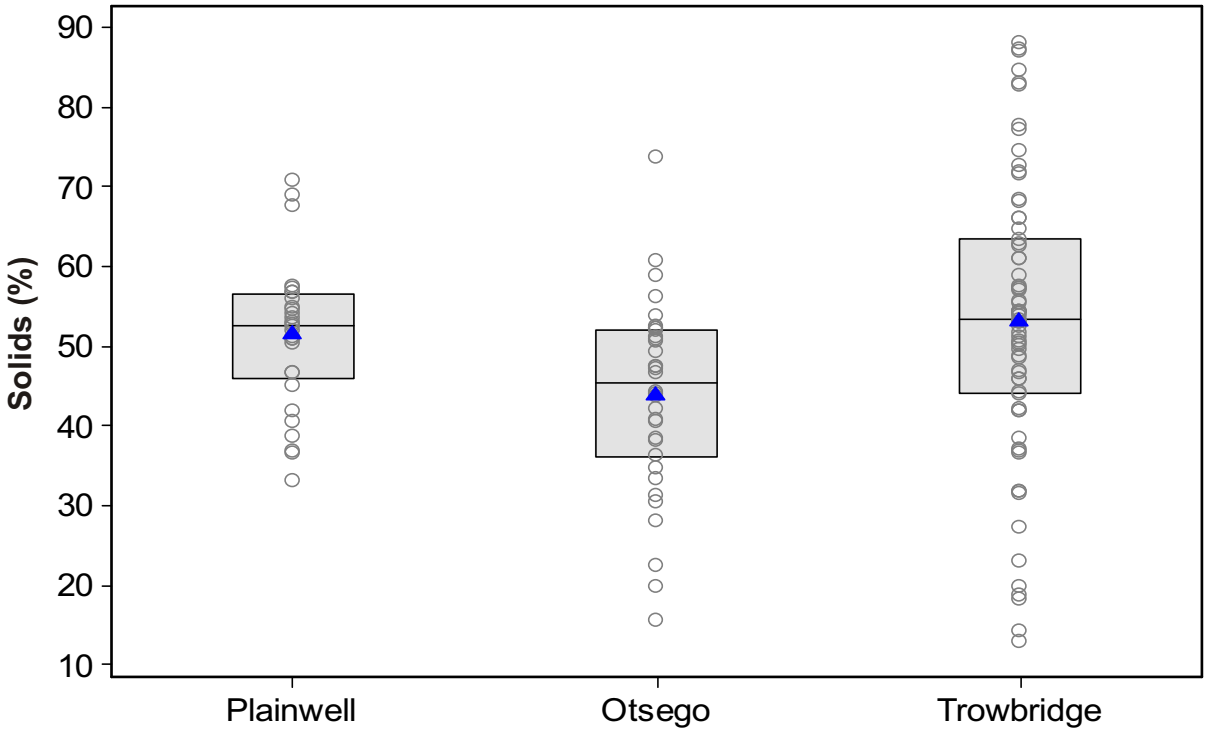
CHARACTERISTICS OF THE FORMERLY IMPOUNDED AREAS

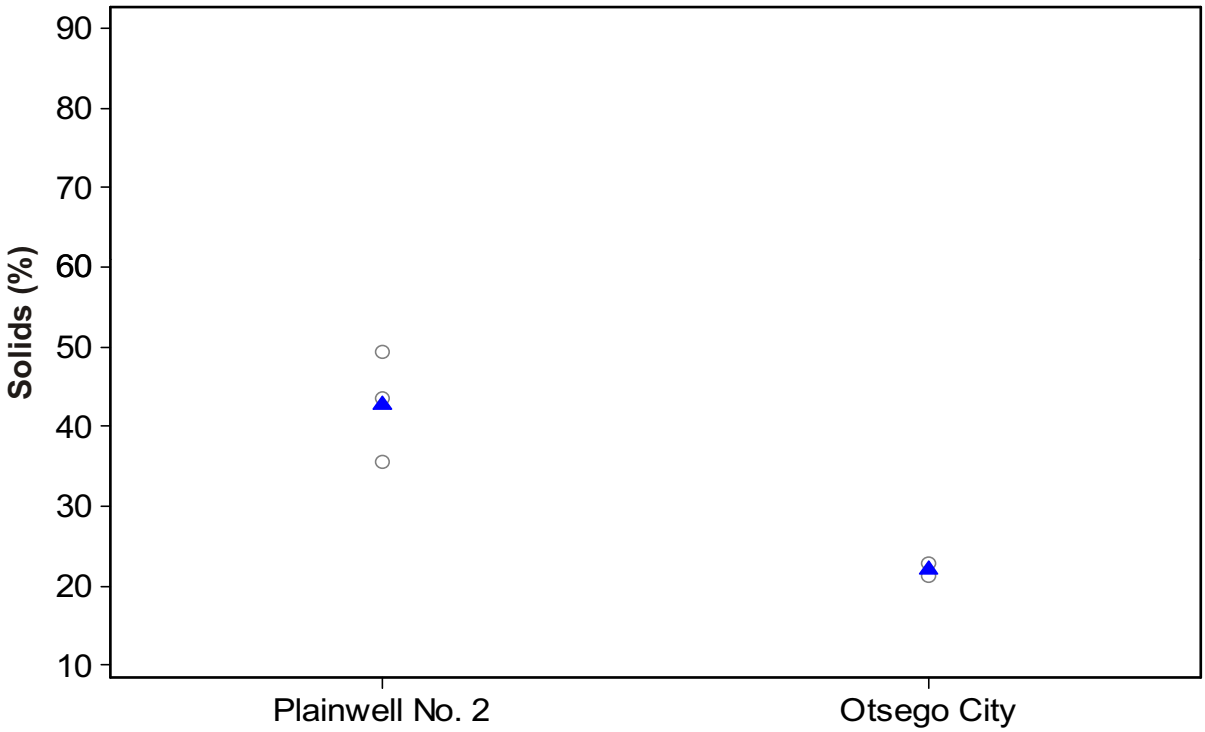
REMAINING DAM STRUCTURES

ARCADIS

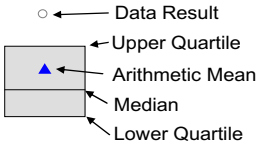
FIGURE
7

05/06/08 SYR-D85-DJH
B0064524/0000/00771/CDR/64524G01.CDR





LEGEND:



NOTE:
Due to small sample size in the former Plainwell No. 2 Area (n = 3) and former Otsego City Impoundment (n = 2), the individual sample results and arithmetic mean are presented for these areas in lieu of statistical box plots

KALAMAZOO RIVER STUDY GROUP
ALLIED PAPER, INC./PORTAGE CREEK/
KALAMAZOO RIVER SUPERFUND SITE

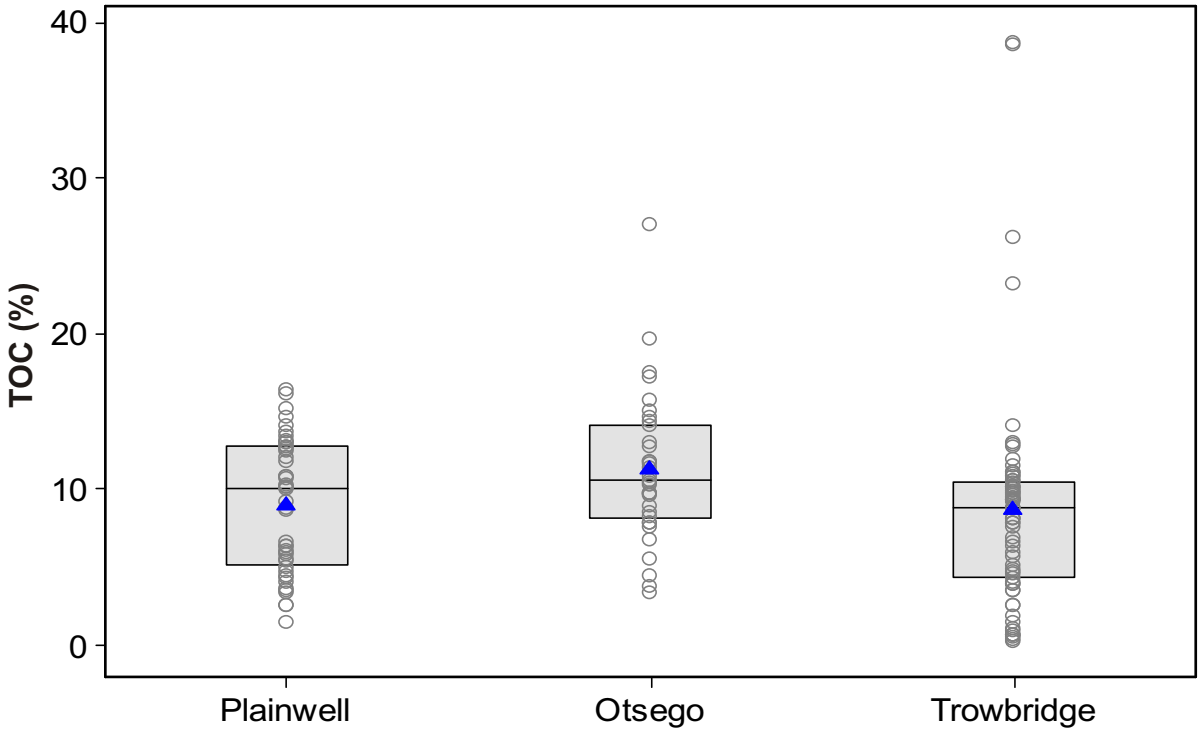
CHARACTERISTICS OF THE FORMERLY IMPOUNDED AREAS

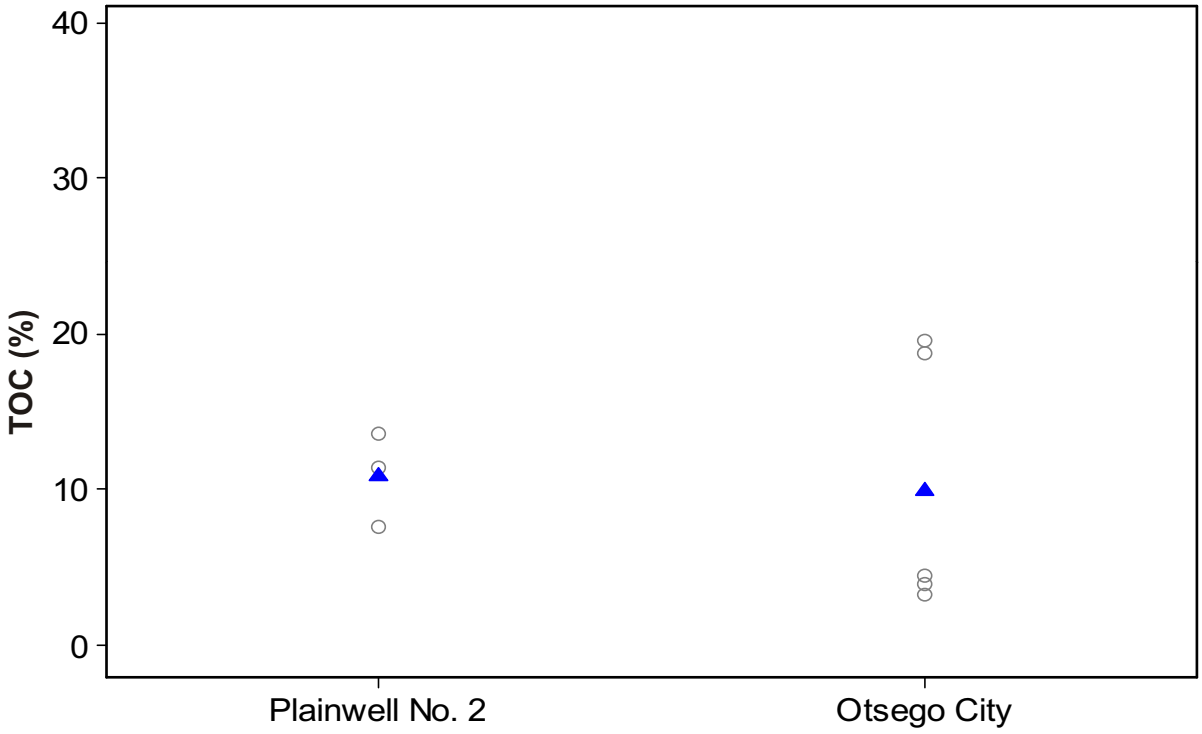
SURFICIAL SOLIDS RESULTS IN FORMER IMPOUNDED AREA EXPOSED SEDIMENT

ARCADIS

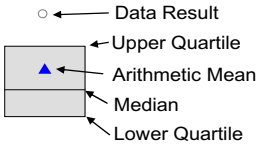
FIGURE
8

05/06/08 SYR-D85-DJH
B0064524/0000/00771/CDR/64524G14.CDR





LEGEND:



NOTE:
Due to small sample size in the former Plainwell No. 2 Area (n = 3) and former Otsego City Impoundment (n = 5), the individual sample results and arithmetic mean are presented for these areas in lieu of statistical box plots

KALAMAZOO RIVER STUDY GROUP
ALLIED PAPER, INC./PORTAGE CREEK/
KALAMAZOO RIVER SUPERFUND SITE

CHARACTERISTICS OF THE FORMERLY IMPOUNDED AREAS

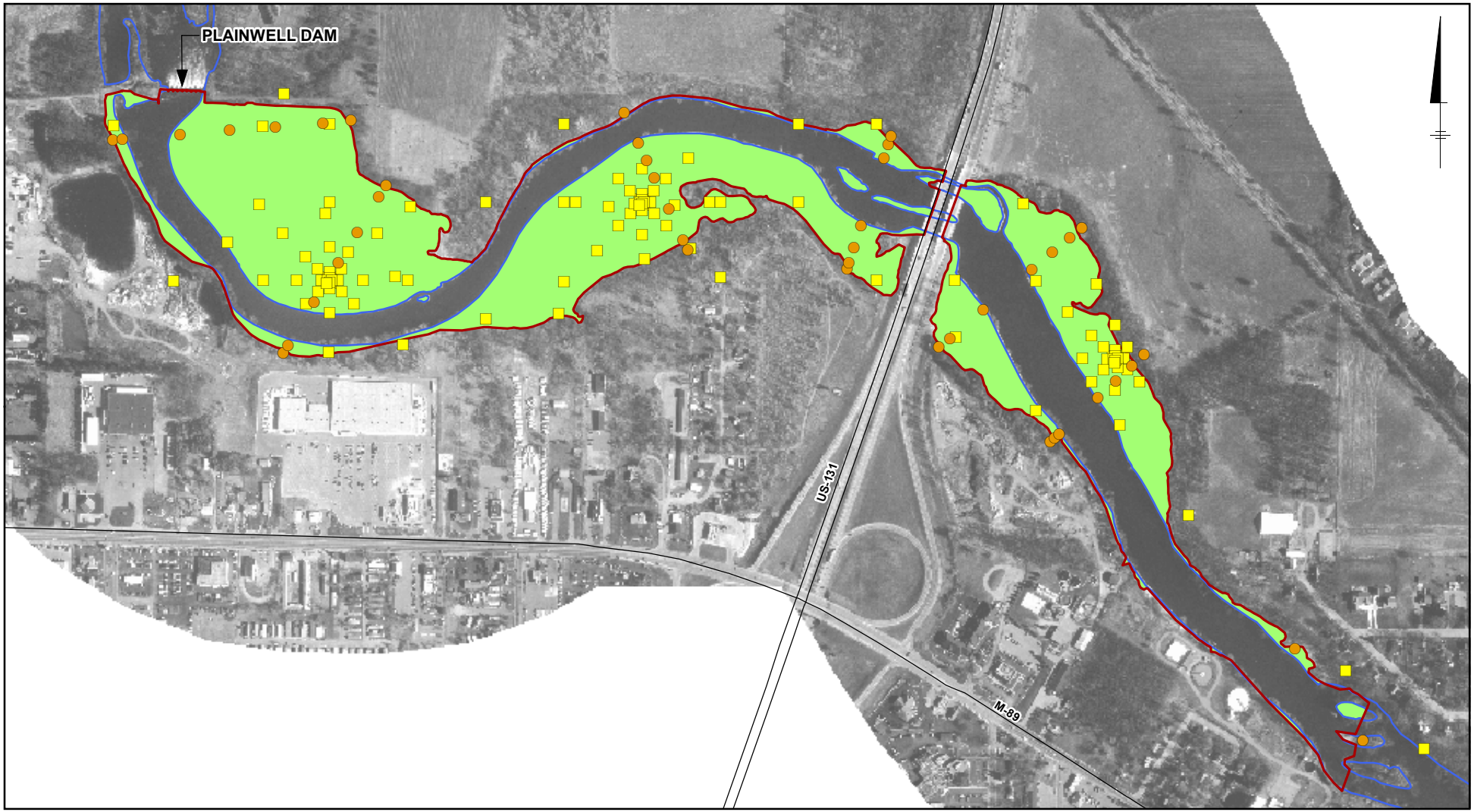
SURFICIAL TOC RESULTS IN FORMER IMPOUNDED AREA EXPOSED SEDIMENT

ARCADIS

FIGURE
9

05/06/08 SYR-D85-DJH
B0064524/0000/00771/CDR/64524G15.CDR

SYR-85 MTK EAL
KRSG (64524.0771)
Q:\KRSG\FormerImpoundments\EcoRiskSupportMemo_01-08-08\mxd\Surface Soil PCB Data - Plainwell Impoundment.mxd - 5/5/2008 @ 5:42:28 PM



LEGEND:

- KRSG SAMPLE LOCATION
- USEPA SAMPLE LOCATION
- EXISTING SHORELINE (APPROXIMATE)
- FORMER PLAINWELL IMPOUNDMENT
- EXPOSED SEDIMENT (APPROXIMATE)

NOTES:

1. FORMER IMPOUNDED AREA BOUNDARIES ARE APPROXIMATE.
2. AERIAL IMAGE DERIVED FROM ORTHOGRAPHIC DATA BY AIR LAND SURVEYS, INC., KALAMAZOO RIVER FLOWN 4/24/99.

0 700 1,400 Feet

GRAPHIC SCALE

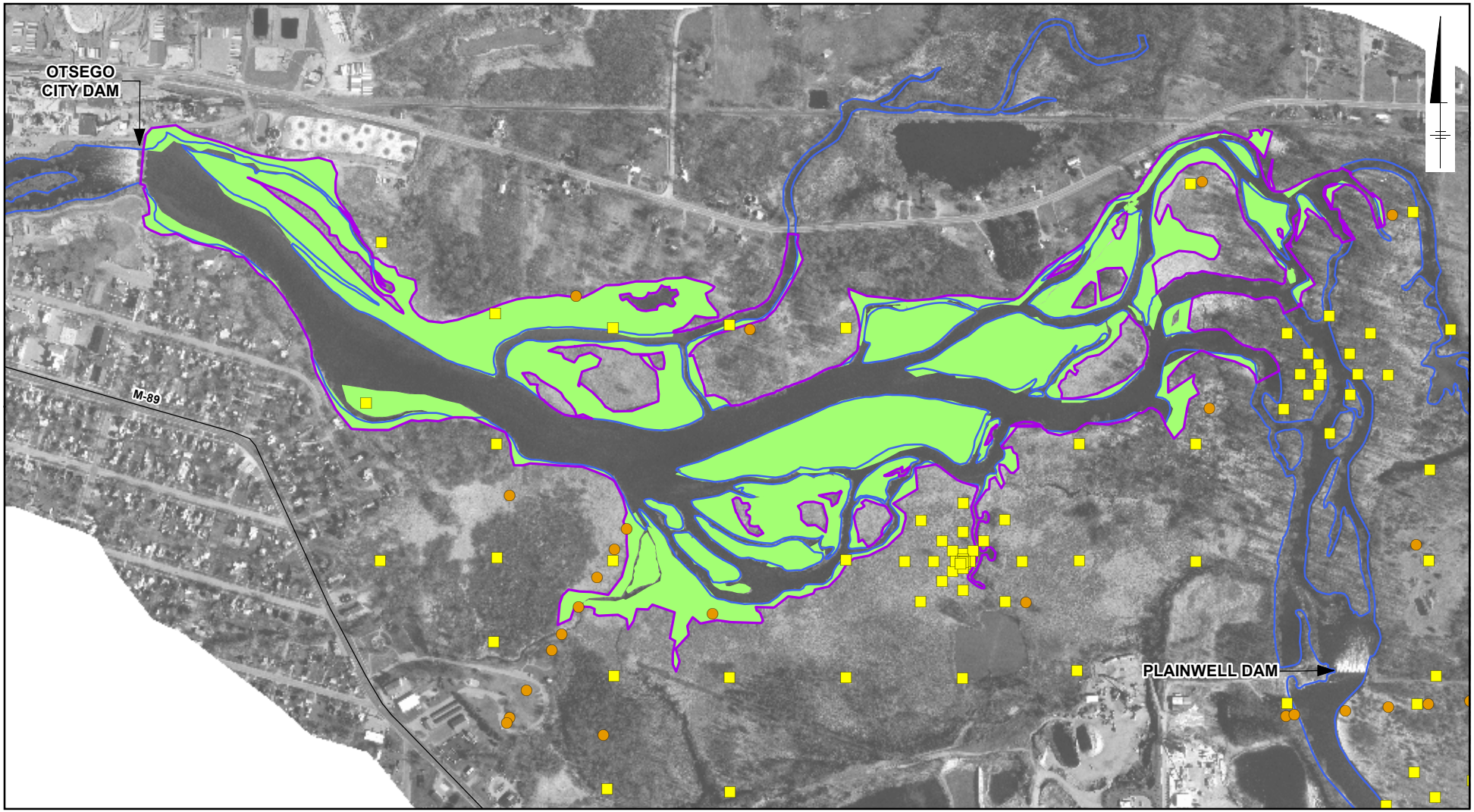
KALAMAZOO RIVER STUDY GROUP
ALLIED PAPER, INC./PORTAGE CREEK/
KALAMAZOO RIVER SUPERFUND SITE
CHARACTERISTICS OF THE FORMERLY IMPOUNDED AREAS

SAMPLE LOCATIONS - FORMER PLAINWELL IMPOUNDMENT

ARCADIS

FIGURE
10

SYR-85 MTK EAL
KRSG (64524.0771)
Q:\KRSG\FormerImpoundments\EcoRiskSupportMemo_01-08-08\mxd\Surface Soil PCB Data - Otsego City Impoundment.mxd - 5/5/2008 @ 5:46:44 PM



LEGEND:

- KRSG SAMPLE LOCATION
- USEPA SAMPLE LOCATION
- EXISTING SHORELINE (APPROXIMATE)
- FORMER OTSEGO CITY IMPOUNDMENT
- EXPOSED SEDIMENT (APPROXIMATE)

NOTES:

1. FORMER IMPOUNDED AREA BOUNDARIES ARE APPROXIMATE.
2. AERIAL IMAGE DERIVED FROM ORTHOGRAPHIC DATA BY AIR LAND SURVEYS, INC., KALAMAZOO RIVER FLOWN 4/24/99.

0 800 1,600 Feet

GRAPHIC SCALE

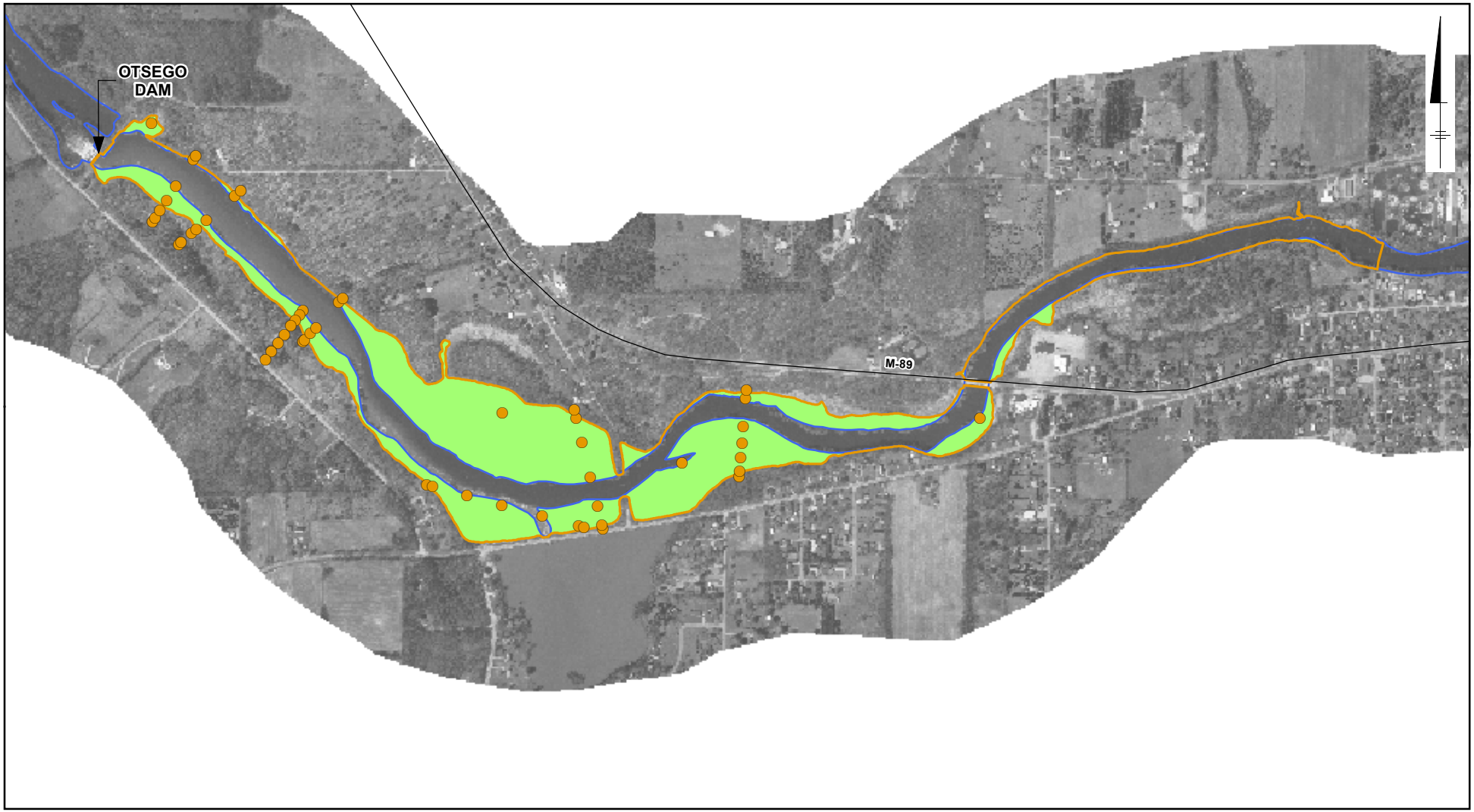
KALAMAZOO RIVER STUDY GROUP
ALLIED PAPER, INC./PORTAGE CREEK/
KALAMAZOO RIVER SUPERFUND SITE
CHARACTERISTICS OF THE FORMERLY IMPOUNDED AREAS

SAMPLE LOCATIONS - FORMER OTSEGO CITY IMPOUNDMENT

ARCADIS

FIGURE
11

SYR-85 MTK EAL
KRSG (64524.0771)
Q:\KRSG\FormerImpoundments\EcoRiskSupportMemo_01-08-08\mxd\Surface Soil PCB Data - Otsego Impoundment.mxd - 5/5/2008 @ 5:47:54 PM



LEGEND:

- KRSG SAMPLE LOCATION
- FORMER OTSEGO IMPOUNDMENT
- EXPOSED SEDIMENT (APPROXIMATE)
- EXISTING SHORELINE (APPROXIMATE)

NOTES:

1. FORMER IMPOUNDED AREA BOUNDARIES ARE APPROXIMATE.
2. AERIAL IMAGE DERIVED FROM ORTHOGRAPHIC DATA BY AIR LAND SURVEYS, INC., KALAMAZOO RIVER FLOWN 4/24/99.

0 1,200 2,400 Feet

GRAPHIC SCALE

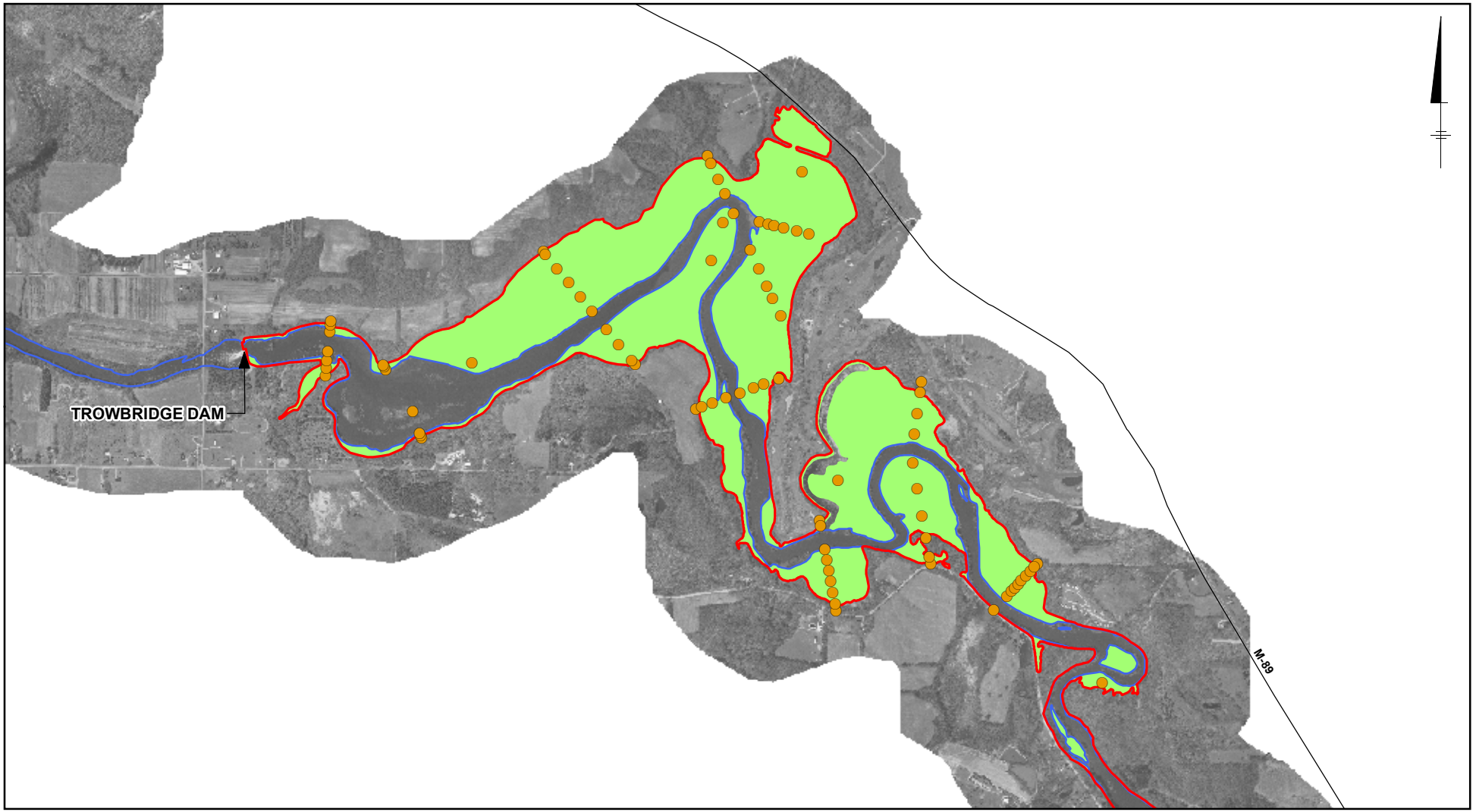
KALAMAZOO RIVER STUDY GROUP
ALLIED PAPER, INC./PORTAGE CREEK/
KALAMAZOO RIVER SUPERFUND SITE
CHARACTERISTICS OF THE FORMERLY IMPOUNDED AREAS

SAMPLE LOCATIONS - FORMER OTSEGO IMPOUNDMENT

ARCADIS

FIGURE
12

SYR-85 MTK EAL
KRSG (64524.0771)
Q:\KRSG\FormerImpoundments\EcoRiskSupportMemo_01-08-08\mxd\Surface Soil PCB Data - Trowbridge Impoundment.mxd - 5/5/2008 @ 5:47:57 PM



LEGEND:

- KRSG SAMPLE LOCATION
- FORMER TROWBRIDGE IMPOUNDMENT
- EXPOSED SEDIMENT (APPROXIMATE)
- EXISTING SHORELINE (APPROXIMATE)

NOTES:

1. FORMER IMPOUNDED AREA BOUNDARIES ARE APPROXIMATE.
2. AERIAL IMAGE DERIVED FROM ORTHOGRAPHIC DATA BY AIR LAND SURVEYS, INC., KALAMAZOO RIVER FLOWN 4/24/99.

0 2,000 4,000 Feet

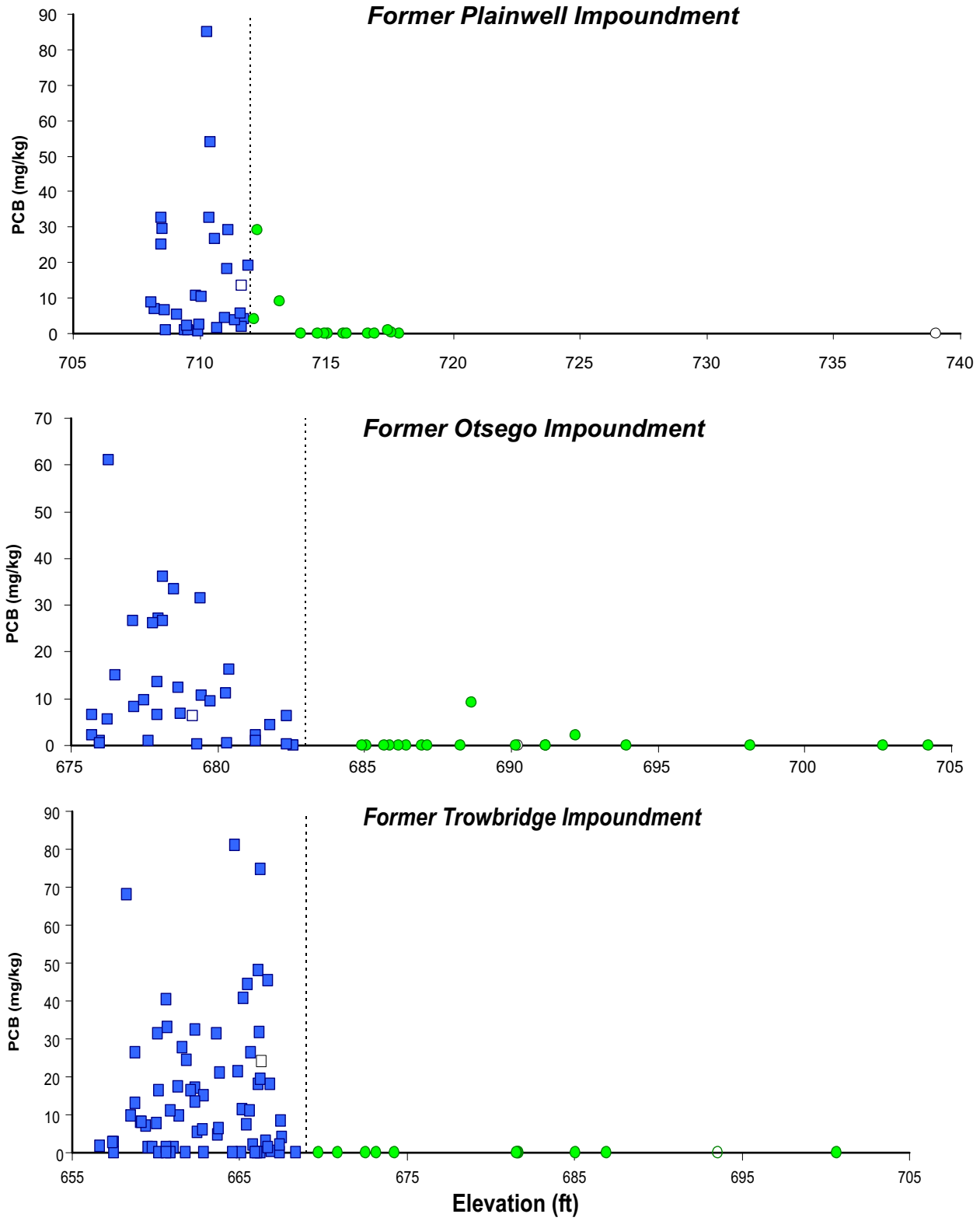
GRAPHIC SCALE

KALAMAZOO RIVER STUDY GROUP
ALLIED PAPER, INC./PORTAGE CREEK/
KALAMAZOO RIVER SUPERFUND SITE
CHARACTERISTICS OF THE FORMERLY IMPOUNDED AREAS

SAMPLE LOCATIONS - FORMER TROWBRIDGE IMPOUNDMENT

ARCADIS

FIGURE
13



LEGEND:

- Outside of the Former Impoundment
- Within the Former Impoundment
- Pre-drawdown normal pool elevation

KALAMAZOO RIVER STUDY GROUP
ALLIED PAPER, INC./PORTAGE CREEK/
KALAMAZOO RIVER SUPERFUND SITE

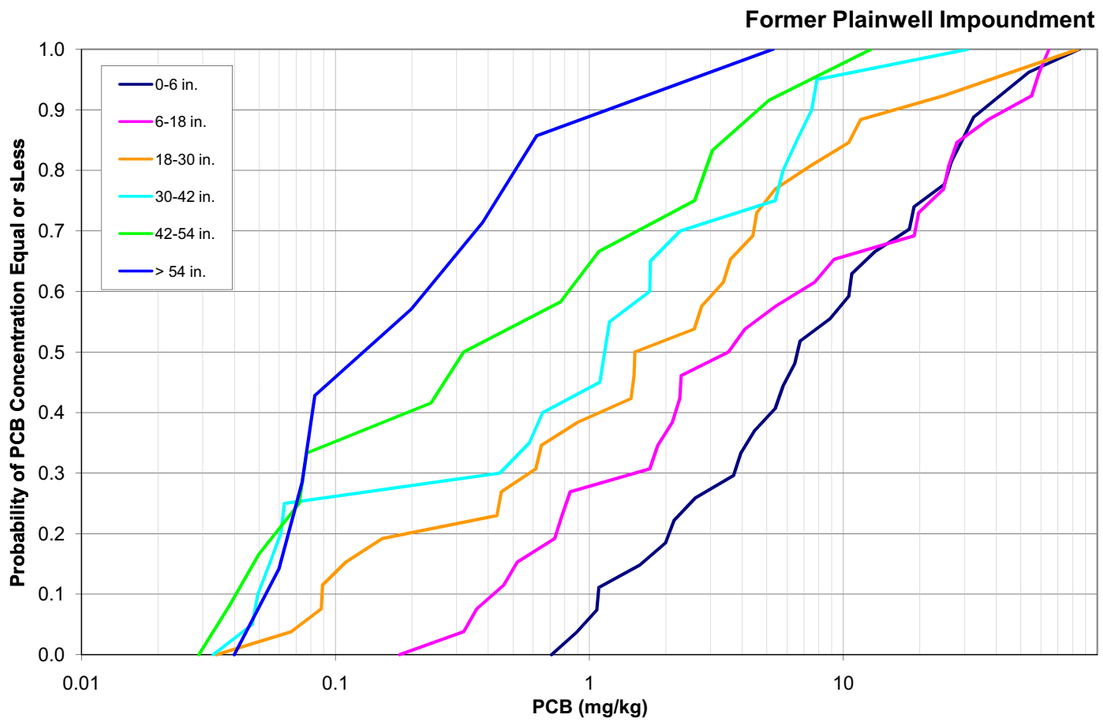
SUMMARY OF THE CHARACTERISTICS OF FORMERLY IMPOUNDED AREAS

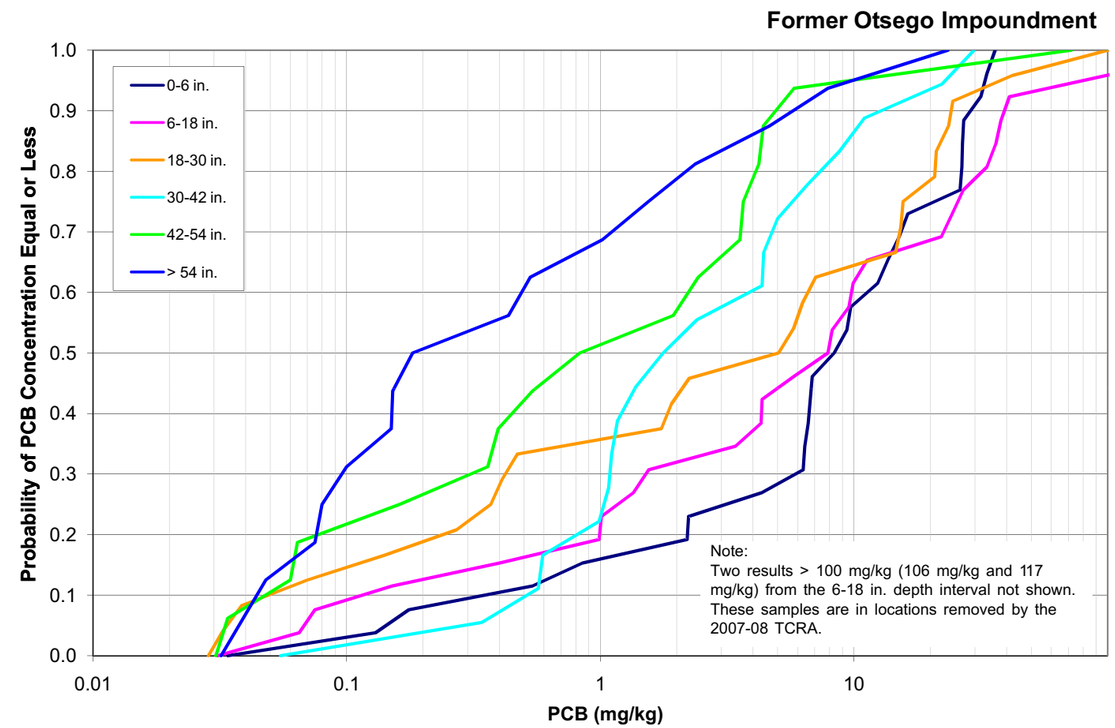
SURFICIAL EXPOSED SEDIMENT AND SURFICIAL FLOODPLAIN SOIL PCB LEVELS IN RELATION TO ELEVATION AND FORMER IMPOUNDMENT POOL ELEVATION

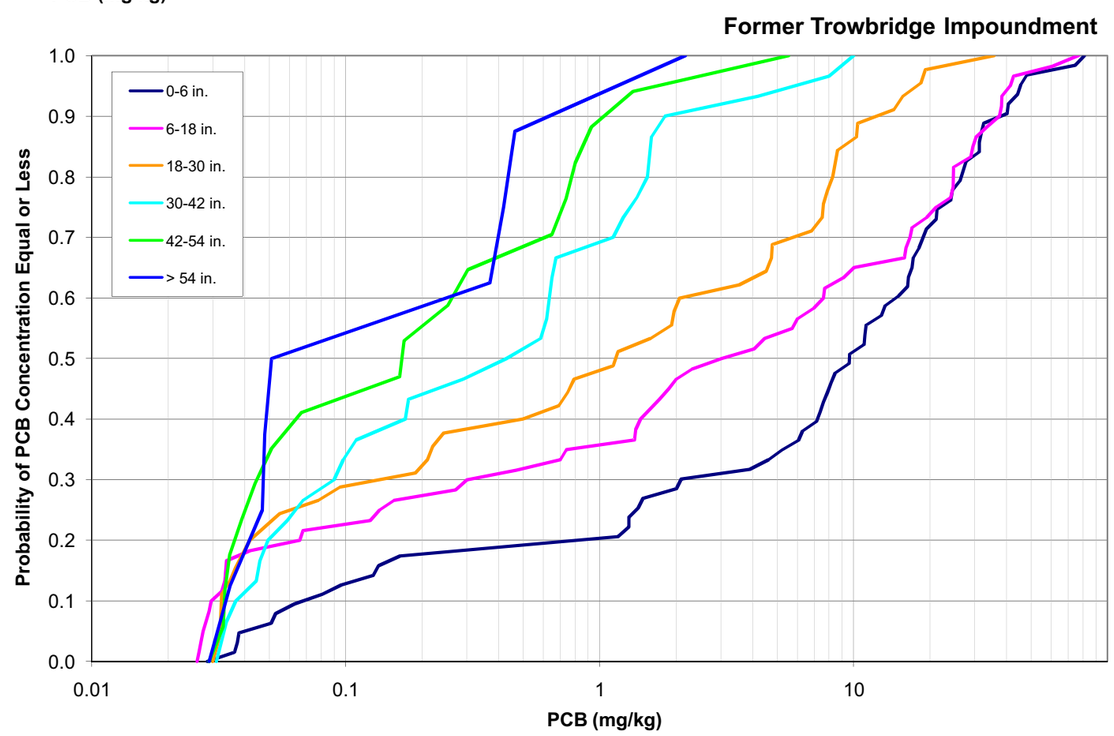
ARCADIS

FIGURE 14

05/06/08 SYR-D85-DJH
B0064524/0000/00771/CDR/64524G23.CDR







NOTE:
1.Data collected in 1993-94.

KALAMAZOO RIVER STUDY GROUP
ALLIED PAPER, INC./PORTAGE CREEK/
KALAMAZOO RIVER SUPERFUND SITE

CHARACTERISTICS OF THE FORMERLY IMPOUNDED AREAS

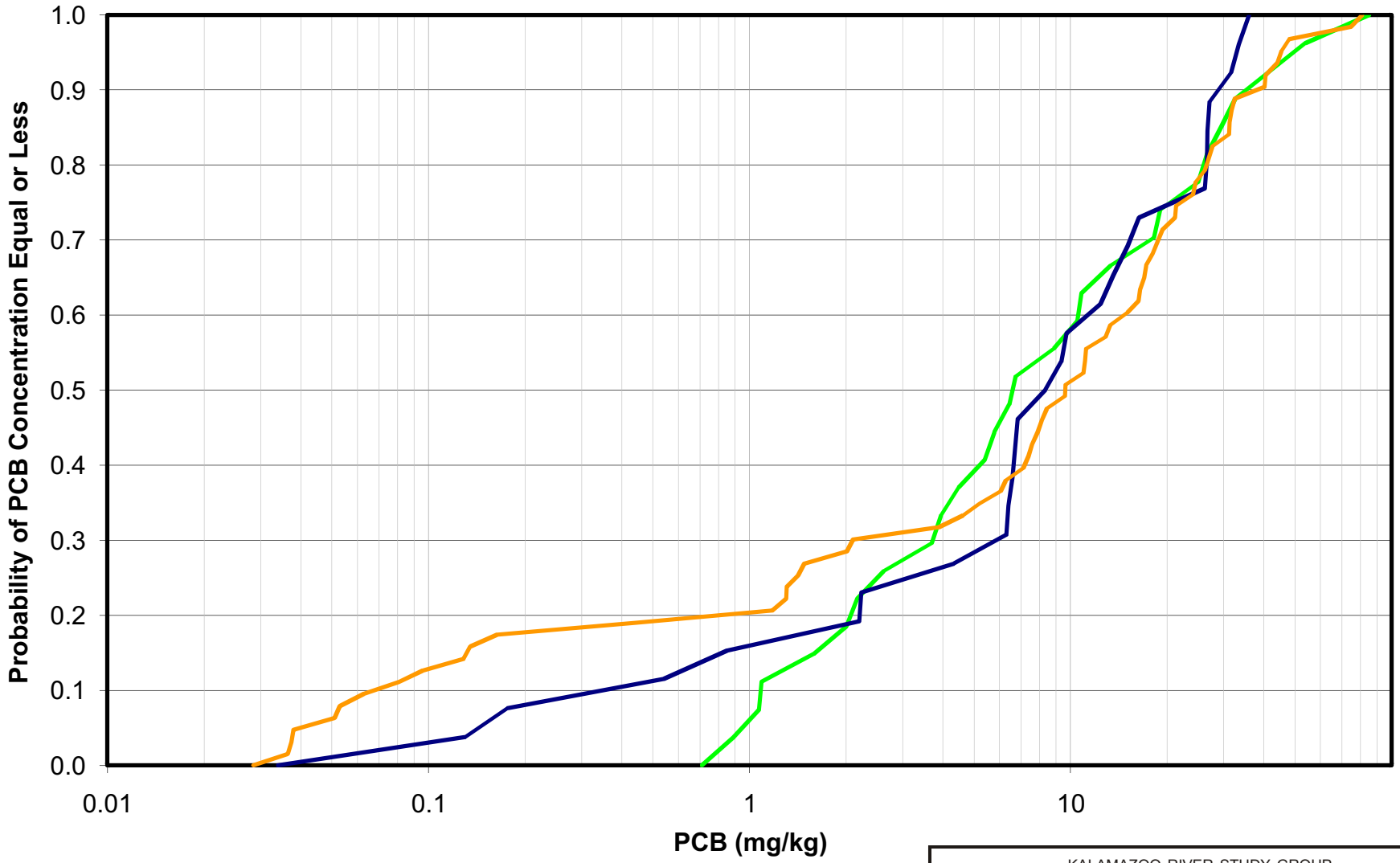
CUMULATIVE FREQUENCY DISTRIBUTION BY DEPTH FOR EACH FORMER IMPOUNDMENT

ARCADIS

FIGURE
15

05/06/08 SYR-D85-DJH
B0064524/0000/00771/CDR/64524G20.CDR

05/06/08 SYR-141ENV-DJH
B0064524/0000/00771/CDR/64524G21.CDR



LEGEND:

- Plainwell Impoundment
- Otsego Impoundment
- Former Trowbridge Impoundment

KALAMAZOO RIVER STUDY GROUP
ALLIED PAPER, INC./PORTAGE CREEK/
KALAMAZOO RIVER SUPERFUND SITE

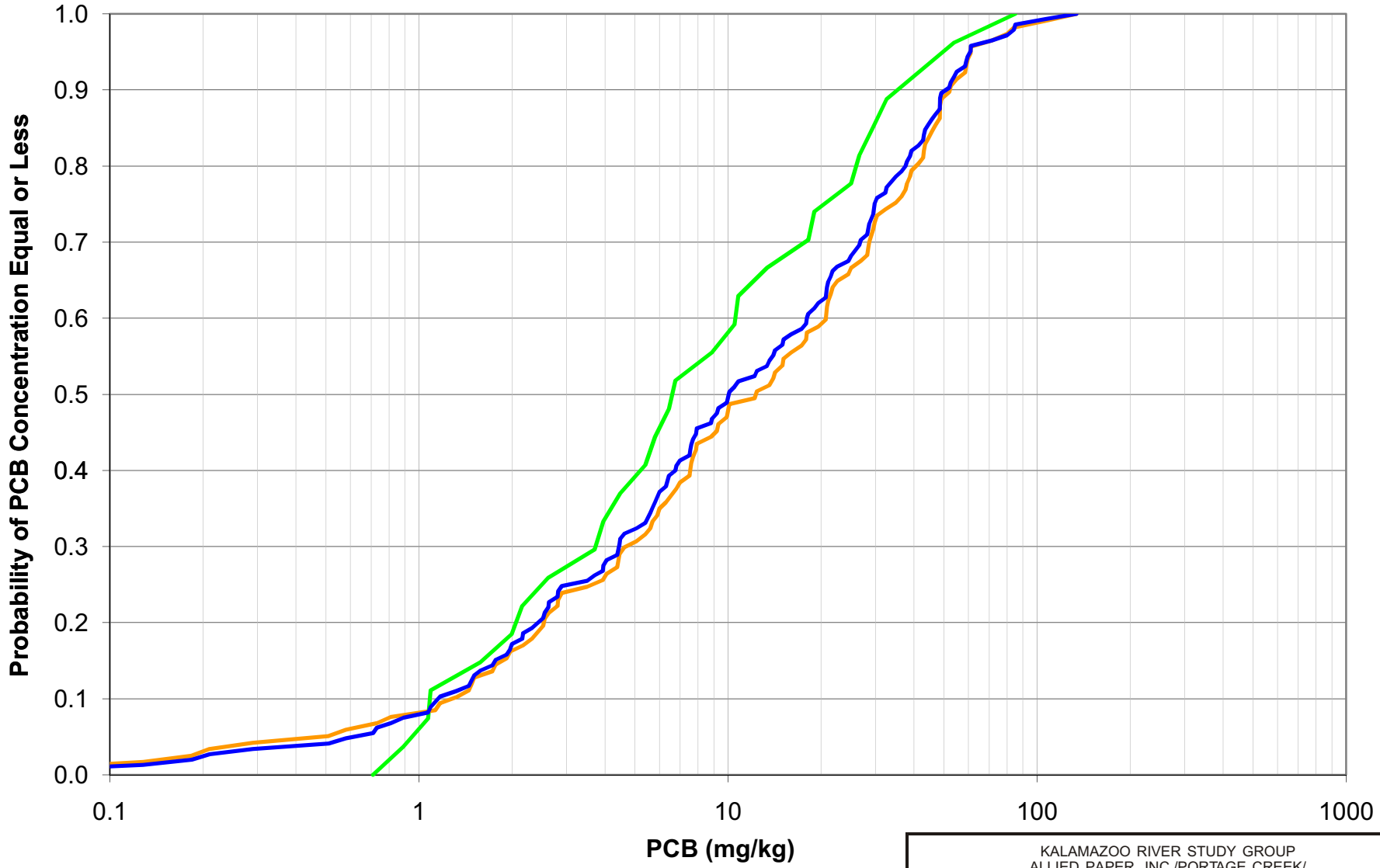
CHARACTERISTICS OF THE FORMERLY IMPOUNDED AREAS

CUMULATIVE FREQUENCY DISTRIBUTION IN SURFICIAL EXPOSED SEDIMENT

ARCADIS

FIGURE
16

05/06/08 SYR-D85-DJH
B0064524/0000/00771/CDR/64524G22.CDR



LEGEND:

- RI Dataset (1993-94)
- USEPA Dataset (2001)
- Combined Dataset

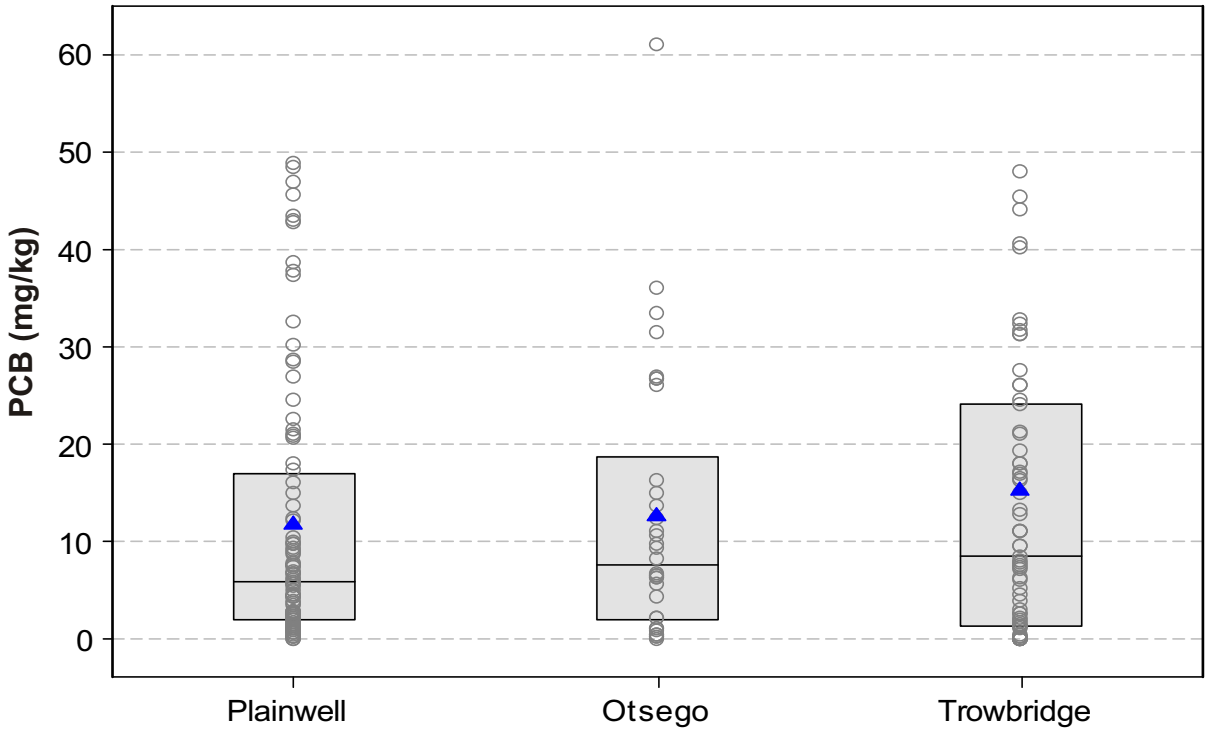
KALAMAZOO RIVER STUDY GROUP
ALLIED PAPER, INC./PORTAGE CREEK/
KALAMAZOO RIVER SUPERFUND SITE

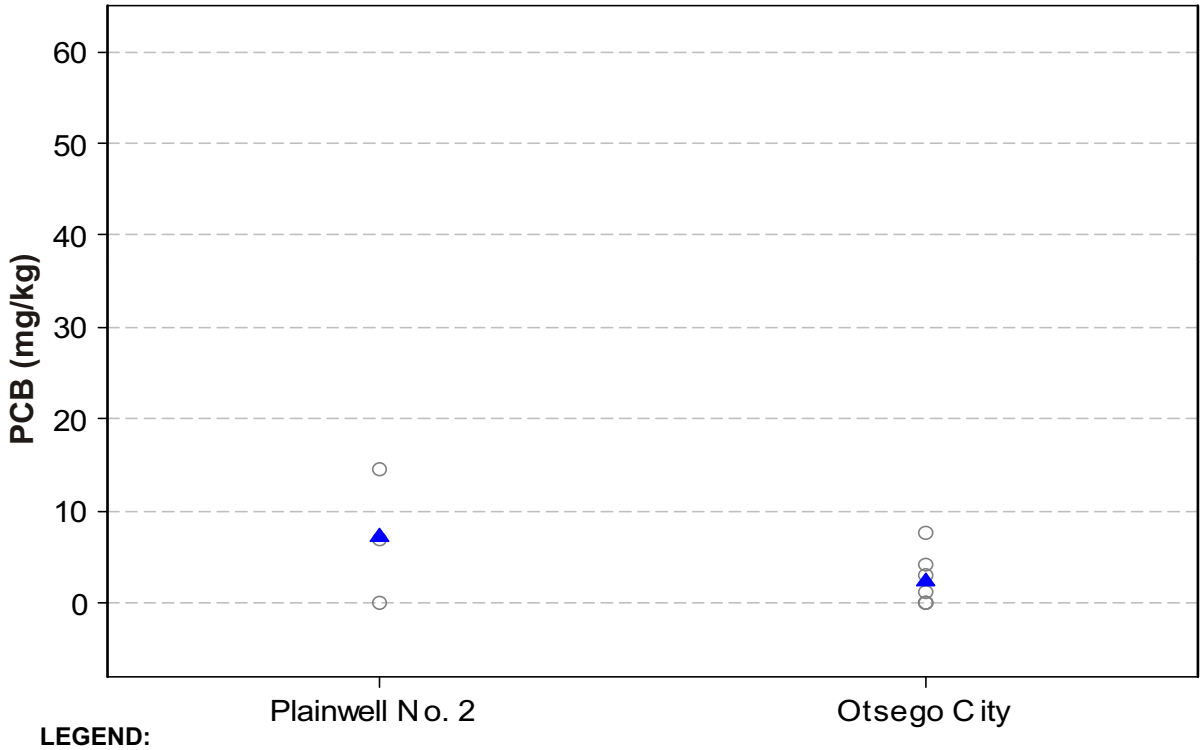
CHARACTERISTICS OF THE FORMERLY IMPOUNDED AREAS

CUMULATIVE FREQUENCY DISTRIBUTION IN FORMER PLAINWELL IMPOUNDMENT EXPOSED SEDIMENT

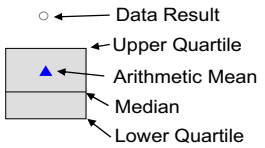
ARCADIS

FIGURE
17





LEGEND:



NOTES:
1. Data for Plainwell represent locations to remain following 2007 and 2008 removal activities. Three PCB concentrations from Trowbridge (68, 75, and 81mg/kg) are not shown on this scale for presentation purposes.
2. Due to small sample size in the former Plainwell No. 2 Area (n = 3) and former Otsego City Impoundment (n = 7), the individual sample results and arithmetic mean are presented for these areas in lieu of statistical box plots

KALAMAZOO RIVER STUDY GROUP
ALLIED PAPER, INC./PORTAGE CREEK/
KALAMAZOO RIVER SUPERFUND SITE

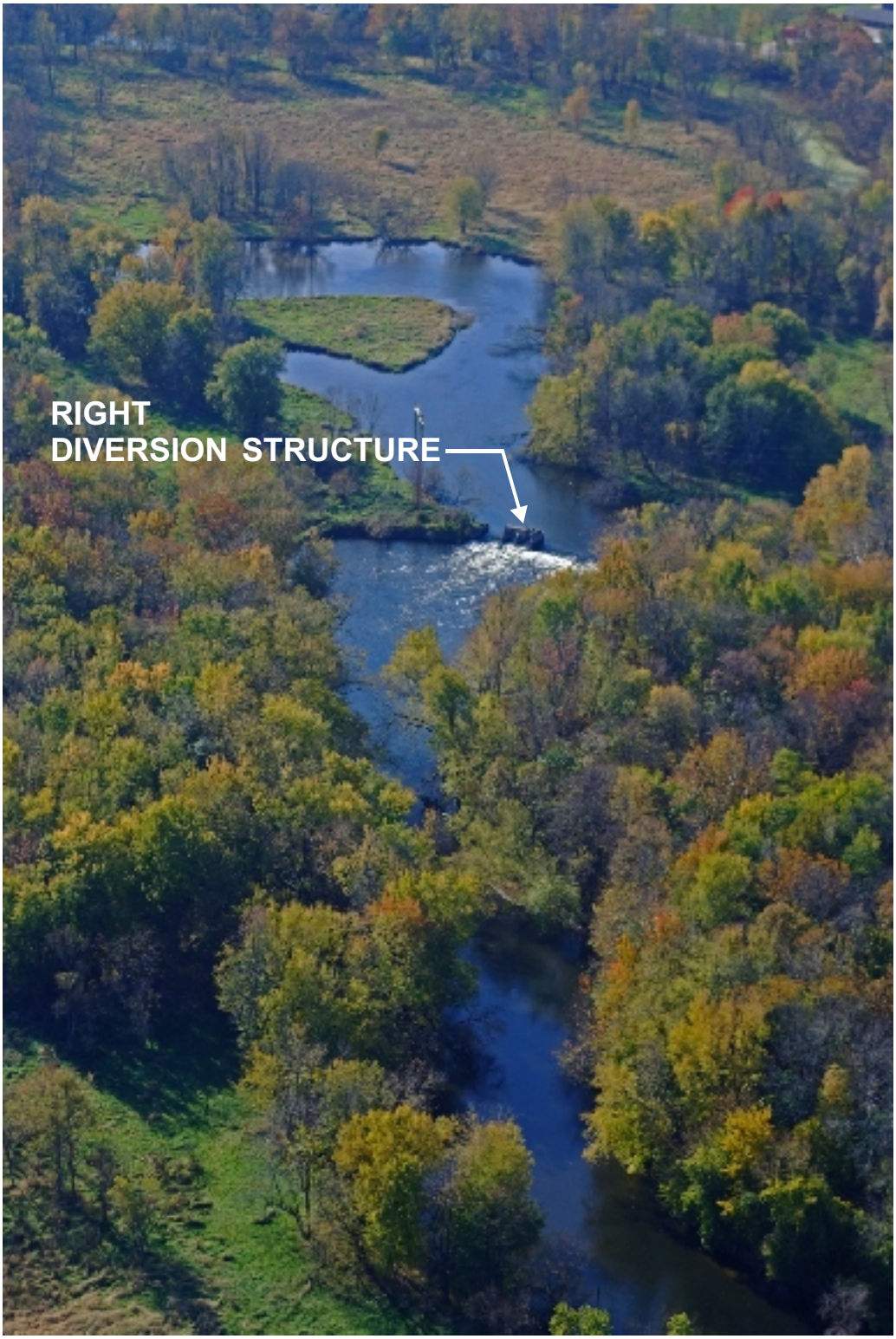
CHARACTERISTICS OF THE FORMERLY IMPOUNDED AREAS

SURFICIAL PCB RESULTS IN FORMER IMPOUNDED AREA EXPOSED SEDIMENT

ARCADIS

FIGURE
18

05/06/08 SYR-141ENV-DJH
B0064524/0000/00771/CDR/64524G19.CDR



NOTES:
1. View looking east.
2. Aerial photograph taken October 30, 2007.

KALAMAZOO RIVER STUDY GROUP
ALLIED PAPER, INC./PORTAGE CREEK/
KALAMAZOO RIVER SUPERFUND SITE
CHARACTERISTICS OF THE FORMERLY IMPOUNDED AREAS

AERIAL PHOTOGRAPH
PLAINWELL NO. 2 DAM AREA

ARCADIS

FIGURE
19

05/06/08 SYR-D85-DJH
B0064524/0000/00771/CDR/64524G07.CDR

NOTES:
1. View looking east.
2. Aerial photograph taken May 25, 2007.

KALAMAZOO RIVER STUDY GROUP
ALLIED PAPER, INC./PORTAGE CREEK/
KALAMAZOO RIVER SUPERFUND SITE
CHARACTERISTICS OF THE FORMERLY IMPOUNDED AREAS

AERIAL PHOTOGRAPH - FORMER PLAINWELL IMPOUNDMENT

ARCADIS

FIGURE
20

05/06/08 SYR-D85-DJH
B0064524/0000/00771/CDR/64524G08.CDR

NOTES:
1. View looking west.
2. Aerial photograph taken October 30, 2007.

KALAMAZOO RIVER STUDY GROUP
ALLIED PAPER, INC./PORTAGE CREEK/
KALAMAZOO RIVER SUPERFUND SITE
CHARACTERISTICS OF THE FORMERLY IMPOUNDED AREAS

**AERIAL PHOTOGRAPH -
FORMER OTSEGO CITY IMPOUNDMENT**

ARCADIS

FIGURE
21

05/06/08 SYR-D85-DJH
B0064524/0000/00771/CDR/64524G09.CDR

NOTES:
1. View looking southwest.
2. Aerial photograph taken October 30, 2007.

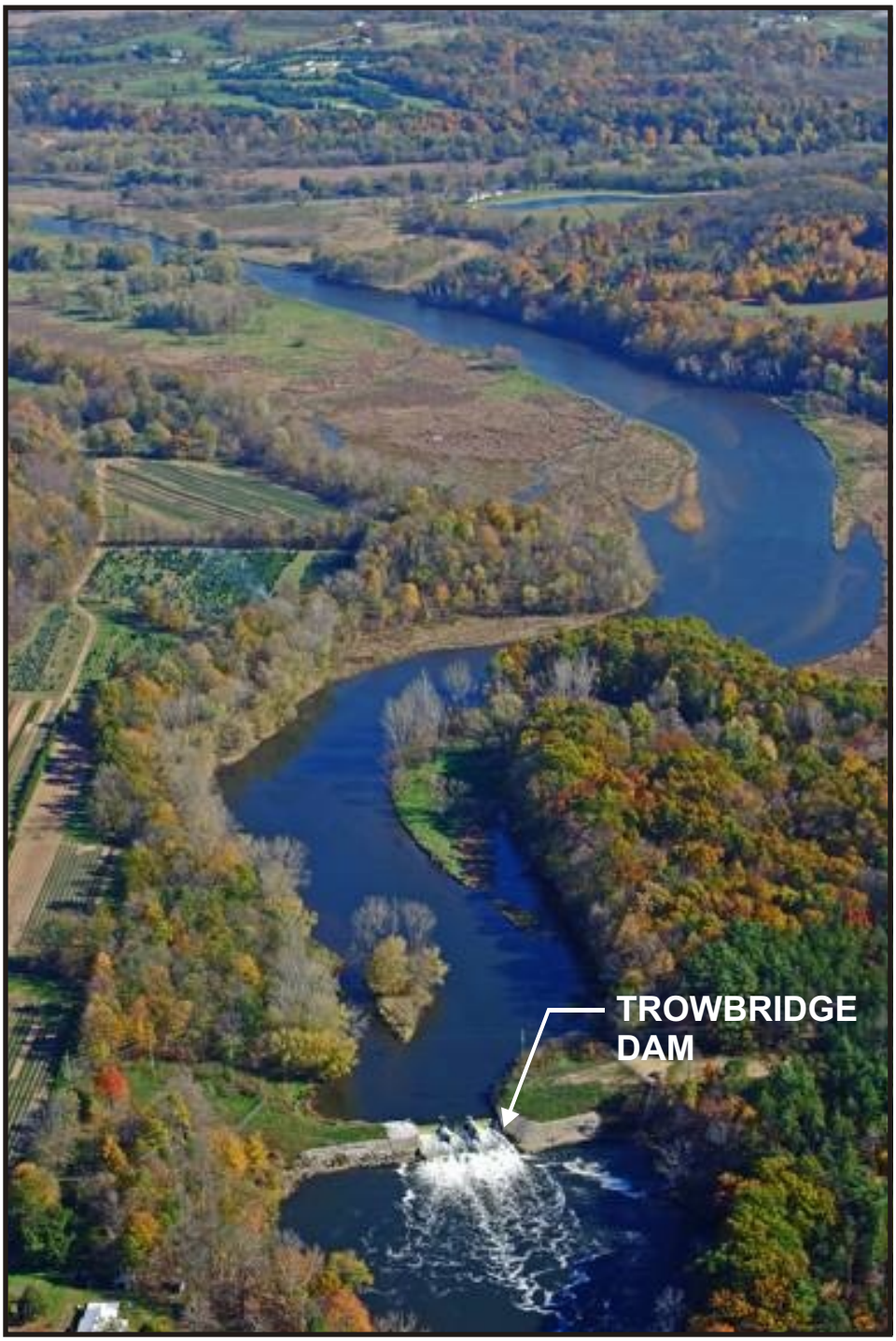
KALAMAZOO RIVER STUDY GROUP
ALLIED PAPER, INC./PORTAGE CREEK/
KALAMAZOO RIVER SUPERFUND SITE
CHARACTERISTICS OF THE FORMERLY IMPOUNDED AREAS

**AERIAL PHOTOGRAPH -
FORMER OTSEGO IMPOUNDMENT**

ARCADIS

FIGURE
22

05/06/08 SYR-D85-DJH
B0064524/0000/00771/CDR/64524G10.CDR



NOTES:
1. View looking east.
2. Aerial photograph taken October 30, 2007.

KALAMAZOO RIVER STUDY GROUP
ALLIED PAPER, INC./PORTAGE CREEK/
KALAMAZOO RIVER SUPERFUND SITE
CHARACTERISTICS OF THE FORMERLY IMPOUNDED AREAS

AERIAL PHOTOGRAPH - DOWNSTREAM END OF THE FORMER TROWBRIDGE IMPOUNDMENT

ARCADIS

FIGURE
23

05/06/08 SYR-D85-DJH
B0064524/0000/00771/CDR/64524G11.CDR

NOTES:
1. View looking northwest.
2. Aerial photograph taken October 30, 2007.

KALAMAZOO RIVER STUDY GROUP
ALLIED PAPER, INC./PORTAGE CREEK/
KALAMAZOO RIVER SUPERFUND SITE

CHARACTERISTICS OF THE FORMERLY IMPOUNDED AREAS

AERIAL PHOTOGRAPH - MIDDLE SECTION OF THE FORMER TROWBRIDGE IMPOUNDMENT

ARCADIS

FIGURE
24

05/06/08 SYR-D85-DJH
B0064524/0000/00771/CDR/64524G17.CDR

NOTES:
1. View looking southeast.
2. Aerial photograph taken October 30, 2007.

KALAMAZOO RIVER STUDY GROUP
ALLIED PAPER, INC./PORTAGE CREEK/
KALAMAZOO RIVER SUPERFUND SITE
CHARACTERISTICS OF THE FORMERLY IMPOUNDED AREAS

AERIAL PHOTOGRAPH - UPSTREAM END OF THE FORMER TROWBRIDGE IMPOUNDMENT

ARCADIS

FIGURE
25

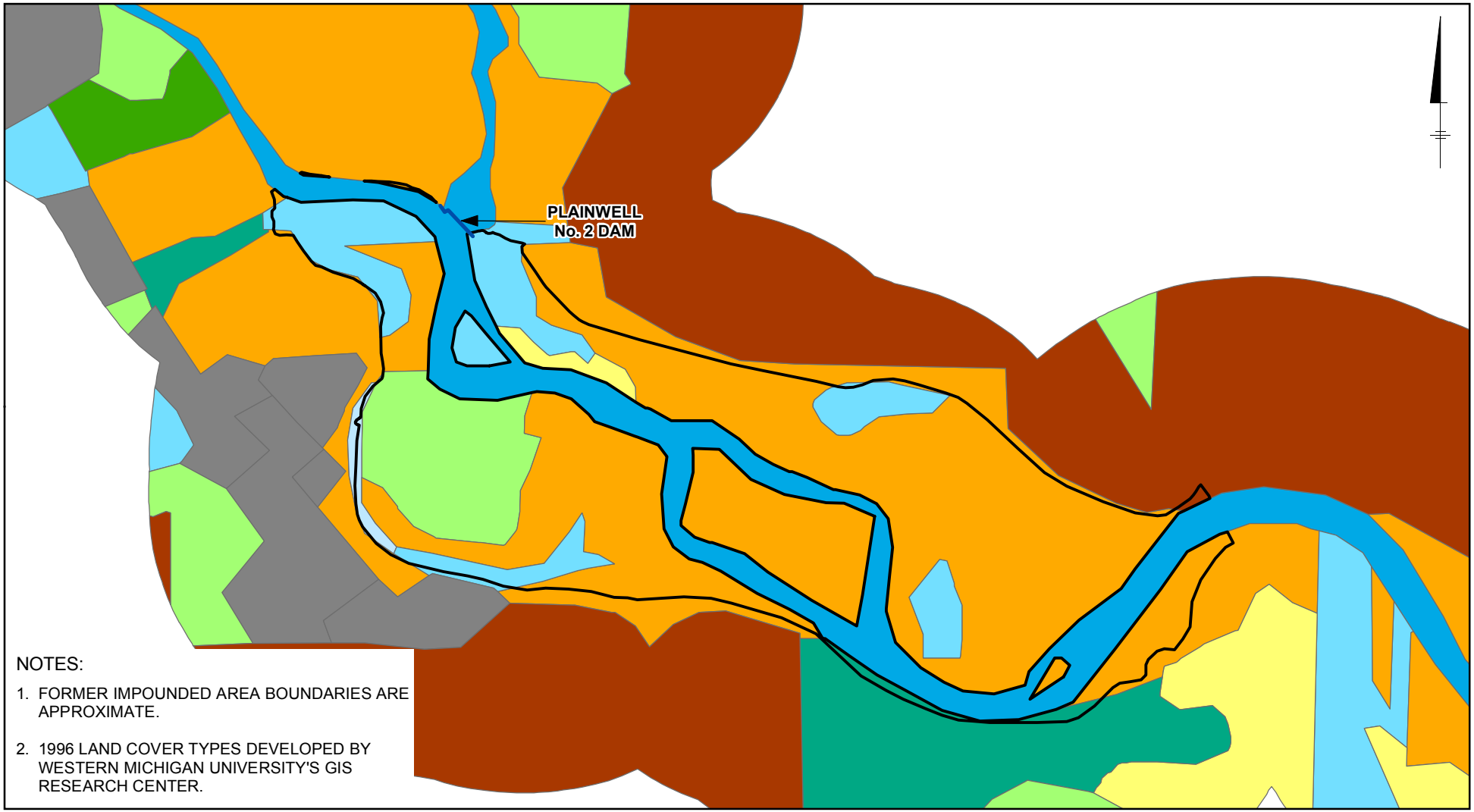
05/06/08 SYR-D85-DJH
B0064524/0000/00771/CDR/64524G18.CDR

ARCADIS

Appendix A

Terrestrial Habitat of the Former Impoundments

SYR-85 MTK EAL
KRSG (64524.0771)
Q:\KRSG\FormerImpoundments\EcoRiskSupportMemo_01-08-08\mxd\Terrestrial Habitat - Plainwell No2 Dam Impoundment.mxd - 5/5/2008 @ 5:48:29 PM



NOTES:

1. FORMER IMPOUNDED AREA BOUNDARIES ARE APPROXIMATE.
2. 1996 LAND COVER TYPES DEVELOPED BY WESTERN MICHIGAN UNIVERSITY'S GIS RESEARCH CENTER.

LEGEND:

1996 LANDCOVER CLASSIFICATION:

- AGRICULTURE/OPEN LAND
- AQUATIC BED WETLANDS
- DECIDUOUS FOREST
- EMERGENT WETLANDS
- FORESTED WETLANDS
- HERBACEOUS OPEN LAND
- SHRUB-SCRUB WETLANDS
- SHRUBLAND
- SURFACE WATER
- URBAN

DAM

APPROXIMATE HISTORICAL FLOODPLAIN LIMITS IN THE FORMER PLAINWELL No. 2 AREA

0 700 1,400 Feet

GRAPHIC SCALE

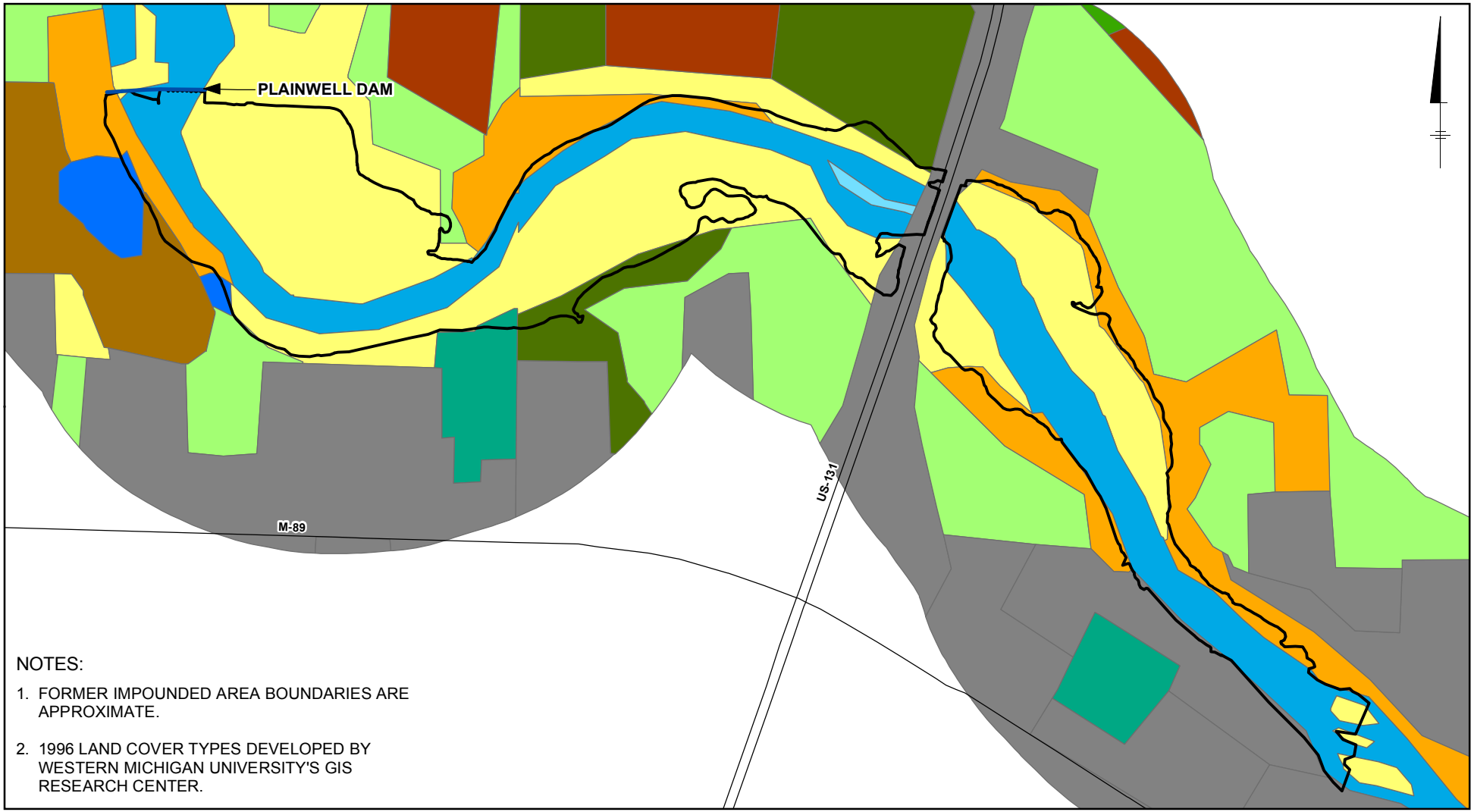
KALAMAZOO RIVER STUDY GROUP
ALLIED PAPER, INC./PORTAGE CREEK/
KALAMAZOO RIVER SUPERFUND SITE
CHARACTERISTICS OF THE FORMERLY IMPOUNDED AREAS

TERRESTRIAL HABITAT - FORMER PLAINWELL No. 2 AREA

ARCADIS

FIGURE
A-1

SYR-85 MTK EAL
KRSG (64524.0771)
Q:\KRSG\FormerImpoundments\EcoRiskSupportMemo_01-08-08\mxd\Terrestrial Habitat - Plainwell Impoundment.mxd - 5/5/2008 @ 5:48:39 PM



NOTES:

1. FORMER IMPOUNDED AREA BOUNDARIES ARE APPROXIMATE.
2. 1996 LAND COVER TYPES DEVELOPED BY WESTERN MICHIGAN UNIVERSITY'S GIS RESEARCH CENTER.

LEGEND:

1996 LANDCOVER CLASSIFICATION:

- AGRICULTURE/OPEN LAND
- DECIDUOUS FOREST
- EMERGENT WETLANDS
- FORESTED WETLANDS
- HERBACEOUS OPEN LAND
- LAKES
- SHRUB-SCRUB WETLANDS
- SHRUBLAND
- SURFACE EXCAVATION
- SURFACE WATER
- UPLAND FOREST
- URBAN
- DAM
- FORMER PLAINWELL IMPOUNDMENT

0 700 1,400 Feet
GRAPHIC SCALE

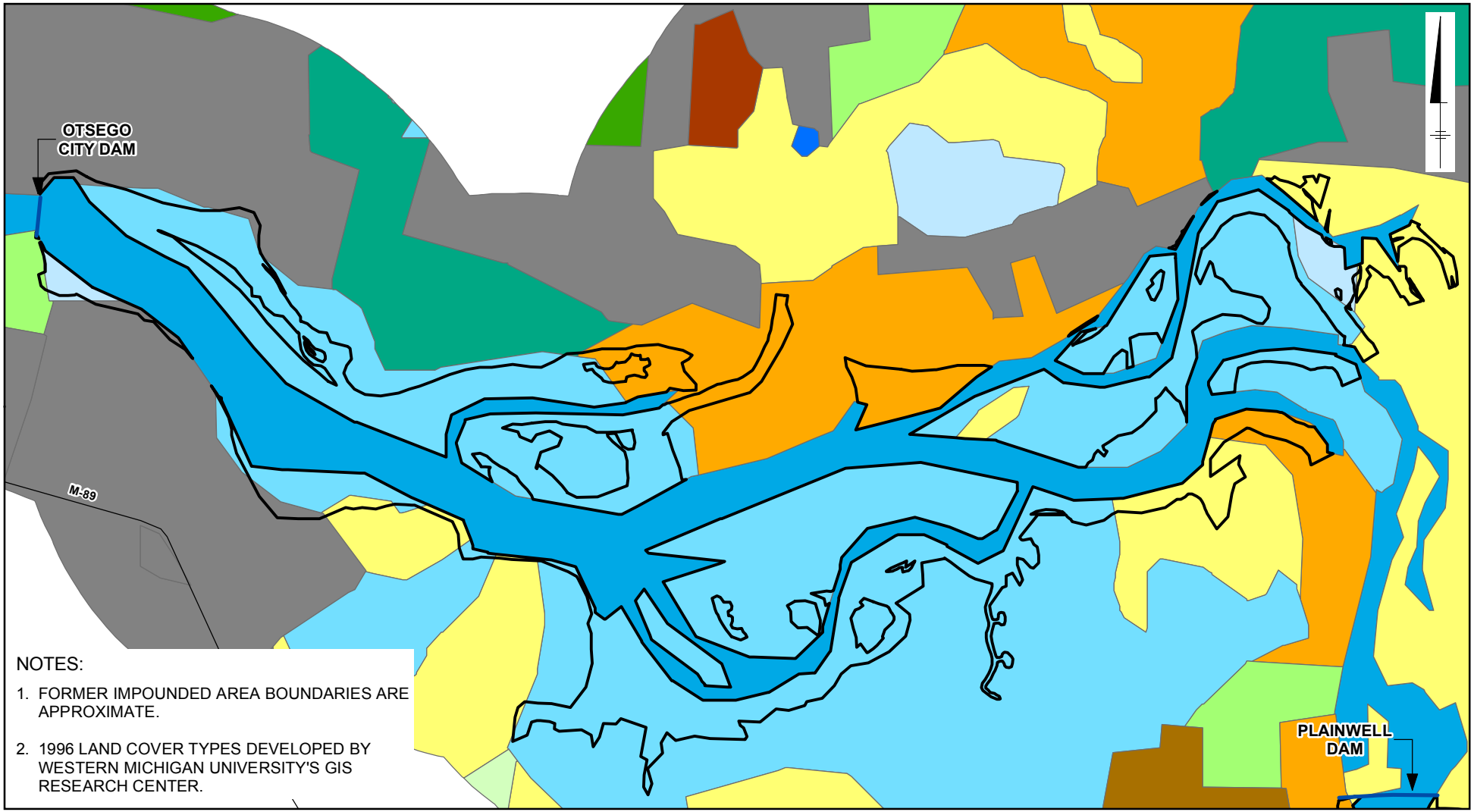
KALAMAZOO RIVER STUDY GROUP
ALLIED PAPER, INC./PORTAGE CREEK/
KALAMAZOO RIVER SUPERFUND SITE
CHARACTERISTICS OF THE FORMERLY IMPOUNDED AREAS

TERRESTRIAL HABITAT - FORMER PLAINWELL IMPOUNDMENT

ARCADIS

FIGURE
A-2

SYR-85 MTK EAL
KRSG (64524.0771)
Q:\KRSG\FormerImpoundments\EcoRiskSupportMemo_01-08-08\mxd\Terrestrial Habitat - Otsego City Impoundment.mxd - 5/5/2008 @ 5:48:46 PM



NOTES:

1. FORMER IMPOUNDED AREA BOUNDARIES ARE APPROXIMATE.
2. 1996 LAND COVER TYPES DEVELOPED BY WESTERN MICHIGAN UNIVERSITY'S GIS RESEARCH CENTER.

LEGEND:

1996 LANDCOVER CLASSIFICATION:

- AGRICULTURE/OPEN LAND
- AQUATIC BED WETLANDS
- DECIDUOUS FOREST
- EMERGENT WETLANDS
- FORESTED WETLANDS
- HERBACEOUS OPEN LAND
- LAKES
- OPEN LAND
- SHRUB-SCRUB WETLANDS
- SHRUBLAND
- SURFACE EXCAVATION
- SURFACE WATER
- URBAN
- DAM
- FORMER OTSEGO CITY IMPOUNDMENT

0 700 1,400 Feet
GRAPHIC SCALE

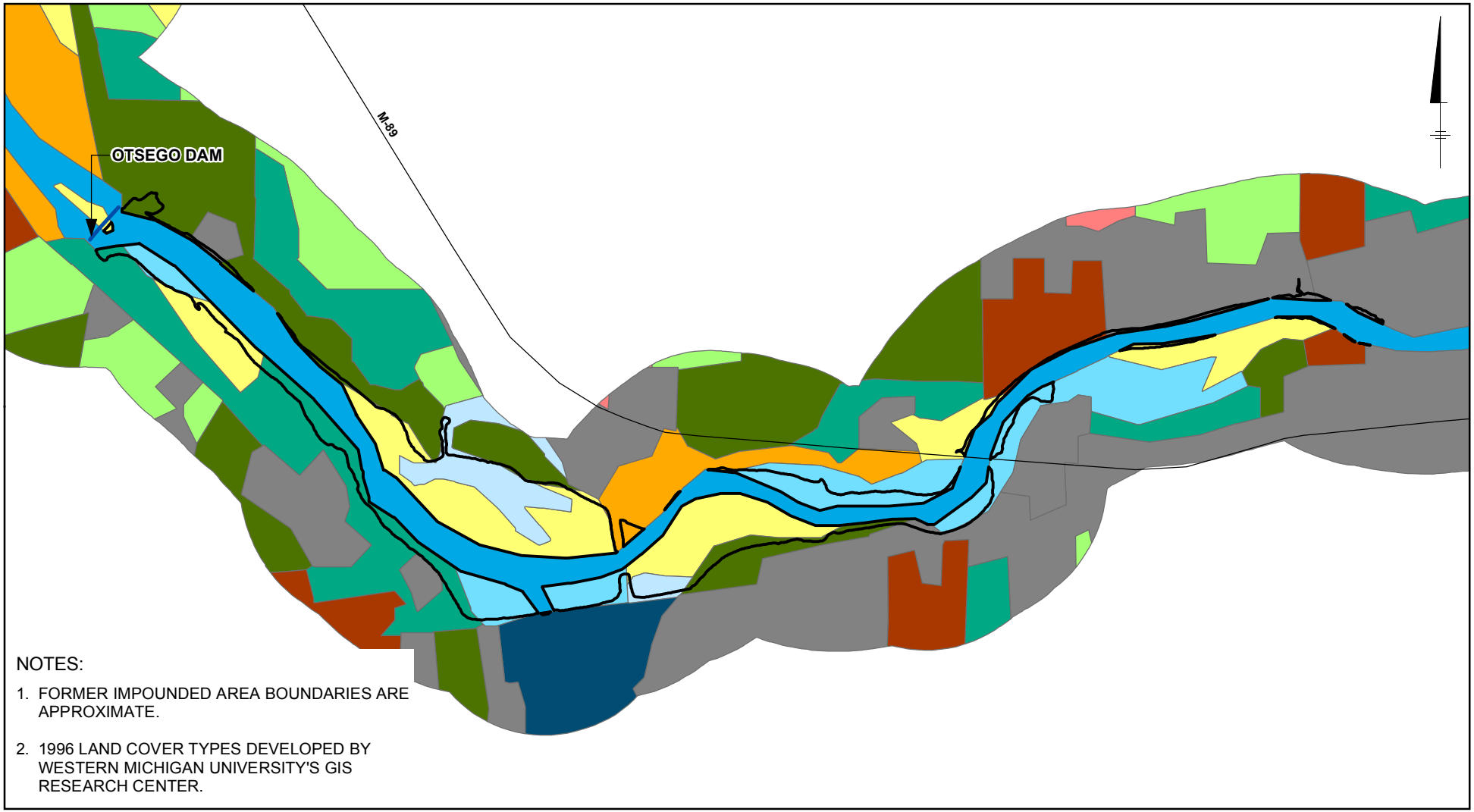
KALAMAZOO RIVER STUDY GROUP
ALLIED PAPER, INC./PORTAGE CREEK/
KALAMAZOO RIVER SUPERFUND SITE
CHARACTERISTICS OF THE FORMERLY IMPOUNDED AREAS

TERRESTRIAL HABITAT - FORMER OTSEGO CITY IMPOUNDMENT

ARCADIS

FIGURE
A-3

SYR-85 MTK EAL
KRSG (64524.0771)
Q:\KRSG\FormerImpoundments\EcoRiskSupportMemo_01-08-08\mxd\Terrestrial Habitat - Otsego Impoundment.mxd - 5/5/2008 @ 5:48:58 PM



NOTES:

1. FORMER IMPOUNDED AREA BOUNDARIES ARE APPROXIMATE.
2. 1996 LAND COVER TYPES DEVELOPED BY WESTERN MICHIGAN UNIVERSITY'S GIS RESEARCH CENTER.

LEGEND:

1996 LANDCOVER CLASSIFICATION:

- AGRICULTURE/OPEN LAND
- AQUATIC BED WETLANDS
- EMERGENT WETLANDS
- EVERGREEN FOREST
- FORESTED WETLANDS
- HERBACEOUS OPEN LAND
- RESERVOIRS
- SHRUB-SCRUB WETLANDS
- SHRUBLAND
- SURFACE WATER
- UPLAND FOREST
- URBAN
- DAM
- FORMER OTSEGO IMPOUNDMENT

0 1,200 2,400 Feet

GRAPHIC SCALE

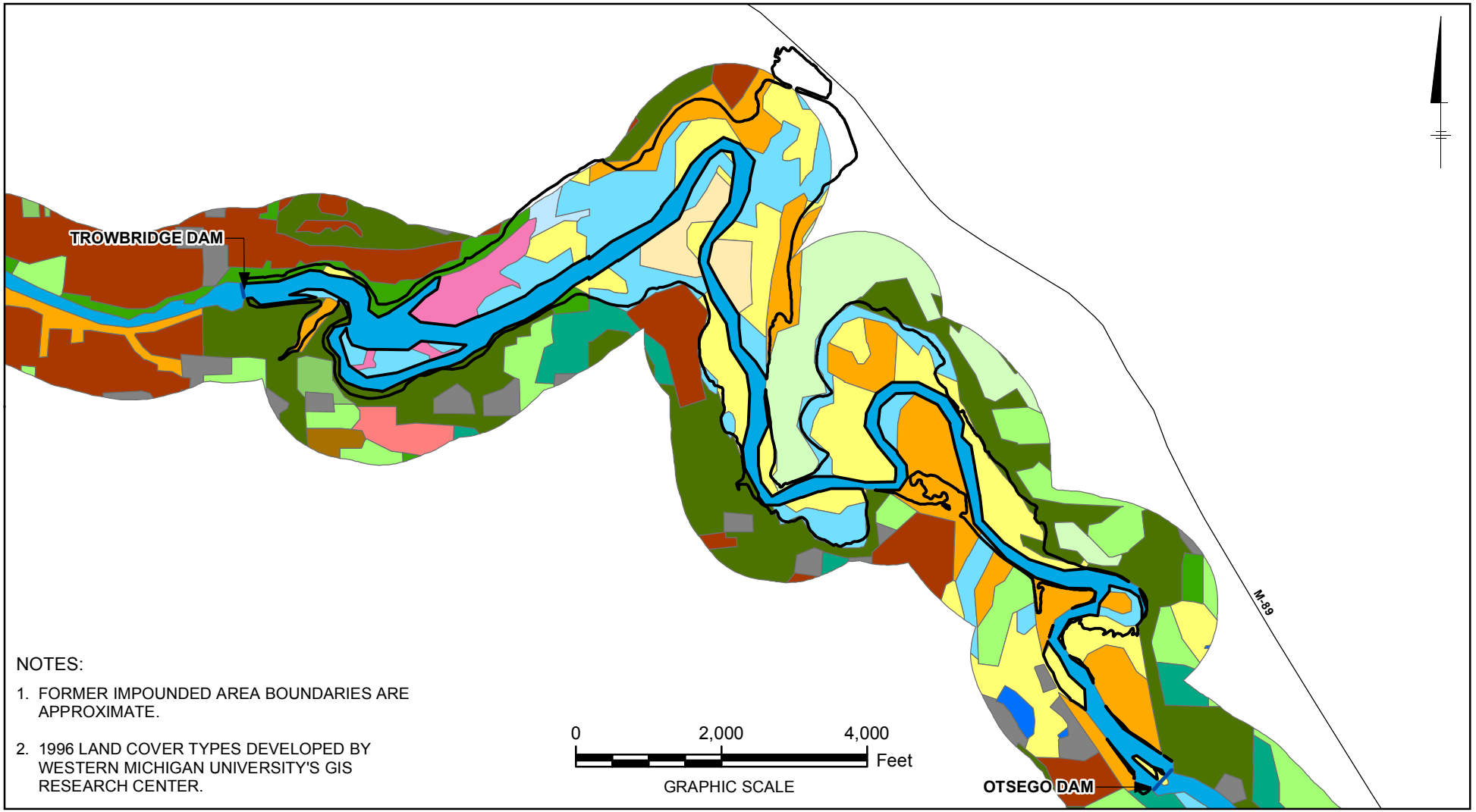
KALAMAZOO RIVER STUDY GROUP
ALLIED PAPER, INC./PORTAGE CREEK/
KALAMAZOO RIVER SUPERFUND SITE
CHARACTERISTICS OF THE FORMERLY IMPOUNDED AREAS

TERRESTRIAL HABITAT - FORMER OTSEGO IMPOUNDMENT

ARCADIS

FIGURE
A-4

SYR-85 MTK EAL
KRSG (64524.0771)
Q:\KRSG\FormerImpoundments\EcoRiskSupportMemo_01-08-08\mxd\Terrestrial Habitat - Trowbridge Impoundment.mxd - 5/5/2008 @ 5:50:51 PM



NOTES:

1. FORMER IMPOUNDED AREA BOUNDARIES ARE APPROXIMATE.
2. 1996 LAND COVER TYPES DEVELOPED BY WESTERN MICHIGAN UNIVERSITY'S GIS RESEARCH CENTER.

LEGEND:

1996 LANDCOVER CLASSIFICATION:

- AGRICULTURE/OPEN LAND
- ALGAL BEDS
- AQUATIC BED WETLANDS
- BEACH AND RIVERBANK
- CHRISTMAS TREE PLANTATIO
- DECIDUOUS FOREST
- EMERGENT WETLANDS
- EVERGREEN FOREST
- FORESTED WETLANDS
- HERBACEOUS OPEN LAND
- LAKES
- OPEN LAND
- SHRUB-SCRUB WETLANDS
- SHRUBLAND
- SURFACE EXCAVATION
- SURFACE WATER
- UPLAND FOREST
- URBAN
- DAM
- FORMER TROWBRIDGE IMPOUNDMENT

KALAMAZOO RIVER STUDY GROUP
ALLIED PAPER, INC./PORTAGE CREEK/
KALAMAZOO RIVER SUPERFUND SITE
CHARACTERISTICS OF THE FORMERLY IMPOUNDED AREAS

TERRESTRIAL HABITAT - FORMER TROWBRIDGE IMPOUNDMENT

ARCADIS

FIGURE
A-5