

# STUDY PLAN FOR MINK INJURY INVESTIGATIONS FOR THE HUDSON RIVER

## HUDSON RIVER NATURAL RESOURCE DAMAGE ASSESSMENT

### HUDSON RIVER NATURAL RESOURCE TRUSTEES

STATE OF NEW YORK

U.S. DEPARTMENT OF COMMERCE

U.S. DEPARTMENT OF THE INTERIOR

### DRAFT FOR PUBLIC REVIEW AND COMMENT PUBLIC RELEASE VERSION\*

JUNE 13, 2006

Available from:

U.S. Department of Commerce

National Oceanic and Atmospheric Administration

Hudson River NRDA, Lead Administrative Trustee

Damage Assessment Center, N/ORR31

1305 East-West Highway, Rm 10219

Silver Spring, MD 20910-3281

*\*Names of certain individuals and affiliations have been removed to maintain confidentiality*



## EXECUTIVE SUMMARY

Natural resources of the Hudson River have been contaminated through past and ongoing discharges of polychlorinated biphenyls (PCBs). The Hudson River Natural Resource Trustees - New York State, the U.S. Department of Commerce, and the U.S. Department of the Interior - are conducting a natural resource damage assessment (NRDA) to assess and restore those natural resources injured by PCBs.

Many species of mammals rely on the Hudson River, including its floodplain, for habitat, food, and as a breeding ground. Mammals that depend on the river for food and habitat include otter, muskrat, raccoon, beaver, and mink. The Hudson River NRDA Plan identified mink and otter health as an area of biological injury investigation. Mink are the subject of this draft Study Plan for an injury determination effort as part of the Hudson River NRDA

Based on the results of preliminary investigations conducted by the Trustees, including the mink and otter work conducted in the upper Hudson River drainage during the 1998-1999 and 1999-2000 trapping seasons, input from a panel of mammal experts, review of the existing mink and otter toxicology literature, and considering factors such as the life history of mink and goals of the NRDA, the Trustees have determined that it is appropriate to conduct further investigations focused on mink to be initiated in the year 2006.

Pursuant to the Hudson River NRDA Plan, the Trustees have developed this Draft Study Plan for a mink injury determination effort. This Draft Study Plan describes a laboratory study the Trustees propose to undertake to evaluate whether mink reproduction and/or development is affected as a result of exposure to PCBs from the Hudson River.

In the future the Trustees may propose additional work to supplement this effort.

In accordance with the Hudson River NRDA Plan, the Trustees are issuing this Draft Study Plan for public review and comment. Comments should be submitted by July 15, 2006 to:

### CONTACT FOR PUBLIC COMMENTS

Ms. Kathryn Jahn  
U.S. Fish and Wildlife Service  
3817 Luker Road  
Cortland, NY 13045  
607-753-9334  
kathryn\_jahn@fws.gov

# TABLE OF CONTENTS

<b>1.0 BACKGROUND.</b> . . . . .	<b>1</b>
<b>2.0 INTRODUCTION.</b> . . . . .	<b>2</b>
<b>3.0 PURPOSE AND OBJECTIVE.</b> . . . . .	<b>3</b>
<b>4.0 METHODS.</b> . . . . .	<b>3</b>
4.1 Dietary Exposure of Mink to Fish from the Hudson River: Effects on Reproduction and Survival .....	3
<b>5.0 QUALITY ASSURANCE/QUALITY CONTROL.</b> . . . . .	<b>4</b>
<b>6.0 LITERATURE CITED.</b> . . . . .	<b>5</b>
 <b>APPENDIX A: WORK PLAN FOR DIETARY EXPOSURE OF MINK TO FISH FROM THE HUDSON RIVER: EFFECTS ON REPRODUCTION AND SURVIVAL</b>	



## 1.0 BACKGROUND

Past and continuing discharges of polychlorinated biphenyls (PCBs) have contaminated the natural resources of the Hudson River. The Hudson River Natural Resource Trustees - New York State, the U.S. Department of Commerce, and the U.S. Department of the Interior - are conducting a natural resource damage assessment (NRDA) to assess and restore those natural resources injured by PCBs (Hudson River Natural Resource Trustees 2002).

Many species of mammals rely on the Hudson River, including its floodplain, for habitat, food, and as a breeding ground. Mammals that depend on the river for food and habitat include otter, muskrat, raccoon, beaver, and mink. The Hudson River NRDA Plan identified mink and otter health as an area of biological injury investigation. Mink are the subject of this draft Study Plan for an injury determination effort as part of the Hudson River NRDA.

Mink are small carnivorous mammals that are associated with aquatic habitats of all kinds, including rivers, lakes, and wetlands (USEPA 1993). They are opportunistic hunters, feeding on any animal material they can find and kill (Linscombe et al. 1982). Mink appear to select prey primarily based on its availability (Gilbert and Nancekivell 1982) and vulnerability (Eagle and Whitman 1987). The mink diet includes other small mammals such as mice, rats, rabbits and muskrats, aquatic prey including frogs, fish, and crayfish, and terrestrial prey including birds, reptiles such as snakes, insects, and other invertebrates. Mink are exposed to PCBs directly through their diet. Mink are also exposed to PCB-contaminated water and soil or sediments as they build dens and forage for food.

The Trustee agencies have assessed PCB concentrations in mink from the Hudson River. PCB concentrations in liver (normalized for the amount of fat, or lipids, in each sample) range from 0.13 ppm to 139 parts per million (ppm) in mink (NYSDEC 2001, 2002). PCB concentrations in Hudson River mink liver on a wet weight basis range from 0.0082 to 3.34 ppm (NYSDEC 2001, 2002).

Those preliminary investigations of mink exposure to PCBs were undertaken to assist the Trustees in determining the extent to which mink in the Hudson River are contaminated with PCBs, to determine if additional pathway and injury assessment studies focused on mink should be conducted as part of the Hudson River NRDA, and for potential use in the design of future studies to assess the health of Hudson River mink.

Several studies have investigated the potential effects of PCB exposure to mammals, including mink. In controlled feeding studies of mink, diets with PCB levels between 0.64 and 5 ppm completely inhibited reproduction (Platonow and Karstad 1973, Bleavins et al. 1980). Moore et al. (1999) predict, based on a dose-response curve, a greater than 99 percent reduction in fecundity (litter size) of ranch mink fed a diet containing 5 ppm PCBs. Bursian et al. (2003), studying the dietary exposure of mink to fish from the Housatonic River, found that a dietary concentration of 3.7 ppm caused a decrease in kit survival and resulted in a maternal hepatic total PCB concentration of 3.1 ppm. Jaw lesions - mandibular and maxillary squamous cell proliferation - were detected in kits fed dietary concentrations as low as 0.96 ppm.

While most of the above-cited studies have focused on adverse effects as a function of contaminant concentrations in the diet, others have evaluated effects as a function of contaminant concentrations in mink tissues. For instance, adverse effects on mink reproduction are expected when PCB concentrations in mink tissues exceed about 0.01 ppm toxic equivalents (TEQs) lipid weight (Leonard et al. 1995, Mason and Wren 2001, Tillitt et al. 1996). In the TEQ approach, the concentration of each dioxin or dioxin-like compound is multiplied by its respective Toxicity Equivalence Factor (TEF), and the products of the concentrations and their respective TEFs are summed in order to obtain a single TCDD TEQ value for the complex mixtures of dioxins or dioxin-like compounds found in the sample (Tillitt 1999, Van den Berg et al. 1998).

Based on Smit et al. (1996), 21 ppm PCBs (lipid normalized) or more is a critical level for health impairment in mink and otter; this is based on the effects of PCBs on hepatic retinol levels in European otter (Smit et al. 1996). Further, 50 ppm or more PCBs (lipid normalized) is a critical level for reproductive impairment in mink and otters; this is based on reduction in litter size in mink (Leonards et al. 1994, 1995).

In January 2002, the Trustees assembled an expert panel to review the exposure and effects information compiled by the NYSDEC for mink and otter, and to provide guidance to the Trustees on appropriate next steps for determining whether PCBs are causing adverse biological effects in Hudson River mammals, particularly mink and otter. The Hudson River NRDA Plan noted that the Trustees planned to build upon the existing mink and otter studies, potentially conducting further studies to determine PCB effects in mink and otter from the Hudson River.

## 2.0 INTRODUCTION

Based on the results of preliminary investigations conducted by the Trustees, including the mink and otter work (NYSDEC 2001, 2002), input from a panel of mammal experts, review of the existing mink and otter toxicology literature, and considering factors such as the life history of mink and goals of the NRDA, the Trustees have determined that it is appropriate to conduct further investigations focused on mink to be initiated in the year 2006.

Pursuant to the Hudson River NRDA Plan, the Trustees have developed this Draft Study Plan for a mink injury determination effort. This Draft Study Plan describes a laboratory study the Trustees propose to undertake to evaluate whether mink reproduction and/or development is affected as a result of exposure to PCBs from the Hudson River.

In accordance with the Hudson River NRDA Plan, the Trustees are issuing this Draft Study Plan for public review and comment. The Trustees are interested in receiving feedback on this Draft Study Plan. To facilitate this process, the Trustees are asking the public and the party or parties responsible for the contamination to review this Draft Study Plan and provide feedback on the proposed approach. Comments should be submitted by July 15, 2006. These comments will help the Trustees plan and conduct an assessment that is scientifically valid and cost effective and that incorporates a broad array of perspectives.

To that end, the Trustees request that you carefully consider this Draft Study Plan and provide any comments you may have to:

### CONTACT FOR PUBLIC COMMENTS

Ms. Kathryn Jahn  
U.S. Fish and Wildlife Service  
3817 Luker Road  
Cortland, NY 13045  
607-753-9334  
kathryn\_jahn@fws.gov

### 3.0 PURPOSE AND OBJECTIVE

The purpose of the present study is to evaluate if ranch mink fed diets containing PCB-contaminated fish from the Hudson River will exhibit impaired reproductive performance, impaired offspring (kit) growth and survival, and/or development of mandibular/maxillary squamous epithelial proliferation. The Trustees will use the results of the study to make determinations regarding injury to mink and guide their future efforts to identify pathways and specific injuries to mink from PCBs, as defined in regulations written by the U.S. Department of the Interior contained in Title 43 of the Code of Federal Regulations Part 11, Natural Resource Damage Assessment. This work will also be used to help determine whether future studies will be performed, and if so, to help in their design.

### 4.0 METHODS

#### **4.1 DIETARY EXPOSURE OF MINK TO FISH FROM THE HUDSON RIVER: EFFECTS ON REPRODUCTION AND SURVIVAL**

On behalf of the Trustees, beginning in 2006, Principal Investigators (PIs) will conduct a study of the effects on reproduction and survival of mink exposed to PCBs via their diet (fish from the Hudson River). This work will be conducted pursuant to a work plan entitled "Dietary Exposure of Mink to Fish from the Hudson River: Effects on Reproduction and Survival" contained in Appendix A.

The purpose of this investigation is to evaluate if ranch mink fed diets containing PCB-contaminated fish from the Hudson River will exhibit impaired reproductive performance, impaired offspring (kit) growth and survival, and/or development of mandibular/maxillary squamous epithelial proliferation (jaw lesions). Data generated by this investigation can then be compared to existing site-specific field data on concentrations of PCBs in typical prey species and hepatic concentrations of PCBs in wild mink to allow evaluation of risk posed to mink residing in the Hudson River watershed.

The following endpoints will be assessed in this investigation:

- Adult body weights;
- Adult feed consumption;
- Number of females mated;
- Length of gestation;
- Number of females whelping/not whelping;
- Total newborn/female whelped;
- Live newborn/female whelped;
- Average kit birth weight;
- Average litter weight;
- Percent kit survival to three weeks of age;
- Kit body weight at three weeks of age;
- Percent kit survival to six weeks of age;
- Kit body weight at six weeks of age;
- Adult and six-week-old kit organ weights;
- Histopathology of adult and six-week-old kit organs and jaws;
- Total PCB and planar PCB, PCDD and PCDF analyses of adult and six-week-old kit livers;
- Monthly body weights of seven-month-old juveniles;
- Organ weights of seven-month-old juveniles;
- Histopathology of seven-month-old juvenile organs and jaws; and,
- Total PCB and planar PCB, PCDD and PCDF analyses of adult and seven-month-old juvenile livers.

This study will enable the Trustees to assess the following injuries to mink: death, disease, cancer, physiological malfunctions (including malfunctions in reproduction), and physical deformations.

As this investigation entails injury endpoints, the Trustees have performed a peer review of the proposed investigation. A draft work plan, prepared by the PIs, has been peer reviewed and changes made as a result of the peer review process. We are seeking public review and comment on this work plan as part of the public review of this draft Study Plan, in accordance with the Hudson River NRDA Plan.

In the future the Trustees may propose additional work to supplement this effort.

## 5.0 QUALITY ASSURANCE/QUALITY CONTROL

This study is being conducted in accordance with the Quality Assurance Management Plan for the Hudson River NRDA (Hudson River Natural Resources Trustees, 2002).

As noted in the Trustees' Responsiveness Summary for the NRDA Plan (Hudson River Natural Resource Trustees, 2003), for each data collection effort that is part of the Hudson River NRDA and is identified in the NRDA Plan, the Trustees will develop a project-specific QA Plan which may be an independent document or may be incorporated into the project Study Plan. Such a QA Plan, in combination with the information on QA management described in the NRDA Plan (Hudson River Natural Resource Trustees, 2002), will ensure that the requirements listed in the National Contingency Plan and applicable EPA guidance for quality control and quality assurance plans are met.

The work plan for the investigation entitled "Dietary Exposure of Mink to Fish from the Hudson River: Effects on Reproduction and Survival" includes a project-specific QA Plan (Section 6).

Chemical analyses will be conducted in accordance with the requirements of the Hudson River NRDA Analytical QA Plan (Hudson River Natural Resource Trustees 2005).

## 6.0 LITERATURE CITED

- Bleavins, M. R., R. J. Aulerich, and R. K. Ringer. 1980. Polychlorinated biphenyls (Aroclors 1016 and 1242): effects on survival and reproduction in mink and ferrets. *Arch. Environ. Contam. Toxicol.* 9: 627-635.
- Bursian, S. J., R. J. Aulerich, B. Yamini, and D. E. Tillitt. 2003. Dietary Exposure of Mink to Fish from the Housatonic River: Effects on Reproduction and Survival. Submitted to Weston Solutions, Inc.
- Eagle, T. C. and J. S. Whitman. 1987. Mink. Pages 615-624 in M. Novak, J. A. Baker, M. E. Obbard, and B. Malloch, eds. *Wildfurbearer Management and Conservation in North America*. Toronto, Ontario: Ontario Ministry of Natural Resources.
- Gilbert, F. F. and E. G. Nancekivell. 1982. Food habits of mink (*Mustela vison*) and otter (*Lutra canadensis*) in northeastern Alberta. *Canadian Journal of Zoology* 60: 1282-1288.
- Hudson River Natural Resource Trustees. 2002. Hudson River Natural Resource Damage Assessment Plan. September 2002. U.S. Department of Commerce, Silver Spring, MD.
- Hudson River Natural Resource Trustees. 2003. Responsiveness Summary for the Hudson River Natural Resource Damage Assessment Plan. July 2003. U.S. Department of Commerce, Silver Spring, MD.
- Hudson River Natural Resource Trustees. 2005. Analytical Quality Assurance Plan: Hudson River Natural Resource Damage Assessment. Public Release Version. September 1, 2005. Version 2.0. U.S. Department of Commerce, Silver Spring, MD.
- NYSDEC. 2001. Fish, Wildlife, and Marine Division. <http://www.dec.state.ny.us/website/prss/presrel/2001-52.html>.
- NYSDEC Biota Database: NYSDEC. 2002. Hudson River PCB Biota Database. NYSDEC, Bureau of Habitat, Albany, New York.
- Leonards, P.E.G., M.D. Smit, A.W.J.J. de Jongh, and B. van Hattum. 1994. Evaluation of dose-response relationships for the effects of PCBs on the reproduction of mink (*Mustela vison*). Institute for Environmental Studies. Vrije Universiteit, Amsterdam. 47 pp.
- Leonards P.E.G., T.H. De Vries, W. Minnaard, S. Stuijzand, P. de Voogt, W.P. Confino, N.M. Van Straalen, and B. van Hattum. 1995. Assessment of experimental data on PCB-induced reproduction inhibition in mink, based on an isomer- and congener-specific approach using 2,3,7,8-tetrachlorodibenzo-p-dioxin toxic equivalency. *Environ. Toxicol. & Chem.* 14(3):639-652.
- Linscombe, G., N. Kinler, and R. J. Aulerich. 1982. Mink. *Mustela vison*. Pages 629-643 in J. A. Chapman and G. A. Feldhamer, eds. *Wild Mammals of North America - Biology, Management, Economics*. Philadelphia, PA: John Hopkins University Press.
- Mason C.F. and C.D. Wren. 2001. Carnivora. In: Shore RF, Rattner BA, eds. *Ecotoxicology of Wild Mammals*. West Sussex, England: John Wiley & Sons Ltd. p 315-370.
- Moore, DR.J., B.E. Sample, G.W. Suter, B.R. Parkhurst, and R.S. Teed. 1999. A probabilistic risk assessment of the effects of methylmercury and PCBs on mink and kingfishers along East Fork Poplar Creek, Oak Ridge Tennessee, USA. *Environ. Toxicol. & Chem.* 18(12): 2941-2953.

- Platonow, N.S. and L.H. Karstad. 1973. Dietary effects of polychlorinated biphenyls on mink. *Can. J. Comp. Med.* 37:391-400.
- Smit, M.D., P.E.G. Leonards, A.J. Murk, A.W.J.J. de Jongh, and B. van Hattum. 1996. Development of otter-based quality objectives for PCBs. Institute for Environmental Studies. Vrije Universiteit, Amsterdam. 129 pp.
- Tillitt D.E., R.W. Gale, C.J. Meadows, J.L. Zajicek, P.H. Peterman, S.N. Heaton, P.D. Jones, S.J. Bursian, T.J. Kubiak, J.P. Giesy and R.L. Aulerich. 1996. Dietary exposure of mink to carp from Saginaw Bay. 3. Characterization of dietary exposure to planar halogenated hydrocarbons, dioxin equivalents, and biomagnification. *Environ. Sci. & Technol.* 30(1):283-291.
- Tillitt, D. E. 1999. The toxic equivalents approach for fish and wildlife. *Human and Ecological Risk Assessment* 5: 25-32.
- United States Environmental Protection Agency. 1993. *Wildlife Exposure Factors Handbook*. Volume I of II. United States Environmental Protection Agency. EPA/600/R-93/187a. Washington, District of Columbia, USEPA Office of Research and Development.
- Van den Berg, M., L. Birnbaum, A. T. C. Bosveld, B. Brunström, P. M. Cook, M. Feeley, J. P. Giesy, A. Hanberg, R. Hasegawa, S. W. Kennedy, T. J. Kubiak, J. C. Larsen, F. X. R. van Leeuwen, A. K. D. Liem, C. Nolt, R. E. Peterson, L. Poellinger, S. Safe, D. Schrenk, D. E. Tillitt, M. Tysklind, M. Younes, F. Waern, and T. Zacharewski. 1998. Toxic Equivalency Factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environ. Health Perspect.* 106: 775-792.

# APPENDIX A

## WORK PLAN FOR DIETARY EXPOSURE OF MINK TO FISH FROM THE HUDSON RIVER: EFFECTS ON REPRODUCTION AND SURVIVAL



**WORK PLAN**

**DIETARY EXPOSURE OF MINK TO FISH FROM THE HUDSON  
RIVER: EFFECTS ON REPRODUCTION AND SURVIVAL**

**DRAFT FOR PUBLIC RELEASE**

**June 2006**

---

**Principal Investigator**

---

**Principal Investigator**

---

**Quality Assurance Coordinator**

**Draft for Public Release**

*[This page intentionally left blank]*

**INVESTIGATION TEAM ACKNOWLEDGEMENT OF WORK PLAN REVIEW AND COMPLIANCE**

By my signature, I acknowledge that I have read this Work Plan and understand it, and will comply with it in performing this work.

Name (printed): \_\_\_\_\_ Name (printed): \_\_\_\_\_

Signature: \_\_\_\_\_ Signature: \_\_\_\_\_

Date: \_\_\_\_\_ Date: \_\_\_\_\_

Title: \_\_\_\_\_ Title: \_\_\_\_\_

Name (printed): \_\_\_\_\_ Name (printed): \_\_\_\_\_

Signature: \_\_\_\_\_ Signature: \_\_\_\_\_

Date: \_\_\_\_\_ Date: \_\_\_\_\_

Title: \_\_\_\_\_ Title: \_\_\_\_\_

Name (printed): \_\_\_\_\_ Name (printed): \_\_\_\_\_

Signature: \_\_\_\_\_ Signature: \_\_\_\_\_

Date: \_\_\_\_\_ Date: \_\_\_\_\_

Title: \_\_\_\_\_ Title: \_\_\_\_\_

Name (printed): \_\_\_\_\_ Name (printed): \_\_\_\_\_

Signature: \_\_\_\_\_ Signature: \_\_\_\_\_

Date: \_\_\_\_\_ Date: \_\_\_\_\_

Title: \_\_\_\_\_ Title: \_\_\_\_\_

Name (printed): \_\_\_\_\_ Name (printed): \_\_\_\_\_

Signature: \_\_\_\_\_ Signature: \_\_\_\_\_

Date: \_\_\_\_\_ Date: \_\_\_\_\_

Title: \_\_\_\_\_ Title: \_\_\_\_\_

*[This page intentionally left blank]*

## TABLE OF CONTENTS

<b>1.</b>	<b>INTRODUCTION.....</b>	<b>1</b>
<b>2.</b>	<b>STUDY DESIGN AND METHODS.....</b>	<b>2</b>
2.1	COLLECTION OF FISH AND FEED PREPARATION .....	2
2.2	DIETARY TREATMENTS.....	3
2.3	PREPARATION OF DIETS.....	4
2.4	ANIMALS .....	5
2.5	MINK FACILITIES.....	5
2.6	ACCLIMATION PERIOD .....	5
2.7	DEFINITIVE TRIAL.....	5
<b>3.</b>	<b>CHEMICAL ANALYSIS.....</b>	<b>7</b>
<b>4.</b>	<b>SUMMARY OF ENDPOINTS .....</b>	<b>9</b>
<b>5.</b>	<b>STATISTICAL ANALYSIS .....</b>	<b>10</b>
5.1	STATISTICAL METHODS.....	10
5.2	SAMPLE SIZE CONSIDERATIONS.....	12
<b>6.</b>	<b>QUALITY ASSURANCE/QUALITY CONTROL .....</b>	<b>14</b>
6.1	DATA QUALITY OBJECTIVES .....	14
6.2	DATA QUALITY INDICATORS .....	15
6.3	SAMPLE HANDLING, TRANSPORTATION AND ANALYTICAL PROCEDURES.....	16
6.4	DATA REDUCTION VALIDATION AND REPORTING .....	17
6.5	SAMPLING METHODOLOGY .....	17
6.6	EQUIPMENT .....	18
6.7	STATISTICAL ANALYSIS OF DATA AND SAMPLING DESIGN .....	18
6.8	CORRECTIVE ACTION .....	18
6.9	TRAINING .....	18
<b>7.</b>	<b>LITERATURE CITED .....</b>	<b>18</b>

**Appendix 1:** Fish Collection Standard Operating Procedure

**Appendix 2:** Ringer et al. 1991

**Appendix 3:** Mink Facility Standard Operating Procedures

**Appendix 4:** Chain of Custody Form

*[This page intentionally left blank]*

## 1. INTRODUCTION

The Hudson River is contaminated with polychlorinated biphenyls (PCBs) from Fort Edward, NY to New York City. General Electric's capacitor manufacturing facilities at Fort Edward and Hudson Falls, NY are considered to be the major source of PCBs in the Upper Hudson River, with discharges beginning in 1947. Between 1966 and 1974, General Electric's Fort Edward and Hudson Falls facilities purchased 35,000 metric tons of PCBs or 15% of domestic sales in the United States. This suggests that General Electric's discharges to the Hudson River Basin could represent approximately 15% of the nationwide total discharges to the environment (Horn et al., 1979).

Foley et al. (1988) reported that mink (*Mustela vison*) collected in the vicinity of the Hudson River contained relatively high concentrations of PCBs in their fat and livers. Comparison of PCB concentrations in the livers of ranch mink fed PCB-contaminated diets and those in wild Hudson River mink suggested that the wild mink could be experiencing similar reproductive impairment with a consequent decrease in abundance (Foley et al., 1988). In a more recent field study, Mayack and Loukmas (2001) reported that there appeared to be no measurable decrease in PCB contamination of mink collected in the vicinity of the Hudson River and that current hepatic PCB concentrations are above the criteria of Leonards et al. (1995) for impairment of mink health and reproduction.

In addition to reproductive impairment, there is concern that mink could develop a squamous epithelial lesion of the mandible and maxilla. Previous studies have indicated that ranch mink exposed to 3,3',4,4',5-pentachlorobiphenyl (PCB 126) or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (Render et al., 2000a, 2000b, 2001), ranch mink fed diets containing PCB-contaminated fish (Bursian et al., 2006a, 2006b), and wild mink trapped in a PCB-contaminated Superfund site (Beckett et al., 2005) developed a lesion characterized by proliferation of squamous epithelial cells into the periodontal ligament that can cause loose and displaced teeth. The maxilla and mandible become markedly porous because of loss of alveolar bone, with concomitant loss of teeth that leads, in severe cases, to aphagia.

The purpose of the present study is to evaluate if ranch mink fed diets containing PCB-contaminated fish from the Hudson River will exhibit impaired reproductive performance, impaired offspring (kit) growth and survival, and/or development of mandibular/maxillary squamous epithelial proliferation. Data generated by this study can then be compared to existing site-specific field data on concentrations of PCBs in typical prey species and hepatic concentrations of PCBs in wild mink to allow evaluation of risk posed to mink residing in the Hudson River watershed.

The following work plan is based on a similar document prepared for a mink feeding study utilizing contaminated fish collected from the Housatonic River, Berkshire County, Massachusetts (Aulerich et al., 2000). The mink is the species of choice for testing this hypothesis because: (1) they are a semi-aquatic piscivorous species native to the area; (2) they are among the most sensitive species to PCBs (Aulerich and Ringer, 1977) and related polychlorinated dibenzo-*p*-dioxins (PCDDs) (Hochstein et al., 1988, 1998); (3) their nutritional requirements are well documented (National Research

Council, 1982); (4) stock of known genetic origin is readily available; (5) all stages of their life cycle can be successfully perpetuated in the laboratory; and (6) mink have a large biological data base (Shump et al., 1976; Scientifur, 1987, 1992; Sundqvist, 1989; Aulerich et al., 1999).

## 2. STUDY DESIGN AND METHODS

Table 1 presents an estimated schedule for the mink feeding study. The following paragraphs describe each step in more detail.

<b>Table 1</b>		
<b>Study Schedule</b>		
<b>Task</b>	<b>Estimated Start Date</b>	<b>Estimated End Date</b>
Collect fish for use in mink feed	6/15/06	6/30/06
Ship to mink study facility and homogenize	6/30/06	7/15/06
PCB analyses of Hudson River fish homogenate	7/15/06	10/15/06
Mix diets	10/15/06	10/31/06
Animal acclimatization	12/15/06	12/31/06
Feeding study implementation	1/1/07	1/1/08
Test diet feeding (adults)	1/1/07	7/1/07
Breeding	3/1/07	3/21/07
Gestation and parturition	3/21/07	5/15/07
Weaning/analysis of six-week kits, adults	7/1/07	12/31/07
Analysis of seven-month kits	11/15/07	6/30/08
PCB analysis of tissues	1/1/07	6/30/08
Data analysis and report generation	6/30/08	12/31/08

### 2.1 COLLECTION OF FISH AND FEED PREPARATION

Fish will be collected from the Hudson River from Fort Edwards to Lock C-1 at Waterford. Collection will begin at the most upstream site (likely to be the most contaminated location) and will move downstream until the required quantity of fish has been obtained. Collection and transport of fish will be handled by New York Department of Environmental Conservation and/or U.S. Fish and Wildlife personnel and in general will follow fish handling and shipping procedures presented in Appendix 1.

When fish arrive at the mink study facility, they will be identified, sorted, and weighed by collection site. All fish will be ground by alternately adding fish to the grinder based on collection site such that the ground product is representative of the total rather than a specific collection site. After grinding, the fish will be placed in a 454 kg capacity mixer and blended into a homogeneous mixture to ensure equal distribution of contaminants. As the ground, blended fish is being emptied from the mixer into storage containers for subsequent diet preparation, six “grab samples” (300 to 500 g each) will be collected over time, placed in chemically clean glass containers, labeled and frozen for subsequent analysis for total PCBs (tPCBs) according to procedures outlined in the Hudson River Natural Resource Damage Assessment Analytical Quality Assurance Plan

(AQAP; Hudson River Natural Resource Trustees, 2005). “Clean” ocean fish will be purchased from a supplier that routinely services the fur industry and will be shipped frozen to the mink study facility. This fish will be processed, sampled, and analyzed in the same manner as the Hudson River fish. Results of the analysis for tPCBs (analyzed by low-resolution mass spectrometry, LRMS) in the fish will determine the proportions of Hudson River fish and ocean fish to be incorporated into the experimental mink diets to achieve the desired dietary concentrations of tPCBs.

## 2.2 DIETARY TREATMENTS

The diets will be conventional mink diets formulated to meet the nutritional requirements of mink (National Research Council, 1982) as described in Ringer et al. (1991; Appendix 2). There will be six dietary treatments, each containing the same percentage of fish (for example, 40%). The control diet will contain 40% “clean” ocean fish. The remaining five diets will contain a mixture of ocean fish and the homogenized fish from the test site(s). Based on past fish sampling efforts, Hudson River carp are anticipated to contain average PCB concentrations in approximately the 10 to 15 mg/kg (ppm) range. The targeted PCB concentrations for use in the mink dietary treatments will depend on the PCB concentrations actually present in the Hudson River fish. For instance, assuming a concentration of 15 ppm in these fish, the highest dose would be 6.0 mg/kg feed (40% \* 15 ppm). Sequentially lower doses are designed to be 0.75x, 0.5x, 0.25x and 0.125x, which would result in targeted doses of 4.5, 3.0, 1.5 and 0.75 mg/kg feed. A concentration of 10 ppm in Hudson River fish would, correspondingly, result in targeted PCB concentrations of 4.0, 3.0, 2.0, 1.0 and 0.5 mg/kg feed. Reproductive impairment has been reported in mink fed diets containing PCB concentrations lower than 5.0 ppm (Heaton et al., 1995a; Restum et al., 1998). However, it should be noted that the congener makeup and non-PCB chemical composition of fish used in those studies differs from fish collected from the Hudson River. Table 2 presents the estimated quantities of Hudson River and ocean fish required for each dietary treatment.

<b>Dietary PCB Concentration, assuming 15 ppm in Hudson fish (ppm)</b>	<b>Dietary PCB Concentration, assuming 10 ppm in Hudson fish (ppm)</b>	<b>Hudson River Fish (kg)</b>	<b>Hudson River Fish (% of Diet)</b>	<b>Ocean Fish (kg)</b>	<b>Ocean Fish (% of Diet)</b>
0	0	0	0%	576	40%
0.75	0.5	52	5%	364	35%
1.5	1.0	104	10%	312	30%
3.0	2.0	208	20%	208	20%
4.5	3.0	432	30%	144	10%
6.0	4.0	576	40%	0	0%
<b>Total</b>		<b>1,372</b>		<b>1,604</b>	
Notes:					
a. These figures assume a mink diet containing 40% fish.					

### 2.3 PREPARATION OF DIETS

It is anticipated that dietary treatments will be prepared two or three times during the trial. Procedures for sampling and analysis will be identical for each batch of feed mixed, with the exception of the number of samples collected.

For the initial batch of feed, after thorough mixing of the dietary ingredients for 30 minutes, six random “grab” samples from each of the six dietary treatments will be collected over time as the feed is being placed in the storage containers. These grab samples will be frozen for subsequent chemical contaminant analysis (organochlorine pesticides [OCs], tPCBs, non-*ortho* PCB congeners, mono-*ortho* PCB congeners, polychlorinated dibenzo-*p*-dioxin [PCDD] isomers, polychlorinated dibenzofuran [PCDF] isomers, polybrominated diphenyl ether [PBDE] isomers and potentially toxic and bioaccumulative metals). Congener-specific analyses will allow calculation of TCDD toxic equivalents (TEQs) in feed samples using mammalian toxic equivalency factors (TEFs) presented in Van den Berg et al. (1998). An additional sample from each dietary treatment will be collected for nutrient (proximate) analysis (moisture, dry matter, fat, crude protein, crude fiber, ash, total digestible nutrients, Ca, K, Mn, Mg, Fe, Na, Cu, Zn and P).

During preparation of subsequent batches of feed, three grab samples from each of the dietary treatments will be collected for PCB analysis by low resolution mass spectrometry (Hudson River Natural Resource Trustees, 2005), and one grab sample will be collected for nutrient analysis. Chemical analyses of grab samples will be completed prior to providing feed from the associated batch to the mink.

In addition to the sampling described above, three grab samples from each treatment will be archived in the event that it is determined later that additional chemical analyses are desired.

Feed will be placed in appropriately labeled, sealed plastic containers and stored frozen in a walk-in freezer at -7°C as described by Ringer et al. (1991). A sufficient quantity of feed for one day will be removed from the freezer in the morning and thawed slowly over the next 24 hours at room temperature, or if conditions require, under a minimal heat source suspended above the material to be thawed. Thawed feed that remains after animals have been fed for the day will be placed in the walk-in cooler for feeding the next day. Thawed feed is kept no longer than 48 hours.

Because the fish species used in the diets are known to contain thiaminase, supplemental thiamine will be provided to the animals on a daily basis to prevent Chastek’s paralysis (National Research Council, 1982). Twenty-five mg thiamine hydrochloride (USB, Cleveland, OH) will be dissolved in 50 ml water and then mixed into 950 g of ranch feed. Each mink will be fed approximately 10 g of the thiamine-containing feed, which provides 0.25 mg thiamine hydrochloride/day, at least two hours before feeding of the treatment diets.

## **2.4 ANIMALS**

There will be 15 uniquely identified, first-year (virgin), natural dark, female mink (*Mustela vison*) and five uniquely identified, first-year, natural dark, male mink from the mink study facility herd randomly assigned to the 1x, 0.75x and control groups and 10 females and five males assigned to each of the 0.5x, 0.25x and 0.125x groups. Litter mates will not be placed in the same treatment group to minimize genetic predisposition to PCB toxicity. If randomization results in any one treatment group being significantly larger (on a mass basis), then additional randomization within groups prior to treatment will be conducted until group masses are comparable. This procedure will ensure that any effects potentially observed are not attributable to treatment group mass differences. All mink will have been immunized against canine distemper, viral enteritis, hemorrhagic pneumonia, and botulism.

## **2.5 MINK FACILITIES**

Mink will be caged individually in an open-sided shed in a manner described by Ringer et al. (1991) that exceeds guidelines specified in the Standard Guidelines for the Operation of Mink Farms in the United States (Fur Commission USA, 1995). As such, mink will be exposed to ambient conditions, which, based on experience, yield superior reproductive performance compared to raising mink in a more controlled indoor environment.

## **2.6 ACCLIMATION PERIOD**

The mink will be acclimated for at least seven days prior to the initiation of the definitive trial as described in Ringer et al. (1991). They will be weighed at the beginning of the acclimation period and an attempt will be made to determine feed consumption as described by Ringer et al. (1991), if weather permits.

## **2.7 DEFINITIVE TRIAL**

Three unexposed females and males from the breeding stock will be euthanized and their livers analyzed for OCs, PCBs (HRMS), PCDDs, PCDFs, PBDEs and potentially toxic and bioaccumulative metals. After the acclimation period, the definitive test will begin on or around 1 January 2007, which is eight weeks prior to the initiation of breeding. Test diets will be fed daily to both females and males for approximately 150 days through the pre-breeding, breeding (March 1 to March 21), gestation, parturition (April 21 to May 15), lactation, and weaning (June 15 to July 1) periods, at which time all the adult females, adult males and 15 kits (approximately evenly split between males and females) randomly selected from each treatment will be euthanized by asphyxiation (CO<sub>2</sub>) and necropsied for analysis. Fifteen kits from each treatment group will be maintained on their respective diets through November to assess possible effects of PCBs on developmental parameters. To the degree possible, the sets of 15 kits will include one kit randomly selected from each female within the treatment group. For treatment groups

of 10 females, one kit will be randomly selected from each female, with the remaining kits being randomly selected from the treatment group as a whole.

Although Aleutian disease has not been observed in the mink study facility breeding stock over the last several years, during the necropsy stage of the study, all individuals will be examined for histopathological abnormalities typically associated with this disease. Should any individual mink be diagnosed with Aleutian disease, it and all of its associated data will be removed from the study analysis.

Husbandry and experimental procedures during the pre-breeding through lactation periods are as described in Ringer et al. (1991). These will include daily observation of mink and determination of body weights every two weeks and feed consumption weekly. Feed consumption will be assessed on a weekly basis by measuring food consumption for two days during this period. Breeding of treated females and males within the same group will begin on or around 1 March 2007 and will follow procedures outlined in Ringer et al. (1991). A ratio of approximately one male for every three females will be used. Attempts will be made to ensure that females will have two or more matings during the breeding period. Determination of body weights and feed consumption will be discontinued at the initiation of breeding. All other procedures related to breeding, gestation, parturition, and lactation are as described in Ringer et al. (1991). Kits will be weighed within 24 hours post-partum and at three and six weeks of age. Their dams will be weighed at the same times.

When the last litter whelped is weaned at six weeks of age, the adult females, males and associated kits from each treatment group will be euthanized with CO<sub>2</sub> and necropsied. Organs (brain, liver, kidneys, spleen, heart, thyroid gland and adrenal glands) will be removed and weighed. Samples of organs will be placed in a 10% formalin-saline solution for subsequent histological examination. Additional liver samples will be frozen for subsequent contaminant analysis (tPCBs, non-*ortho* PCB congeners, mono-*ortho* PCB congeners, PCDD isomers, and PCDF isomers). Congener specific analyses will allow calculation of TEQs in liver samples using mammalian TEFs presented in Van den Berg et al. (1998). The remaining portion of each liver will be archived in the event that additional analyses (such as retinoid analyses) are desired at a later date. Heads will also be collected and placed in 10% formalin-saline for subsequent examination of mandibular and maxillary squamous epithelial proliferation. All collected materials will be appropriately labeled (type of tissue, identification of the individual animal that the tissue came from, date of collection, and project identification).

Fifteen kits from each treatment group will be maintained on their respective diets through November 2007. These kits will be immunized against canine distemper, viral enteritis, hemorrhagic pneumonia, and botulism at 10 weeks of age. Body weights will be determined every four weeks. At the end of the growth period in November, 15 juveniles from each of the treatment groups will be euthanized by CO<sub>2</sub> and necropsied with tissues being handled as described above. In addition to the organs collected from the six-week-old kits, the reproductive tracts of all male and female juveniles will be removed and processed for subsequent histological examination. Any mink (except

unweaned kits) that die during the trial period will be evaluated by a board certified veterinary pathologist.

Scat samples will be collected from each adult female and each seven-month-old juvenile just prior to necropsy. These samples will be archived in the event that contaminant analysis of these samples is deemed desirable.

### **3. CHEMICAL ANALYSIS**

Chemical analyses will be conducted in accordance with the Hudson River AQAP (Hudson River Natural Resource Trustees, 2005). Table 3 indicates the types and numbers of samples to be taken for each analysis.

Table 3 Anticipated Sample Analyses											
	Sample	No. Samples	OCs	PCBs LRMS HRMS		PCDDs/ PCDFs	PBDEs	Metals	Lipids	Necropsy/ Histopathology	Nutrient Analysis (feed)
<b>Feed Preparation</b>											
	HR fish	6	0	6	0	0	0	0	6	N/A	0
	Ocean fish	6	0	6	0	0	0	0	6	N/A	0
	Dietary mix - first batch (6 treatments * 3 samples)	18	18	0	18	18	18	18	18	N/A	18
	Dietary mix - second batch (6 treatments * 2 samples)	12	0	0	12	0	0	0	12	N/A	0
	Dietary mix - third batch (6 treatments * 2 samples)	12	0	0	12	0	0	0	12	N/A	0
<b>Experimental Results</b>											
<i>Pre-Trial</i>	Adult livers, individual	6	6	0	6	6	6	6	6	N/A	N/A
<i>Weaning</i>	Adult individuals (3 treatments of 15F and 5M, plus 3 of 10F and 5M)	105	N/A	N/A	N/A	N/A	N/A	N/A	N/A	105	N/A
	Adult livers, individual (3 treatments of 15F and 5M, plus 3 of 10F and 5M)	105	0	0	105	105	0	0	105	N/A	N/A
	Kits @ weaning (15 kits * 6 treatments)	90	N/A	N/A	N/A	N/A	N/A	N/A	N/A	90	N/A
	Kit livers @ weaning, individual (15 kits * 6 treatments)	90	0	0	90	90	0	0	90	N/A	N/A
<i>7 mos.</i>	Kits @ 7 mos. (15 kits * 6 treatments)	90	N/A	N/A	N/A	N/A	N/A	N/A	N/A	90	N/A
	Kits livers @ 7 mos., individual (15 kits * 6 treatments)	90	0	0	90	90	0	0	90	N/A	N/A
<p><b>Note:</b> The adult individuals evaluated at the pre-trial stage include three males and three females. The adult individuals evaluated at weaning include both females (10-15 per treatment) and males (5 per treatment). All kit evaluations include approximately equal numbers of males and females. As indicated in Hudson River Natural Resource Trustees (2005), organochlorine (OC) pesticides include: aldrin, <math>\alpha</math>-BHC, <math>\beta</math>-BHC, <math>\gamma</math>-BHC, <math>\alpha</math>-chlordane, <math>\gamma</math>-chlordane, chlordane, 2,4'-DDD, 2,4'-DDE, 2,4'-DDT, 4,4'-DDD, 4,4'-DDE, 4,4'-DDT, dieldrin, endosulfan I, endosulfan II, endosulfan sulfate, endrin, endrin aldehyde, endrine ketone, heptachlor, heptachlor epoxide, hexachlorobenzene, methoxychlor, cis-nonachlor, trans-nonachlor, oxychlordane, and toxaphene. Congeners measured using LRMS include: 8, 18, 28, 31, 44, 45, 47, 49, 52, 56, 66, 70, 74, 77, 81, 87, 95, 99, 101, 105, 110, 114, 118, 123, 126, 128, 138, 146, 149, 151, 153, 156, 157, 158, 167, 169, 170, 174, 177, 180, 183, 187, 189, 194, 195, 201, 206, 206, plus homologues and tPCBs. HRMS measurements include all LRMS values plus: 105, 114, 118, 123, 156, 157, 167, and 189. Metals include aluminum, antimony, arsenic, barium, beryllium, cadmium, calcium, chromium, cobalt, copper, iron, lead, magnesium, manganese, mercury, nickel, potassium, selenium, silver, sodium, thallium, vanadium, and zinc.</p>											

#### 4. SUMMARY OF ENDPOINTS

Adult body weights:	At beginning of the acclimation period; at beginning of the definitive trial; every other week thereafter until initiation of breeding; at whelping; at time when kits are three weeks old; at time when kits are six weeks old; at necropsy (Ringer et al., 1991)
Adult feed consumption:	During the acclimation period; weekly (two consecutive days/week) during the definitive trial (if the temperature is above 0°C) until initiation of breeding (Ringer et al., 1991)
Number of females mated:	(Ringer et al., 1991)
Length of gestation:	(Ringer et al., 1991)
Number of females whelping/ not whelping:	(Ringer et al., 1991)
Total newborn/female whelped:	(Ringer et al., 1991)
Live newborn/female whelped:	(Ringer et al., 1991)
Average kit birth weight:	(Ringer et al., 1991)
Average litter weight:	(Ringer et al., 1991)
Percent kit survival to three weeks of age:	(Ringer et al., 1991)
Kit body weights at three weeks of age:	(Ringer et al., 1991)
Percent kit survival to six weeks of age:	(Ringer et al., 1991)
Kit body weights at six weeks of age	(Ringer et al., 1991)
Adult and six-week-old kit organ weights:	(Heaton et al., 1995a)
Histopathology of adult and	

six-week-old kit organs and jaws:	(Heaton et al., 1995b; Bursian et al., 2006a,b)
Total PCB and planar PCB, PCDD and PCDF analyses of adult and six-week-old kit livers:	(Hudson River Natural Resource Trustees, 2005)
Monthly body weights of seven-month-old juveniles:	(Heaton et al., 1995a)
Organ weights of seven-month-old juveniles:	(Heaton et al., 1995a)
Histopathology of seven-month-old juvenile organs and jaws:	(Heaton et al., 1995b; Bursian et al., 2006a,b)
Total PCB and planar PCB, PCDD and PCDF analyses of seven-month-old juvenile livers:	(Hudson River Natural Resource Trustees, 2005)

## **5. STATISTICAL ANALYSIS**

### **5.1 STATISTICAL METHODS**

Twenty measurement endpoints of interest are identified in Section 4. These endpoints can be classified into three data types: continuous measurements such as total PCB concentrations in livers; counts, such as the number of mandibular lesions per mink; or binary outcomes such as whether or not an individual kit survived to three weeks. Statistical analyses will be conducted using a generalized linear model framework (McCullagh and Nelder, 1989), where each data type and specific distributional characteristics will be used to select the most appropriate class of linear model. In general, continuous endpoints will be analyzed using normal-theory linear models (Neter et al., 1996) such as analysis of variance or repeated measures analysis of variance (Miliken and Johnson, 1984). Count variables will be analyzed using Poisson or overdispersed Poisson regression models (McCullagh and Nelder, 1989), and binary variables will be analyzed using logistic regression models for clustered sampling designs (McCullagh and Nelder, 1989). Each of the endpoints is classified by data-type and anticipated analysis method in Table 4. For endpoints measured at three or more points in time, repeated measures analyses will be used to test for differences in growth profiles (*i.e.*, profile analysis, Seber 1984).

<b>Table 4</b>		
<b>Summary of Data Types and Anticipated Statistical Analyses</b>		
<b>Endpoint</b>	<b>Data Type</b>	<b>Statistical Methods</b>
Number females mated	Binary	Logistic Regression; Spearman Karber LCp
Number of females whelping	Binary	Logistic Regression; Spearman Karber LCp
Kit survival at three and six weeks	Binary	Logistic Regression; Spearman-Karber LCp
Adult body weight	Continuous	ANOVA / Regression
Adult feed consumption	Continuous	ANOVA / Regression
Length of gestation	Continuous	ANOVA / Regression
Kit weight at birth, three and six weeks	Continuous	Repeated Measures ANOVA / Regression (Profile Analysis)
Average litter weight	Continuous	ANOVA / Regression
Adult and six-week-old kit organ weights	Continuous	ANOVA / Regression
Total PCB and planar PCB, PCDD and PCDF analyses of adult and six-week-old kit livers	Continuous	ANOVA / Regression
Monthly body weights of seven-month-old juveniles	Continuous	Repeated Measures ANOVA / Regression (Profile Analysis)
Organ weights of seven-month-old juveniles	Continuous	ANOVA / Regression
Total PCB and planar PCB , PCDD and PCDF analyses of seven-month-old juveniles livers	Continuous	ANOVA / Regression
Number whelped per female	Count	Poisson Regression (log transform instead of logit)
Number whelped live per female	Count	Poisson Regression
Histopathology of adult and six-week-old kit organs and jaws	Count/Binary	Poisson/Logistic Regression
Histopathology of seven-month-old juveniles	Count/Binary	Poisson/Logistic Regression

The minimum dose necessary to induce a specified proportion (p) of kit mortality (LCp) will be estimated based on the maximum likelihood estimates provided by the generalized linear model analysis (i.e., logit or probit analysis), as well as using the nonparametric Spearman-Kärber method (Spearman 1908, USEPA 1993). Estimated LCp from both methods will be compared, although based on simulation studies conducted by Miller and Ulrich (2001), it is anticipated that the Spearman-Kärber method will provide the most robust estimates. Dose response relationships will be estimated for total PCB concentrations as well as TEQs. Statistical analyses will include both hypothesis testing and estimation of confidence intervals for parameter estimates and effect sizes.

In addition to estimating the dose response relationships, differences in endpoints among dosing groups will also be estimated. The precision of estimates will be quantified using confidence limits for differences. Point estimates combined with

confidence limits express both the magnitude of effects as well as the precision with which they are estimated (Cherry,1998 and Johnson, 1999). Additionally, lower confidence limits for differences can be interpreted as tests for no difference among treatments, while upper confidence limits can be interpreted as tests against a pre-specified minimal difference of interest. For example, when an upper confidence limit for the difference is less than a pre-specified effect size of interest, this is equivalent to rejecting a test of bioequivalence (e.g., the reverse null hypothesis) (McDonald and Erickson, 1994). Additional statistical evaluations may also be employed.

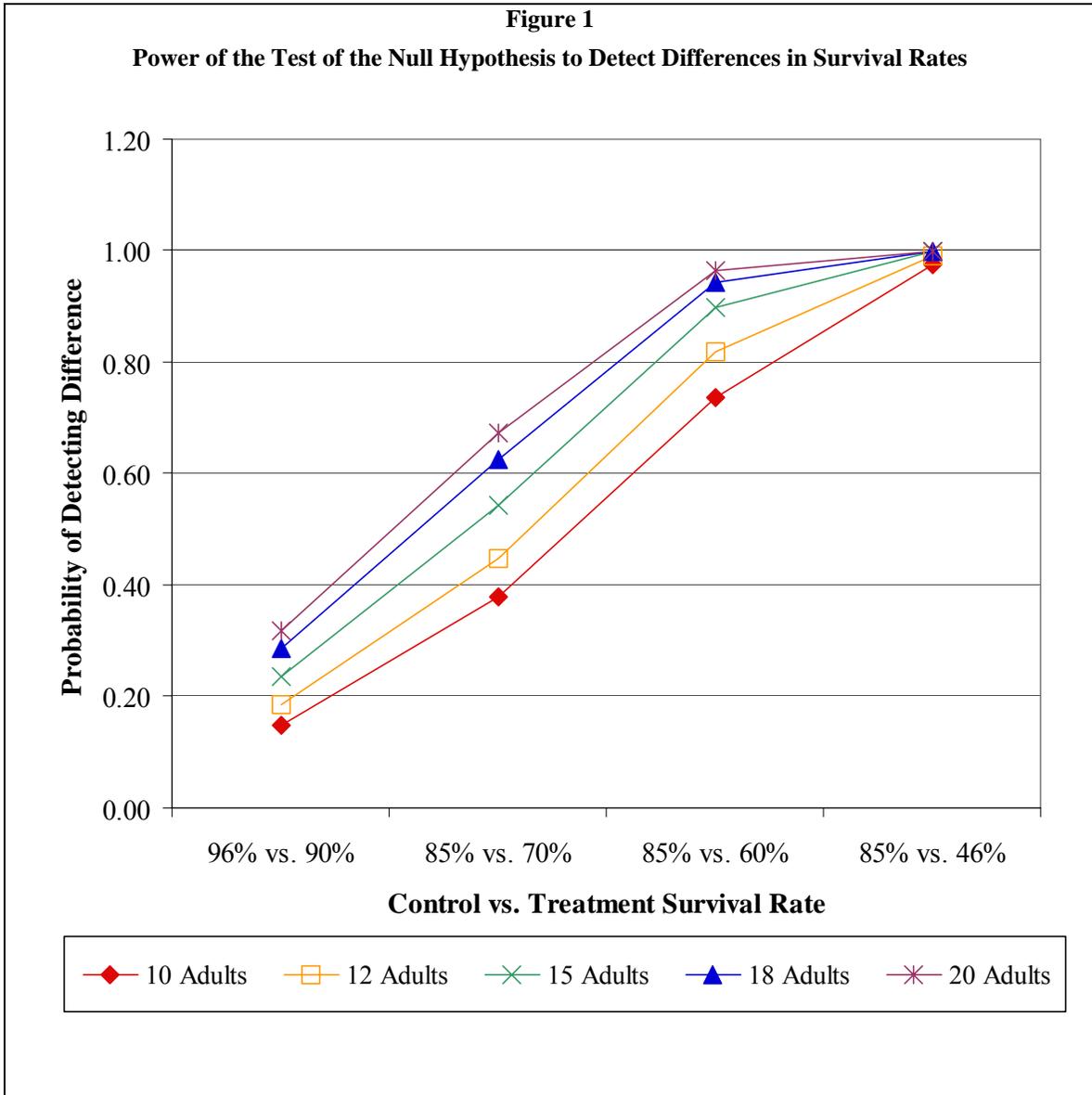
## **5.2 SAMPLE SIZE CONSIDERATIONS**

The number of mink to be placed on trial will balance a reasonable expectation of detecting biologically meaningful effects subject to the limitations of available time and resources to conduct the study. One of the objectives of this study is to identify relationships between dietary PCB doses in adult females and kit survival rates. Survival rates are estimated from binary data summarizing kit survival. Effects are indicated by differences in control and treatment survival rates. In this section, a power analysis is conducted to provide estimates of the probability of detecting differences in survival rates among control and treatment mink. Conducting a power analysis with respect to this particular endpoint (i.e., kit survival) is reasonable not only because of the importance of the endpoint from a biological perspective but also because, assuming similar effect sizes, detecting differences amongst groups requires the largest sample sizes when the measurement metric is binary in nature. As a result, the power associated with the other endpoints proposed in the study will be higher given the same sample size.

In general, to conduct a prospective power analysis one requires estimates of the nature of the anticipated data and the effect sizes (differences in survival rates) of interest. In this study, the null hypothesis ( $H_0$ ) is that the survival rates are equal among control and treatment groups. The alternative hypothesis ( $H_a$ ) is that treatment survival rates are lower than the control rate. For this power analysis, we used the results of a similar study conducted by Bursian et al. (2003) as a source of data to estimate expected control and treatment survival rates and variability. Bursian et al. (2003) report control survival rates of 96% at birth and 85% at three and six months. They also reported that each female whelped approximately 4 to 6 kits and that survival of kits whelped from PCB dosed females ranged from 46% to 99% depending on the dose.

Based on these results we developed four scenarios to calculate the power to test  $H_0$ . The first scenario represents the comparison of survival rates at birth for which the control survival rate was assumed to be 96% and the dosed survival rate was assumed to be approximately 90%. The additional three scenarios represent comparison of control survival rate (85%) with dosed survival rates assumed to be approximately 46%, 60% and 70%. These are representative of the range of reduced survival rates observed by Bursian et al. (2003) in kits whelped from dosed adult females. For each of these four scenarios, power was estimated for samples of 10, 12, 15, 18 and 20 adult females. It was assumed that on average five kits would result from each female in the test. A group of 15 females, for example, would therefore contribute approximately 75 (5x15) kits to be

monitored for survival and other endpoints. For each combination of the four scenarios and five sample sizes, we calculated the power of a one sided test of the null hypothesis of equal survival rates (Fleiss, 1981). Calculations were conducted using an internet based Java Applet developed by Lenth (2005). The results of these calculations are summarized in Figure 1 and Table 5.



<b>Table 5</b>					
<b>Power to Detect Differences in Proportions</b>					
	<b>Number of Adult Females</b>				
<b>Control vs. Treatment Survival</b>	<b>10</b>	<b>12</b>	<b>15</b>	<b>18</b>	<b>20</b>
96% vs. 90%	0.1495	0.1843	0.2359	0.2862	0.3189
85% vs. 70%	0.3778	0.4476	0.5421	0.6240	0.6718
85% vs. 60%	0.7370	0.8174	0.8976	0.9443	0.9633
85% vs. 46%	0.9751	0.9911	0.9982	0.9997	0.9999

It is anticipated that kits from the most heavily dosed females will have survival rates ranging from 60% to 70%. Assuming these survival rates, the number of females required per treatment to maximize the probability of detecting differences between the control and treatment groups is in the range of 15 to 20. However, the mink study facility does not have capacity for more than the proposed number of adult females per treatment (*i.e.*, 10 or 15), for the contemplated number of treatments.

It should be noted that the power analysis we conducted is approximate and not completely aligned with the analyses that are anticipated. These power estimates are based on standard statistical methods for comparing proportions (Fleiss, 1981) from independent trials, while it is anticipated that litter mates may not be statistically independent. Therefore, these power estimates may overestimate the actual power that will be realized.

## **6. QUALITY ASSURANCE/QUALITY CONTROL**

The objectives of the quality assurance (QA) plan for the proposed study are: 1) to ensure that the mink reproductive toxicity tests are conducted and properly documented according to protocols and the standard operating procedures (SOP) of the mink study facility (Appendix 3), and in accordance with all applicable animal use and care requirements of the facility, and 2) to ensure that the analytical measurements and biological/toxicological assays are accurate and precise. The general protocol includes replication of various stages, comparison and calibration against known standards, proper maintenance and calibration of equipment, accurate sample tracking and custody, proper documentation at all steps of sample processing, and other considerations of Good Laboratory Practice (GLP).

### **6.1 DATA QUALITY OBJECTIVES**

The data quality objectives for the mink dietary exposure study are directly linked to endpoints presented in Section 4 and study objectives discussed in Section 1. In summary, the measurement endpoints in the study will be evaluated to determine if the assessment endpoints of survival, reproduction or development of mink are being

impacted by dietary exposure to PCBs. To achieve these objectives, the following types of data will be required:

- Reproduction, growth and survival data for control and treatment groups
- Dietary exposure chemistry
- Mink liver chemistry
- Pathological evaluations

The data developed as part of the mink dietary exposure study must achieve acceptable standards of accuracy, completeness, representativeness and comparability. The purpose of this section of the work plan is to further document the measures being taken to ensure that these standards are met.

## **6.2 DATA QUALITY INDICATORS**

Data developed in the mink dietary study must meet acceptable standards of precision, accuracy, completeness, representativeness, comparability and sensitivity. Each of these data quality indicators, some of which are not readily quantifiable, is discussed below with specific reference to the mink dietary study.

Precision is defined as the level of agreement among repeated independent measurements of the same characteristics. Precision for this study is assessed by the performance of several replicates (up to 15) per treatment. For the measurements that are not unique to the mink dietary study, such as diet and tissue chemistries, precision is evaluated as described in the Hudson River AQAP (Hudson River Natural Resource Trustees, 2005).

Accuracy is defined as the agreement of a measure with its true value. For the parameters unique to this study (tissue weights, reproductive effects and pathology), accuracy is defined as meaning that tissues are correctly weighed, and reproductive effects and tissue pathology are correctly assessed. The data generated by this study may be evaluated for accuracy via comparison with reference organisms, and results observed in similar dietary studies. For parameters such as diet and tissue chemistry and dietary nutrient content, accuracy is defined as the degree of agreement of an analytical measurement with the true or expected concentration.

Completeness is defined as the percentage of the planned samples actually evaluated and processed. Completeness can be evaluated for all components of the mink dietary study. To ensure that the desired statistical resolution is achieved, it is important that a high level of completeness be achieved for all components of this study. Mink toxicity studies have been conducted by researchers at the selected mink study facility for over 35 years. During this time, no studies have been discontinued or significantly impacted by non-treatment-related mortalities or sample exclusions (e.g., >30% weight loss) to such a degree that the remaining data were deemed incomplete or unacceptable for use in accessing treatment related effects. The current statistical design of this study (i.e., 10 or 15 replicates per treatment) is adequate to account for typical non-treatment-

related losses while still maintaining sufficient sample size required for a high level of data completeness.

Representativeness refers to the degree to which the data accurately reflect the effects that would be observed if a wild mink would ingest a similar diet. This data quality indicator is addressed through implementation of proper experimental design and sampling processing design and may be evaluated via comparison with expected results.

Comparability is a measure of the confidence with which the study data may be compared to another similar data set. Comparability may be evaluated for this data set through comparison with previous mink dietary studies with similar contamination levels.

Sensitivity, the ability of a measurement technique or instrument to operate at a level sufficient to measure the parameter of interest, is largely not applicable to the biological parameters. The detection limits for chemistry parameters are specified in Hudson River Natural Resource Trustees (2005). These, in conjunction with reproductive and pathological effects, will provide sufficient sensitivity for the purpose of providing insight into the potential for the measured contaminants to impact resident mink populations.

### 6.3 SAMPLE HANDLING, TRANSPORTATION AND ANALYTICAL PROCEDURES

Samples of fish, diets, and livers will be collected at the mink study facility and sent to Alpha Woods Hole Lab (AWHL) and/or Axys Analytical Services, Limited (Axys), as appropriate, for chemical analyses. Table 6 sets forth which laboratories will conduct which chemical analyses. The laboratory project managers are:

**Peter Kane**  
 Alpha Woods Hole Lab  
 375 Paramount Drive, Suite 2  
 Raynham, MA 02767-5154  
 (508) 822-9300; FAX (508) 822-3288  
*pkane@alphalab.com*

**Pam Riley**  
 Axys Analytical Services, Limited  
 2045 Mills Road West  
 Sidney, British Columbia, Canada V8L358  
 (250) 655-5800; FAX (250) 655-5811  
*priley@axys.com*

<b>Sample</b>	<b>Alpha Woods Hole Lab</b>	<b>Axys Analytical Services</b>
Ocean fish blend	PCBs, lipids, moisture	--
Hudson River fish blend	PCBs, lipids, moisture	--
Dietary mix - initial batch	Metals, moisture	PCBs, OCs, PCDDs/PCDFs, PBDEs, lipids, moisture
Dietary mix - subsequent batches	--	PCBs, lipids, moisture
Pre-trial adult livers	Metals, moisture	PCBs, OCs, PCDDs/PCDFs, PBDEs, lipids
Adult and kit livers at weaning	--	PCBs, PCDDs/PCDFs, lipids
Kit livers at 7 months	--	PCBs, PCDDs/PCDFs, lipids

Fish, diet and tissue samples for chemical and nutritional analyses will be stored in I-Chem jars at -80°C prior to shipment. Fish, diet and tissue samples for chemical analysis will be shipped by overnight courier frozen on dry ice. Diet samples for nutritional analysis will be shipped by overnight courier to Litchfield Analytical Services<sup>1</sup> frozen on dry ice. Chain of custody documentation (Appendix 4) will accompany all shipped samples.

Chemical analyses of fish, diet, and tissue samples will be performed in conformance with the Hudson River Natural Resource Damage Assessment AQAP (Hudson River Natural Resource Trustees, 2005).

Tissue samples preserved in formalin for histopathological analysis will be transported under Chain of Custody by the Principal Investigator from the mink study facility at the end of each necropsy session (at weaning [adult females and males and six-week-old kits] and when juveniles are seven months old) to a board certified veterinary pathologist where they will be processed. All tissues are assigned a unique number upon receipt by the pathology lab, which follows the tissue through processing and reading of the slides. Tissue blocks are returned to the Principal Investigator when the pathology report is submitted. A subset of slides will also be reviewed by a second pathologist to confirm interpretations.

#### **6.4 DATA REDUCTION VALIDATION AND REPORTING**

All experimental information is recorded in bound notebooks or on forms kept in loose leaf notebooks and will be signed and dated. Copies are maintained in a separate, secured area. Instrument printouts and computerized data tables are uniquely labeled and cross-referenced to the project notebook. The accuracy of all such measurements will be checked internally by the Principal Investigator on a weekly basis. Copies of the computerized data files are maintained in a project notebook and on CD in the project file. During the course of the experiment, an external audit will be conducted by the Hudson River Quality Assurance Coordinator to evaluate adherence to relevant protocols and ensure that procedures are in place for proper sample handling, processing, and documentation of results. Prior to use by the Principal Investigator, analytical data will be validated as described in the Hudson River AQAP (Hudson River Natural Resource Trustees, 2005).

#### **6.5 SAMPLING METHODOLOGY**

Fish sampling in the Hudson River will be conducted according to procedures outlined in Appendix 1. Carp (*Cyprinus carpio*) is the primary target of this sampling

---

<sup>1</sup> Contact information for Litchfield is as follows: Stan W. Force, President, Litchfield Analytical Services. P.O. Box 457, 535 Marshall Street, Litchfield, MI 49252. Telephone: 517-542-2915.

activity because previous fish sampling activities identified populations of sufficient size and number so that collecting these species at these locations would have minimal impact on the resident populations and could be accomplished in a time-efficient manner.

## **6.6 EQUIPMENT**

All equipment used in these studies (grinder, feed mixer, freezers, cooler and balances) is routinely inspected, calibrated, and preventive maintenance is performed. A logbook is kept for each instrument to document its use, performance, calibration, and maintenance.

## **6.7 STATISTICAL ANALYSIS OF DATA AND SAMPLING DESIGN**

The statistical treatment of the data is described in Section 5 of the work plan. Sampling design in general follows procedures described by Ringer et al. (1991) (Appendix 2).

## **6.8 CORRECTIVE ACTION**

Problems will be identified as they occur or through weekly staff meetings. Remedial actions will be taken as deemed appropriate and in accordance with the QA performance criteria. All such problems and corrective actions will be recorded in the project notebook and reported to the Principal Investigator.

## **6.9 TRAINING**

All sampling and analyses will be directed by the Principal Investigator or by the appropriate supervisor, depending upon the task, who have experience in the collection and shipping of samples, the analyses of tissue and diet chemistry, and the evaluation of mink reproductive endpoints and pathology. Supporting staff will receive training from the Principal Investigator in overall goals of the study and in techniques to be followed to ensure collection of quality data.

## **7. LITERATURE CITED**

Aulerich, R.J. and R.K. Ringer, 1977. Current status of PCB toxicity to mink, and effect on their reproduction. *Archives of Environmental Contamination and Toxicology* 6:279-292.

Aulerich, R.J., D.C. Powell, and S.J. Bursian, 1999. *Handbook of Biological Data for Mink*. Department of Animal Science, Michigan State University, East Lansing, MI. 138 pp.

- Aulerich, R.J., S.J. Bursian, B. Yamini, and D.E. Tillitt, 2000. Dietary exposure of mink to fish from the Housatonic River: Effects on reproduction and survival. Final Supplemental Investigation Work Plan for the Lower Housatonic River, Appendix A.26. U.S. Army Corps of Engineers, New England District, Concord, MA. 109 pp.
- Beckett, K.J., S.D. Millsap, A.L. Blankenship, M.J. Zwiernik, J.P. Giesy and S.J. Bursian, 2005. Squamous epithelial lesion of the mandibles and maxillae of wild mink (*Mustela vison*) naturally exposed to polychlorinated biphenyls. *Environmental Toxicology and Chemistry* 24:674-677.
- Bursian, S.J., R.J. Aulerich, B. Yamini and D.E. Tillitt, 2003. Dietary exposure of mink to fish from the Housatonic river: effects on reproduction and survival. Revised final report submitted to Weston Solutions, Inc. One Weston Way, West Chester, PA 19380-1499.
- Bursian, S.J., K.J. Beckett, B. Yamini, P.A. Martin, K. Kannan, K.L. Shields and F.C. Mohr, 2006a. Assessment of effects in mink caused by consumption of carp collected from the Saginaw River, Michigan, USA. *Archives of Environmental Contamination and Toxicology* (accepted).
- Bursian, S.J., C. Sharma, R.J. Aulerich, B. Yamini, R.R. Mitchell, K.J. Beckett, C. E. Orazio, D. Moore, S. Svirsky and D.E. Tillitt, 2006b. Dietary exposure of mink (*Mustela vison*) to fish from the Housatonic River, Berkshire County, MA, USA: Effects on organ weights and histology and hepatic concentrations of polychlorinated biphenyls and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxic equivalents. *Environmental Toxicology and Chemistry* (accepted).
- Cherry, S., 1998. Statistical tests in publications of The Wildlife Society. *Wildlife Society Bulletin* 26:947-953.
- Fleiss, J.L., 1981. *Statistical Methods for Rates and Proportions*. Second Edition. Wiley series in probability and mathematical statistics. John Wiley and Sons. New York.
- Foley, R.E., S.J. Jackling, R.L. Sloan, and M.K. Brown, 1988. Organochlorine and mercury residues in wild mink and otter: Comparison with fish. *Environmental Toxicology and Chemistry* 7:363-374.
- Fur Commission USA, 1995. *Standard Guidelines for the Operation of Mink Farms in the United States*. Fur Commission USA, St. Paul, MN. 16pp.

- Heaton, S.N., S.J. Bursian, J.P. Giesy, D.E. Tillitt, J.A. Render, P.D. Jones, D.A. Verbrugge, T.J. Kubiak, and R.J. Aulerich, 1995a. Dietary exposure of mink to carp from Saginaw Bay, Michigan. I. Effects on reproduction and survival and the potential risks to wild mink populations. *Archives of Environmental Contamination and Toxicology* 28:334-343.
- Heaton, S.N., S.J. Bursian, J.P. Giesy, D.E. Tillitt, J.A. Render, P.D. Jones, D.A. Verbrugge, T.J. Kubiak, and R.J. Aulerich, 1995b. Dietary exposure of mink to carp from Saginaw Bay, Michigan. 2. Hematology and liver pathology. *Archives of Environmental Contamination and Toxicology* 29:411-417.
- Hochstein, J.R., R.J. Aulerich, and S.J. Bursian, 1988. Acute toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin to mink. *Archives of Environmental Contamination and Toxicology* 17:33-37.
- Hochstein, J.R., S.J. Bursian, and R.J. Aulerich, 1998. Effects of dietary exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in adult female mink. *Archives of Environmental Contamination and Toxicology* 15:348-353.
- Horn, E.G., L.J. Hetling, and T.J. Tofflemire, 1979. The problem of PCBs in the Hudson River system. *Annals New York Academy of Sciences* 320:591-609.
- Hudson River Natural Resource Trustees, 2005. *Analytical Quality Assurance Plan: Hudson River Natural Resource Damage Assessment, Public Release Version 2.0*. September 1, 2005. Department of Commerce, Silver Spring, MD. 31 pp.
- Johnson, D. H., 1999. The insignificance of statistical significance testing. *Journal of Wildlife Management* 63:763-772.
- Lenth R. 2005. Java applets for power and sample size. University of Iowa. <http://www.stat.uiowa.edu/%7Erlenth/Power/>
- Leonards, P.E.G., T.H. Vries, W. Minnaard, S. Stuijzand, P. deVoogt, W.P. Cofino, N.M. van Straalen, and B. van Hattam, 1995. Assessment of experimental data on PCB-induced reproduction inhibition in mink, based on isomer- and congener-specific approach using 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxic equivalency. *Environmental Toxicology and Chemistry* 14:639-652.
- Mayack, D.T. and J. Loukmas, 2001. Progress report on Hudson River mammals: Polychlorinated biphenyl (PCB) levels in mink, otter, and muskrat and trapping results for mink, the upper Hudson River drainage, 1998-2000. Progress Report to the New York Department of Environmental Conservation. 24 pp.
- McCullagh, P. and J.A. Nelder, 1989. *Generalized Linear Models*. Second Edition. Monographs on Statistics and Applied Probability 37. Chapman and Hall. New York.

- McDonald, L. L. and W. P. Erickson, 1994. Testing for bioequivalence in field studies: Has a disturbed site been adequately reclaimed? In *Statistics in Ecology and Environmental Monitoring*, D. J. Fletcher and B. F. J. Manly (eds.), 183-197. Dunedin, New Zealand: University of Otago Press.
- Miller, J. and F. Ulrich, 2001. On the analysis of psychometric functions: The Spearman—Kärber method. *Perception and Psychophysics* 63(8):1399-1420.
- Milliken, G.A. and D.E. Johnson, 1984. Analysis of messy data. Volume I: Designed Experiments. Van Nostrand Reinhold. New York.
- National Research Council, 1982. Nutrient requirements of mink and foxes. In *Nutritional Requirements of Domestic Animals*, No. 7. National Academies Press, Washington, DC. 72 pp.
- Neter, J., M. H. Kutner, C. J. Nachtsheim, and W. Wasserman, 1996. *Applied Linear Statistical Models, Fourth Edition*. Irwin: Chicago.
- Render, J.A., R.J. Aulerich, S.J. Bursian, and R.F. Nachreiner, 2000a. Proliferation of maxillary and mandibular periodontal squamous cells in mink fed 3,3',4,4',5-pentachlorobiphenyl (PCB 126). *Journal of Veterinary Diagnostic Investigation* 12:477-479.
- Render, J.A., J.R. Hochstein, R.J. Aulerich, and S.J. Bursian, 2000b. Proliferation of periodontal squamous epithelium in mink fed 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). *Veterinary and Human Toxicology* 42:85-86.
- Render, J.A., S.J. Bursian, D.S. Rosenstein, and R.J. Aulerich, 2001. Squamous epithelial proliferation in the jaws of mink fed diets containing 3,3',4,4',5-pentachlorobiphenyl (PCB 126) or 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). *Veterinary and Human Toxicology* 43:22-26.
- Restum, J.C., S.J. Bursian, J.P. Giesy, J.A. Render, W.G. Helferich, E.B. Shipp, D.A. Verbrugge, and R.J. Aulerich, 1998. A multigenerational study of the effects of consumption of PCB-contaminated carp from Saginaw Bay, Lake Huron, on mink. I. Effects on mink reproduction, kit growth and survival, and selected biological parameters. *Journal of Toxicology and Environmental Health, Part A* 54:343-375.
- Ringer, R.K., T.C. Hornshaw, and R.J. Aulerich, 1991. Mammalian wildlife (mink and ferret) toxicity test protocols (LC<sub>50</sub>, reproduction, and secondary toxicity). Report #600/3-91/043. U.S. Environmental Protection Agency, Washington, DC.
- Scientificur, 1987. Scientific Index I. I.G. Jorgensen (ed.). Scientificur, Hilleroed, Denmark. 196 pp.

- Scientifur, 1992. Scientific Index II. G. Jorgensen (ed.). Scientifur, Tjele, Denmark. 164 pp.
- Seber, G.A.F., 1984. *Multivariate Observations*. Wiley Series in Probability and Mathematical Statistics. John Wiley and Sons, New York.
- Shump, A.U., K.A. Shump, Jr., G.A. Heidt, and R.J. Aulerich, 1976. *A Bibliography of Mustelids, Part II: Mink*. Department of Poultry Science, Michigan State University, East Lansing, MI. 156 pp.
- Spearman, C., 1908. The method of “right and wrong cases” (“consistent stimuli”) without Gauss’s formulae. *British Journal of Psychology* 2:227-242.
- Sundqvist, C., 1989. *Mink Encyclopedia*. Release 1.0, Department of Biology, Abo Akademi, Porthansgatan 3, Turku, Finland.
- U.S. Environmental Protection Agency (USEPA), 1993. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Fourth Edition. EPA/600/4-90/027F.
- Van den Berg M, L. Birnbaum, A.T. Bosveld, B. Brunstrom, P. Cook, M. Feeley, J.P. Giesy, A. Hanberg, R. Hasegawa, S.W. Kennedy, T. Kubiak, J.C. Larsen, F.X. van Leeuwen, A.K. Liem, C. Nolt, R.E. Peterson, L. Poellinger, S. Safe, D. Schrenk, D. Tillitt, M. Tysklind, M. Younes, F. Woern, and T. Zacharewski, 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environmental Health Perspectives* 106:775-792.