

**Histological Assessment of Mink Jaws Collected during GLPF Project C30239**  
RAP Progress in the Rochester Embayment of Lake Ontario: Population Monitoring, Trophic Relationships, and Levels of Bioaccumulative Chemicals of Concern in Mink, a Sentinel Species

A Supplementary Report by

Kerrie J. Beckett  
Stantec Consulting Services, Inc.  
30 Park Drive  
Topsham, ME 04086

and

James M. Haynes  
Department of Environmental Science and Biology  
State University of New York  
College at Brockport  
350 New Campus Drive  
Brockport, NY 14420-2973

## **1.0 Introduction**

The Lake Ontario food web is contaminated with persistent organic pollutants, including polychlorinated biphenyls (PCBs), dioxins and furans, mirex, etc. The purpose of the study for which mink were collected originally was to determine: 1) if contaminants in mink tissue come primarily from sources in the Rochester Embayment Area of Concern (AOC) or from sources in Lake Ontario, and 2) if contaminant concentrations in mink are high enough to cause reproductive impairment. While doing the literature review for the original study (Haynes et al. 2007), it was discovered that the most sensitive known indicator of mink exposure to persistent organic pollutants is jaw lesions and associated histopathology; hence, this work.

Assessment of mandibular and maxillary squamous epithelial proliferation offers one of the most sensitive methods to detect piscivorous mammalian exposure to certain environmental chemicals, including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), polychlorinated dibenzofurans (PCDF), and TCDD-like PCBs. Environmental exposure to these pollutants manifests as a histologically detectable lesion identified as mandibular and maxillary squamous epithelial proliferation or hyperplasia in the gingival tissue of the maxilla and mandible of mink (*Mustela vison*) (Beckett et al., 2005; Render et al. 2001, 2000a, b). The lesion is characterized by proliferation of squamous cells that form nests or cords that infiltrate into the periodontal ligament and alveolar bone causing osteolysis of the mandible and maxilla (Beckett et al., 2005). The lesion, identified as squamous epithelial proliferation, appears to progress to squamous cell carcinoma (SCC) in mink gingival tissue (Bursian et al., 2006a, b; Beckett et al., 2005). This SCC is extremely invasive and destructive, meeting certain criteria of malignant carcinogenicity, and has been shown to be fatal in mink in laboratory studies. The chemical, dose, and duration of exposure will contribute to the level or stage of progression of the identified lesion. In this study, the prevalence of the oral lesion was assessed in wild, trapped mink that were naturally exposed to environmental contaminants in the Lake Ontario watershed.

## 2.0 Methods

Previously frozen mink heads were received in 10% buffered formalin. All mink heads were examined clinically prior to the decalcifying process. Mink heads were placed in Surgipath® Decalcifier II (hydrochloric acid) (Surgipath Medical Industries, Inc., Richmond, IL) and tissues were trimmed during the decalcifying process. The mandible and maxilla (i.e., lower and upper jaw, respectively) were sectioned and processed for paraffin embedding, and then sectioned to 5 microns ( $\mu\text{m}$ ), mounted on microscopic slides, and stained with hematoxylin and eosin (H & E) for subsequent histological assessment. If the histopathological assessment revealed any cellular alterations or anomalies, the lesion was rated against a severity scale (Beckett et al., 2005). Suspected lesions were verified by a certified pathologist (Dr. S. Fitzgerald, Diagnostic Center for Population and Animal Health, Michigan State University, East Lansing, MI, pers. comm.).

## 3.0 Results and Discussion

Twelve mink heads were processed and jaws were histologically analyzed for exposure to environmental contaminants (Table 1). Three mink came from each of four areas in relation to the Rochester (New York) Embayment AOC: 1) near Lake Ontario in the AOC; 2) inland (i.e., not in contact with the Lake Ontario food web) in the AOC; 3) near Lake Ontario out of the AOC; and 4) inland out of the AOC. The three mink from each area were the individuals with the highest and lowest levels of total PCBs in liver tissue and the individual closest to the average PCB concentration among 10 mink collected in each area.

One of the 12 mink (mink #17), captured near Lake Ontario within the Rochester AOC, exhibited gross and histological evidence of oral neoplasia, and also had the highest hepatic concentration of total PCBs (5870.8 ng/g-wet weight [ww]) in the original study (Haynes et al. 2007). Gross evaluation of mink #17 revealed separation of the maxillary incisors, with some missing incisors. Although both mandibular canines were broken off near or below the gum-line (due to tooth extraction for aging in the original study), there was an overall healthy appearance and condition of teeth. Both the maxilla and the mandible bones were broken also, and therefore were sectioned off-center of the midline. The lesion was grossly apparent during the processing stages, particularly in the left maxilla in which small proliferative areas containing nodular-like growths and swelling were recognized along the outside of the last premolar along the dental arcade. Mink #17 was diagnosed with severe lesions in all available jaw quadrants (right and left maxillae and mandible). Histopathologically, cellular cords and nests extended deep into the jaw bone in which the lesion was apparent. Greater than about 50% of the jaw bone had been replaced with cords and/or nests of squamous epithelial cells. This is the first report of the lesion being grossly detectable in a naturally exposed mink, and therefore suggests increased credibility for its use as a monitoring tool for exposure to TCDD-like environmental contaminants.

Three other mink (mink #21, #63, and #46) each had suspicious areas within the gingival tissue suggestive of the lesion. However, the degree of freeze/thaw artifact and tissue damage from tooth extraction for aging was too extensive to confirm the etiology of the tissue damage and whether or not it was caused by contaminant exposure.

Mink #21 had discoloration of the premolars and was missing some maxillary incisors. Additionally, there was evidence of tissue and tooth damage to some incisors in the right maxilla

that presented as ruffled edges of the teeth. No obvious squamous epithelial cysts were identified. The total hepatic PCB concentration in mink #21 was 682.0 ng/g-ww. This mink was captured near Lake Ontario within the Rochester AOC, and had the third highest PCB concentration among the 40 mink analyzed in the original study.

Mink #63 presented pronounced incisor separation in the maxilla, as well as some incisor separation in the mandible. The mandible also had clear evidence of tissue damage around the incisors, as well as increased cellular spacing in this region. No obvious cysts comprised of squamous cells were noted. However, an abnormal cellular lesion in the molar region was suspicious, but evidence of its origin was difficult to determine due to extensive freeze/thaw artifact and tissue damage. The total hepatic PCB concentration in mink #63 was 554.4 ng/g-ww. This mink was captured inland in the Rochester AOC, and had the fourth highest PCB concentration among the 40 mink analyzed in the original study.

Mink #46 had apparent tissue damage around the incisors of the mandible, with moderate freeze/thaw artifact throughout. The last mandibular molar region had suspicious tissue damage that was suggestive of a lesion, although this could not be confirmed. The total hepatic PCB concentration in mink #46 was 229.8 ng/g-ww. This mink was captured near Lake Ontario outside of the Rochester AOC, and had the fifth highest PCB concentration among the 40 mink analyzed in the original study.

#### **4.0 Conclusions**

Among the 12 mink analyzed for the jaw lesion, four of the five with the highest levels of total PCB in liver had definite (mink #17) or suspected (mink #21, #63, and #46) histopathologic appearance of the lesion that suggests squamous cell carcinoma. The characteristics of the lesion observed in mink from the current study were consistent with some of the criteria for malignant neoplasia, including invasiveness of atypical cell type, tissue destruction, increased proliferative activity, and diffuse infiltration. The data presented here and elsewhere suggest 1) a strong correlation between the presence and severity of the lesion with total PCB concentrations measured in tissue; 2) that the lesion is an extremely sensitive indicator of environmental exposure of mink to the most toxic of persistent organic pollutants; and 3) that mink near the shore of Lake Ontario and, perhaps, inland in the AOC are exposed to levels of persistent organic pollutants sufficient to cause the lesion.

#### **5.0 Acknowledgments**

Randall Baase collected most of the mink used for this study. James Pagano, SUNY College at Oswego, performed PCB analyses. Columbia Analytical Services, Houston, TX performed dioxin-furan analyses. Sara Wellman conducted the literature search that revealed jaw lesions as the most sensitive indicator of exposure to persistent organic pollutants that led to this study.

## 6.0 References

- 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**(3):674-677.
- 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* **50**(4):614-623.
- 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* **25**(6):1541-1550.
- Haynes, J.M., S.T. Wellman and J.J. Pagano. 2007. RAP Progress in the Rochester Embayment of Lake Ontario: Population Monitoring, Trophic Relationships, and Levels of Bioaccumulative Chemicals of Concern in Mink, a Sentinel Species. Final report to the NY Great Lakes Protection Fund. NY Department of Environmental Conservation, Buffalo, NY.
- Render, J.A., J.R. Hochstein, R.J. Aulerich and S.J. Bursian. 2000a. Proliferation of periodontal squamous epithelium in mink fed 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). *Veterinary and Human Toxicology* **42**(2):85-86.
- Render, J.A., R.J. Aulerich, S.J. Bursian and R.F. Nachreiner. 2000b. Proliferation of maxillary and mandibular periodontal squamous cells in mink fed 3,3',4,4',5-pentachlorobiphenyl (PCB 126). *J Vet Diagn Invest* **12**:477-479.
- 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**(1):22-26.

**Table 1.** Tissue concentrations of selected persistent organic pollutants in liver and adipose tissue of mink collected near Lake Ontario and inland, in and out of the Rochester Embayment Area of Concern (AOC), and corresponding presence and severity of jaw lesion.

<b>Collection Location</b>	<b>Total PCB Liver (ng/g-ww)</b>	<b>Adipose TEQ (ng/kg-ww)</b>	<b>Liver TEQ (ng/kg-ww)</b>	<b>Jaw Lesion</b>
<i><b>AOC-Lake</b></i>				
Mink #17	5870.8	339.98	21.26	Severe
Mink #21	682.0	na	3.50	Suggestive
Mink #56	14.7	7.66	nd	nd
<i><b>AOC-Inland</b></i>				
Mink #1	8.5	0.03	0.10	nd
Mink #61	64.2	8.96	nd	nd
Mink #63	554.4	9.13	nd	Suggestive
<i><b>Out of AOC-Lake</b></i>				
Mink #46	229.8	19.63	2.09	Suggestive
Mink #49	755.0	38.29	38.31	nd
Mink #53	13.6	5.30	0.92	nd
<i><b>Out of AOC-Inland</b></i>				
Mink #14	7.0	nd	0.03	nd
Mink #30	45.0	na	na	nd
Mink #33	19.3	na	na	nd
na = not analyzed; nd = not detected; ww = wet weight				