Lack of Jaw Pathology, a Sensitive Indicator of Exposure to TCDD-like Chemicals, in Mink Collected from the Tittabawassee River

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Overview: Outline

- Mink as a Sensitive Species
- Study Areas
- Jaw Lesion — Characteristics and Description
- Convergence and Findings of Field and Lab Studies —
  - Michigan State University (Lab)
  - Kalamazoo River (Field)
  - Lake Ontario Ecosystem (Field)
  - Tittabawassee River (Field)
Introduction – Mink (*Mustela vison*)

- Semi-aquatic, live along the edges of waterways
- Apex predators
- Highly sensitive species to environmental contaminants
Introduction – Study Areas

- Tittabawassee River Field Study
- Kalamazoo River Field Study
- Lake Ontario Field Study
- Lab Studies – PCBs, Furans, Dioxin
Mandibular and maxillary squamous epithelial proliferation appears to be one of the most sensitive biomarkers to evaluate exposure of mink to 2,3,7,8-tetrachlorodibenzo-\textit{p}-dioxin (TCDD)-like contaminants.
In a study designed to examine the effects of PCB 126 and TCDD on growing mink, an unexpected lesion of the mandibles and maxillae was observed.

Jaw Lesion is Characterized by

- Swelling of the upper and lower muzzle
- Islands and/or cysts of stratified squamous epithelial cells
- Loss of periodontal ligature
- Periodontoclasia (loose and displaced teeth)
- Osteolysis of the mandible and maxilla
- Bleeding gums and receding gingiva
- Increased gingival surface area, may lead to nodular proliferation
- Eventual loss of teeth, leading to aphagia

(aphagia is the inability to eat)
Histological Examination Revealing Levels of Jaw Lesion Development

Control: Note consistent alveolar bone around teeth, and no nests of squamous epithelial cells are present. HE.

Mild Lesion: Note the focal islands of squamous epithelial cells and cysts beginning to form adjacent to the teeth. HE.

Severe Lesion: Extensive infiltration of large islands of squamous epithelial cells have developed and formed cysts, destroyed tissue of the periodontal ligament, which then lead to loosening of the teeth. These cysts may contain centers of exfoliated epithelia and sometimes keratin. HE.

*Extensive osteoinvasive periodontal squamous cell proliferation results in focal loss of alveolar bone or osteolysis.*
Histopathology of the Jaw Lesion

Prior to clinical signs, the lesion can be detected histologically.

- Histologic evidence with no clinical signs
- Histologic evidence in which clinical signs were pronounced
Additional Pathologic Signs

* Note gingival thickening along dental arcade of the mandible
Clinical Signs of the Lesion

Gums are inflamed and receding, and incisors continue to spread.

Loose and spreading of the teeth is the first clinical sign of the lesion.
Pathologic Signs in Bone

Mink skulls demonstrating normal alveolar structure in a control mink (left skull [A] and upper skull [B]) and osteolysis of alveolar bones in a PCB 126 exposed mink (right skull [A] and lower skull [B]).
Mink skull demonstrating osteolysis of alveolar bone in a PCB 126 exposed mink.
Meeting Malignant Criteria

Commonly accepted criteria for malignant neoplasia

1) Lack of dependence on the continued presence of the stimulus (irreversible)
2) Proliferation
3) Diffuse infiltration
4) Invasiveness and tissue destruction
5) Metastasis
Reversibility Studies to Determine Malignancy

• Phase I (Reversibility Study) was to determine if the histologically detectable lesion progressed or regressed in mink after withdrawal of the stimulus, the PCB 126 contaminated diet.

• Phase II (Cell Harvest Study) was a preliminary study to determine if PCB 126 induced tumor growth by implanting the cells from the mink lesion into athymic mice, producing a tumor growth (*not being presented*).
Ranch strain of standard dark mink were exposed to 24.0 μg PCB 126/kg feed via their diet.

Reversibility Study

Weeks of exposure per group of mink: O = 1 week

The remaining mink were euthanized to compare histology at 6 months post-exposure to mink histology taken after the designated week(s) of exposure.

Jaws were collected and processed for histology.
Reversibility Study

Serial exposures and grow-outs. Following the specified exposure (in weeks), the mink were given control feed for an additional 6 months.

Top photomicrographs are exposure weeks
Bottom photomicrographs are 6-months post exposure
Results

Reversibility Study

*Histological lesions in all exposure groups became more severe and continued to progress following the 6-month grow-out period.

*Dose response and short duration of exposure led to lesion even with removal of stimulus.
Convergence and Findings of Field and Lab Studies
Kalamazoo River

Squamous Epithelial Lesion of the Mandibles and Maxillae of Wild Mink (*Mustela vison*) Naturally Exposed to Polychlorinated Biphenyls

Beckett et al., 2005
This was the first report of this jaw lesion type observed from a wild mink population – trapped in the Kalamazoo River basin, Michigan.

- 4 of 9 mink trapped had evidence of the jaw lesion; the ratings of the detectable lesions ranged from moderate (1) moderate-mild (1) to mild (2)
- Calculated hepatic TEQs averaged 0.3 ug TEQ/kg ww (mink from KR)
- The mink with the moderate rating had [Hepatic total PCB] of 6.0 mg/kg
- PCB 126 comprised 91% of the total TEQs
Results
Kalamazoo

The incidence and severity of the jaw lesion was positively correlated with TEQs (in a dose-response manner) in this wild mink population.
Histological Lesions in Mink Jaws are a Highly Sensitive Biomarker of Effect after Exposure to TCDD-like Chemicals: Field and Literature-based Confirmations

Hayes et al., 2009
Results

Rochester Embayment of Lake Ontario Ecosystem

First report of wild mink demonstrating gross or clinical signs of the jaw lesion.

- Mink #17: separation of the maxillary incisors, nodular-like growths and swelling, severe histologic evidence
- [Hepatic total PCB] was 5.87 mg/kg ww
Exposure and effects assessment of resident mink (Mustela vison) exposed to polychlorinated dibenzofurans and other dioxin-like compounds in the Tittabawassee River Basin, Midland, Michigan, USA

Zwiernik et al., 2008
Results
Tittabawassee River

48 wild mink collected in the field
- 26 from the Tittabawassee River (TR)
- 22 from an upstream reference area (RA)

* Only one mink had histologic evidence of mild jaw lesion, a female from the RA, with moderate to high hepatic TEQ concentration ~400 ng TEQ/kg (2 to 35-fold higher than the TR mink).

* NO lesions were observed in mink from TR.
Results
Tittabawassee River

Major contributing contaminants reported as TEQs (ng TEQ /kg ww) in hepatic tissue in mink from the Tittabawassee River:

- **Dioxins**: 4.2 RA vs. 21 TR
- **Furans**: 5.6 RA vs. 290 TR
- **Co-planar PCBs**: 8.4 RA vs. 80 TR
- **Total TEQs**: 20 RA vs. 400 TR

RA – Reference Area
TR – Tittabawassee River

Data from Zwiernik et al. 2008
Additional Lab Study

Multi-Generation Furan

Pastel ranch mink received 2,3,7,8-TCDF in feed

Generation 0: No Lesions
Generation 1: No Lesions
Generation 2: 2 mild lesions were detected in 2 mink following *in utero* and dietary exposure
<table>
<thead>
<tr>
<th>Mink Study - Location</th>
<th>Lesion Rating</th>
<th>Major Contaminant</th>
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<tr>
<td>Lab Studies</td>
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<td>Furan</td>
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<td>PCB 126 Reversibility Study</td>
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<td>Feeding Studies:</td>
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<td>Lake Ontario</td>
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<td>Tittabawassee River</td>
<td>No Lesion</td>
<td>Furans</td>
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Conclusions

• Exposure to co-planar TCDD-like compounds (TCDDs, PCDFs, PCBs) cause the sensitive jaw lesion observed in mink.

  *In particular, PCB 126 is the most toxic contaminant contributing to the jaw lesion, as was observed in the lab and field.*

• Increased severity of the lesion is observed with dose and duration of exposure

• Observations in the field also demonstrate increased severity as related to dose

• This unique jaw lesion is potentially lethal, and may pose a serious threat to wild populations of mink both in respect to inability to catch and consume food.