

Short Communication

SQUAMOUS EPITHELIAL LESION OF THE MANDIBLES AND MAXILLAE OF WILD MINK (*MUSTELA VISON*) NATURALLY EXPOSED TO POLYCHLORINATED BIPHENYLS

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Abstract—Approximately 125 km of the Kalamazoo River, located in southwestern Michigan (USA), are designated as a Superfund site, with polychlorinated biphenyls (PCBs) as the contaminant of concern. Mink (*Mustela vison*) are a naturally occurring predator in this area and also a species of concern because of their known sensitivity to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and structurally similar compounds, such as PCBs. Four of nine mink trapped from the Kalamazoo River area of concern (KRAOC) exhibited histological evidence of a jaw lesion previously identified in ranch mink. The jaw lesion, hyperplasia of squamous epithelium in the mandible and maxilla, is known to be caused by 3,3',4,4',5-pentachlorobiphenyl (PCB 126) and TCDD. Mink trapped from an upstream reference area (Fort Custer Recreation Area [FCRA]) did not exhibit the lesion. Mean concentrations of total PCBs were 2.8 and 2.3 mg/kg wet weight in the livers of mink from the KRAOC and FCRA, respectively, and TCDD toxic equivalent (TEQ) concentrations were 0.30 and 0.11 µg/kg wet weight, respectively. Significant correlations were found between the severity of the lesion and the hepatic concentrations of total PCBs and TEQs. To our knowledge, this is the first published report of the lesion occurring in wild mink.

Keywords—Polychlorinated biphenyls Mink Jaw Pathology

INTRODUCTION

The Kalamazoo River Superfund site is located in southwestern Michigan (USA) and encompasses approximately 125 km (80 miles) of the Kalamazoo River, from Morrow Dam in Kalamazoo County to Lake Michigan. The Kalamazoo River area of concern (KRAOC) became contaminated with polychlorinated biphenyls (PCBs) by waste discharged from the recycling and processing of carbonless copy paper, with the primary source of the PCBs being Aroclor 1242 and, to a lesser degree, Aroclor 1254 [1].

Mink are a naturally occurring predator in the Kalamazoo River area and also a species of concern because of their known sensitivity to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and compounds of similar structure, such as PCBs [2–7]. To assess the possible effects of PCBs on wildlife residing in the Kalamazoo River basin, wild mink were trapped from two areas, the KRAOC and the Fort Custer Recreation Area (FCRA; a reference area with lesser concentrations of PCBs) [7].

The potential for adverse effects of PCBs on mink in the KRAOC was estimated using two approaches: By applying dietary exposure models, and by measuring liver concentrations of TCDD toxic equivalents (TEQs) and total PCBs in trapped mink [7]. The concentrations of total PCBs and TEQs measured in the livers of the wild mink were in the range of those found in ranch mink that had expressed hyperplasia of squamous epithelium in the mandibles and maxillae when exposed to PCBs in their diet [8].

Previous studies have indicated that ranch mink of different

ages exposed to 3,3',4,4',5-pentachlorobiphenyl (PCB 126) at 24.0 µg/kg feed or to TCDD at 2.4 µg/kg feed developed clinical signs of mandibular and maxillary squamous epithelial hyperplasia that, in severe cases, resulted in the loss of teeth [9–11]. In an unpublished study (K. Beckett), mink fed diets containing PCB 126 concentrations as low as 0.24 µg/kg feed developed the clinical lesion, although the required period of exposure was longer. The lesion develops when squamous epithelial cells form infiltrating nests and cords into the alveolar bone, which results in osteolysis. Infiltration of squamous cells into the periodontal ligament eventually causes loose and displaced teeth. The maxillae and mandibles became 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 was to determine if wild mink inhabiting a PCB-contaminated environment exhibited squamous epithelial lesions of the mandibles and maxillae that, until now, have been reported only in ranch mink exposed to PCB 126 or TCDD under laboratory conditions.

MATERIALS AND METHODS

Wild mink were trapped throughout the KRAOC and FCRA during the winters of 2000, 2001, and 2002 [7]. Nine male mink were collected within the KRAOC from D Avenue downstream to the former Trowbridge impoundment, a distance of approximately 25 river km. Three mink (two males and one female) were collected from the upstream reference area at Fort Custer, which is located approximately 25 river km upstream of D Avenue, in Kalamazoo (MI, USA). Frozen mink carcasses were brought to the laboratory and thawed. A gross

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necropsy was performed by a board-certified veterinary pathologist. The weight (without pelt) and the length (nose to base of tail) of each mink were determined. Organ condition was assessed during necropsy to determine any gross abnormalities. Samples of the livers were collected for histological assessment as well as for determination of total PCB and TEQ concentrations.

For chemical analysis, PCBs were extracted from liver tissue based on U.S. Environmental Protection Agency Method 3540 (Soxhlet extraction) [12]. An aliquot of the extract was analyzed for total PCBs with a gas chromatograph (Perkin-Elmer Autosystem, Boston, MA, USA) equipped with a ^{63}Ni electron-capture detector according to the method described by Nakata et al. [13]. A second aliquot of the extract was subjected to carbon-column chromatography for the separation of non-*ortho*-substituted (i.e., coplanar) PCB congeners. The resulting extracts were analyzed by gas chromatography–mass spectroscopy on a Hewlett-Packard 5890 Series II gas chromatograph (Rolling Meadows, IL, USA) equipped with a Hewlett-Packard 5972 series mass-selective detector [7].

The TEQ method relates the toxicity of TCDD-like chemicals, including certain polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans, and PCBs, to the toxicity of TCDD, which is considered to be the most toxic of this group of chemicals. In the present study, TEQs were calculated by multiplying the concentration of an individual PCB congener by its toxicity equivalency factor (TEF) as presented by Van den Berg et al. [14]. The TEF denotes a congener's toxicity relative to TCDD, which has a toxicity value of 1.0. Total TEQ concentrations in liver samples were determined by summing the concentrations of TEQs of individual congeners detected in each sample. The PCB congeners 77, 81, 105, 118, 126, 156, 157, 167, and 169 were used for the calculation of TEQs [7].

In all cases, heads were removed from carcasses and fixed in 10% neutral-buffered formalin. Heads were then decalcified in Surgipath® Decalcifier II (Surgipath Medical Industries, Richmond, IL, USA) and trimmed, and the mandibles and maxillae were collected. Jaws were processed for histological examination, sectioned (thickness, 5 μm), and stained with hematoxylin and eosin. The slides were examined using light microscopy at 40 to 400 \times (Olympus model BX40F4, Olympus, Tokyo, Japan).

Lesions, the development of which is a dynamic process, were rated from mild to severe based on the following criteria: A rating of mild is characterized by one or a few small, focal cysts, which is the initial phase of the lesion; a rating of moderate is characterized by the presence of several foci of cysts or islands of squamous epithelial cells, or a few larger cysts, that invade the periodontal pockets and disrupt the teeth; a rating of severe is assigned when cysts, increasing in size and invasiveness, infiltrate throughout the mandible and/or maxilla and destroy all surrounding tissues, including the alveolar bone that supports tooth structure. Correlation between the total PCB and TEQ concentrations in the liver and the severity of the lesion (numerically scored from one (mild) to three (severe) based on the above-described rating description) was assessed by the Pearson correlation analysis using SAS® PROC CORR (Release 8.0; Statistical Analysis System, Cary, NC, USA).

RESULTS

Mink from both the KRAOC and FCRA appeared to be healthy based on body weight, gross examination, and histo-

logical assessment of liver tissue. However, four of the nine mink from the KRAOC had histological evidence of mandibular and maxillary squamous epithelial hyperplasia (Fig. 1), and none of the mink in the FCRA had the lesion. The severity of the lesion ranged from mild to moderate (Table 1).

Total concentrations of PCBs in the livers of the four mink from the KRAOC that expressed the lesion ranged from 2.9 to 6.0 mg/kg wet weight. Total PCB concentrations in the livers of the other five mink from the KRAOC ranged from 0.05 to 3.4 mg/kg wet weight. Concentrations of TEQs ranged from 0.21 to 1.3 $\mu\text{g}/\text{kg}$ wet weight in the livers of the four mink from the KRAOC that exhibited the lesion and from 0.02 to 0.25 $\mu\text{g}/\text{kg}$ wet weight in the five mink from the KRAOC not expressing the lesion (Table 1). Concentrations of total PCBs in the livers of the three mink from the FCRA ranged from 1.6 to 3.7 mg/kg wet weight, and concentrations of TEQs ranged from 0.05 to 0.20 $\mu\text{g}/\text{kg}$ wet weight (Table 1). Congener PCB 126 is known to be a potent inducer of squamous hyperplasia of the mandibles and maxillae [9–11], and in the present study, the primary PCB congener contributing to the TEQ concentrations in livers of the mink trapped in the Kalamazoo River Basin was PCB 126, with an average contribution of 77% at the KROAC and 69% at the FCRA (Table 1).

Lesion severity was significantly and positively correlated with concentrations of both total PCBs and TEQs in the livers of mink collected from the KRAOC. Correlation coefficients (r^2) were 0.88 ($p < 0.001$) and 0.89 ($p < 0.001$) for total PCBs and TEQs, respectively.

DISCUSSION

When six- and 12-week-old ranch mink kits were exposed to TCDD at 2.4 $\mu\text{g}/\text{kg}$ feed or to PCB 126 at 24.0 $\mu\text{g}/\text{kg}$ feed, they displayed gross displacement of the incisor and canine teeth, with nodular swelling of the mandibular and maxillary gingiva within four weeks of exposure. The PCB 126- and TCDD-induced lesion reported in these initial studies was characterized by hyperplasia of squamous epithelial cells that formed infiltrating nests and cords into the alveolar bone, causing osteolysis. The maxillae and mandibles became markedly porous because of loss of alveolar bone, with concomitant loss of teeth, which led to aphagia and rapid weight loss [9–11].

The lesion subsequently was reported in an unpublished study to develop by 24 weeks of age in ranch mink kits exposed to PCB 126 at 0.24 $\mu\text{g}/\text{kg}$ feed in utero through sexual maturity (K. Beckett). Histological evidence of hyperplasia of squamous epithelial cells in the mandibles and maxillae also was observed in the same study in adult mink fed a diet containing PCB 126 at 0.24 $\mu\text{g}/\text{kg}$ feed (K. Beckett), which is an environmentally relevant concentration [4,8,15]. Hepatic concentrations of PCB 126 and TCDD were not measured in the initial jaw lesion studies by Render et al. [9–11].

Concentrations of total PCBs and TEQs in the liver also were measured in a recent study in which ranch mink that were fed diets containing PCB-contaminated fish collected from the Housatonic River in Massachusetts (USA) developed the jaw lesion [8]. In that laboratory study, in which mink were exposed to a total PCB concentration of 1.6 mg/kg feed (TEQs, 0.02 $\mu\text{g}/\text{kg}$ feed) from conception through 31 weeks of age, the lesion was detected at a similar frequency as that reported in the present study. The average concentrations of total PCBs and TEQs in the livers of mink fed the diet containing Housatonic River fish were 3.5 mg/kg wet weight and 0.10 $\mu\text{g}/\text{kg}$

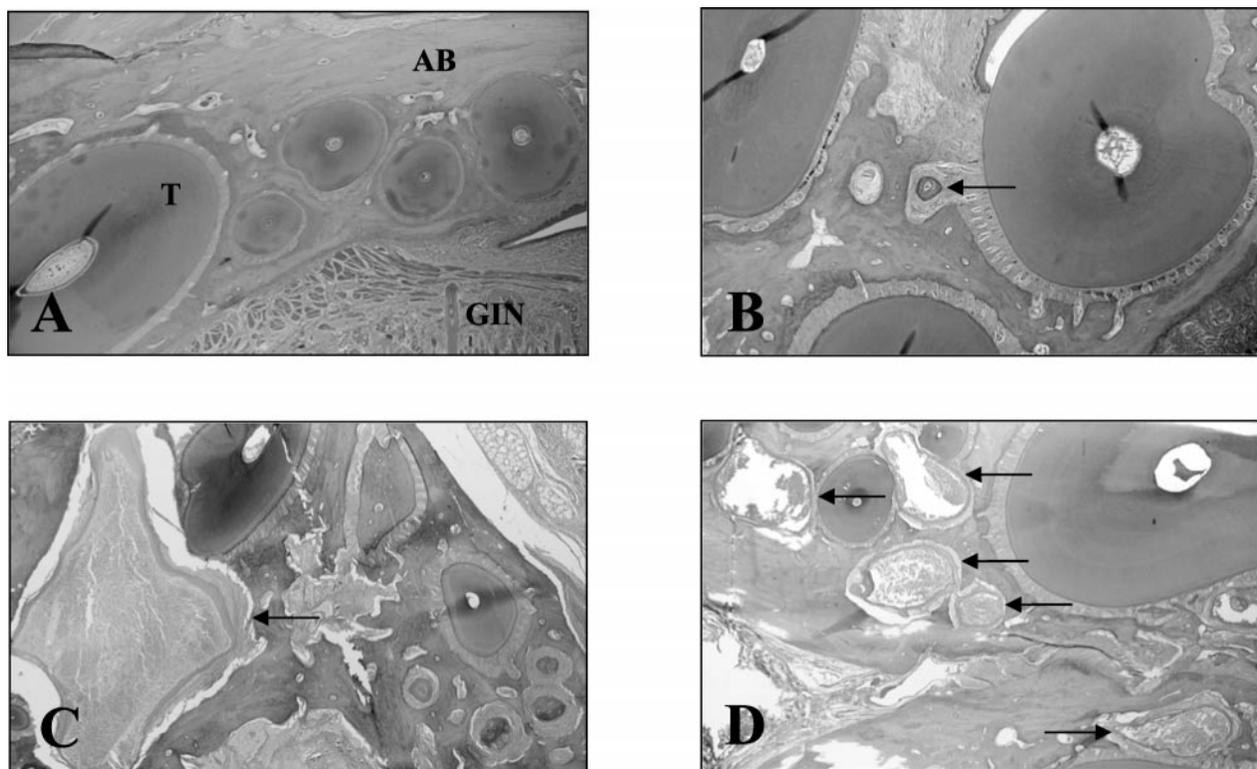


Fig. 1. Mandibular and maxillary squamous epithelial hyperplasia in wild mink exposed to polychlorinated biphenyls in the Kalamazoo River area of concern (KRAOC). (A) A section from a control mink is shown for reference. Note the solid-appearing alveolar bone (AB) surrounding the teeth (T), which is above the gingiva (GIN). (B) KRAOC mink 03 had a lesion rating of mild, with one focus (arrow). (C) KRAOC mink 06 had a lesion rating of mild–moderate, with a large invasive cyst (arrow) and small, multifocal, diffuse nests. (D) KRAOC mink 08 had a lesion rating of moderate, with diffuse squamous cysts throughout the jaw (arrows). Extensive infiltration by large islands of squamous epithelial cells has occurred and formed cysts that have resulted in the loosening of teeth. These cysts may contain centers of exfoliated epithelia and, sometimes, keratin.

wet weight, respectively. Of the mink trapped in the KRAOC, 44% had histological evidence of mandibular and maxillary squamous epithelial hyperplasia. The average concentrations of total PCBs and TEQs in the livers of the KRAOC mink exhibiting the lesion were 4.3 mg/kg wet weight and 0.60 $\mu\text{g}/\text{kg}$ wet weight, respectively. The average total PCB concentration in the livers of the KRAOC mink is comparable to the

value reported by Bursian et al. [8] in mink exposed to PCBs through consumption of fish collected from the Housatonic River (3.5 mg/kg wet wt). Concentrations of TEQs in the livers of mink from the KRAOC were sixfold greater than those in the livers of mink fed diets containing fish from the Housatonic River (0.10 $\mu\text{g}/\text{kg}$ wet wt). Hepatic concentrations of total PCBs and TEQs in livers of mink fed Housatonic River fish

Table 1. Incidence of mandibular and maxillary squamous hyperplasia, hepatic concentrations of total polychlorinated biphenyls (PCBs), toxic equivalents (TEQs), and percentage contribution toward toxic equivalents by 3,3',4,4',5-pentachlorobiphenyl (PCB 126) in wild mink trapped in the Kalamazoo River Basin (MI, USA)^a

Mink no.	Trapping location	PCBs (mg/kg wet wt)	TEQs ($\mu\text{g}/\text{kg}$ wet wt)	PCB 126 (%)	Lesion rating
08	KRAOC	6.0	1.3	91	Moderate
06	KRAOC	5.0	0.58	90	Mild–moderate
02	KRAOC	2.9	0.33	83	Mild
01	KRAOC	3.4	0.25	86	No lesion
03	KRAOC	3.3	0.21	72	Mild
07	KRAOC	1.0	0.03	68	No lesion
09	KRAOC	0.05	0.03	99	No lesion
11	KRAOC	1.6	0.03	0	No lesion
25	KRAOC	1.1	0.02	26	No lesion
		<i>2.8 ± 0.7</i>	<i>0.30 ± 0.14</i>		
1002	FCRA	3.68	0.20	80	No lesion
1003	FCRA	1.55	0.08	59	No lesion
1001	FCRA	1.59	0.05	68	No lesion
		<i>2.3 ± 0.7</i>	<i>0.11 ± 0.04</i>		

^a KRAOC refers to the contaminated Kalamazoo River area of concern, and FCRA refers to the Fort Custer recreation area, which is the upstream reference site. Data for total PCB and TEQ concentrations in the liver are presented for individual animals and as the mean \pm standard error (in italics) for each of the two sites.

were determined in a pooled sample that was comprised of tissue from all animals in the group, regardless of lesion status. This could explain the difference in TEQ concentrations between these two studies. It is possible that hepatic concentrations of TEQs in only those mink in the feeding study exhibiting the lesion were greater than the group average (0.10 µg/kg wet wt) and closer to the average reported for the KRAOC mink with the lesion.

One mink each from the KRAOC and the FCRA did not develop the lesion (Table 1) yet had concentrations of total PCBs and TEQs in the liver expected to be associated with the expression of mandibular and maxillary squamous epithelial hyperplasia. One explanation is individual variation in sensitivity to TCDD-like chemicals. In addition, the duration of exposure, the age during exposure, and/or the time required for the lesion to develop could have been suboptimal for its manifestation in these individuals.

In conclusion, the data presented here indicate that wild mink trapped in the KRAOC exposed to environmentally derived PCBs developed the lesion characterized as mandibular and maxillary squamous epithelial hyperplasia. The average concentrations of total PCBs and TEQs in livers of mink trapped from the KRAOC that expressed the lesion were 4.3 mg/kg wet weight and 0.60 µg/kg wet weight, respectively. A positive correlation was found between hepatic total PCB and TEQ concentrations and the presence and severity of the jaw lesion observed in wild mink. Therefore, mink exposed to relevant concentrations of TCDD-like compounds could experience increased severity of the lesion, leading to erosion of the mandibles and maxillae, with concomitant loss of teeth and, eventually, aphagia. This jaw lesion, now identified in a wild mink population, poses a threat to wildlife health and survival. The results of the present study are consistent with the predicted hazard quotients based on concentrations of both total PCBs and TEQs in the liver and effects on survival and weight gain in mink kits whelped of mink exposed to these compounds in the diet. However, even though the lesion was observed in this population of mink, the body conditions of the mink did not indicate that it was resulting in effects on body weight [7]. These results indicate that the occurrence of the lesion in mink is a sensitive, functional measure of exposure to PCBs in the diet, but also that it may not translate into population-level effects. This may result from several compensatory factors, including sufficient reproductive capacity to offset adverse effects on the longevity of individual mink or the fact that the lesion does not become sufficiently severe during the normal lifetime of mink living in the wild.

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