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Chronic Effects of Polychlorinated Dibenzofurans on Mink in Laboratory and Field Environments

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Original Research

ABSTRACT

Mink are often used as a sentinel species in ecological risk assessments of chemicals such as polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), and dibenzofurans (PCDFs) that cause toxicity mediated through the aromatic hydrocarbon receptor. Considerable toxicological information is available on the effects of PCBs and PCDDs on mink, but limited toxicological information is available for PCDFs. Thus, exposure concentrations at which adverse effects occur could not be determined reliably for complex mixtures in which PCDFs dominate the total calculated concentration of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalent (TEQ). Two studies were conducted to evaluate the potential toxicity of PCDFs to mink. The first was a chronic exposure, conducted under controlled laboratory conditions, in which mink were exposed to 2,3,7,8-tetrachlorodibenzofuran (2,3,7,8-TCDF) concentrations as great as 2.4×10^3 ng 2,3,7,8-TCDF/kg wet-weight (ww) diet or 2.4×10^2 ng $TEQ_{2006\text{-WHO-mammal}}$/kg ww diet. In that study, transient decreases in body masses of kits relative to the controls was the only statistically significant effect observed. The second study was a 3-y field study during which indicators of individual health, including hematological and morphological parameters, were determined for mink exposed chronically to a mixture of PCDDs and PCDFs under field conditions. In the field study, there were no statistically significant differences in any of the measured parameters between mink exposed to a median estimated dietary dose of 31 ng $TEQ_{2006\text{-WHO-mammal}}$/kg ww and mink from an upstream reference area where they had a median dietary exposure of 0.68 ng $TEQ_{2006\text{-WHO-mammal}}$/kg ww. In both studies, concentrations of $TEQ_{2006\text{-WHO-mammal}}$ to which the mink were exposed exceeded those at which adverse effects, based on studies with PCDD and PCB congeners, would have been expected. Yet in both instances where PCDF congeners were the sole or predominant source of the $TEQ_{2006\text{-WHO-mammal}}$, predicted adverse effects were not observed. Taken together, the results of these studies suggest that the values of the mammalian-specific toxicity equivalency factors suggested by the World Health Organization overestimate the toxic potency of PCDFs to mink. Therefore, hazard cannot be accurately predicted by making comparisons to toxicity reference values derived from exposure studies conducted with PCBs or PCDDs in situations where mink are exposed to TEQ mixtures dominated by PCDFs.

Keywords: *Mustela vison* 2,3,7,8-TCDF 2,3,4,7,8-PeCDF TEFs Furan

INTRODUCTION

The Tittabawassee River, which flows through mid-Michigan into the Saginaw River and then into Saginaw Bay, contains detectable concentrations of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs). The concentrations of PCDF are significantly greater than those in other watersheds of the region and include some of the greatest concentrations of PCDF ever reported (Hilscherova et al. 2003). In particular, 2 congeners, 2,3,7,8-tetrachlorodibenzofuran (2,3,7,8-TCDF) and 2,3,4,7,8-pentachlorodibenzofuran (2,3,4,7,8-PeCDF), contributed most of the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalent ($TEQ_{2006\text{-WHO-mammal}}$) in floodplain soils, sediments, and dietary items of the mink (Zwiernik et al. 2008b).

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Mink are the preferred receptor species in ecological risk assessments where PCDDs, PCDFs, PCBs, and other structurally similar, dioxin-like compounds that interact with the aryl hydrocarbon receptor (AhR) (Giesy et al. 1994b). This is because mink, as apical carnivores, consume a great amount of food relative to their body mass and are among the mammals that are more sensitive to AhR-mediated effects (Aulerich et al. 1985; Hochstein et al. 1988; Tillitt et al. 1996). As such, mink are often predicted to have the greatest potential for adverse effects in multispecies risk calculations for sites with a substantial aquatic habitat (Basu et al. 2007). Thus, risk-based cleanup goals are often derived for mink in situations where risks are predicted to occur because of AhR-active compounds (Kannan et al. 2000).

An initial hazard assessment based on concentrations of PCDD/DF in fish from the Tittabawassee River and toxicity reference values (TRVs) based on compounds other than 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF resulted in values of hazard quotients (HQs) that were greater than 1.0, which suggested potential adverse effects on mink (Galbraith

Table 1. Concentrations of 2,3,7,8-tetrachlorodibenzofuran (2,3,7,8-TCDF) and $TEQ_{2006\text{-WHO-mammal}}$ measured in the diet and predicted in the liver of mink exposed under laboratory conditions[a]

Concn.	Control	Low	High
Diet (ng 2,3,7,8,-TCDF/kg, diet ww)	0.0	2.4×10^2	2.4×10^3
Diet (ng $TEQ_{2006\text{-WHO-mammal}}$/kg diet, ww)[b]	2.0	2.6×10^1	2.4×10^2
Liver (ng 2,3,7,8-TCDF/kg liver, ww)[c]	0.0	3.6×10^1	9.8×10^2
Liver (ng $TEQ_{2006\text{-WHO-mammal}}$ kg liver, ww)[bc]	2.8×10^{-1}	3.6	9.9

[a] TEQ = TCDD equivalent.
[b] $TEF_{2006\text{-WHO-mammal}} = 0.1$.
[c] Predicted from bioaccumulation factor.

Environmental Services 2003). However, surveys of the conditions of individual mink and the mink population, including track surveys, trapping, and age distributions, and sex ratios, indicated that the mink population was not being adversely affected. Therefore, field and laboratory investigations were conducted to determine the reason for this apparent inconsistency between the predicted and observed responses of mink to these AhR-active compounds (Zwiernik et al. 2008b). Here we present the results of 2 studies designed to provide insight into toxicity of 2,3,7,8-TCDF to mink. The first was a controlled laboratory study designed to identify measurement endpoints that were affected by exposure to 2,3,7,8-TCDF and to develop dose–response relationships. The second was a 3-y field study of wild mink from the Tittabawassee River, Michigan, USA, where they were chronically exposed to a mixture of AhR-active compounds, dominated by 2,3,7,8-TCDF and 2,3,4,7,8-PCDF. Effect concentrations expressed as $TEQ_{2006\text{-WHO-mammal}}$ in either the tissues or the diets of mink determined from the results of these 2 studies were compared to TRVs derived from studies with PCDDs and PCBs.

MATERIALS AND METHODS

Laboratory study

The laboratory study described herein was designed to determine the threshold for toxic effects to mink exposed to 2,3,7,8-TCDF (Ultra Scientific, North Kingstown, RI, USA; >99% purity) through the diet (Table 1). Methodologies were based on previously established protocols for determining the effects of chemicals on mink by evaluating ecologically relevant parameters of survival, health, and reproduction (USEPA 1991). Adults and kits were examined for sublethal effects including kit growth, organ masses, and tissue histology. Thirty randomly selected 10-month-old adult (P_0) pastel female mink were fed diets containing <MDL (non-detect or less than the method detection limit [MDL] of 0.30 ng/kg, ww), 2.4×10^2, or 2.4×10^3 ng 2,3,7,8-TCDF/kg on a wet-weight (ww) basis (Table 1). The dietary exposure to TCDF was started in early February for P_0 females, 3 weeks prior to the initiation of breeding, and continued for the duration of the study. At the end of the exposure period, necropsies were conducted on all P_0 and a randomly selected subset of F_1 mink. Following processing, the jaws were examined histologically for the presence of squamous epithelial cell proliferation as described in Beckett et al. (2005).

Female mink were housed individually in wire-meshed breeder cages (61 cm L × 76 cm W × 46 cm H) with an attached wooden nest box (30 cm L × 22.5 cm W × 25 cm H) lined with aspen shavings at the Michigan State University (MSU) Experimental Fur Farm containment facility. Lighting in the room simulated the natural light/dark cycle for the Eastern Standard Time zone. Mink were observed daily for the duration of the study for any clinical signs of toxicity including but not limited to refusal to eat, changes in physical appearance, and behavior. Unexposed males were used for breeding, which began in late February. All potential matings were verified by checking postcoital vaginal aspirations microscopically for sperm. An initial positive mating was followed by a second mating the next day and additional breeding attempts on days 8 and 9. Each female had at least 1 positive mating. The kits, herein referred to as F_1 kits, were exposed to 2,3,7,8-TCDF in utero and throughout lactation. The F_1 kits began to eat solid food at approximately 4 weeks and received the same diet that their dams had been fed (0.0, 2.4×10^2, or 2.4×10^3 ng 2,3,7,8-TCDF/kg ww diet). The F_1 kits were weaned by 6 to 8 weeks of age and maintained on their respective diets for an additional 64 weeks.

Dietary exposure to TCDF

A standard dietary mix was used with and without spiking with 2,3,7,8-TCDF. The base diet was used as the control, while 2,3,7,8-TCDF was added to create a "low" dose and a "high" dose that was nominally 10-fold greater. The measured concentrations in the control, "low," and "high" dose treatment groups were <MDL (MDL = 0.30 ng/kg, ww), 2.4×10^2, and 2.4×10^3 ng 2,3,7,8-TCDF/kg, ww diet, respectively (Table 1). The base feed used in all diets contained a total concentration of 2.0 ng $TEQ_{2006\text{-WHO-mammal}}$/kg ww based on 17 2,3,7,8-substituted PCFD and PCDD congeners and 12 individual PCB congeners. Feed was frozen (−7°C) in 2-L containers (1–2-d supply) and thawed in a walk-in cooler (4°C) as needed. Water was available ad libitum. Dams and their offspring were maintained on their treatment diet throughout the course of the study. Dietary assignment was based on equal mass distribution of females over the specified dietary treatments.

Necropsies and histological assessment

Each mink was weighed and its length determined (with and without tails) and then was examined by a board-certified veterinary pathologist (MSU Diagnostic Center for Population and Animal Health) both internally and externally for overall health, nutritional status (scored on a scale of 1 [poor] to 4 [excellent]) based on body condition, and the presence of fat and the presence of gross abnormalities. The reproductive status of each animal was assessed, and major organs and tissues (brain, kidney, liver, spleen, heart, lung, adrenal glands, thyroid, gastrointestinal [GI] tract, and reproductive tissues) were removed, examined, and collected. Tissues were fixed in 10% buffered formalin (pH 7.4) for subsequent histological assessment using hematoxylin and eosin–stained sections.

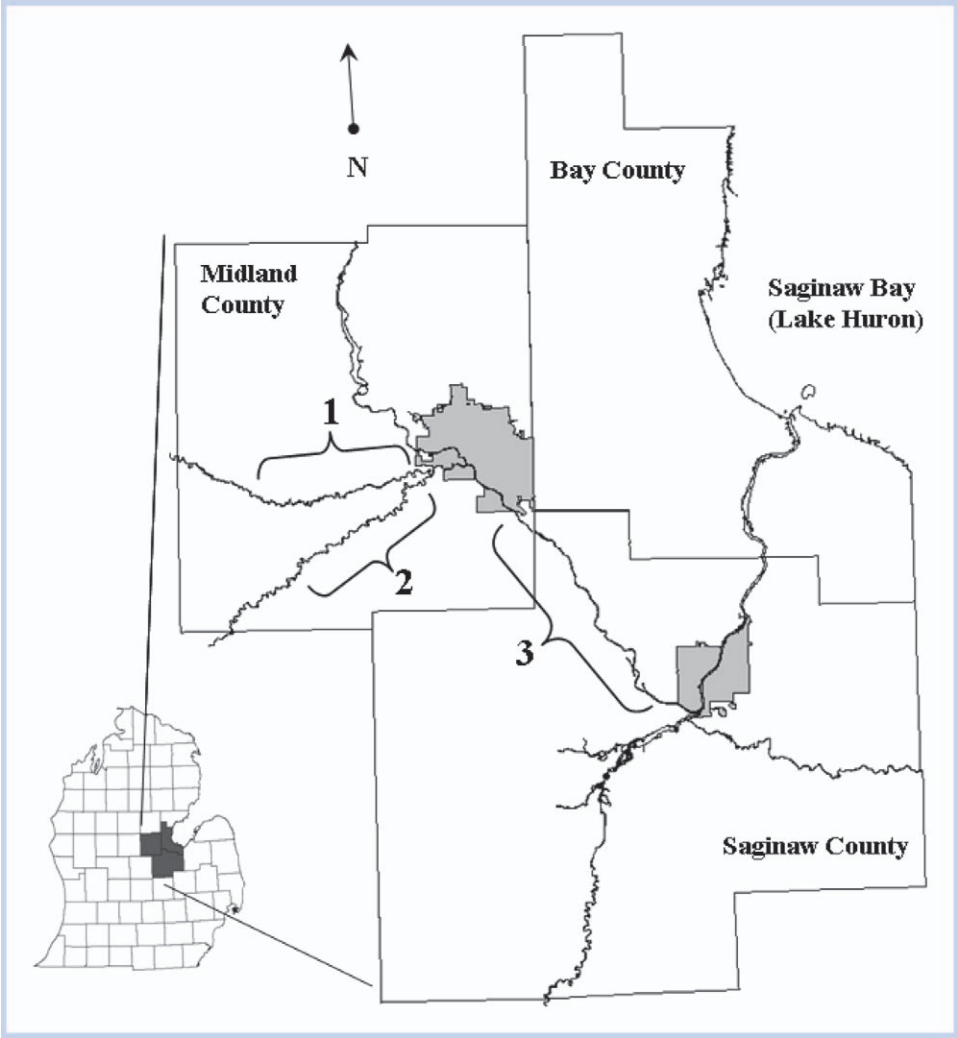

Figure 1. Tittabawassee River study area. (**1**) Chippewa River (reference); (**2**) Pine River (reference); (**3**) Tittabawassee River (target).

Uteri were grossly and histologically examined for implantation sites as well as for signs of early embryonic death or late fetal death. In addition, ovaries were histologically examined for signs of ovulation and degree of follicular development. Mink heads were removed and decalcified in Decal II solution (SurgiPath, Medical Industries, Richmond, IL, USA). Following processing, the jaws were examined histologically for the presence of squamous epithelial cell proliferation as described in Beckett et al. (2005). Lesions were graded as mild, moderate, or severe based on the number and size of foci of squamous cell proliferation in the maxilla and mandible.

FIELD STUDY

Forty-eight wild mink, 22 from the target area and 26 from the reference areas, were collected throughout the Tittabawassee River drainage basin (Figure 1) during the winters of 2003 to 2005. Habitat suitability was determined by use of the US Fish and Wildlife Service (USFWS) mink habitat suitability index model, which has been validated for the Great Lakes region (Allen 1986). Gross and histological examinations were made. Exposure of mink to TEQ was assessed by comparing the concentrations of 17 individual 2,3,7,8-substituted PCDF and PCDD congeners and 12 individual PCB congeners measured in the livers of collected mink to the concentrations of these same compounds in colocated dietary items, including fish, muskrats, small mammals, and frogs.

Adult mink collection (trapping)

Approximately 100 traps were set and checked on a daily basis. The location of each trap was determined by global positioning system. Identification tags were affixed to the carcass of each trapped mink, and field conditions (e.g., weather and trap set details) were recorded before transporting the mink to a secure field location for processing and subsequent delivery to the MSU Aquatic Toxicology Laboratory.

Determination of mink abundance and habitat suitability

Suitability of habitat to support mink as well as the abundance of mink and their population age structure were determined for both the target (greater concentrations of PCDDs and PCDFs) (Tittabawassee River, MI, USA) and the reference (Chippewa and Pine rivers, MI, USA) areas. Methods to estimate mink abundance included surveys of the presence and extent of scat and tracks as well as visual observations of mink presence and trapping (Zwiernik et al. 2008b). In addition standard operating procedures that

contain very detailed methods for all aspects of mink collection, sampling, necropsy, record keeping, and archiving are available from the corresponding author. Observations were recorded prior to and throughout the study (2003–2005).

Necropsy of mink collected from the field

Prior to necropsy, mink were examined for overall health, nutritional status, and the presence of gross abnormalities by a board-certified veterinary pathologist. Necropsies were performed as described in the laboratory methods except for the following differences. Once removed, the masses of individual mink livers were determined and apportioned for analytical and histological procedures. A 2-g sample of liver tissue was placed in a 10% formalin–saline solution (10% formalin in 0.9% sodium chloride) for histological examination. The remaining liver tissue was divided into 3 10-g aliquots for determination of concentrations of PCDDs, PCDFs, and PCBs. These samples were placed in I-Chem® jars (I-Chem, New Castle, DE, USA) and frozen at −20°C until used for quantification of residues or placed into archive. Kidneys, GI tract, 2 premolar teeth, baculum (os penis), or uterine horns/ovaries were removed and massed, and heads were collected. The entire GI tract contents were removed and rinsed through a stacking sieve (mesh numbers 5–230; Hubbard Scientific, Fort Collins, CO, USA), transferred to a glass tray, and dried at 90°C for 24 h. Afterward, the contents were hand separated into their major components—bone, feathers, exoskeleton, hair, teeth, scales, and miscellaneous—and compared to multiple identification keys, including vertebrate collections from the MSU museum. Dietary items were identified down to the lowest practical taxonomic classification and grouped by species or genus. Mean values for occurrence, excluding plant material, were converted to biomass based on the site-specific mean weights for collected individual prey items (small mammals, shrew, crayfish, frogs) or by comparisons to site-specific individuals when possible (Zwiernik et al. 2008b). An upper and lower premolar tooth was used to age each mink by microscopic analysis of the tooth's cementum annuli. The teeth were prepared as described by Fancy (1980) and aged based on Matson (1981). The uterus and ovaries were removed and the uterus examined for placental scaring.

Exposure assessment

Concentrations of 17 individual 2,3,7,8-substituted PCDF and PCDD congeners and 12 individual PCB congeners were measured in the dietary items and livers of mink collected from the Tittabawassee River by use of USEPA methods 8290 or 1668, respectively (USEPA 1994). Concentrations of TCDD equivalents ($TEQ_{2006\text{-}WHO\text{-}mammal}$) were calculated as the sum of the products of the concentrations of congeners multiplied by their respective toxic equivalency factor ($TEF_{2006\text{-}WHO\text{-}mammal}$) given by the World Health Organization (WHO) (Van den Berg et al. 2006). A surrogate value of one-half the detection limit (MDL) was used for concentrations less than the MDL.

Estimates of daily exposure of mink to $TEQ_{2006\text{-}WHO\text{-}mammal}$ were calculated using a modification of a generalized USEPA exposure model (Zwiernik et al. 2008b). The central tendency and upper centile dietary exposure concentrations were estimated by use of Monte Carlo (or resampling) (Zwiernik et al. 2008b). This procedure involved calculating the estimated daily dose 135500 times using a randomly sampled (with replacement) concentration in a dietary item from the complete data set for each dietary category. This procedure resulted in estimates of the daily dose that were created from site-specific dietary composition and measured dietary item contaminant concentrations. The dietary dose was set to be equivalent to the median and upper 95th percentile of the frequency distribution.

Because of limited quantities of liver, concentrations of 2,3,7,8-TCDF as well as histological examination and measurement of specific biochemical parameters could not be measured simultaneously in mink exposed under laboratory conditions. Therefore, to compare concentrations in liver from mink exposed in the laboratory to those collected from the field, concentrations of 2,3,7,8-TCDF in the livers of mink fed in the laboratory were estimated by use of a predictive relationship developed in a controlled laboratory study (Zwiernik et al. 2008a, 2008c). In that study, concentrations in mink liver were measured as a function of time, and it was determined that the mink had achieved more than 98% of steady state by 180 d. Kinetic parameters as well as a steady-state bioaccumulation factor (BAF) were extracted by curve fitting and used to predict concentrations in livers based on the 2 dietary concentrations. The BAF (liver:diet) for mink exposed to 9.9×10^1 or 1.9×10^3 ng 2,3,7,8-TCDF/kg diet ww were 1.4×10^{-1} and 4.1×10^{-2}, respectively. These values were used to predict the concentration of 2,3,7,8-TCDD in the livers of adult female mink.

Statistical analyses

Statistical analyses of all data were conducted with SAS software (Statistical Analysis System, Ver 9.1 Cary, NC, USA). Sample sets were analyzed for normal distribution by the Kolmogorov–Smirnov 1-sample test with Lilliefors transformation and for homogeneity of variance by F test. Samples were generally lognormally distributed, and therefore if assumptions of normality were not met, the data were log transformed. Parameters for adult mink, including body mass, organ masses, and morphological measurements, were analyzed by analysis of variance with significant differences subsequently verified using Dunnett's t test. Differences were considered to be significant with a type I error rate (α) of 5% level or less ($p \leq 0.05$). For the field data, reference areas were evaluated for similarity using a t test with the Satterwaite approximation. When there was not a statistically significant difference, the populations were pooled for comparison with the target area.

Permits and approvals

All the research was conducted under the guidance and permission of the MSU Institutional Animal Care and Use Committee (IACUC) and an attending veterinarian. Standard operating procedures for all activities were reviewed and approved by the IACUC. All the research was conducted under the necessary and appropriate state and federal permits. Copies of all permits and IACUC approvals are on file and are available from the corresponding author.

RESULTS AND DISCUSSION

2,3,7,8-TCDF laboratory mink feeding study

Exposure to 2.4×10^2 or 2.4×10^3 ng 2,3,7,8-TCDF/kg feed ww in the diet was predicted to result in concentrations

Table 2. Reproductive parameters of adult female mink and survival of mink kits exposed to 0.0, 2.4×10^2, or 2.4×10^3 ng 2,3,7,8-tetrachlorodibenzofuran/kg wet weight (2,3,7,8-TCDF/kg ww) in the diet under laboratory conditions[ab]

Treatment ng TCDF/kg diet ww[c]	Females	Females whelped	Gestation (d)	Kits whelped	Live born (%)	Survival to 3 weeks	Survival to 6 weeks	Survival to weaning
Control	10	8	51.5	57	46 (81)	29 (63)	29 (63)	29 (63)
2.4×10^2	10	10	51.0	67	45 (69)	24 (52)	24 (52)	24 (52)
2.4×10^3	10	8	53.5	52	35 (67)	27 (77)	26 (74)	26 (74)

[a] No statistically significant differences for any parameter at $p < 0.05$.
[b] Numbers in parentheses represent percent survival.
[c] Based on $TEQ_{2006\text{-WHO-mammal}}$, would be 2.0, 2.6×10^1, and 2.4×10^2 ng/kg ww, respectively.

of 3.4×10^1 and 9.8×10^2 ng 2,3,7,8-TCDF/kg liver ww. Based on the $TEF_{2006\text{-WHO-mammal}}$ value of 0.1, this would be equivalent to 3.4 and 9.8 ng $TEQ_{2006\text{-WHO-mammal}}$/kg liver ww for TCDF, respectively (Table 1).

Neither the lesser or the greater concentration of 2,3,7,8-TCDF resulted in any statistically significantly ($p < 0.05$) effects on any of the ecologically relevant measurement endpoints, including survival of adults or kits, adult body masses, number of adult females bred per treatment, number of adult females whelped per treatment, gestation length, kits whelped per female, number of kits live at birth, percent kits alive at birth, and kit survival to weaning (Table 2).

Among the more sensitive measurement endpoints, including kit masses, organ masses, relative organ masses, and organ and jaw histology, the only measurement endpoint that was statistically significantly different from controls was kit masses, but the effect was transient, being observed at 1 of 7 time points for 3 of 4 treatment groups and 4 consecutive time points for a single treatment and only when kits were separated by sex within treatments and time points (Table 3). Masses of 3-week old male kits that had been fed 26 or 2.4×10^2 ng $TEQ_{2006\text{-WHO-mammal}}$/kg ww diet were 17% and 26% less than the mass of the of the unexposed kits, respectively. Masses of female kits exposed to the lesser dose were 17% less than that of controls after 24 weeks. A sustained reduction in kit mass ranged from 15% to 20% and was observed at weeks 6, 12, 24, and 36 in female kits fed 2.4×10^2 ng $TEQ_{2006\text{-WHO-mammal}}$/kg ww diet. All statistically significant mass reductions were transient and no longer significant relative to controls at

Table 3. Masses (g) of mink kits (mean ± SD) and sample sizes at 7 time points during exposure. The adults and their respective offspring were exposed to 2 different concentrations of 2,3,7,8-tetrachlorodibenzofuran (2,3,7,8-TCDF)

	Control (*n*)[a]	2.4×10^2 ng 2,3,7,8-TCDF/kg ww[b] (*n*)	2.4×10^3 ng 2,3,7,8-TCDF/kg ww (*n*)
Female			
Birth	8.0 ± 1.0 (22)	7.0 ± 2.0 (19)	8.0 ± 1.0 (17)
Week 3	115 ± 18 (13)	101 ± 13 (9)	98 ± 25 (14)
Week 6	284 ± 65 (13)	256 ± 47 (9)	227* ± 30 (13)
Week 12	840 ± 48 (10)	810 ± 192 (7)	700* ± 53 (11)
Week 24	1170 ± 130 (6)	970* ± 115 (5)	940* ± 90 (6)
Week 36	1160 ± 107 (6)	990 ± 107 (4)	970* ± 123 (5)
Adult (≥48 weeks)	1000 ± 196 (6)	760 ± 29 (3)	850 ± 39 (5)
Male			
Birth	9.0 ± 2.0 (24)	8 ± 2.0 (25)	8.0 ± 2.0 (18)
Week 3	126 ± 14 (16)	104* ± 10 (15)	94* ± 20 (13)
Week 6	320 ± 53 (16)	302 ± 57 (15)	273 ± 93 (13)
Week 12	1190 ± 134 (13)	1220 ± 207 (12)	1040 ± 154 (7)
Week 24	1890 ± 205 (10)	1840 ± 422 (8)	1510 ± 364 (5)
Week 36	2160 ± 229 (7)	2240 ± 358 (8)	2080 ± 156 (3)
Adult (≥48 weeks)	1660 ± 142 (6)	1810 ± 203 (7)	1650 ± 123 (3)

[a] *n* = number of kits.
[b] ww = wet weight.
* Significantly different ($p < 0.05$) from control.

Table 4. Morphological data (mean ± SD) from wild mink trapped in the Tittabawassee River Basin, MI, USA

	Males		Females	
	Reference ($n = 18$)	Target ($n = 15$)	Reference ($n = 8$)	Target ($n = 7$)
Body mass (g)	910 ± 142	962 ± 218	481 ± 73.2	517 ± 26.4
Body length (cm)	58.8 ± 2.57	58.7 ± 4.08	47.9 ± 2.82	50.8 ± 1.04
Liver mass (g)	50.6 ± 10.1	55.7 ± 18.4	27.2 ± 3.03	31.6 ± 4.48
Brain mass (g)	8.29 ± 0.59	8.05 ± 0.76[a]	6.34 ± 0.41	6.31 ± 0.59
Liver:brain ratio	6.14:1	7.21:1[a]	4.31:1	5.06:1
Age (y)	1.8 ± 0.8	2.2 ± 1.1	2.3 ± 0.7	2.7 ± 0.9
Nutritional status[b]	2.9	3.0	2.3	2.7
Number placental scars[c]	NA[d]	NA	3.9	4.3
Baculum length (mm)	42.6 ± 3.65	42.7 ± 2.79[e]	NA	NA
Sex ratio (M:F)	2.25:1 (reference)		2.14:1 (target)	

[a] $n = 14$ (brain from 1 mink not suitable for analysis).
[b] Average nutritional status value. Scale: 1 = poor, 4 = very good.
[c] Average number of scars per female mink.
[d] NA = not applicable.
[e] $n = 13$ (baculum from 2 mink lost during pelting process).

adulthood (22–64 weeks). Overall, both dams and offspring appeared normal and healthy regardless of treatment.

Field study of mink chronically exposed to furans

Suitable habitat for mink was found in the drainage basins of the Tittabawassee, Pine, and Chippewa Rivers. The habitat suitability indices, based on a scale of 0 to 100 (0 = poor, 100 = excellent mink habitat), were 57%, 67%, and 70% for the 3 rivers, respectively. These values were consistent with estimates of mink abundance based on track survey data on the 3 rivers, which were 1.3, 3.2, and 1.2 mink/km of river, respectively (Zwiernik et al. 2008b).

Neither sex ratio, age, nor any of the measures of morphology of captured mink were statistically different between the target and reference areas. The sex ratios of mink (M:F) of 2.3 in the target area (Tittabawassee River) and 2.1 in the reference areas (Pine and Chippewa Rivers) were not statistically significantly different ($p \geq 0.05$). The average age of adult mink trapped on the 3 river systems for the reference and target areas, respectively, were 1.8 ± 0.8 y, 2.2 ± 1.1 y for males and 2.3 ± 0.7 y, 2.7 ± 0.9 y for females. This age demographic is indicative of an established lightly harvested or low turnover mink population (Whitman 2003). No statistically significant differences ($p \geq 0.05$) were observed in morphological measures, including body mass, length, liver or brain mass, brain:liver mass ratio, nutritional status, placental scars, or baculum length, between mink trapped from the target area ($n = 22$) when compared to mink from the reference area ($n = 26$) (Table 4).

Assessments of potential effects of AhR-active compounds, such as the PCDF congeners that account for most of the $TEQ_{2006\text{-WHO-mammal}}$ at the Tittabawassee River site, are complicated by the fact that these compounds can cause a number of effects, the compounds occur in mixtures, and the individual compounds have different absolute and relative potencies to elicit adverse effects; 2,3,7,8-TCDD and related compounds have been implicated in many biological alterations (Giesy et al. 1994a, 1998). To simplify the risk assessment process, it has been assumed that the effects mediated through the AhR are the critical responses, occurring at the least concentrations in mixtures. The predicted AhR response is calculated by multiplying the relative potency or toxic equivalency factor (TEF) of each congener in the mixture when normalized to 2,3,7,8-TCDD, which is summed in an additive model (Van den Berg et al. 1998, 2006). Toxic equivalency factors provide the framework for potency normalization whereby the concentration of 1 or more AhR-active compound can be multiplied by the appropriate TEF and then added to describe the sum toxicity of an environmental mixture in terms of total 2,3,7,8-TCDD dioxin equivalents or TEQ (Van den Berg et al. 1998; Blankenship and Giesy 2002; USEPA 2003; Van den Berg et al. 2006). Twenty-nine of the 419 congeners associated with PCDDs, PCDFs, and PCBs, which are known to cause AhR-mediated toxicity, have been assigned TEF values. These include 17 2,3,7,8-substituted dioxin and furan congeners and 4 non-*ortho*- and 10 mono-*ortho*-substituted PCB congeners. The TEF values have been derived specifically to be protective estimates for use in risk assessments. If TEFs are accurate and assumptions of additive effects are met, then the normalized dioxin-like potency (TEQ) should predict the toxic effects of AhR-active compounds regardless of origin (individual congener or environmental mixture). The benefit of such an approach is that it allows for comparisons among studies and predictions of toxicological response (USEPA 2003).

In risk assessments, the responding parameter is referred to as a "measurement endpoint," which is then used to evaluate an ecologically based hypothesis or "assessment endpoint." Dose–response relationships for these alterations are used to develop TRVs of no-observable-adverse-effect level (NOAEL) and the lowest-observed-adverse-effect level (LOAEL). In particular, TRVs have been derived for the effects of AhR-active compounds, expressed as $TEQ_{2006\text{-WHO-mammal}}$, on

mink ($TRV_{TEQ\text{-}mink}$) (Blankenship et al. 2008). Risk of harm is assessed by comparing these TEQ-normalized TRVs to similarly normalized exposure calculations. Thus, accurately predicting effects requires accurate estimates of both TEF values and TRVs, which are not necessarily mixture, species, or endpoint specific. While there are a number of assumptions involved in applying the TEF approach, one of the implicit assumptions is that the dose–response curve of 1 AhR-active compound can be predicted from the normalized relative potency of a different compound and that this relative relationship remains constant regardless of the measurement endpoint (Giesy et al. 1998a; Blankenship et al. 2008). An additional complication is that some TEF values are based on acute cellular assays that may or may not include effects of toxicokinetics when applied to complex organisms.

The primary goal of this study was to test the hypothesis that the current $TEF_{2006\text{-}WHO\text{-}mammal}$ and $TRV_{TEQ\text{-}mink}$ derived from studies of other AhR-active compounds, such as congeners of PCBs and PCDDs, would accurately predict effects for mink in the wild. To do this, concentrations of $TEQ_{2006\text{-}WHO\text{-}mammal}$ in the diets and livers of mink collected from the field were compared to $TRV_{TEQ\text{-}mink}$ determined from studies of the effects of congeners other than 2,3,7,8-TCDF as well as responses to known concentrations of 2,3,7,8-TCDF in the diet of mink under laboratory conditions.

Effect of dietary 2,3,7,8-TCDF under laboratory conditions

Lesser masses of mink kits and appearance of mandibular and maxillary minor squamous epithelial cell proliferation have been identified as sensitive measurement endpoints that occur at concentrations less than those required to affect fertility, fecundity, survival of adults or kits, and mass of F_1 adults when exposed in utero and as kits (Restum et al. 1998; Beckett et al. 2005; Bursian et al. 2006b; Bursian et al. 2006b). Proliferation of both mandibular and maxillary squamous epithelial cells has been shown to be caused by 2,3,7,8-TCDD, PCB 126 as well as a number of environmental mixtures of dioxin-like compounds (Render et al. 2000a,b, 2001). In all cases where multiple endpoints were measured, mandibular and maxillary squamous epithelial cell proliferation occurred at doses less than those that caused adverse effects on reproduction or survival (Beckett et al. 2005; Bursian et al. 2006b). Similarly, lesser masses of kits were transient and were not correlated with long-term survival in the results reported here as well as in other studies (Bursian et al. 2006c). Thus, these 2 endpoints can be considered "early warning" functional measures of exposure. Ecologically significant effects would not be expected to occur if concentrations associated with these endpoints are not exceeded.

The NOAEL and LOAEL for mink exposed to 2,3,7,8-TCDF in the study reported herein were determined to be 2.6×10^1 and 2.4×10^2 ng $TEQ_{2006\text{-}WHO\text{-}mammal}$/kg diet ww, respectively. The lesser dietary concentration was classified as the NOAEL because only 1 of the 17 measurement endpoints resulted in a statistically significant effect. This measurement endpoint, mean body mass of kits, was statistically significant only when kits were separated by sex, and the response occurred at only 1 of 7 time points. Despite the notation as significant in this study, the body masses of kits were not outside the normal range of masses observed at the facility for unexposed kits of the same age (Heaton et al. 1995a; Bursian et al. 2006a, 2006b; Beckett et al. 2008). No differences in body mass when the kits became adults or effects on any of the other measurement endpoints studied were observed, including jaw lesions, which are a very sensitive response (Table 2). This dietary exposure resulted in a predicted steady-state liver concentration of 3.4×10^1 ng $TEQ_{2006\text{-}WHO\text{-}mammal}$/kg ww.

Exposure to 2.4×10^2 ng $TEQ_{2006\text{-}WHO\text{-}mammal}$/kg diet ww resulted in more consistent statistically significant effects on mass of the kits. Therefore, even though the effects on kit mass did not result in statistically significant effects on masses of the F_1 adults or survival, a value of 2.4×10^2 ng $TEQ_{2006\text{-}WHO\text{-}mammal}$/kg diet ww was designated as the LOAEL. Both the NOAEL and the LOAEL derived from this study are consistent with the range of TRVs given by Blankenship et al. (2008).

Effect concentrations observed in wild mink chronically exposed to PCDFs

No statistically significant differences or adverse effects were observed for any of the measurement endpoints, including squamous epithelial cell proliferation, the most sensitive endpoint examined, even though mink inhabiting the Tittabawassee River are exposed to a median predicted dietary concentration of 31 ng $TEQ_{2006\text{-}WHO\text{-}mammal}$/kg ww. The ranges of HQ for dietary exposure are 0.4 to 1.7 and 0.1 to 0.4 based on the NOAEL and LOAEL given by Blankenship et al. (2008), respectively, and using the 5th and 95th percentiles of the ADD to represent the range of dietary doses (Zwiernik et al. 2008b). This result suggests that one might expect some adverse effects on mink but that the effects might be subtle and of minimal severity. In fact, based on the results of the field study, a dietary NOAEL of >31 ng $TEQ_{2006\text{-}WHO\text{-}mammal}$/kg ww would be justified for chronic exposure. This finding is consistent with the NOAEL and LOAEL values, which were determined for 2,3,7,8-TCDF of 2.6×10^1 and 2.4×10^2 ng $TEQ_{2006\text{-}WHO\text{-}mammal}$/kg diet ww, respectively, during the laboratory study, because the threshold for effects would be expected to fall somewhere between these 2 values.

Risk assessment accuracy

Part of the reason for the wide range of values for the $TRV_{TEQ\text{-}mink}$, some of which would predict effects that were not observed in the field study, are uncertainties associated with the relative potencies of individual components that would differentially affect mixtures of varying composition. The uncertainty associated with the utilization of a $TRV_{TEQ\text{-}mink}$ based on exposure to dissimilar compounds, although each is AhR active, can be highlighted by comparing dose responses for similar measurement endpoints across studies where different congeners or AhR-active mixtures were utilized. The most direct comparison of relative potencies of 2 AhR-active congeners relevant to the field study can be made by comparing the data collected from the 2,3,7,8-TCDF study reported herein to a parallel study conducted at the same facility (MSU Experimental Fur Farm) and using the same methodologies, of 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) (Beckett et al. 2008) that met many of the WHO criteria of an ideal study design for determining relative effect potency (Van den Berg et al. 2006). Mink were exposed to concentrations of PCB 126 that were equivalent on both a mass or a $TEQ_{2006\text{-}WHO\text{-}mammal}$/kg diet basis to the dietary concentrations used in the 2,3,7,8-TCDF

laboratory study (0, 26, or 2.4×10^2 ng $TEQ_{2006\text{-}WHO\text{-}mammal}$/kg ww). The concentrations expressed as TEQ were equivalent because both compounds have been assigned a $TEF_{2006\text{-}WHO}$ value of 0.1. An additional dose of 2.4×10^3 ng $TEQ_{2006\text{-}WHO\text{-}mammal}$/kg ww was included in the PCB 126 study. Exposure of adult, female mink to 2.4×10^2 ng or 2.4×10^3 ng $TEQ_{2006\text{-}WHO\text{-}mammal}$/kg ww resulted in complete reproductive failure. However, when adult female mink were exposed to the same dose (2.4×10^2 ng $TEQ_{2006\text{-}WHO\text{-}mammal}$/kg ww) of 2,3,7,8-TCDF, they had a whelping rate (80%) that was not different from that of the controls ($p < 0.5$). Furthermore for the lesser dose (2.4×10^1 ng $TEQ_{2006\text{-}WHO\text{-}mammal}$/kg ww), kits in the PCB 126 study displayed an 80% incidence of mandibular and maxillary squamous epithelial cell proliferation or jaw lesions (K. Beckett, personal communication), while no jaw lesions were identified in the 2,3,7,8-TCDF study, even at a 10-fold greater exposure (Beckett et al. 2005). These comparisons demonstrate that there is a difference between the toxic potency for these 2 compounds for both reproductive and the more sensitive jaw lesion endpoints in mink.

Comparisons can also be made among threshold concentrations of other dioxin-like compounds to both absolute liver mass and relative liver mass, expressed as a percentage of brain mass (Aulerich and Ringer 1977; Heaton et al. 1995b; Restum et al. 1998). While this endpoint has been quantified in several studies, it is possible to express the NOAEL and LOAEL as TEQ in only 1 study (Heaton et al. 1995a, 1995b). Based on a dose-dependent change in relative adult liver mass, the reported NOAEL and LOAEL values were 0.7 and 16.8 ng $TEQ_{2006\text{-}WHO\text{-}mammal}$/kg ww, respectively. Even though concentrations of $TEQ_{2006\text{-}WHO\text{-}mammal}$ to which mink were exposed in the laboratory 2,3,7,8-TCDF feeding study and the field study were as much as 14-fold greater than the LOAEL and as much as 345-fold greater than the NOAEL, no statistically significant differences in absolute or relative liver masses were observed. The reason for this is likely the difference in the relative contributions of AhR-active congeners to the mixture. In the study conducted by Heaton et al. (1995a, 1995b), PCB 126 contributed 64% of the total TEQs, while TCDF contributed less than 2% to the diet. In the studies reported here, the dietary TEQ exposure of Tittabawassee River mink is made up of 75% furans and 10% PCBs, while for the associated laboratory study mink, 100% of their TEQ exposure originated from 2,3,7,8-TCDF.

Comparison of mixture effects on toxic potency of AhR-active compounds can be made by comparing masses of kits and survival of kits compared between the Heaton et al. (1995a) study and a study by Bursian et al. (2006a) where fish from the same area of Saginaw Bay collected at a later time were fed to mink. This comparison is particularly relevant because both studies were preformed at the same facility and utilized similar methods. In addition, both these studies are directly related to 2 studies described herein because the Tittabawassee River flows into the Saginaw River and into Saginaw Bay, resulting in exposure mixtures influenced by the same sources. The TEQ in the fish used in the study conducted by Heaton et al. (1995a) were contributed primarily by PCBs with the greatest contribution from PCB 126. Twelve years later, after PCB-oriented remediation in the Lower Saginaw River and Saginaw Bay, the percent contribution of PCBs to total $TEQ_{2006\text{-}WHO\text{-}mammal}$ was less (38%), likely because of localized source controls, while the relative contribution by furans was greater (24% relative to less than 10%). The mixture in the fish fed by Heaton et al. (1995a, 1995b) was more potent, resulting in lesser kit mass and survival at the least dose of 16.8 ng $TEQ_{2006\text{-}WHO\text{-}mammal}$/kg (ww) with complete reproductive failure at 67.5 ng $TEQ_{2006\text{-}WHO\text{-}mammal}$/kg ww. These results are contrary to those reported by Bursian et al. (2006a), where neither kit weights nor kit survival were adversely effected at doses of 22, 37, or 57 ng $TEQ_{2006\text{-}WHO\text{-}mammal}$/kg ww. One explanation for this outcome is that the reduction in PCB-relative percent contribution to the environmental mixture was responsible for the reduction in toxicological potency.

The most comprehensive comparison of mixture and congener toxicological potency can be made by comparing all the available dose–response relationships between concentrations of TEQ and occurrence of squamous epithelial cell proliferation or jaw lesions. As described previously, jaw lesions are a sensitive response of mink to 2,3,7,8-TCDD, PCB 126 and mixtures of dioxin-like compounds. The response intensity or percent occurrence of jaw lesions as well as $TEQ_{2006\text{-}WHO\text{-}mammal}$ has been compiled for 5 studies in which mink were exposed to various AhR-active compounds or combinations (Table 5). The presence and increasing frequency of jaw lesions is a direct function of the concentration of $TEQ_{2006\text{-}WHO\text{-}mammal}$ due to PCB 126 and non-*ortho* PCB. No clear relationship exists between the presence or frequency of jaw lesions and the total concentration of $TEQ_{2006\text{-}WHO\text{-}mammal}$, contributed by PCDD or PCDF, 2,3,7,8-TCDF, or mono-*ortho* PCBs. This does not mean that there is not a dose response for these compounds but rather that the data set is limiting. The environmental mixtures that resulted in jaw lesions had relatively great proportions of PCBs, specifically PCB 126, which may have confounded the correlation for other AhR-active compounds or groups. Furthermore, the response range may be limiting for some compounds, such as 2,3,7,8-TCDF, that did not induce a response at a $TEQ_{2006\text{-}WHO\text{-}mammal}$-normalized exposure, 35-fold greater than the least dose for a PCB-dominated mixture.

The results of other studies in which mink were exposed to PCDFs also suggest that observed responses were not consistent with what would be predicted from the $TEF_{2006\text{-}WHO\text{-}mammal}$ for PCDF congeners (Kihlstrom et al. 1992). In the study by Kihlstrom et al. (1992), a PCDF contaminant isolated from a commercial PCB mixture was added to the diet of mink either alone or in combination with PCBs. Exposure to the PCDFs, either singly or in combination, did not cause any adverse effects on any of the parameters measured. In fact, mink exposed to feed containing 15 ng Clophen A50–derived PCDFs/kg feed (ww) produced a significantly greater number of live kits, and the survival rate was greater than that of kits from unexposed dams. In a second, related experiment, mink exposed to feed containing 16 ng Aroclor 1254–derived PCDFs/kg feed (ww) appeared to reverse the adverse effects of PCBs to mink. The apparent discrepancies in dose response and additive effects observed in these experiments appear to be caused by 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF (Hagenmaier 1987; Brown et al. 1988), the 2 predominant furan congeners in mink collected from the Tittabawassee.

Mink from the Tittabawassee River were exposed to dietary concentrations of 31 ng $TEQ_{2006\text{-}WHO\text{-}mammal}$/kg ww, 75% of which was due to PCDFs, with a majority of that originating

Table 5. Effect levels for dietary exposure of mink to dioxin-like compounds in ng $TEQ_{2006\text{-}WHO\text{-}mammal}$/kg diet wet weight

Study	Sum TEQs[a]	PCB 126 TEQ[b]	2,3,7,8-TCDF TEQs[c]	% Jaw lesions	Dioxin TEQs	Furan TEQs	Non-ortho PCB TEQs	Mono-ortho PCB TEQs
Housatonic River fish lab study (Bursian et al. 2006b)	50	41	0.3	100% (6/6)	0.9	1.9	44	3.4
PCB 126 lab study (Beckett et al. 2008)	24	24	0	80% (12/15)	0	0	24	0
Saginaw River fish lab study (Bursian et al. 2006a)	57	19	2.1	75% (6/8)	20	14	20	3.2
Saginaw River fish lab study (Bursian et al. 2006a)	36	11	0.9	57% (4/7)	14	8.8	12	2.0
Housatonic River fish lab study (Bursian et al. 2006b)	12	9.8	0.1	33% (2/6)	0.3	0.5	10	0.8
Saginaw River fish lab study (Bursian et al. 2006a)	22	7.2	0.7	0% (0/8)	11	3.0	7.2	1.2
Housatonic River fish lab study (Bursian et al. 2006b)	6.8	5.4	0.1	17% (1/6)	0.3	0.3	5.8	0.5
Housatonic River fish lab study (Bursian et al. 2006b)	4.3	3.2	0.1	0% (0/6)	0.3	0.3	3.4	0.3
Tittabawassee River wild mink (Zwiernik et al. 2008b)	31	2.4	8.8	0% (0/22)	4.4	22	2.5	0.2
2,3,7,8-TCDF lab study (reported here)	2.4×10^2	0	2.4×10^2	0% (0/8)	0	2.4×10^2	0	0

[a] TEQs = 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents.
[b] PCB = polychlorinated biphenyl.
[c] 2,3,7,8-TCDF = 2,3,7,8-tetrachlorodibenzofuran.

from 2,3,7,8-TCDF (31%) and 2,3,4,7,8-PeCDF (37%). The PCB congeners contributed 10% of the $TEQ_{2006\text{-}WHO\text{-}mammal}$/kg diet (ww), and 11% was due specifically to 2,3,7,8-TCDD (Zwiernik et al. 2008b). Significant uncertainty seems to be associated with using $TRV_{TEQ\text{-}mink}$ based on an AhR exposure to a compound or a mixture that is dissimilar from the exposure to which the TRV is being compared. This is especially true for mixtures that differ greatly in the relative contribution of PCBs and furans and more specifically PCB 126 compared to 2,3,7,8-TCDF. Therefore, it would be expected that the most accurate TRV would be one developed from an exposure study that most closely resembles the AhR-active components to which it is to be compared. The absence of observable effects in mink from the Tittabawassee River is consistent with the HQ predicted based on the results of the dietary exposure assessment and TRVs based on a single primary compound, 2,3,7,8-TCDF, as well as the most applicable environmental mixture from just downstream of the site (Bursian et al. 2006a). A recent study of the kinetics of select AhR-active compounds in mink show that 2,3,7,8-TCDF is quickly metabolized compared to TCDD and 2,3,4,7,8-TCDF (Zwiernik et al. 2008b). Thus, the apparent discrepancy between calculated and realized toxic potency for 2,3,7,8-TCDF and mixtures containing 2,3,7,8-TCDF as compared to TCDD- and PCB 126–containing mixtures may be in part due to dissimilar metabolic degradation and elimination. Additional studies directly investigating the relative potency of 2,3,7,8-TCDF and 2,3,4,7,8-PeDCF to 2,3,7,8-TCDD are presently under way.

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