BIOCHEMICAL, HISTOLOGICAL AND REPRODUCTIVE EFFECTS IN MINK (*MUSTELA VISON*) EXPOSED TO POLYCHLORINATED DIBENZOFURANS (PCDFs) AND 2,3,7,8 TETRACHLORODIBENZO-*P*-DIOXIN (TCDD)

By

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Animal Science – Master of Science

2013

ABSTRACT

BIOCHEMICAL, HISTOLOGICAL AND REPRODUCTIVE EFFECTS IN MINK (*MUSTELA VISON*) EXPOSED TO POLYCHLORINATED DIBENZOFURANS (PCDFs) AND 2,3,7,8 TETRACHLORODIBENZO-*P*-DIOXIN (TCDD)

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In the Tittabawassee River basin, the major proportion of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-like exposure to mammals is from 2,3,7,8-tetrachlorodibenzofuran (TCDF) and 2,3,4,7,8-pentachlorodibenzofuran (PeCDF). Mink tissues collected from the Tittabawassee River had concentrations of TCDF and PeCDF that exceeded toxicity reference values (TRV), suggesting the potential for adverse effects. However, field evaluation of mink residing in the area indicated that the population was healthy. Two mink feeding studies were conducted to investigate the toxic potencies of TCDF and PeCDF in attempt to explain the unexpected lack of effects in the field. The first study was a toxicokinetic study that indicated hepatic cytochrome P450 activity can be used as an index of exposure to TCDF and PeCDF. The second study assessed the effects of feeding TCDD, TCDF or PeCDF at doses expected to cause adverse effects on reproduction and offspring viability and growth. The lack of significant effects on reproduction and offspring viability was unexpected based on TRVs established from other mammalian studies. Results suggest that the World Health Organization (WHO) Toxic Equivalency Factor (TEF) for TCDF requires further evaluation, and in the case of mink, the TEF for PCB 126 is underestimated or should be standardized outside the TCDD-centric TEF approach.

DEDICATION

In Memory of
Greg Markham
&
Robert "Bio Bob" Collins
&
Marie B. Moore

ACKNOWLEDGMENTS

Dr. Steven Bursian

Dr. Scott Fitzgerald

Dr. John Newsted

Dr. Matthew Zwiernik

Dr. John P. Giesy

Ms. Jane Link

Mr. Angelo Napolitano

Mr. Patrick Bradley

Dr. Marcus Hecker

Dr. Kerrie Beckett

Dr. Ben Yamini

Dr. Karl Strause

Mr. Andrew Christofferson

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Ms. Nozomi Ikeda

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ABBREVIATIONS

AhR	Aryl hydrocarbon receptor
ATL	Aquatic Toxicology Laboratory
EROD	Ethoxyresorufin-*O*-deethylase
EFF	Experimental Fur Farm
CYP1A1	Cytochrome P450, family 1, member A1
CYP1A2	Cytochrome P450, family 1, member A2
LOAEL	Lowest observed adverse effect level
MROD	Methoxy resorufin-o-deethylase
MSU	Michigan State University
NOAEL	No observed adverse effect level
PeCDF	2,3,4,7,8-Polychlorinated dibenzofuran
PCBs	Polychlorinated biphenyls
PCDD	Polychlorinated dibenzo-*p*-dioxin
PCDF	Polychlorinated dibenzofuran
PCB 126	3,3',4,4',5-Pentachlorobiphenyl
PAHs	Polycyclic aromatic hydrocarbons
SE	Standard error
TCDD	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin
TCDF	2,3,7,8-Tetrachlorodibenzofuran
TEF	Toxic equivalency factor
TEQ	TCDD Toxic equivalents
TR	Tittabawasssee River
TRV	Toxicity reference value

CHAPTER 1

INTRODUCTION

TCDD and TCDD-like compounds

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are primarily byproducts of commercial processes, but they are also naturally occurring compounds (Safe, 1990). Polychlorinated biphenyls (PCBs) are man-made structurally related compounds that along with PCDDs and PCDFs are ubiquitous, persistent, and toxic (Safe, 1998, Van den Berg et al., 1994). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the most studied, and considered the most potent, of these structurally related compounds (Van den Berg et al., 1998, 2006). TCDD-like compounds are chemicals with structures and mechanism of action similar to TCDD. There are 17 TCDD-like PCDDs and PCDFs, and 12 TCDD-like PCB congeners. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and TCDD-like compounds are located throughout the food chain (Giesy and Kannan, 1998). The persistence of these compounds is due to their lipophilic nature, which allows them to bioaccumulate (Safe, 1990) in tissues of fish, wildlife and humans. Effects of TCDD and TCDD-like chemicals in living organisms include enzyme induction, developmental deformities, reproductive failure, liver damage, wasting syndrome, and death (Giesy et al., 1994, Blankenship and Giesy, 2002). Further study of these compounds is warranted due to their ubiquitous presence, persistence and toxicity so that humans may avoid, minimize and/or manage the impacts that these compounds pose to all living organisms.

TCDD, PCDF and PCB configuration and mechanism of action

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and TCDD-like compounds are classified as polycyclic aromatic hydrocarbons (PAHs). These compounds are distinguished by two six-carbon ring structures connected by one or two "bridge bonds". TCDD has two "bridge bonds" each containing a single oxygen atom (Figure 1.1). TCDF and PeCDF have one "bridge bond" containing a single oxygen atom and another "bridge bond" linking two carbons from each ring (Figure 1.2a and 1.2b). The two rings comprising the PCBs are directly linked by a single carbon-to-carbon bond between the two rings (Figure 1.3). The various PCDD, PCDF and PCB congeners are further distinguished by the location and number of chlorine atoms on the carbon atoms of the rings.

There are eight positions on the carbon ring skeletons of PCDDs and PCDFs to which chlorine atoms may bind and there are ten potential binding sites on PCBs. Compounds that differ from one another only by number and/or location of chlorine atoms are called *congeners.* There are 209 possible PCB congeners, 135 PCDF congeners, and 75 PCDD congeners (Erickson, 1997). The chemical properties and toxic potencies of individual congeners are dependent on the number and positions of chlorine atoms on the two rings.

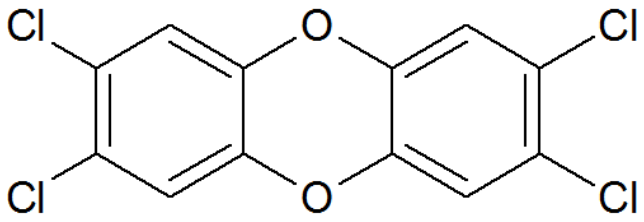


Figure 1.1. Configuration of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)

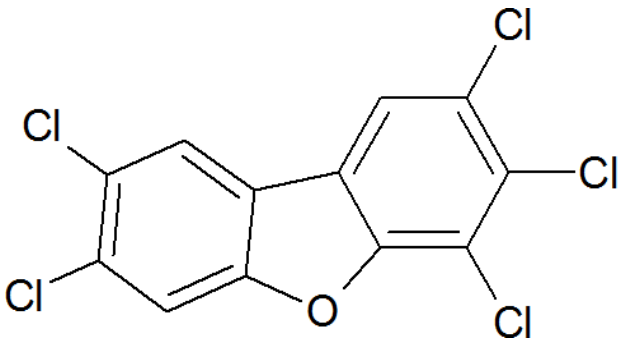


Figure 1.2a. Configuration of 2,3,4,7,8-pentachlorodibenzofuran (PeCDF)

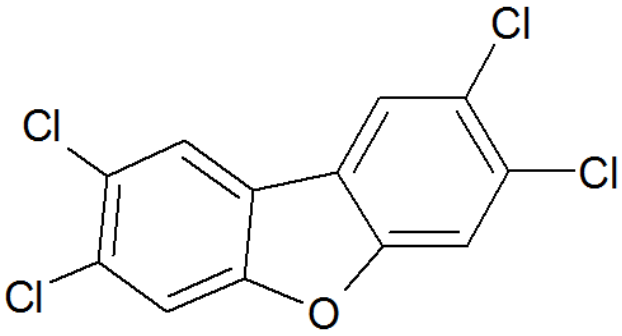


Figure 1.2b. Configuration of 2,3,7,8-tetrachlorodibenzofuran (TCDF)

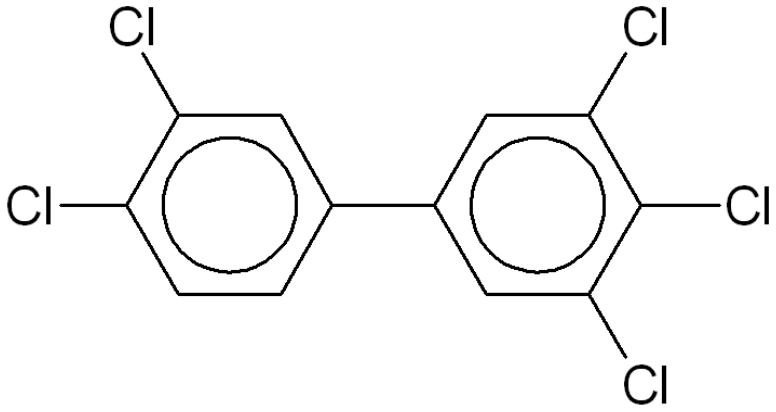


Figure 1.3. Configuration of 3,3’,4,4’,5-pentachlorobiphenyl (PCB 126)

Due to their co-planar structure, these TCDD-like compounds are known to induce a common suite of effects through a shared mechanism of action. This mechanism is mediated by binding of each planar ligand to a specific high-affinity cytosolic protein, known as the aryl hydrocarbon receptor (AhR) (Zwiernik et al., 2012). Once bound, the ligand and receptor complex translocate into the nucleus, and activates transcription of several genes including cytochrome P450 1A1 (CYP1A1) (Denison and Nagy, 2003).

PCDD, PCDF and PCB classification

The coplanar structure and lipophilic nature of PCDDs, PCDFs and PCBs place these contaminants in a class of compounds that are environmentally persistent and toxic to living organisms. The World Health Organization (WHO) developed a standardized approach known as the Toxic Equivalence (TEQ) method (Van den Berg et al., 2006) to quantify risk of harm to living organisms when these compounds are present. Since TCDD is believed to be the most toxic of these compounds, the toxicity of specific PCDD, PCDF and PCB congeners are evaluated relative to TCDD. Each congener is assigned a Toxic Equivalency Factor (TEF) value. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin is assigned a TEF value of 1.0, while TCDF is assigned a TEF value of 0.1, indicating that this furan is considered to be 10% as potent as TCDD. 2,3,4,7,8-Pentachlorodibenzofuran is assigned a TEF value of 0.3, thus it is thought to be 30% as potent as TCDD. 3,3',4,4',5-Pentachlorobiphenyl (PCB 126) is assigned a TEF value of 0.1, implying it is 10% as potent as TCDD. The TCDD-like activity contributed by each congener in a mixture expressed as TEQ is determined by multiplying the concentration of the congener by its TEF value. The total TEQ present in a mixture is the sum of the products of each congener's specific concentration and its specific TEF value

(Figure 1.4). For example, if a sample of liver contains concentrations of 10 ng TCDF/kg and 10 ng PeCDF/kg, then the TEQ contributed by TCDF and PeCDF are 1 ng (10 ng x 0.1) TEQ/kg and 3 ng (10 ng x 0.3) TEQ/kg, respectively, or a total TEQ sum of 4 ng/kg (1 ng TEQ/kg + 3 ng TEQ/kg).

$$TEQ = \sum_{i \to n} [(Congener_i \times TEF_i) + ...(Congener_n \times TEF_n)]$$

Figure 1.4. The TCDD Toxic Equivalents (TEQ) equation (Zwiernik et al., 2008)

Model for examining toxic effects

Because humans and other vertebrate species that share the same environment often have similar responses to toxic substances, mink (*Mustela vison*) may be used as surrogates to monitor environmental contaminant exposure and effects (Zwiernik et al., 2011). Basu et al. (2007) defines mink as a sentinel species because they meet certain criteria, these include: a (1) widespread distribution, (2) high trophic status, the (3) ability to accumulate contaminants, may be (4) maintained and studied in captivity, (5) captured in sufficient numbers, reside within (6) restricted home ranges, have a (7) well-known biology, and are (8) sensitive to contaminants. Laboratory and field studies of mink exposed to TCDD and/or TCDD-like compounds has shown adverse effects based on examination of morphological, histological, biochemical and reproductive characteristics. Mink are a model mammal to evaluate the risk of harm caused by TCDD and TCDD-like compounds because: (1) they are among the most sensitive species to PCBs (Aulerich and Ringer, 1977, Beckett et al., 2008) and related PCDDs (Hochstein et al., 1988, 1998); (2) their nutritional requirements are well

documented (National Research Council, 1982); a (3) stock of known genetic origin is readily available; (4) all stages of their life cycle can be successfully perpetuated in the laboratory; and (5) mink have a large biological and toxicological response data base (Shump et al., 1976, Scientifur, 1987, 1992; Sundqvist, 1989; Aulerich et al., 1999).

The Tittabawassee River and a sentinel species and laboratory model

The Tittabawassee River (TR) is the largest tributary of the Saginaw River/Bay watershed, Michigan, USA. The city of Midland is a major industrial and population center on the TR, where significant concentrations of polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-*p*-dioxins (PCDDs) have been found in sediments and floodplain soils (Hilsherova et al., 2003). The Dow Chemical Company (Dow) is a corporation headquartered in Midland since 1867 with the history of producing vast amounts of chemicals that have been exported throughout the world. Products of Dow have included agricultural chemicals, caustic soda, elemental chlorine, and bleach, as well as Agent Orange produced during the Vietnam War. The improper disposal of graphite anodes used in the chloralkali process has led to environmental contamination by 2,3,7,8-tetrachloridbenzo-p-dioxin (TCDD) and other TCDD-like compounds, including PCDFs.

Zwiernik et al. (2008a) reported concentrations of PCDDs and PCDFs in tissues of mammals collected in the TR (Michigan, USA) basin in 2003, 2004, and 2005. The average TEQ-adjusted TCDD-like concentrations in the livers from 22 wild mink, harvested downstream of Midland, Michigan averaged 400 ng TEQ/kg, of which 290 ng TEQ/kg was contributed by PCDFs and 21 ng TEQ/kg was contributed by PCDDs. The upstream control mink had average liver concentrations of 20 ng TEQ/kg that were more evenly distributed

among the PCDDs, PCDFs, and TCDD-like PCBs (Zwiernik et al., 2008a). Upstream median dietary exposure was 0.68 ng TEQ/kg and median dietary exposure in a downstream study area was 31 ng TEQ/kg (Zwiernik et al., 2008a). As one of the most highly exposed and most sensitive species based on the toxicological potency of these furan mixtures, dietary- and tissue-based exposure data suggested that mink residing in the TR basin, should be experiencing adverse effects. However, no pathology was reported for any of the 22 wild mink collected from within the study area and population measures including abundance and demographics, indicated that mink populations were stable and at, or close to, carrying capacity for the TR. Mink did not exhibit any adverse effects despite exposure to TEQ concentrations that exceeded dietary and hepatic concentration toxicity reference values (TRVs). In light of this disparity, it was concluded that additional information on the potency of the environmentally relevant toxic mixture of compounds found in the TR soils, sediments, and wildlife were needed. While it may be feasible to trap mink in order to evaluate morphological, histological and population characteristics, trapping live mink, and then studying their reproduction and the viability of their offspring is not. To provide risk managers with the best possible information pertaining to the potency of the site-specific contaminant mixture, two controlled feeding studies were conducted in which ranch mink were exposed to relevant PCDD and PCDF congeners at concentrations bracketing those observed in the field to determine dose-and-time dependent effects and to examine whether these congeners effect reproduction and offspring viability and growth.

Two mink feeding studies were conducted at the Michigan State University Experimental Fur Farm (EFF) to elucidate the toxic potencies of the two most prevalent TCDD-like PCDF compounds in TR sediment, soils, and mink. In the first study, adult

female ranch mink were fed TCDF, PeCDF, or a mixture of TCDF and PeCDF for 180 d. Doses were approximately eight times greater than doses in wild mink estimated in the TR field study (Moore et al., 2009). This study was conducted to assess: (1) the dosages of TCDF and PeCDF necessary to achieve liver concentrations bracketing those observed in wild mink, (2) time to achieve steady-state concentrations of the two congeners, and (3) effect of co-administration of TCDF and PeCDF on the toxicokinetics and distribution of each congener (Zwiernik et al., 2008b). This study also evaluated dose- and time-dependent effects of TCDF, PeCDF, or a mixture of these two congeners on hepatic P450 enzyme activity and tissue morphology, including jaw histology (Moore et al., 2009 and Chapter 2). Since TCDD, PeCDF and TCDF made up the majority of the calculated toxic potency based on TEQ using current WHO TEFs for the TR, the second study (Moore et al., 2012 and Chapter 3) assessed the reproductive performance of female mink fed diets containing TCDD, PeCDF or TCDF and the growth and viability of their offspring. In addition to bracketing field exposures, the dosing regime was expanded to cover a range of concentrations including those expected to elicit effects previously reported in mink exposed to TCDD-like compounds. Lesser doses were set to mimic nominal, environmentally relevant concentrations and were expected to result in no effects except for the most sensitive responses at the molecular level. In contrast, the highest dose for each congener expressed as TEQ exceeded median predicted environmental exposures for the TR. This highest dose was expected to cause reproductive effects based on results of laboratory studies in which mink fed TEQ-normalized concentrations of PCBs (Beckett et al., 2008, Bursian et al., 2006a,b,c, Heaton et al., 1995a, Heaton et al., 1995b, Tillitt et al., 1996) at similar levels experienced decreased litter size and/or reduce offspring viability.

The two studies presented herein contribute to the current body of knowledge used by risk managers to assess risk of harm to mink exposed to site-specific or environmentally relevant concentrations of TCDD-like compounds. Exposures at TEQ-normalized concentrations of TCDF and PeCDF in both studies resulted in adverse effects that were less than expected based on the TEFs assigned by the WHO as well as TRVs from other mammalian studies. In addition, the additive assumption of the TEQ method conflicts with results from the first study where two PCDFs were coadministered. Finally, results from the second study described herein and other reproductive feeding studies performed at the same facility with similar methodology suggest that the TEF for PCB 126 is underestimated and that PCB 126 should be evaluated relative to TCDD and TCDD-like compounds, or consideration should be given to standardize the toxicity of PCB 126 outside the TCDD-centric TEF approach. It is recommended that interactions of TCDD-like congeners be evaluated further while relative potency studies are necessary to compare single congener exposures of TCDD-like compounds to TCDD as well as PCB 126 to derive species-specific TEFs for a sensitive environmentally relevant wildlife receptor.

CHAPTER 2

HEPATIC P450 ENZYME ACTIVITY, TISSUE MORPHOLOGY AND HISTOLOGY OF MINK (*MUSTELA VISON*) EXPOSED TO POLYCHLORINATED DIBENZOFURANS (PCDFS)

ABSTRACT

Dose- and time-dependent effects of environmentally relevant concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TEQ) of 2,3,7,8-tetrachlorodibenzofuran (TCDF), 2,3,4,7,8-pentachlorodibenzofuran (PeCDF), or a mixture of these two congeners on hepatic P450 enzyme activity and tissue morphology, including jaw histology, of adult ranch mink were determined under controlled conditions. Adult female ranch mink were fed either TCDF (0.98, 3.8, or 20 ng TEQ_{TCDF}/kg body wt/d) or PeCDF (0.62, 2.2, or 9.5 ng TEQ_{PeCDF}/kg body wt/d) or a mixture of TCDF and PeCDF (4.1 ng TEQ_{TCDF}/kg body wt/d and 2.8 ng TEQ_{PeCDF}/kg body wt/d, respectively) for 180 d. Doses used in this study were approximately eight times greater than those reported in a parallel field study. Activities of the cytochrome P450 1A enzymes, ethoxyresorufin *O*-deethylase (EROD) and methoxyresorufin *O*-deethylase (MROD) were significantly greater in livers of mink exposed to TCDF, PeCDF and a mixture of the two congeners, however, there were no significant histological or morphological effects observed. It was determined that EROD and MROD activity can be used as sensitive biomarkers of exposure to PeCDF and TCDF in adult female mink, however, under the conditions of this study the response of EROD/MROD induction occurred at doses that were less than those required to cause histological or morphological changes.

INTRODUCTION

Recently, there has been concern about the concentrations of polychlorinated dibenzofurans (PCDFs), polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated biphenyls (PCBs) in floodplain soil and sediment from the Tittabawassee River (Hilscherova et al., 2003). The Tittabawassee River flows into the Saginaw River and Saginaw Bay, Michigan, USA, as part of the Lake Huron watershed. Both field and laboratory-based studies have been conducted to assess the potential risks of these concentrations of PCDD, PCDF and PCBs on terrestrial and aquatic organisms (Zwiernik et al., 2008a).

The mink (*Mustela vison*) has been utilized as a sentinel species for ecological risk assessments at sites where contaminants of concern are chemicals that can bind to the aromatic hydrocarbon receptor (AhR) such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and structurally similar compounds (Giesy et al., 1994; Tillitt et al., 1996; Blankenship et al., 2008; Basu et al., 2007). The mink is considered to be among the more sensitive mammals to TCDD and related compounds (Hochstein et al., 1988, 1998; Beckett et al., 2008). Mink have a relatively great potential for exposure to these persistent, bioaccumulative chemicals (Basu et al., 2007).

An ecological risk assessment using previously-established toxicity reference values (TRVs) derived primarily from studies of the effects of TCDD and other AhR-active compounds on mink (Blankenship et al., 2008) and concentrations of TCDD equivalents (TEQ) in the dietary and tissues of mink inhabiting the Tittabawassee River has been conducted (Zwiernik et al., 2008a). This study indicated that mink might be at risk of being adversely affected by these compounds with hazard quotients between <1 to 10 being

calculated. However, despite accumulating relatively great concentrations of 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) and 2,3,7,8-tetrachlorodibenzofuran (TCDF) in their livers the conditions of individual mink from the more highly contaminated areas of the Tittabawassee River was comparable or superior to that of mink collected in reference areas and that the population was robust (Zwiernik et al., 2008a). The inconsistency between the apparent healthy population and the elevated hazard quotient (HQ) estimates may due to several factors including: (1) World Health Organization (WHO) toxic equivalent factor (TEF) values and resulting TEQ concentrations are conservative and may have overestimated risk; (2) The toxicity reference values (TRVs) used to estimate the HQs may not have been accurate for mink due to lack of toxicological information for the dominant PCDF congeners identified in mink at the site relative to data available in the literature to derive TEFs: and (3) Uptake rates, metabolism, excretion and disposition of TCDF and PeCDF may differ from TCDD or PCBs that have been studied in mink (Beckett et al., 2008; Zwiernik et al., 2008b).

A 180-day dietary study was conducted to: (1) determine rates of assimilation and distribution of environmentally relevant doses of TCDF, PeCDF or a combination of the two congeners in liver tissue of mink (Zwiernik et al., 2008b); (2) examine the relationship between chemical exposure and hepatic cytochrome P4501A enzyme activities, potential functional indicators of exposure to AhR agonists (Hahn, 1998; Whitlock Jr., 1999; Kawajiri et al., 2007). Ethoxyresorufin *O*-deethylase (EROD) activity is most directly associated with the induction of hepatic activity of the cytochrome P4501A1 enzymes whereas methoxyresorufin *O*-deethylase (MROD) activity is more associated with P4501A2 enzymes. However, while both enzymes can metabolize either substrate to some extent, metabolism of both substrates provides valuable information as to P4501A activity in an organism relative

to its exposure to xenobiotics; and (3) examine relationships between EROD and MROD activity in liver to other morphological and histological changes in mink. This chapter presents the results of the effects of TCDF and PeCDF on hepatic EROD and MROD activities and selected morphological and histological parameters in mink.

MATERIALS & METHODS

Mink husbandry, exposure and necropsy

Adult, female, ranch mink were randomly assigned and housed individually in wire mesh breeder cages (61 cm L x 76 cm W x 46 cm H) with wooden nest boxes (30 cm L x 22.5 cm W x 25 cm H) within an indoor facility at Michigan State University (MSU). A total of 50 female mink were distributed among eight treatments with six individuals in each of seven furan-dosed groups (three TCDF groups, three PeCDF groups and one TCDF plus PeCDF group) and eight female mink in the control group. Doses were expressed as TEQ (Table 2.1) calculated by use of toxic equivalency factors (TEFs) reported by Van den Berg et al. (2006).

Table 2.1. Daily dose and concentrations of 2,3,7,8-tetrachlorodibenzofuran (TCDF) and/or 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) in the liver of mink (*Mustela vison*) [a].

Treatment	Daily dose (ng TEQ/kg body wt/d)	Liver concentration (ng TEQ/kg, ww) [b] 0 d	90 & 180 d
Control			
TCDF	<LOD[d]	<LOD[d]	0.79 ± 0.24
PeCDF	<LOD[d]	<LOD[c]	0.61 ± 0.46
TCDF	0.98	NA	1.2 ± 0.27
	3.8	NA	2.3 ± 0.22
	20	NA	7.1 ± 1.1
PeCDF	0.62	NA	52 ± 18
	2.2	NA	270 ± 25
	9.5	NA	1600 ± 530
Mixture			
TCDF	4.1	NA	1.4 ± 0.24
PeCDF	2.8	NA	360 ± 80

[a] Each treatment group had six mink while the control group had eight mink. Control animals were sampled at 0, 90 and 180 d; three treated animals per dose group were sampled at 90 and 180 d. All concentrations were converted to 2,3,7,8-TCDD equivalent

[b] Liver concentrations are presented as mean ±1 SD.

LOD = Limit of detection

[c] LOD = 0.1 ng TEQ/kg, ww

[d] LOD = 0.01 ng TEQ/kg, ww

[e] n=2, so no SD was calculated; one mink was euthanized because of kidney failure that was not treatment related.

NA indicates that samples were not collected.

The test chemical for each treatment was dissolved in hexane to produce a stock solution and aliquots of the stock were then diluted appropriately with 100 ml corn oil. The corn oil containing test chemical was added to the water component of the mink diet and mixed well in a paddle mixer prior to addition of the other feed ingredients. After addition of all of the dietary ingredients, the feed was mixed for an additional 20 minutes. Each morning

for 180 d, 25 g of feed containing the furan congener(s) was given to each animal. This procedure ensured complete ingestion of the contaminated feed, eliminating the need to measure daily feed consumption in order to estimate doses. After this feed was consumed, an additional 100 g of uncontaminated feed was given to each animal. Water was provided *ad libitum*. Full-spectrum lighting controlled by a timer simulated the natural light/dark cycle for the Eastern Standard Time Zone. Temperature was maintained between 13º C and 28º C and humidity ranged from 26% to 91%. Mink were observed daily for signs of toxicity including a decrease in feed consumption and lethargy. Individual body masses (g) were measured at the beginning of the study (January 31, 2006) and every 30 d thereafter.

Three animals from the control group were euthanized by asphyxiation with carbon dioxide at initiation of the exposure (0 d) and three animals from each of the eight treatment groups were euthanized at 90 and 180 d of exposure for subsequent necropsy. Body mass (g) and length (cm) including and excluding the tail were recorded for each female mink. Mink were examined externally and internally for overall condition, nutritional status and the presence of gross abnormalities. Livers were removed and weighed. Sub-samples of liver were frozen in liquid nitrogen for subsequent measurement of EROD and MROD activities. Approximately 2.0 g of liver tissue was placed in a 10% formalin-saline solution (10% formalin in 0.9% sodium chloride) for histological examination. The remaining liver was placed in I-Chem® jars (I-Chem, New Castle, DE, USA) and frozen at -20˚ C for subsequent determination of TCDF and PeCDF concentrations using High Resolution-Gas Chromatography/Mass Spectrometry (HR-GC/MS). In addition, the spleen, kidney, thymus, mesenteric lymph node, and brain were removed and preserved for subsequent histological examination. The head was placed in formalin-saline solution for subsequent histological

examination of mandibular and maxillary squamous epithelial cell proliferation as described by Beckett et al. (2005). The lesion was graded as mild, moderate, or severe based on the number and size of foci of squamous cell proliferation in the maxilla and mandible (Beckett et al., 2005). The MSU Institutional Animal Care and Use Committee approved this study (AUF 12/05 – 165 – 00).

Chemicals and reagents

PeCDF and TCDF were obtained from Accustandard Inc. (New Haven, CT, USA) and dissolved in hexane to produce a stock solution. Working solutions and dilutions of PeCDF and TCDF were prepared in pesticide residue analysis grade OmniSolv n-hexane (EM Science, Lawrence, KS, USA). For biochemical analyses, 7-ethoxyresorufin (7-ER) was obtained from Molecular Probes (Eugene, OR, USA) while 7-methoxyresorufin (7-MR) and resorufin were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other biochemical reagents including NADPH were obtained from Sigma-Aldrich and were reagent grade or better unless stated otherwise.

EROD and MROD quantification

Liver microsomes were prepared by homogenizing 0.5 g of liver in Tris buffer (0.05 M Tris and 1.15% KCl, pH 7.5) and centrifuged to obtain the microsomal fraction. The microsomal pellet was resuspended in microsomal stabilization buffer (20% glycerol, 0.1 M KH_2PO_4, 1mM EDTA, and 1mM dithiothreitol, pH 6.25) and aliquots were stored at -80^{o} C. EROD and MROD activities measured using a modification of methods described by Kennedy and Jones (1994). The assays were optimized and conducted in 96-well plates

(Corning Costar Corp., Corning, NY, USA) where both microsomal cytochrome P450 activity and protein concentration were measured simultaneously using a Fluoroscan Ascent microplate fluorometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). For EROD assays, the range of the working resorufin standards was 0 to 210 pmol/well. The reaction mixture included 3.0 µl of microsome preparation in 0.05 M HEPES buffer (pH 7.8), 0.3 mM NADPH and 5mM ethoxyresorufin (7-ER) per well. For MROD assays, the working resorufin standard range was 0 to 180 pmol/well. The reaction mixture included 8 µl of microsome preparation in 0.05 HEPES buffer (pH 7.8), 0.3 mM NADPH and 2.5 mM methoxyresorufin (7-MR) per well. Following the addition of the substrates (7-ER or 7-MR), all assay plates were pre-incubated for 10 min at 37°C prior to the addition of NADPH to initiate the reaction. EROD and MROD activities were determined kinetically by measuring the formation of resorufin every 2 min for 30 min. The reaction was terminated by adding 60 ml acetonitrile (Burdick and Jackson, Muskegon, MI, USA) containing 0.4 mM fluorescamine (Sigma-Aldrich, St. Louis, MO, USA) to each well followed by the determination of protein concentrations (Kennedy and Jones, 1994). EROD and MROD activities were determined from the linear range of the time-curves for each well and the results were expressed as pmol substrate converted per min per mg protein (pmol/min/mg).

Quantification of PCDD, PCDF and TEQ

To insure that co-contaminants were not a factor in the study, the 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 mink tissues as described in Zwiernik et al. (2008b). Concentrations of TEQ were calculated as the sum of the products of the concentrations of

congeners multiplied by their respective TEF (Van den Berg et al., 2006). A surrogate value of one-half the method detection limit (MDL) was used for concentrations less than the MDL

Data analysis

All statistical analyses were performed with SAS (SAS, Ver. 9.1; Cary, NC, USA). Because of the nature of the parameters, several statistical models were used for data analyses. The study was designed for the application of both fixed effects models (test for differences among exposure groups) and regression analysis (correlation of liver PeCDF and TCDF concentrations and EROD and MROD enzyme activities). Prior to conducting statistical comparisons, data were tested for normality using the Shapiro-Wilkes test and probability plots. If necessary, values were log-transformed to approximate normality. Differences among exposure groups were tested using a one-way ANOVA followed by Dunnett's test (PROC ANOVA). A sensitivity analysis was conducted to determine the bias introduced by assuming a value of half the limit of quantification (LOQ) for censured data sets.

RESULTS

PCDF concentration in liver

Concentrations of TCDF and PeCDF in livers of mink fed daily doses of TCDF, PeCDF or a mixture of the two congeners did not differ between 90 or 180 d, thus a single mean concentration is presented (Table 2.1).

Concentrations of TCDF in the liver varied among doses dose, ranging from 30% greater than the daily dose (0.98 TEQ_{TCDF}/kg body wt/d) to 65% less than the daily dose (20

TEQ_{TCDF}/kg body wt/d). Concentrations of PeCDF in mink liver increased significantly with dose, with bioaccumulation factors (BAFs) of 9.5 and 17 for the two doses 0.62 and 9.5 ng TEQ_{PeCDF}/kg body wt/d, respectively. Concentrations of TCDF and PeCDF in livers of mink fed the TCDF/PeCDF mixture were similar to concentrations in the livers of mink receiving a similar dose of the individual congeners. Hepatic BAFs based on TEQ concentration were 0.032 for TCDF (4.1 ng TEQ_{TCDF}/kg body wt/d) and 12 for PeCDF (2.8 ng TEQ_{PeCDF}/kg body wt/d).

Gross morphology and histology

There were no treatment-related changes in gross morphology or histology. No external lesions or abnormalities that were attributable to treatment were observed and the nutritional status of all mink, except for one individual was classified as "good" to "very good". There were no significant changes in body mass or liver mass over the course of the study (data not presented). The most frequent histological alteration was hepatocellular vacuolation that occurred in all groups, and thus, was not considered to be treatment-related (Table 2.2).

Table 2.2. Incidence of gross and histological effects in female mink exposed to either TCDF, PeCDF singly or as a mixture through the diet for up to 180 d [a].

		TCDF (ng TEQ/kg body wt/d)			PeCDF (ng TEQ/kg body wt/d)			TCDF/ PeCDF
Pathological Endpoints[b]	Control	0.98	3.8	20	0.62	2.2	9.5	Mixture[c]
Oral Lesions								
Squamous epithelial osteoinvasion	0	0	0	0	0	0	1	0
Osetoclasts and bone resorption	0	0	0	0	0	0	1	0
Periodontitis	1	0	0	0	0	0	0	0
Liver								
Hepatocellular vacoulation	5	6	6	6	6	6	6	6
Periportal lymphocytic/plasmytic	0	0	1	0	0	0	0	0
Fatty liver	0	1	0	1	1	3	2	0
Bile duct hyperplasia	0	0	1	0	1	1	0	0
Kidney								
Medullary tubules or uroliths	1	1	1	1	0	1	1	2
Infection	0	0	1	0	0	0	0	1
Nephritis	0	1	0	0	0	0	0	0
Lymphoid aggregates	0	0	0	0	0	2	0	0
Spleen								
Hemorrhage	0	0	0	0	0	0	0	1
Scar fissure	1	0	0	0	0	0	0	0

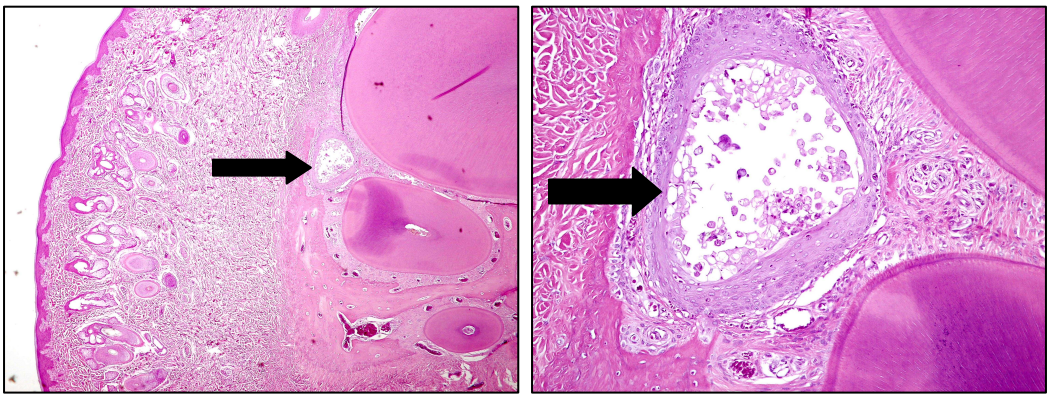
[a] Treatment concentrations are estimated daily doses reported as TEQ values. Mammalian TEF used were 0.3 for PeCDF and 0.1 for TCDF (Van den Berg et al. 2006). Mixture consisted of 4.1 and 2.8 ng TEQ/kg bw/d for TCDF and PeCDF, respectively.

[b] Values given for each endpoint represent the number findings (mink) associated with each treatment group (n=6 mink per treatment).

[c] Mixture consisted of 4.1 and 2.8 ng TEQ/kg body wt/d for TCDF and PeCDF, respectively.

There were a few cases of bile duct hyperplasia and minimal to mild mineralization of renal medullar tubules that occurred across all treatments. There was a numerically greater incidence of fatty liver in mink fed only PeCDF, compared to the other groups (Table 2.2). Periodontitis was observed in one mink from the control group, but this was considered incidental and not treatment-related. Jaw lesions classified as mild were observed at the termination of the study in two mink from the 9.5 ng TEQ_{PeCDF}/kg body wt/d treatment group (Table 2.2). One of these mink exhibited a single cyst consisting of squamous epithelial cells (Figure 2.1). However, the presence and severity of this lesion was not dose-dependent, and therefore, was considered incidental.

A B



For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis.

Figure 2.1. Single cyst consisting of squamous epithelial cells

EROD and MROD activities

Mink fed TCDF alone had significantly greater EROD and MROD activities in the liver compared to controls. Because there were no significant treatment by time interactions, enzyme activities measured after 90 and 180 d of exposure were averaged. Exposure to TCDF resulted in significantly greater activities of both EROD and MROD in mink at doses of 3.8 and 20 ng TEQ_{TCDF}/kg body wt/d (Figure 2.2).

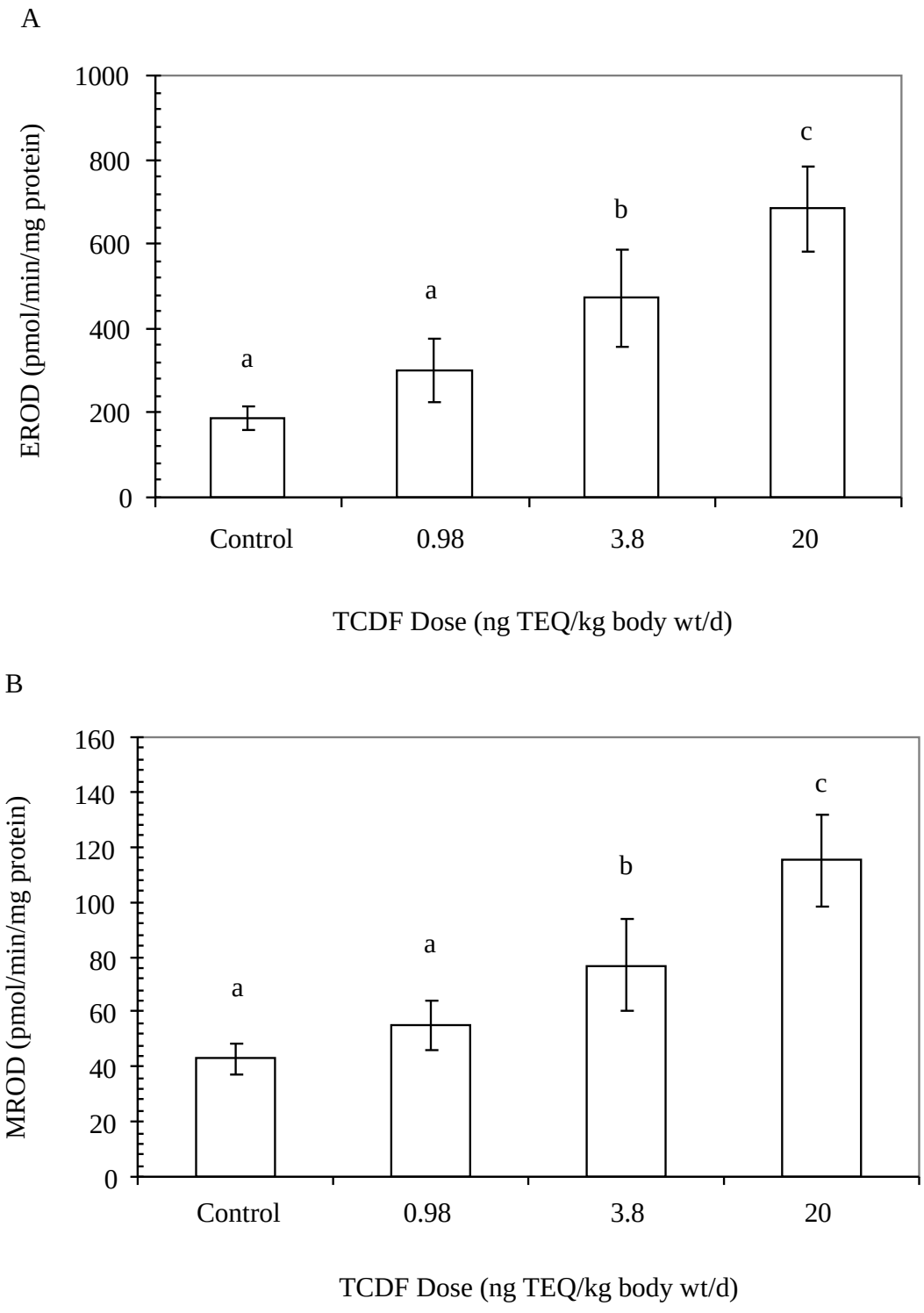


Figure 2.2. TCDF exposure and EROD and MROD activity in mink

Additionally, EROD and MROD activities in mink fed 20 ng TEQ_{TCDF}/kg body wt/d were significantly greater than activities of those fed 3.8 ng TEQ_{TCDF}/kg body wt/d group. Both EROD (Figure 2.3A) and MROD (Figure 2.3B) activities were positively correlated with concentrations of TCDF expressed as TEQ in the liver.

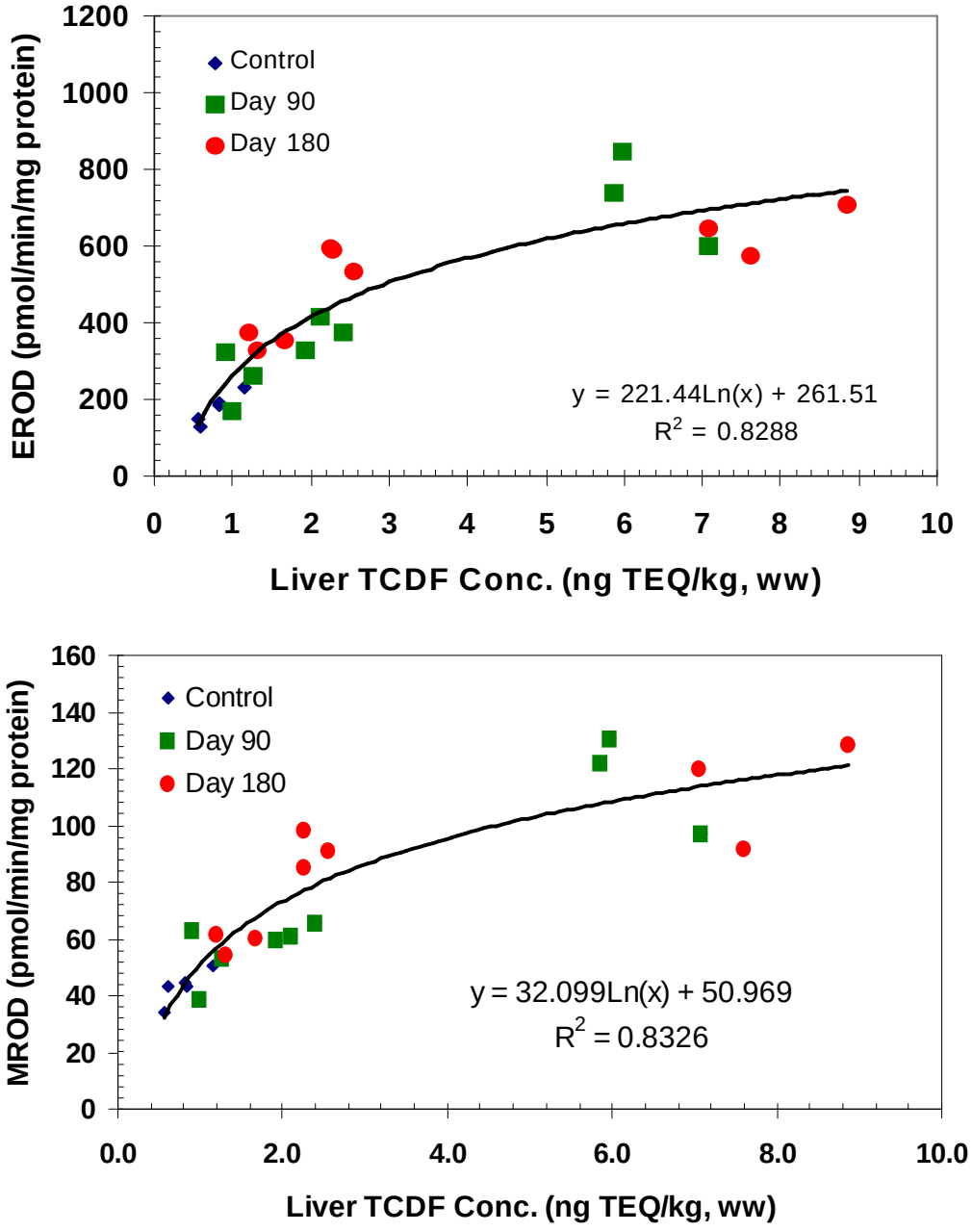


Figure 2.3. TCDF liver concentration and EROD and MROD activity in mink (Moore et al.. 2009)

Exposure to PeCDF resulted in statistically significant greater EROD and MROD activities relative to controls (Figure 2.4). Because there were no statistically significant differences in either EROD or MROD enzyme activities at 90 and 180 d and there were no interactions between treatment and time the values of each of these enzyme activities at the two times were averaged. EROD activities in all PeCDF-dosed groups were significantly greater than control activity (Figure 2.4A).

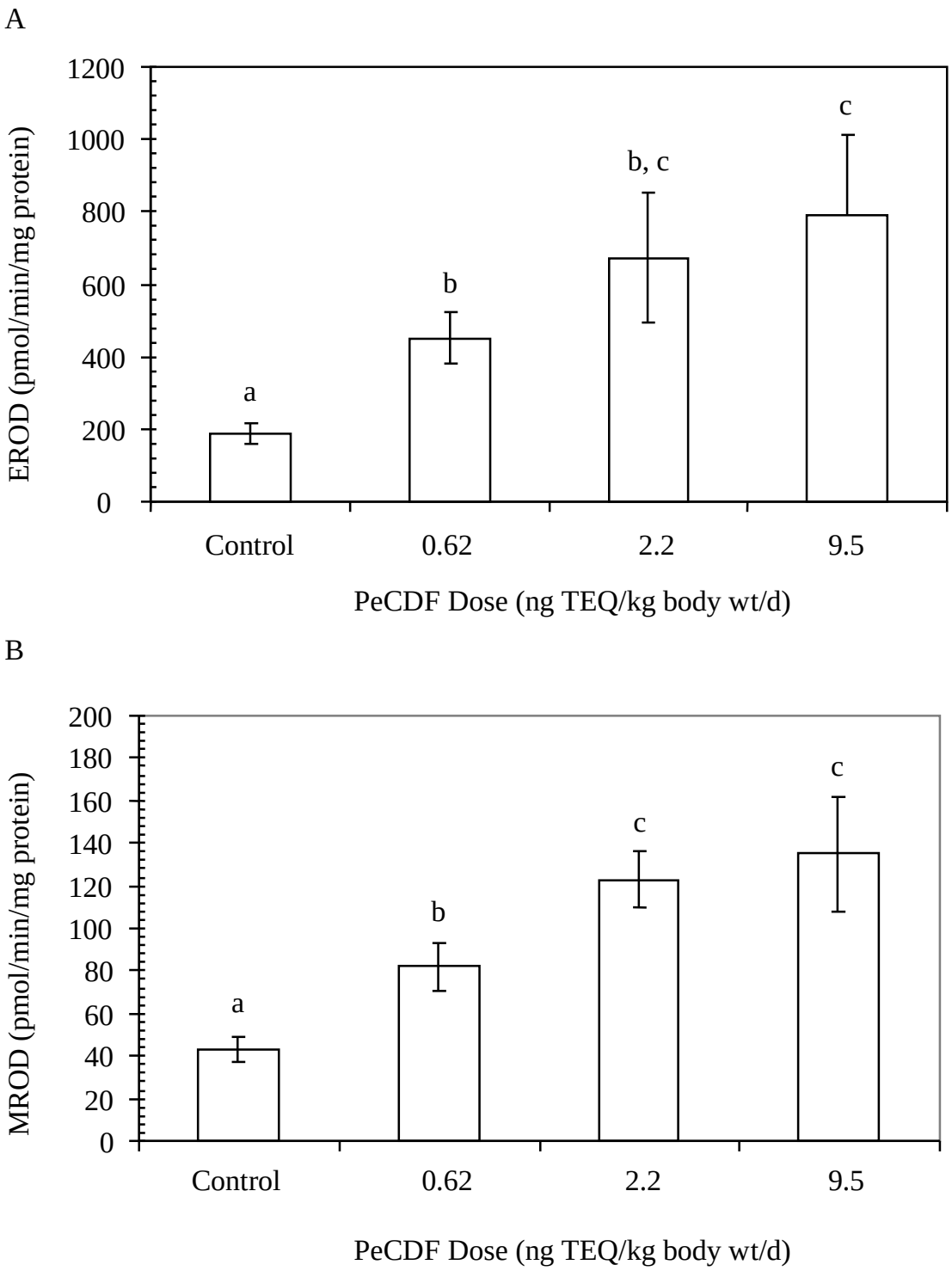


Figure 2.4. PeCDF exposure and EROD and MROD activity in mink

EROD activity in the 9.5 ng TEQ_{PeCDF}/kg body wt/d group was significantly greater than enzyme activities in the 0.62 and 2.3 ng TEQ_{PeCDF}/kg body wt/d dose groups. MROD activities were also significantly greater than control activities at all PeCDF doses with activities in livers of mink fed 2.2 or 9.5 ng TEQ_{PeCDF}/kg body wt/d being significantly greater than activities in livers of mink fed 0.62 ng TEQ_{PeCDF}/kg body wt/d. Both EROD and MROD activities were positively correlated with concentrations of PeCDF expressed as TEQ in the liver (Figure 2.5A, B).

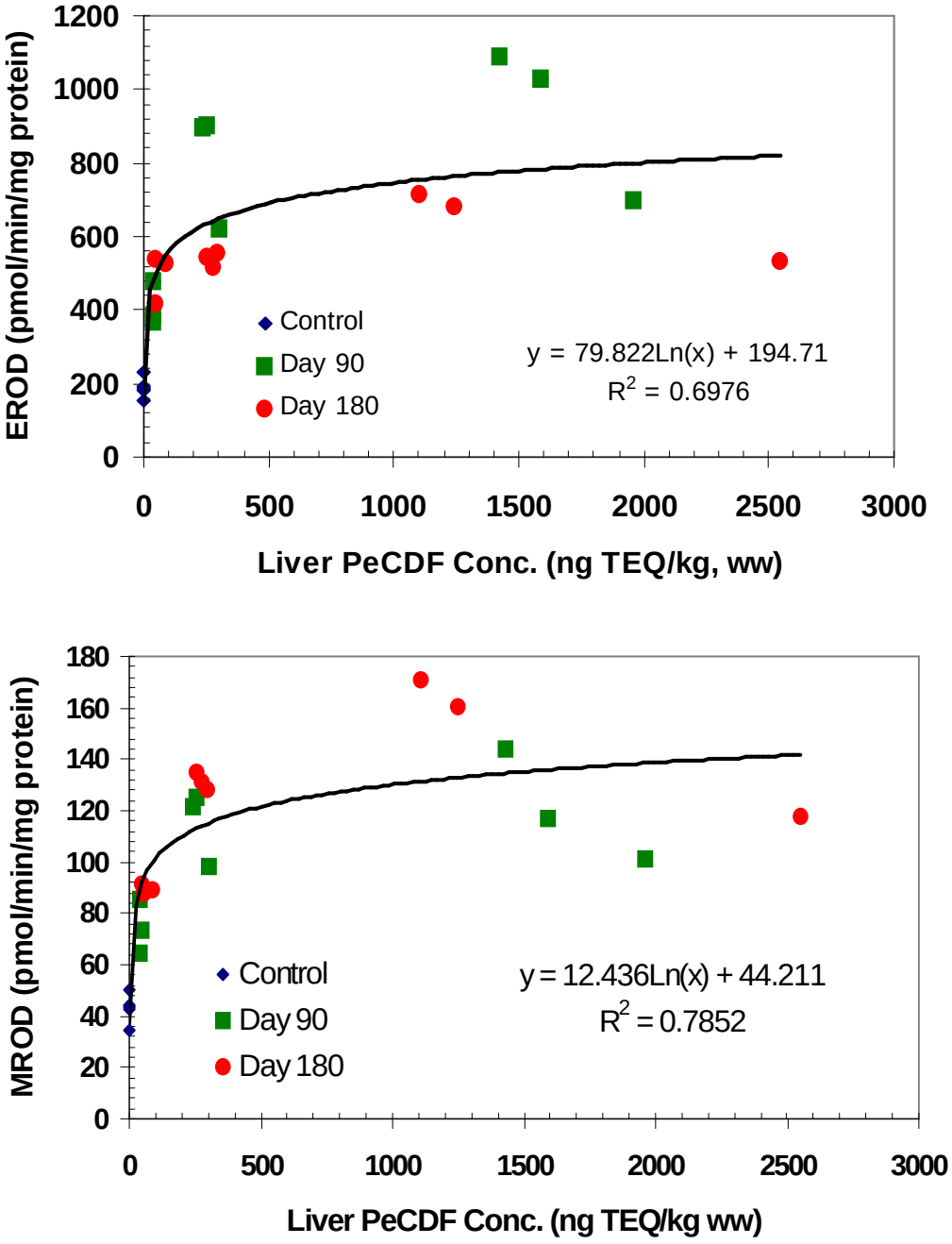


Figure 2.5. PeCDF liver concentrations and EROD and MROD activity in mink (Moore et al., 2009)

EROD and MROD activities in livers of mink fed a mixture of TCDF (4.1 ng TEQ_{TCDF}/kg body wt/d) and PeCDF (2.8 TEQ_{PeCDF}/kg body wt/d) were significantly greater than activities in livers of control mink (Figure 2.6). EROD activity in the livers of mink fed the mixture of TCDF and PeCDF were similar to the activities in mink fed 3.8 TEQ_{TCDF}/kg body wt/d and those fed 2.2 ng TEQ_{PeCDF}/kg body wt/d (Figure 2.6A). MROD activity in livers of mink fed the mixture was significantly greater than enzyme activity in livers of mink fed 3.8 ng TEQ_{TCDF}/kg body wt/d, but did not differ from activity in livers of mink fed 2.2 ng TEQ_{PeCDF}/kg body wt/d PeCDF (Figure 2.6B).

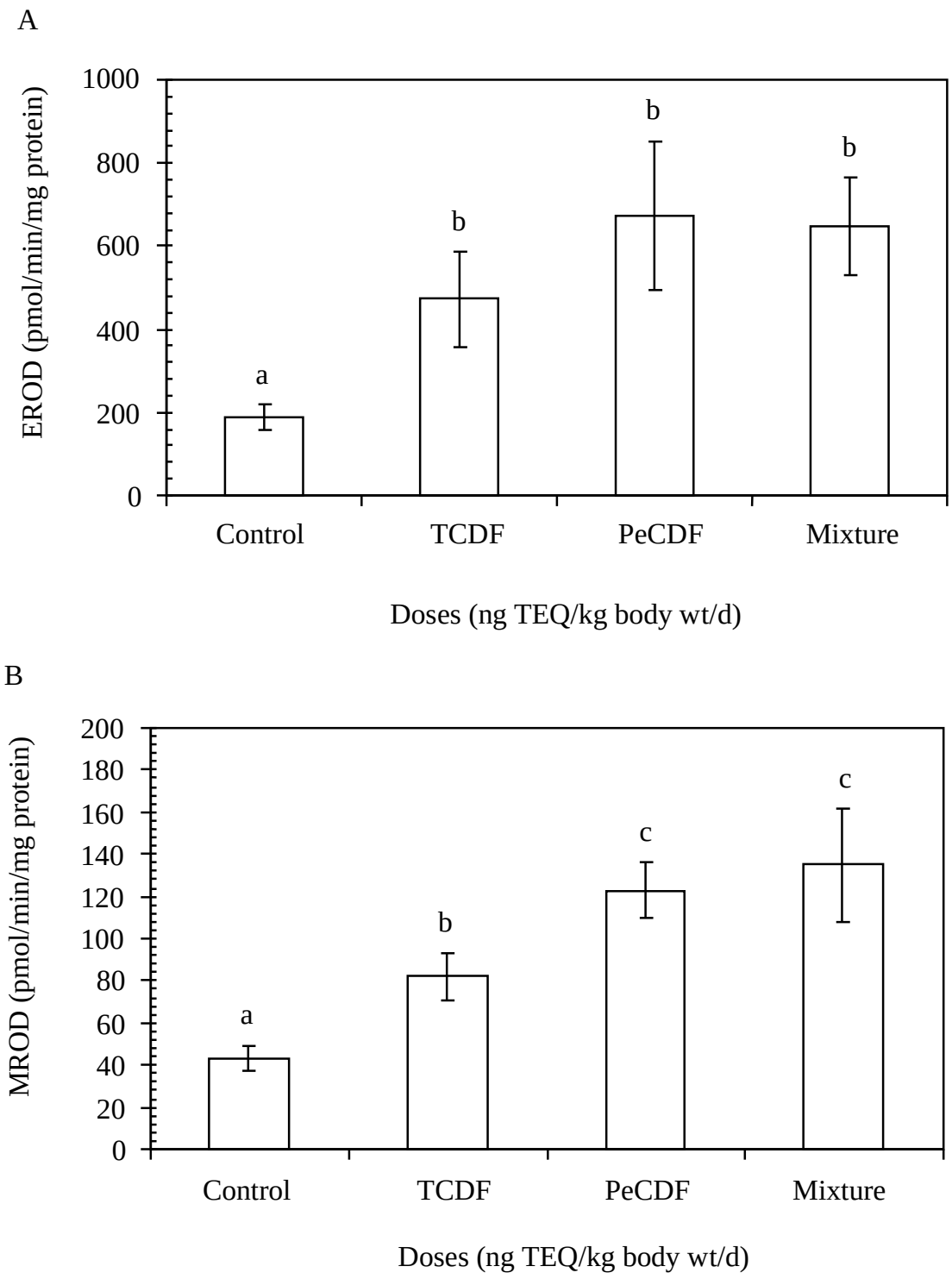


Figure 2.6. TCDF and PeCDF exposure and EROD and MROD activity in mink

DISCUSSION

PCDF concentrations in liver

2,3,4,7,8-Pentachlorodibenzofuran accumulated in the liver of the mink to a much greater extent than did TCDF when administered as a single congener or in combination with TCDF (Table 2.1). Hepatic sequestration of PeCDF relative to that of PCDDs and other PCDFs including TCDF is consistent with what has been reported in other studies with mammals (Brewster and Birnbaum 1987, 1988; Devito et al., 1997). These studies have shown that PeCDF accumulates in the liver of rodents by binding to hepatic CYP1A2 protein (Dilberto et al., 1999) and presumably, PeCDF could be sequestered in livers of mink by the same mechanism (Zwiernik et al., 2008b). The lesser concentrations of TCDF accumulated in livers of the mink suggest an efficient elimination and/or metabolism of the congener. The BAF of this congener has been reported to be inversely proportional to dose (Zwiernik et al., 2008b), which suggests inducible metabolism of TCDF. This is similar to what has been reported in rodents (Tai et al., 1993). The fact that the presence of PeCDF reduced the accumulation of TCDF in the liver of the mink to an even greater extent strengthens the argument that induction of CYP1A1 reduced accumulation of TCDF (Zwiernik et al., 2008b).

The whole-body half-time for elimination of PeCDF observed for mink in this study was estimated to be approximately 8 d while the half-time for elimination of TCDF was less than half a day in mink (Zwiernik et al., 2008b). The half-time for elimination for TCDF and PeCDF in the mink are less than those reported for rodents. The half-time of TCDF is approximately 2 d in mice (Devito et al., 1997) and the half-time of PeCDF in the rat is more than 60 d (Brewster and Birnbaum, 1987).

Histology

In this study, TCDF and PeCDF, administered singly or in combination, at environmentally relevant doses for 180 d did not result in changes in gross morphological or histological endpoints (Table 2.2) that have been reported for other studies in which mink were exposed to dioxin or dioxin-like compounds (Hochstein et al., 1988, 1998; Render et al., 2000a,b, 2001). Recent studies (Bursian et al., 2006b,c) suggest that a very sensitive indicator of exposure of mink to environmentally relevant concentrations of TCDD-like compounds is proliferation of mandibular and maxillary squamous epithelia. Previous studies have indicated that ranch mink exposed to 24.0 μg 3,3',4,4',5-pentachlorobiphenyl (PCB 126)/kg or 2.4 μg TCDD/kg feed (2.4 μg TEQ/kg feed or approximately 300 ng TEQ/kg body wt/d) developed clinical signs of mandibular and maxillary squamous epithelial hyperplasia that in severe cases resulted in the loss of teeth (Render et al., 2000a; 2001). Mink fed diets containing concentrations as little as 0.24 μg PCB 126/kg feed (0.024 μg TEQ/kg feed or 3 ng TEQ/kg body wt/d) (Beckett et al., 2008) exhibited the lesion (K. Beckett, personal communication) as did mink fed a diet containing fish containing PCBs, PCDDs, and PCDFs that provided an estimated daily dose of 1 ng TEQ/kg body wt/d (Bursian et al., 2006b). In the present study, only one animal, which had been fed 9.5 ng TEQ_{PeCDF}/kg body wt/d had a single cyst of squamous epithelial cells at 180 d. The concentration of PeCDF in the liver of that mink was 1.3 ng TEQ/g, ww. In those mink studies where jaw lesion incidence and liver TEQ concentrations were assessed, results indicated that histological lesions were evident in animals with hepatic TEQ concentrations ranging from 40 to 75 ng/kg, ww in the liver (Bursian et al., 2006b,c). Wild mink with

histological evidence of proliferation of mandibular and maxillary squamous epithelia had an average concentration of 610 ng TEQ/kg, ww (Beckett et al., 2005). There are two possible explanations for the scarcity of the jaw lesions in the present study. One possibility is that the age at which exposure was initiated was too late and/or the duration of exposure was not sufficient. In the studies with ranch mink in which effects were observed at concentrations similar or less than those tested in this study, exposure began *in utero* and continued until mink were approximately 7 mo old (Bursian et al., 2006b,c). In those studies where exposure periods ranged from 30 to 60 d (Render et al., 2000a, 2001), the mink were approximately 6 wk old and the dose was approximately 30-fold greater than the dose in the present study (300 ng TEQ/kg body wt/d versus 9.5 ng TEQ/kg body wt/d). A second possibility is related to the specific PCB/PCDD/PCDF congeners contributing to the TEQs. In studies of ranch mink utilizing individual congeners, TEQs were provided by either TCDD or PCB 126 (Render et al., 2000a,b, 2001). In those studies of mink fed diets containing contaminated fish, the majority of TEQs were contributed by congeners other than furans. For example, in a study that assessed the effects of feeding diets containing fish from the Housatonic River, PCB 126 and TCDD contributed 61% of the total TEQs while TCDF and PeCDF contributed 4% (Bursian et al., 2006a,b). In a similar study utilizing fish from the Saginaw River, PCB 126 and TCDD contributed 39% of the total while TCDF and PeCDF accounted for 25% of the total. It is possible that TCDF and PeCDF are less effective than PCB 126 and TCDD in inducing proliferation of mandibular and maxillary squamous epithelia. Furthermore, it has been determined that the effects of PCDFs can not be accurately predicted from the use of TEQ-based TRVs developed from studies of PCDDs and PCBs (Blankenship et al., 2008). This suggests that there are differences in the

sensitivity of mink to PCBs and PCDFs that are not appropriately reflected by the currently utilized TEQ approach (Van den Berg et al., 2006).

Enzyme Induction

Basal EROD activities measured in livers of mink during this study fell within a range of control activities that have been reported in other studies with mink (Smits et al., 1995; Shipp et al., 1998; Brunström et al., 2001; Käkelä et al., 2001; Martin et al., 2007). Values in this study were similar to those reported by Smits et al. (1995), Kakela et al. (2001) and Martin et al. (2007), but were less than those values reported by Brunström et al. (2001). However, given the inconsistencies between all of these studies relative to experimental design, age, and sex of animals, as well as potential contaminants associated with their feed, a direct comparison between these studies is not possible. Given that the basal EROD activities in our study are similar to those enzyme activities measured in other studies, it can be assumed that the cytochrome P4501A1 system was functioning properly.

To our knowledge, there have been no reports of MROD enzyme activities in mink to date. Basal MROD activity was less than that reported for EROD, which is in accordance with studies in other mammals such as rats or monkeys (Lubet et al., 1990; Weaver et al., 1994; Suzuki et al., 2001) but opposite to reports on other species such as various mice strains, hamster, or humans (Weaver et al., 1994; Hamm et al., 1998). The relative difference between EROD and MROD activities was greater (~5-fold) when compared to that reported for rats or monkey (<2- to 3-fold). It has been previously reported that the specificities of orthologous forms of P450s are expressed differently among mammalian species. In rats *CYP1A1* and *CYP1A2* selectively catalyze EROD and MROD, respectively, while in humans

CYP1A2 has similar activities for both EROD and MROD. From the data presented here it appears that mink are associated more closely with rat or monkey regarding their basal EROD/MROD profiles. However, further elucidation of the specificities of different forms of P450s for the different alkylresorufin *O*-dealkylases (AROD) is necessary to be able to assign mink to a certain mammalian metabolism type.

There were no significant differences in enzyme activity in mink receiving daily doses of TCDF and/or PeCDF between 90 and 180 d. This suggests that maximum induction of CYP1As in livers of mink as a function of time in response to the exposure with TCDF and PeCDF occurs earlier than the first sampling time point at 90 d.

The EROD activity in mink dosed with the mixture of TCDF and PeCDF was similar to enzyme activity in those mink dosed with either TCDF or PeCDF while MROD activity was similar to activity in those mink dosed with PeCDF. This suggests that induction resulting from the combination of the two furan congeners may not have been additive and perhaps was due primarily to the action of only one of the congeners. Based on liver concentration data indicating greater concentration of PeCDF compared to TCDF, it is possible that enzyme induction in those animals receiving the mixture was due primarily to PeCDF. Alternatively, TCDF may also have contributed to the increase in enzyme activities, but due to metabolism, its concentration in the liver was less than that of PeCDF.

ACKNOWLEDGEMENTS

This research was supported by an unrestricted grant from ENTRIX Inc. to Steven J. Bursian, John P. Giesy and Matthew J. Zwiernik. Special thanks go to Angelo Napolitano, Jeff Greenlee, C.P. Napolitano, David Hamman, Patrick Bradley, Michael Kramer, Nozomi

Ikeda, Molly Wiersema, and Melissa Shotwell for the expertise and support they provided during the study.

CHAPTER 3

EFFECTS OF DIETARY EXPOSURE OF MINK (MUSTELA VISON) TO 2,3,7,8 – TETRACHLORODIBENZO-P-DIOXIN (TCDD), 2,3,4,7,8-PENTACHLORODIBENZOFURAN (PECDF) AND 2,3,7,8-TETRACHLORODIBENZOFURAN (TCDF) ON REPRODUCTON AND OFFSPRING VIABILITY AND GROWTH

ABSTRACT

This study assessed the effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) and 2,3,7,8 tetrachlorodibenzofuran (TCDF) on the reproductive performance of female mink (*Mustela vison*) and the viability and growth of their offspring. Nine adult female mink each were randomly assigned to one of 13 dietary treatments (one control and four doses each of TCDD, PeCDF and TCDF [2.1-8.4, 4.0-15 and 5.2-25 ng TCDD toxic equivalents (TEQ)/kg body wt/d]. Diets were fed from two months prior to breeding through weaning of offspring at six weeks of age. At least nine kits per treatment group were maintained on their diets through 27 weeks of age. There were no effects on litter size or viability of offspring. No consistent effects were observed on body mass or relative organ masses of animals at any age. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and PeCDF accumulated in the liver and adipose tissue, but TCDF was rapidly cleared. The lack of significant effects on reproduction and offspring viability contrasts with effects reported for mink exposed to environmentally derived PCB mixtures with equivalent TCDD potencies. This suggests that it may be inappropriate to apply toxicity reference values associated with PCB mixtures to animals also exposed to TCDD, PeCDF or TCDF and the World Heath Organization TCDD toxic equivalency factors for some congeners may not be appropriate for mink.

INTRODUCTION

Elevated concentrations of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) have been detected in sediments, floodplain soils, and fish of the Tittabawassee River (MI, USA) (Hilsherova et al., 2003). Polychlorinated dibenzo-*p*-dioxins and PCDFs are persistent, bioaccumulative compounds; therefore, top trophic level predators have the greatest potential for exposure. Mink (*Mustela vison*) are a species of special interest because they forage within the riparian zone and have a prey base consisting of both terrestrial and aquatic organisms. The home range of an adult male is estimated to average 2.6 km in stream length and that of an adult female averages 1.9 km (Linscombe et al., 1982). In addition, laboratory studies have shown that mink are among the most sensitive species to the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and TCDD-like compounds (Hochstein et al., 1988, Hochstein et al., 1998, Beckett et al., 2008). The combination of exposure potential and sensitivity to the site-specific contaminants of concern make the mink a good species for interpreting risk of harm to piscivorous mammalian wildlife species, as discussed by Basu et al. (2007) residing within the Tittabawassee River floodplain.

Concentrations of PCDDs and PCDFs in tissues of mammals residing within the Tittabawassee River basin are among the highest ever reported (Zwiernik et al., 2008a). When concentrations are expressed as TCDD toxic equivalents (TEQ) using World Health Organization toxic equivalency factors (TEFs) (Van den Berg et al., 2006), livers from 22 wild mink, collected downstream of Midland, MI, USA, had an average of 400 ng TEQ/kg (wet wt), of which 290 ng TEQ/kg (wet wt) was contributed by PCDFs and 21 ng TEQ/kg (wet wt) was contributed by PCDDs. Mink collected upstream of the study area had a

concentration of 20 ng TEQ/kg (wet wt) in liver tissue, which was distributed more evenly among the PCDDs, PCDFs, and TCDD-like polychlorinated biphenyls (PCBs) (Zwiernik et al., 2008a). Based on the present understanding of the toxicological potency of these mixtures, dietary- and tissue-based exposure data suggest that mink, as one of the most highly exposed and most sensitive species, should be experiencing adverse effects (Bursian et al., 2006a,b,c) along the Tittabawassee River. Conversely, selected measures of individual health, including histological and morphological measures, as well as measures of population conditions such as abundance and demographics, indicate that mink appear to be healthy and populations are stable and at or close to carrying capacity for the Tittabawassee River (Zwiernik et al., 2009). From this apparent disparity between the predicted and observed condition of resident mink, it was concluded that additional information on the potency of the toxic mixture of compounds found in the Tittabawassee River soils, sediments, and wildlife was needed.

To provide risk managers with the best possible information pertaining to the potency of the site-specific contaminant mixture, a controlled feeding study was conducted in which ranch mink were exposed to relevant PCDD and PCDF congeners at concentrations bracketing those observed in the field. These included TCDD, 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) and 2,3,7,8-tetrachlorodibenzofuran (TCDF), which were the three compounds that made up the majority of calculated toxic potency based on TEQ using current World Health Organization TEFs (Van den Berg et al., 2006). Because the present study design included TCDD in a side-by-side comparison of toxicity to the two furans, the results also provide animal-based relative potency data that can be used by the World Health Organization for calculating the mammalian TEFs for PeCDF and TCDF. In

addition to bracketing field exposures, the dosing regime was expanded to cover a range of concentrations including those expected to elicit effects previously reported for mink exposed to TCDD-like compounds. Lesser doses were set to mimic nominal environmentally relevant concentrations and were expected to result in no effects except for the most sensitive responses at the molecular level. In contrast, the highest dose for each congener expressed as TEQ using the current World Health Organization TEFs (Bursian et al., 2006a) (TCDD = 8.4 ng TEQ_{TCDD}/kg body wt/d, PeCDF = 15 ng TEQ_{PeCDF}/kg body wt/d, and TCDF = 25 ng TEQ_{TCDF}/kg body wt/d) exceeded the median predicted environmental exposures for the Tittabawassee River of 3.9 ng TEQ/kg body wt/d. This highest dose was expected to cause reproductive effects based on the results of laboratory studies where mink fed TEQ-normalized concentrations of PCBs (Beckett et al., 2008, Bursian et al., 2006a,b,c, Heaton et al., 1995a, 1995b, Tillitt et al., 1996) at similar levels experienced decreased litter size and/or reduced offspring viability.

The present report describes the effects of consumption of diets containing various concentrations of TCDD, PeCDF or TCDF on adult female reproductive performance and offspring viability and growth through 27 weeks of age.

MATERIALS and METHODS

Chemicals and reagents

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin, PeCDF and TCDF were obtained from AccuStandard and dissolved in hexane (OmniSolv, EMD Chemicals) to produce a stock solution for each congener. Working solutions of TCDD, PeCDF and TCDF were then

prepared by serial dilution in hexane. One ml of each working solution was added to 100 ml corn oil for incorporation into the feed.

Dietary treatments

The treatment diets were based on the Michigan State University (MSU) Experimental Fur Farm ranch diet formulated to meet the nutritional requirements of mink (Table 3.1) (National Research Council, 1982). The treatment diets were prepared by adding water to a 500-kg-capacity paddle mixer, followed by fishmeal, wheat middlings, and soybean oil. These ingredients were thoroughly mixed prior to addition of the working solutions, which had been diluted 1:100 with corn oil. A solution of 1 ml hexane and 100 ml corn oil was added to the control feed. After an additional period of mixing to allow the hexane to evaporate, the remaining ingredients were added and mixed thoroughly. Three grab samples consisting of five subsamples per grab sample were collected for each diet for congener analysis (Vista Laboratories), as well as a sample for nutrient analysis (Litchfield Analytical Services). The treatment diets were packaged in labeled, one-gallon aluminum containers that were stored in a walk-in freezer (-20º C) at the MSU Experimental Fur Farm. Twenty-fours hours prior to use, containers were transferred from the walk-in freezer to a walk-in cooler (4º C) to allow the feed to thaw. One container was sufficient to feed a group of nine mink for approximately 3 d. Feed was mixed and sampled a second time halfway through the trial as described above.

Table 3.1. Composition and nutrient analysis of basal experimental diets (as fed basis).

Ingredient	Composition (%)
Water	34.0
Soybean oil[a]	6.0
Spray-dried poultry liver[b]	4.0
Spray-dried eggs[b]	5.0
Spray-dried blood cells[c]	4.0
Chicken[d]	26.0
Wheat middlings[a]	15.0
Fishmeal[a]	4.0
Vitamin premix[e]	0.5
Mineral premix[f]	0.5
Phosphoric acid[g]	1.0
Larvacide[h] (ml/kg feed)	0.2
d-biotin[i] (mg/kg feed)	2.4
Nutrient analysis (%)	
Moisture	53.8
Protein	17.6
Fat	11.0
Ash	4.7
Crude fiber	1.7
Total digestible nutrients	43.9

[a]North American Nutrition, Lewisburg, OH, USA.

[b]VanElderen, Martin, MI, USA.

[c]California Spray Dry, Stockton, CA, USA.

[d]Whole ground chicken, Whalen Foods, Chaska, MN, USA.

[e]Calcium, 13.40%; copper, 2000 mg/kg; iodine, 30 mg/kg; iron, 2.0 %; manganese, 2000 mg/kg; selenium, 60 mg/kg; zinc, 2.0 %; Akey, Louisburg, OH, USA.

Table 3.1. Cont'd.

[f]Vitamin A, 916,652 IU/kg; vitamin D3, 91,674 IU/kg; activity, vitamin E, 11,000 IU/kg; vitamin K 2200 mg/kg; menadione, 733 mg/kg; vitamin B12, 5.5 mg/kg; riboflavin, 733 mg/kg; d-pantothenic acid, 2935 mg/kg; niacin, 4400 mg/kg; thiamine, 183 mg/kg; pyridoxine, 33 mg/kg; Akey, Louisburg, OH.
[g]Astaris, St. Louis, MO, USA.
[h]Active ingredient: cyromazine (N-cyclopropyl-1,3,5- triazine-2,4,6-triamine, 2%), Novartis Animal Health, Greensboro, NC, USA.
[i]Biotin 100 (100 mg/lb), ADM, Des Moines, IA, USA.

Targeted dietary concentrations were 21, 42, 73 and 104 ng TCDD/kg feed; 139, 243, 347 and 533 ng PeCDF/kg feed; 728, 1600, 2560 and 3120 ng TCDF/kg feed. Actual dietary concentrations reflecting both mixes, as determined by high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS), and daily doses of TCDD, PeCDF, and TCDF as well as the corresponding TEQs (based on TEFs reported by Van den Berg et al., 2006) are presented in Table 3.2. The TEQ concentration for each of the TCDD, PeCDF, and TCDF groups reflects the concentration provided by that congener only because the TEQs contributed by other congeners were less than 1% of the total. Dose calculations were based on the average estimated feed consumption and body mass of adult females in each treatment group through the first 15 weeks of the trial. Feed consumption was estimated by providing each animal with a daily allotment of 125 g of feed, which was slightly greater than the consumption of 115 g/d previously reported for adult female ranch mink (Bleavins et al., 1981), and determining the amount feed remaining at the time of next feeding.

Table 3.2. Dietary concentrations and corresponding doses of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 2,3,4,7,8-pentachlorodibenzofuran (PeCDF), and 2,3,7,8-tetrachlorodibenzofuran (TCDF).

	Dietary concentration							Estimated dose[a]	
Treatment	ng/kg feed	SE[b]	ng TEQ[c]/kg feed	Mean estimated daily feed intake wk 1-15 (g)	SE	Mean body weight wk 1-15 (g)	SE	ng/kg body wt/d	ng TEQ/kg body wt/d
TCDD	23	0.6	23	99.5	1.1	1088	27	2.1	2.1
	53	1.6	53	103.2	0.5	1187	31	4.6	4.6
	77	2.6	77	100.3	0.9	1286	21	6.0	6.0
	101	3.9	101	102.3	0.8	1226	23	8.4	8.4
PeCDF	166	3.1	50	99.1	0.9	1241	14	13	4.0
	288	4.7	86	102.8	0.6	1172	23	25	7.6
	363	23.2	109	99.3	0.9	1207	29	30	9.0
	619	12.2	186	96.8	0.9	1215	20	49	15
TCDF	679	21.3	68	100.0	1.0	1318	26	52	5.2
	1464	35.3	146	99.3	1.0	1254	26	116	12
	2402	150.5	240	99.1	1.0	1110	19	214	21
	2866	163.6	287	93.5	1.2	1091	30	246	25

[a]Dose based on estimated feed consumed from a daily allotment of 125 g feed and mean body weights of adult female mink through week 15 of the study.

[b]SE refers to standard error.

[c]TEQ refers to toxic equivalents that are based on toxic equivalency factors of 1.0, 0.3 and 0.1 for TCDD, PeCDF and TCDF, respectively [Van den Berg et al., 2006].

Animals

One hundred seventeen first-year (virgin) and second-year (proven breeder), natural dark, female mink from the MSU Experimental Fur Farm herd were assigned randomly on November 20, 2006 to 13 dietary treatment groups (nine mink per group) with the exception that littermates were not placed in the same treatment group to minimize genetic predisposition to compound toxicity. Untreated, natural dark, male mink were used for breeding purposes only. The MSU Institutional Animal Care and Use Committee approved the use of animals for this trial.

Housing

Female mink were housed individually in wire breeder cages (76 cm L x 46 cm W x 38 cm H) suspended above the ground in an open-sided mink shed. Nine animals per treatment group were assigned randomly to a bank of nine cages separated from the next bank of nine cages by an empty cage. Assignment of treatments to banks of cages was done to minimize the potential for cross-contamination between groups. A wooden nest box (38 cm L x 25 cm W x 29 cm H) bedded with excelsior (wood wool) prebreeding or aspen shavings postbreeding was attached to the outside of each cage. The standard guidelines for the operation of mink farms in the United States (Fur Commission U.S.A., 2003) were followed to house and maintain the animals.

Exposure period

Mink were started on their treatment diets on December 30, 2006, after a one-week acclimation period. The daily allotment of feed (125 g) was placed on a cleaned grid on the

top of the cage. Water was available ad libitum. Animals were weighed every four weeks until the initiation of breeding (March 1, 2007).

Adult females were mated to untreated males between March 1 and March 26, 2007. Each female was given an opportunity to mate every fourth day until a successful mating was obtained. Females were assumed to have bred successfully if evidence of vulvar swelling appeared following a copulation period of at least 10 minutes. Mated females were given an opportunity to breed with a different male the day following a successful mating and on the eighth and ninth days after the first successful mating (a common commercial mink breeding practice).

Whelping began on April 15, 2007 and ended on May 15, 2007. Nest boxes were checked on a daily basis for the presence of mink kits. Live kits were enumerated, and body masses were recorded at birth and at three and six weeks of age. Body masses of adult females were recorded at the time their litters were weighed.

All surviving adults and a representative number of kits from each treatment group were euthanized (CO_2) when kits were six weeks old (May 22 to June 25, 2007). These individuals were necropsied and samples of selected tissues were taken for analytical and histological assessment. At least nine kits per treatment were maintained on their diets until they were 27 weeks old (October 22 to November 2, 2007), at which time they were euthanized and processed as above. At least four males and four females, but no more than nine mink, were selected randomly from each treatment for the final necropsy and tissue analysis. The thyroid gland, thymus, heart, adrenal glands, kidneys, spleen, reproductive organs (uterus with ovaries/testes), liver, and brain were removed, weighed, and placed in 10% neutral buffered formalin for subsequent histological assessment.

Chemical analysis

To ensure that cocontaminants were not a factor in the present study, concentrations of 17 2,3,7,8-substituted PCDF and PCDD congeners and 12 TCDD-like PCB congeners were measured in the dietary items, feed samples, and liver tissue as described by Zwiernik et al. (2008b). Cocontainments accounted for less than 1% of the TEQ contributed by TCDD, PeCDF, or TCDF. Thus, concentrations of dietary and hepatic TEQ for each of the TCDD, PeCDF, and TCDF treatment groups were calculated as the product of the concentration of that congener only multiplied by its respective TEF (Van den Berg et al., 2006). A surrogate value of one-half the method detection limit (MDL) was used for concentrations less than the MDL. Liver tissues were extracted following a modification of U.S. Environmental Protection Agency (U.S. EPA) Method 1613B (Telliard, 1994)). Liver tissue extracts were shipped on dry ice to Vista Laboratories for congener analysis by high-resolution gas chromatography/high-resolution mass spectrometry according to U.S. EPA Method 1613B (Telliard, 1994).

Histological analysis

Histological examination of tissues was performed at MSU's Diagnostic Center for Population and Animal Health. Tissues were embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin. A board-certified veterinary pathologist examined slides of the thyroid gland, thymus, heart, adrenal glands, kidneys, spleen, reproductive organs (uterus with ovaries/testes), liver, brain, and maxilla and mandible of each mink sampled at necropsy.

Statistical analysis

All statistical analyses were performed with SAS Version 9.1. Because of the nature of the parameters, several statistical models were used for data analyses. The present study was designed for the application of fixed effects models to test for differences among exposure groups. Prior to conducting statistical comparisons, data were tested for normality using the Shapiro-Wilkes test and probability plots. If necessary, values were log transformed to approximate normality. Differences among treatment groups were evaluated by analysis of variance using SAS PROC Mixed. Because of the unbalanced experimental design (unequal sample sizes), least square means were used in the analyses. When group effects were statistically significant, differences among treatment groups were tested with Tukey-Kramer test to account for differences in sample size among the groups. Differences among groups were considered significant at $p < 0.05$.

RESULTS

Reproductive performance and offspring viability

All females bred at least once. The percent of bred females whelping ranged from 78% to 100% with the exception of the greatest PeCDF treatment group (49 ng PeCDF/kg body wt/d or 15 ng TEQ_{PeCDF}/kg body wt/d), which had a 56% whelping rate. Mean litter sizes at birth for females that whelped were not significantly different from the control group irrespective of the treatment compound or dose. Similarly, no significant differences were observed in kit viability among treatment groups compared with controls through six weeks of age (Table 3.3). Although differences in kit viability among dose groups were not statistically significant because of sample size and variability, the percentages of viable kits

in the control and low-dose PeCDF groups were numerically greater compared to the other groups.

Table 3.3. Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 2,3,4,7,8-pentachlorodibenzofuran (PeCDF), and 2,3,7,8-tetrachlorodibenzofuran (TCDF) on reproduction and kit growth and survivability through six weeks of age[a].

Treatment	Dose (ng/kg body wt/d)	Number of females whelping/total number of females	Litter size (live kits)	Mean body mass (g) at birth	Mean body mass (g) at six weeks	Survivability through six weeks of age (%)
Control	0	9 of 9	6.1 (0.70)	9.91 (0.60)	250.13 (23.50)	80.3 (0.5 - 1.1)
TCDD	2.1	7 of 9[b]	5.6 (0.80)	7.57 (0.70)	215.6 (33.30)	46.3 (0.0 – 0.9)
	4.6	7 of 9	4.9 (0.80)	8.39 (0.70)	182.06 (33.30)	53.1 (0.1 - 1.0)
	6.0	8 of 9[c]	5.7 (0.70)	9.3 (0.60)	255.27 (27.27)	51.6 (0.2 – 0.8)
	8.4	8 of 9	4.6 (0.70)	9.95 (0.60)	181.12 (27.20)	67.7 (0.3 – 1.0)
PeCDF	13	8 of 9	5.4 (0.80)	8.65 (0.60)	275.39 (23.50)	81.3 (0.6 – 1.0)
	25	8 of 9	4.8 (0.80)	9.53 (0.60)	184.04 (29.80)	36.3 (0.0 – 0.7)
	30	8 of 9	5.4 (0.80)	9.9 (0.70)	172.78 (27.20)	51.5 (0.2 – 0.9)
	49	5 of 9[d]	4.4 (1.00)	9.68 (0.80)	180.5 (33.30)	65.0 (0.1 – 1.2)

Table 3.3. Cont'd.

TCDF	52	9 of 9	4.4	10.19	228.73	51.8
			(0.70)	(0.60)	(25.10)	(0.2 – 0.9)
	116	9 of 9	5.9	9.22	238.13	61.1
			(0.70)	(0.60)	(22.20)	(0.4 – 0.9)
	214	8 of 9	4.6	9.04	228.08	57.1
			(0.80)	(0.70)	(29.80)	(0.2 – 1.0)
	246	7 of 9	5.1	7.7	181.75	66.7
			(0.80)	(0.70)	(27.20)	(0.3 – 1.0)

[a]Data are presented as means with (standard error) or (95% confidence interval) beneath.

[b]One female died due to renal failure caused by bacterial pyelonephritits. Uterus contained six fetuses.

[c]One female died due to bacterial pneumonia. Uterus did not contain fetuses.

[d]One female died due to ruptured uterus. Uterus contained five fetuses.

Body mass

Mean body masses of adult females prior to the whelping period were not significantly different compared to controls (Table 3.4). Similarly, no significant differences were noted in mean body masses of kits at birth and six weeks of age compared with controls (Table 3.3). Conversely, some significant treatment-related differences were observed in juvenile male mean body masses compared to controls at week 14 and 27. Treatment differences were generally not consistent in terms of age and/or dose (Table 3.4). The mean body mass of male juveniles exposed to the highest dose of TCDD (8.4 ng/kg body wt/d; 8.4 ng TEQ_{TCDD}/kg body wt/d) was significantly less than the mean body mass of the control group counterparts at week 14 of the trial; however, by week 27, masses were no longer different. For the next-lesser-dose TCDD group (6.0 ng TCDD/kg body wt/d; 6.0 ng TEQ_{TCDD}/kg body wt/d), male mean body mass did not differ from the control group counterparts at week 14 but did differ at week 27. In males exposed to PeCDF, mean body masses were significantly less compared with controls at weeks 14 and 27 in the 25 and 49 ng PeCDF/kg body wt/d (7.6 and 15 ng TEQ_{PeCDF}/kg body wt/d) treatment groups but not in the 30 ng PeCDF/kg body wt/d (9.0 ng TEQ_{TCDD}/kg body wt/d) group. Only at the highest dose of TCDF (246 ng TCDF/kg body wt/d; 25 ng TEQ_{TCDF}/kg body wt/d) was mean body mass of juvenile males significantly different compared with controls at 14 weeks of age but not at 27 weeks of age. Regardless of congener or dose, mean body mass in juvenile females did not differ significantly from controls (Table 3.4).

Table 3.4. Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 2,3,4,7,8-pentachlorodibenzofuran (PeCDF), and 2,3,7,8-tetrachlorodibenzofuran (TCDF) on adult female pre-whelping mass (g) and juvenile male and female mass (g) from 14 to 27 weeks of age[a,b].

		Adult Females			Juvenile males			Juvenile females	
Treatment	Dose (ng/kg body wt/d)	*n*	Wk 0 pre-whelping	Wk 15 pre-whelping	*n*	Wk 14 of age	Wk 27 of age	*n*	Wk 14 of age	Wk 27 of age
Control	0	9	1275 (62)	1290 (49)	12	1164 (58)	1537 (79)	17	802 (41)	1164 (53)
TCDD	2.1	8	1274 (62)	1018 (49)	7	972 (72)	1355 (96)	7	836 (66)	999 (86)
	4.6	9	1311 (62)	1225 (49)	7	1066 (72)	1285 (96)	7	765 (60)	983 (78)
	6.0	8	1326 (62)	1362 (49)	6	950 (75)	1073 A (102)	16	815 (76)	1013 (60)
	8.4	9	1264 (62)	1285 (49)	9	841 A (58)	1226 (81)	7	689 (60)	952 (78)
PeCDF	13	9	1250 (62)	1284 (49)	10	1037 (51)	1307 (70)	13	795 (44)	1123 (63)
	25	9	1303 (62)	1254 (49)	3	866 A (75)	1093 A (123)	7	733 (63)	938 (90)
	30	9	1244 (62)	1270 (49)	7	1066 (56)	1494 (83)	8	762 (55)	1043 (79)
	49	8	1272 (62)	1215 (49)	7	805 A (70)	1119 A (88)	2	651 (109)	821 (155)

Table 3.4. Cont'd.

TCDF	52	9	1387	1373	9	1102	1354	4	869	1026
			(62)	(49)		(58)	(89)		(61)	(79)
	116	9	1350	1249	12	1055	1572	12	816	1144
			(62)	(49)		(52)	(79)		(45)	(57)
	214	9	1256	1096	9	926	1357	9	712	1008
			(62)	(49)		(61)	(92)		(46)	(59)
	246	9	1248	1095	9	911 A	1304	9	737	1035
			(62)	(49)		(56)	(86)		(49)	(63)

[a]Data are presented as means with (standard error) beneath.

[b]Means that are significantly different then the control mean at $p < 0.05$ are designated with an A.

Organ mass

Relative masses (percent of body mass) of the spleen and liver were greater compared to controls at the highest doses of the three congeners, depending on age, whereas changes in relative masses of other organs were inconsistent across doses (Table 3.5). Mean relative spleen mass in the adult females receiving the greatest dose of TCDD (8.4 ng TCDD/kg body wt/d; 8.4 ng TEQ/kg body wt/d) was significantly greater compared to controls (mean [95% confidence interval]; 0.34 [0.30-0.42] vs 0.25 [0.22-0.28]) as was mean relative spleen mass in the juvenile males dosed with 8.4 ng TCDD/kg body wt/d (8.4 ng TEQ_{TCDD}/kg body wt/d). Mean relative liver masses of juvenile males at the highest PeCDF (49 ng PeCDF/kg body wt/d; 15 ng TEQ_{PeCDF}/kg body wt/d) and TCDF (246 ng TCDF/kg body wt/d; 25 ng TEQ_{TCDF}/kg body wt/d) doses were significantly greater compared to controls. There appeared to be a dose-related trend of increasing relative liver masses in the TCDD and PeCDF groups. Other significant changes in relative organ masses included increased mean relative kidney masses at 4.6 ng TCDD/kg body wt/d (4.6 ng TEQ_{TCDD}/kg body wt/d) and 25 and 49 ng PeCDF/kg body wt/d (7.6 and 15 ng TEQ_{PeCDF}/kg body wt/d) in juvenile males. In juvenile females, mean relative thymus masses were significantly decreased at 6.0 ng TCDD/kg body wt/d (6.0ng TEQ_{TCDD}/kg body wt/d) and 52 ng TCDF/kg body wt/d (5.2 ng TEQ_{TCDF}/kg body wt/d), mean relative heart masses were significantly increased at 6.0 ng TCDD/kg body wt/d (6.0 ng TEQ_{TCDD}/kg body wt/d) and 25 ng PeCDF/kg body wt/d (7.6 ng TEQ_{PeCDF}/kg body wt/d) and mean relative adrenal gland mass was significantly

increased at 8.4 ng TCDD/kg body wt/d (8.4 ng TEQ_{TCDD}/kg body wt/d) compared with controls. Absolute and relative masses for all adult female, kit, and juvenile organs are presented in Supplemental Data (Appendix).

Table 3.5. Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 2,3,4,7,8-pentachlorodibenzofuran (PeCDF), and 2,3,7,8-tetrachlorodibenzofuran (TCDF) on juvenile male and female relative organ mass (% of body mass) at 27 weeks of age[a,b,c].

			Males			
Treatment	Dose (ng/kg body wt/d)	*n*	Body mass-necropsy (g)	Liver	Spleen	Kidneys
Control	0	12	1536	5.1	0.19	0.66
			(79)	4.79-5.42	0.16-0.21	0.60-0.72
TCDD	2.1	7	1355	5.19	0.18	0.75
			(96)	4.28-6.11	0.16-0.20	0.07-0.84
	4.6	6	1295	5.54	0.22	0.83 A
			(103)	4.73-6.36	0.20-0.25	0.70-0.95
	6.0	6	1072 A	5.99	0.18	0.83
			(103)	5.23-6.75	0.16-0.20	0.74-0.92
	8.4	9	1225	5.97	0.26 A	0.76
			(81)	5.50-6.45	0.21-0.31	0.70-0.82
PeCDF	13	10	1307	5.38	0.19	0.72
			(71)	4.62-6.15	0.17-0.22	0.65-0.80
	25	3	1093 A	6.67	0.78	0.86 A
			(128)	1.78-11.56	0.00-3.25	0.56-1.17
	30	7	1497	5.70	0.27	0.68
			(85)	5.35-6.06	0.23-0.32	0.62-0.74
	49	8	1057 A	6.83 A	0.31	0.91 A
			(82)	6.01-7.64	0.25-0.38	0.82-0.10

Table 3.5. Cont'd.

TCDF	52	9	1357	4.99	0.21	0.73
			(90)	4.50-5.48	0.14-0.28	0.65-0.80
	116	12	1574	4.98	0.20	0.68
			(79)	1.77-5.19	0.17-0.24	0.65-0.70
	214	10	1323	5.31	0.24	0.75
			(90)	4.87-5.75	0.19-0.28	0.67-0.82
	246	9	1302	5.85 A	0.27	0.74
			(87)	5.48-6.22	0.24-0.29	0.67-1.80

Females

Treatment	Dose (ng/kg body wt/d)	*n*	Body mass-necropsy (g)	Thymus	Heart	Adrenal glands
Control	0	17	1164	0.11	0.71	0.026
			(54)	0.09-0.12	0.65-0.77	0.021-0.030
TCDD	2.1	7	999	0.08	0.83	0.035
			(87)	0.06-0.11	0.70-0.97	0.024-0.047
	4.6	8	990	0.09	0.85	0.028
			(78)	0.06-0.11	0.77-0.93	0.020-0.036
	6.0	16	1013	0.07 A	0.92 A	0.029
			(60)	0.06-0.08	0.87-0.96	0.025-0.032
	8.4	7	952	0.08	0.86	0.038 A
			(79)	0.06-0.10	0.72-0.99	0.033-0.043

Table 3.5. Cont'd.

PeCDF	13	13	1123	0.09	0.78	0.027
			(63)	0.08-0.11	0.72-0.84	0.020-0.034
	25	7	938	0.07	0.96 A	0.039
			(90)	0.04-0.09	0.74-1.18	0.025-0.053
	30	8	1043	0.07	0.76	0.032
			(79)	0.05-0.09	0.68-0.84	0.022-0.042
	49	1	967	0.06	0.76	0.028
TCDF	52	4	1025	0.06 A	0.89	0.027
			(80)	0.01-0.11	0.63-1.15	0.018-0.037
	116	12	1144	0.08	0.70	0.031
			(58)	0.06-0.09	0.62-0.78	0.026-0.037
	214	8	1019	0.07	0.84	0.030
			(61)	0.06-0.09	0.72-0.96	0.021-0.039
	246	9	1035	0.07	0.80	0.027
			(64)	0.06-0.09	0.70-0.89	0.021-0.032

[a]Body mass data are presented as the least squares mean with (standard error) beneath.

[b]Relative organ relative mass data are presented as the mean with 95% confidence interval beneath.

[c] Means that are significantly different than the control mean at $p < 0.05$ are designated with an A.

Pathology

Exposure to TCDD, PeCDF, and TCDF did not induce any consistent treatment-related histological changes in the tissues examined, with the exception of mandibular and maxillary squamous epithelial proliferation in six-week-old kits and 27-week-old juveniles (Bursian et al., 2012) and significant mineralization of the liver, heart, and thyroid gland in juveniles exposed to the greatest dose of TCDF (246 ng TCDF/kg body wt/d; 25 ng TEQ_{TCDF}/kg body wt/d) (Table 3.6). Additionally, in both six-week-old kits (data not shown) and 27-week-old juveniles, evidence of mild renal mineralization and hepatic vacuolation was observed in all dose groups, including the controls.

Table 3.6. Effects of dietary 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 2,3,4,7,8-pentachlorodibenzofuran (PeCDF), and 2,3,7,8-tetrachlorodibenzofuran (TCDF) on organ histology of juvenile mink.

Treatment	Dose (ng/kg body wt/d)	*n*	Kidney mineralization[a]	Hepatic vacuolation[b]	Hepatic mineralization[a,c]	Cardiac mineralization[a,c]	Thyroid mineralization[a,c]
Control		14	1.07	1.86	0	0	0
TCDD	2.1	9	1.11	1.89	0	0	0
	4.6	9	1.00	1.75	0	0	0
	6.0	9	1.11	1.67	0	0	0
	8.4	9	1.11	2.00	0	0.11	0
PeCDF	13	10	1.00	1.70	0	0.10	0
	25	7	1.00	2.00	0	0.14	0
	30	9	1.00	1.89	0	0	0
	49	9	1.00	1.89	0	0	0
TCDF	52	9	1.00	2.00	0	0.22	0
	116	10	0.91	1.89	0	0.20	0
	214	9	1.00	2.00	0.11	0.56	0.11
	246	10	1.00	2.00	0.60 A	0.90 A	0.60 A

[a] A value of 1 = mild mineralization; 2 = moderate mineralization

[b] A value of 1 = mild fatty vacuolation; 2 = moderate fatty vacuolation.

[c] Means that are significantly different than the control mean at $P < 0.05$ are designated with an A.

Hepatic and adipose TCDD/PeCDF/TCDF concentrations

Concentrations of TCDD, PeCDF, and TCDF in liver and adipose of adult females and 27-week-old juveniles generally increased with dose (Table 3.7). Concentrations of the three congeners in livers of adults were significantly different from those in livers of controls at the two highest doses of TCDF, all doses of PeCDF, and the two highest doses of TCDF. In adult female adipose tissue, concentrations of TCDD, PeCDF, and TCDF were significantly greater than control concentrations at all doses. Congener concentrations in livers of juvenile mink fed TCDD, PeCDF, and TCDF were significantly greater than those in livers of controls at all doses except the lowest doses of TCDD and TCDF. Concentrations of TCDD, PeCDF, and TCDF in adipose tissue of juveniles were significantly greater at all doses than those in adipose tissue of unexposed juvenile mink. Concentrations were generally similar between adults and juveniles.

Bioaccumulation factors were generally consistent across treatment groups for each congener (Table 3.7). Bioaccumulation factors were greater than one for TCDD and PeCDF in both liver and adipose tissue of adults and juveniles but less than one for TCDF in all treatment groups. The bioaccumulation factors indicated that TCDD bioaccumulated to a greater extent in adipose tissue than in the liver, whereas PeCDF bioaccumluated to a greater extent in liver than in adipose tissue. TCDF did not bioaccumulate in either tissue relative to the diet being fed.

Table 3.7. Hepatic and adipose concentrations of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 2,3,4,7,8-pentachlorodibenzofuran (PeCDF), and 2,3,7,8-tetrachlorodibenzofuran (TCDF) and bioaccumulation factors in adult female mink and their juvenile offspring.

Adults						
		Liver				
Dietary treatment	Dose (ng/kg body wt/d)	*n*	Concentration (ng/kg ww)	SE[a]	*p* value dose vs. control	Bioaccumulation factor[b]
Control		9	0.14	23.6		2.33
TCDD	2.1	9	56	23.6	0.4660	2.46
	4.6	7	157[c] A	26.7	0.0009	2.93
	6.0	7	250 A	26.7	< 0.0001	3.23
	8.4	7	364 A	26.7	< 0.0001	3.61
Control		9	0.53	336		4.08
PeCDF	13	9	1851 A	336	0.0036	11.2
	25	7	3066 A	381	< 0.0001	10.7
	30	8	4078 A	356	< 0.0001	11.2
	49	7	7209 A	381	< 0.0001	11.7
Control		9	0.66	13.2		0.250
TCDF	52	3	46	22.9	0.4560	0.068
	116	5	58	17.7	0.1080	0.040
	214	4	109 A	19.8	0.0014	0.045
	246	5	125 A	17.7	< 0.0001	0.044

Table 3.7. Cont'd.

Dietary treatment	Dose (ng/kg body wt/d)	Adipose				
		n	Concentration (ng/kg ww)	SE	*p* value dose vs. control	Bioaccumulation factor[b]
Control		6	0.89	51.9		14.8
TCDD	2.1	7	344 A	48.0	< 0.0001	15.1
	4.6	7	540 A	48.0	< 0.0001	10.1
	6.0	7	969 A	48.0	< 0.0001	12.5
	8.4	5	1418 A	56.8	< 0.0001	14.1
Control		6	3.7	190		26.2
PeCDF	13	6	1314 A	190	< 0.0001	7.92
	25	5	1704 A	208	< 0.0001	5.93
	30	7	2396 A	176	< 0.0001	6.59
	49	7	3008 A	176	< 0.0001	4.86
Control		6	0.44	44.1		0.170
TCDF	52	3	289 A	62.4	0.0004	0.425
	116	3	318 A	62.4	0.0002	0.217
	214	4	568 A	54.0	< 0.0001	0.237
	246	3	791 A	62.4	< 0.0001	0.276

Table 3.7. Cont'd.

Juveniles						
		Liver				
Dietary treatment	Dose (ng/kg body wt/d)	*n*	Concentration (ng/kg ww)	SE[a]	*p* value dose vs. control	Bioaccumulation factor[b]
Control		13	0.18	21.9		3.00
TCDD	2.1	11	59	32.1	0.0788	2.60
	4.6	9	165 A	25.8	< 0.0001	3.08
	6.0	9	289 A	22.4	< 0.0001	3.74
	8.4	9	338 A	23.9	< 0.0001	3.34
Control		13	0.94	341		7.28
PeCDF	13	9	1662 A	364	0.0002	10.0
	25	11	3161 A	431	< 0.0001	11.0
	30	9	5261 A	381	< 0.0001	14.5
	49	10	8167 A	457	< 0.0001	13.2
Control		13	0.17	18.0		0.064
TCDF	52	9	39	21.6	0.0824	0.057
	116	11	125 A	19.6	< 0.0001	0.085
	214	9	207 A	21.6	< 0.0001	0.086
	246	10	207 A	20.5	< 0.0001	0.072

Table 3.7. Cont'd.

Dietary treatment	Dose (ng/kg body wt/d)	Adipose				
		n	Concentration (ng/kg ww)	SE	*p* value dose vs. control	Bioaccumulation factor[b]
Control		7	0.09	86.5		15.5
TCDD	2.1	3	371 A	113	0.0065	16.4
	4.6	4	825 A	112	< 0.0001	15.5
	6.0	3	1105 A	113	< 0.0001	14.3
	8.4	3	1560 A	113	< 0.0001	15.5
Control		7	0.75	141		5.36
PeCDF	13	6	966 A	152	< 0.0001	5.82
	25	5	1536 A	166	< 0.0001	5.34
	30	3	1918 A	214	< 0.0001	5.28
	49	3	2903 A	214	< 0.0001	4.69
Control		7	0.53	23.2		0.200
TCDF	52	3	323 A	29.9	< 0.0001	0.475
	116	3	544 A	29.9	< 0.0001	0.372
	214	3	633 A	29.9	< 0.0001	0.264
	246	3	756 A	29.9	< 0.0001	0.264

[a]SE refers to standard error.

[b]Bioaccumulation factor = (liver or adipose concentration/feed concentration).

[c]Means that are significantly different than the control mean at $p < 0.05$ are designated with an A.

DISCUSSION

Reproductive performance and offspring viability

The reproductive performance of the control mink and viability of their offspring in the present study were comparable to those of control mink in two other reproduction trials (Bursian et al., 2006a,c) conducted at the MSU Experimental Farm using similar methodology. Average litter size at birth in the present study was 6.1 kits per litter compared to 5.7 (Bursian et al., 2006c) and 4.6 (Bursian et al., 2006a) kits per litter. Kit survivability through six weeks of age was 80.3% in the present study compared to 88.9% (Bursian et al., 2006c) and 85.0% (Bursian et al., 2006a).

Toxic equivalent doses of TCDD, PeCDF and TCDF as great as 8.4 ng TEQ_{TCDD}/kg body wt/d, 15 ng TEQ_{PeCDF}/kg body wt/d and 25 ng TEQ_{TCDF}/kg body wt/d, respectively, had no significant effect on reproductive performance of mink and viability of their offspring. These doses corresponded to maternal hepatic TEQ concentrations of 364, 2163 and 13 ng /kg ww, respectively. However, only 56% of the bred females in the highest PeCDF dose group whelped compared to 100% in the control group. It is possible that the dose of 15 ng TEQ_{PeCDF}/kg body wt/d affected the whelping rate, although one of the four females not whelping died of a ruptured uterus that contained five fetuses, which was not considered treatment related. The females that did whelp had a mean litter size that did not differ from the mean litter size of control females. In a mink feeding study utilizing 3,3',4,4'5-pentachlorobiphenyl (PCB 126), females fed diets containing 240 ng $TEQ_{PCB\ 126}$/kg feed (30 ng $TEQ_{PCB\ 126}$/kg body wt/d) and higher experienced complete reproductive

failure, whereas animals fed diet containing 24 ng $TEQ_{PCB\ 126}$/kg feed (3.0 ng $TEQ_{PCB\ 126}$/kg body wt/d) were not affected (Beckett et al., 2008).

The general lack of an effect on reproductive performance and offspring viability was unexpected in that reproductive impairment and reduced offspring viability have been associated with similar or lesser TEQ doses in other mink feeding studies using a similar exposure scenario (Beckett et al., 2008, Bursian et al., 2006a,b, Zwiernik et al., 2009, Heaton et al., 1995a, Tillitt et al., 1996, Hochstein et al., 2001). From studies utilzing single congeners, Hochstein et al. (1998) reported a 125 d LC50 value for TCDD in mink of 47 ng TEQ_{TCDD}/kg body wt/d, which is less than twice the highest TEQ dose provided by TCDF. Hochstein et al. (2001) also attempted a mink reproduction study utilizing TCDD at estimated daily doses of 2.0, 6.6, 22.5 and 175 ng TEQ_{TCDD}/kg body wt/d (16, 53, 180 and 1,400 ng TEQ_{TCDD}/kg feed, respectively). An effect on reproduction could not be clearly determined because of subnormal reproductive performance of the control group, which was attributed to the fact that the trial was conducted indoors. The highest dose resulted in 17% adult mortality and a 26% decrease in body weight. Significant dose-dependent decreases were noted in kit birth mass and survival from birth to three weeks of age in the groups that had reproduction (animals in the 16 ng TEQ_{TCDD}/kg feed [2.0 ng TEQ_{TCDD}/kg body wt/d] did not reproduce). Zwiernik et al. (2009) reported that dietary concentrations of 240 and 2,400 ng TCDF/kg feed (26 and 240 ng TEQ_{TCDF}/kg feed or estimated doses of 3.3 and 30 ng TEQ_{TCDF}/kg body wt/d) did not affect reproduction and kit viability, but body masses of

offspring through 36 weeks of age were decreased compared with controls at various time points.

Several mink feeding studies have been conducted using contaminated fish collected from specific bodies of water. In one such study, mink were fed diets containing fish collected from Saginaw Bay (MI, USA) that were contaminated with a mixture of PCB, PCDF and PCDD congeners (Heaton et al., 1995a, Tillitt et al., 1996). Mated females exposed to a dose of 8.3 ng $TEQ_{PCBs/PCDDs/PCDFs}$/kg body wt/d (based on a dietary concentration of 66 ng $TEQ_{PCBs/PCDDs/PCDFs}$/kg feed with TEQ recalculated using TEFs presented by Van den Berg et al. (2006) produced fewer live kits compared to controls, while a dose of 2.1 ng $TEQ_{PCBs/PCDDs/PCDFs}$/kg body wt/d (dietary concentration of 17 ng $TEQ_{PCBs/PCDDs/PCDFs}$/kg feed) significantly reduced kit viability through six weeks of age compared with controls. In another study of similar design, PCB/PCDF/PCDD-contaminated fish collected from the Housatonic River (MA, USA) resulting in a dose of 6.4 ng $TEQ_{PCBs/PCDDs/PCDFs}$/kg body wt/d (dietary concentration of 51 ng $TEQ_{PCBs/PCDDs/PCDFs}$/kg feed; TEQ recalculated using TEFs presented by Van den berg et al. (2006)) also had reduced kit viability at six weeks of age (Bursian et al., 2006a). The corresponding maternal hepatic concentrations were 226 ng $TEQ_{PCBs/PCDDs/PCDFs}$/kg and 189 ng $TEQ_{PCBs/PCDDs/PCDFs}$/kg for the Saginaw Bay (Heaton et al., 1995a, Tillitt et al., 1996) and Housatonic River (Bursian et al., 2006b) studies. In contrast to the Saginaw Bay (Heaton et al., 1995a, Tillitt et al., 1996) and Housatonic River (Bursian et al., 2006b) studies

and similar to the results in the present study, mink fed diets containing fish collected from the Saginaw River (MI, USA) at dietary concentrations of 22, 36 and 57 ng $TEQ_{PCBs/PCDDs/PCDFs}$/kg feed (TEQs were recalculated using TEFs presented by Van den berg et al., 2006), which correspond to estimated doses of 2.8, 4.5 and 7.1 ng $TEQ_{PCBs/PCDDs/PCDFs}$/kg body wt/d, experienced no effects on reproduction or kit viability. The doses of TEQs in the present study at which no effects on reproduction and survival were noted were up to fourfold greater than doses of TEQ in those fish feeding studies that reported such effects.

This apparent difference in toxicity between studies could be a reflection of the source of TEQ. Environmentally derived mixtures contain quantifiable PCBs and other identified TCDD-like contaminants that contribute the calculated sum TEQ value, whereas single congener studies provide a single congener source and subsequent TEQ value. In the Saginaw Bay study (Heaton et al., 1995a, Tillitt et al., 1996), PCB 126 contributed 62 and 53%, TCDD contributed 11 and 8% and PeCDF contributed 6 and 24% of the dietary and hepatic TEQs, respectively. In the Saginaw River study (Bursian et al., 2006c), PCB 126 contributed 33 and 34%, TCDD contributed 16 and 8%, and PeCDF contributed 17 and 44% of the dietary and hepatic TEQ, respectively. In the Housatonic River study (Bursian et al., 2006a,b), PCB 126 contributed 81 and 85%, TCDD contributed less than 1%, and PeCDF contributed 7 and 6% of the dietary and hepatic TEQs, respectively. In mink feeding studies using dietary TEQ provided exclusively by PCB 126 (Beckett et al., 2008) or TCDF (Bursian et al., 2006b), PCB 126 caused complete reproductive failure at a concentration that was one order of magnitude less than the greatest concentration of TCDF that resulted in no

reproductive effects. Thus, despite the fact that PCB 126 and TCDF have an identical TEF of 0.1, it is apparent that when provided individually, PCB 126 is considerably more toxic to mink than is TCDF. The same relationship could also be true for PCB 126 compared to TCDD and PeCDF, explaining reproductive effects at lesser doses of TEQ that are provided primarily by PCB 126, as in the Saginaw Bay (Heaton et al., 1995a, Tillitt et al., 1996), and Housatonic River (Bursian et al., 2006a) studies when compared to the present study.

Body mass

Exposure of mink to TCDD, PeCDF, and TCDF did not have a significant effect on body masses of adult females or male and female kits through six weeks of age and juvenile females through 27 weeks of age and had an inconsistent effect on juvenile male body mass at dietary TEQ concentrations as great as 287 ng/kg feed and TEQ doses up to 25 ng/kg body wt/d. The results of other mink feeding studies have indicated variable effects of TCDD-like chemicals on body mass. Adult female mink fed diets containing fish collected from Saginaw Bay that provided $TEQ_{PCBs/PCDDs/PCDFs}$ concentrations as little as 17 ng/kg feed (2.1 ng/kg body wt/d) produced kits of significantly lesser body mass at three and six weeks of age compared with control animals (Heaton et al., 1995a). Similarly, feeding mink diets containing 51 ng $TEQ_{PCBs/PCDDs/PCDFs}$/kg feed (6.4 ng $TEQ_{PCBs/PCDDs/PCDFs}$ /kg body wt/d) derived from fish collected from the Housatonic River resulted in a transient decrease in kit body masses at three weeks of age, but body masses of adult females and juveniles were not affected (Bursian et al., 2006a). Body masses of adult female mink and their offspring that were fed diets containing fish collected from the Saginaw River that provided up to 57 ng $TEQ_{PCBs/PCDDs/PCDFs}$/kg feed (7.1 ng $TEQ_{PCBs/PCDDs/PCDFs}$/kg body wt/d)

were not adversely affected (Bursian et al., 2006c). Body masses of offspring of mink fed a diet containing 24 ng $TEQ_{PCB\ 126}$/kg feed (3.0 ng $TEQ_{PCB\ 126}$/kg body wt/d) were not significantly different compared to controls (Beckett et al., 2008). Body masses of male mink kits exposed to TCDF in utero and during lactation at dietary concentrations of 24 and 240 ng TEQ/g feed (3.0 and 30 ng TEQ_{TCDF}/kg body wt/d) were less than those of controls at three weeks of age, and body masses of female offspring were less compared with those of controls from six to 36 weeks of age (Zwiernik et al., 2009).

Organ mass

Mink exposed to TCDD, PeCDF or TCDF had relative organ masses that were different compared to controls in some cases, and, although the differences were not strictly dose dependent, with the exception of what appeared to be a trend of increasing relative liver masses with dose in juvenile males exposed to TCDD and TCDF, the changes were comparable to those reported in other mink feeding studies involving TCDD-like chemicals. In the present study, mean relative liver, heart, spleen, kidney and adrenal gland masses were greater compared to controls for various doses of the three chemicals in the three age groups, and mean relative thymus masses were reduced. Adult female mink fed diets containing fish collected from Saginaw Bay that provided from 17 to 66 ng $TEQ_{PCBs/PCDDs/PCDFs}$/kg feed (2.1 to 8.3 ng $TEQ_{PCBs/PCDDs/PCDFs}$/kg body wt/d, respectively) exhibited greater mean relative spleen and liver masses at all doses, greater mean relative adrenal gland masses at 33 and 66 ng $TEQ_{PCBs/PCDDs/PCDFs}$/kg feed (4.1 and 8.3 ng $TEQ_{PCBs/PCDDs/PCDFs}$/kg body wt/day, respectively) and greater mean relative kidney mass at 66 ng

$TEQ_{PCBs/PCDDs/PCDFs}$/kg feed (8.3 ng $TEQ_{PCBs/PCDDs/PCDFs}$/kg body wt/d) compared with controls (Heaton et al., 1995a). Conversely, six-week-old kits generally had reduced mean relative organ masses at 17 and 33 ng $TEQ_{PCBs/PCDDs/PCDFs}$/kg feed (2.1 and 4.1 ng $TEQ_{PCBs/PCDDs/PCDFs}$/kg body wt/day, respectively). No kits survived in the 66 ng $TEQ_{PCBs/PCDDs/PCDFs}$/kg feed (8.3 ng $TEQ_{PCBs/PCDDs/PCDFs}$/kg body wt/d) treatment group (Heaton et al., 1995a). The mean relative liver mass in six-week-old kits whelped by dams exposed to 36 and 57 ng $TEQ_{PCBs/PCDDs/PCDFs}$/kg feed (4.5 and 7.1 ng $TEQ_{PCBs/PCDDs/PCDFs}$/kg body wt/d) provided by fish collected from the Saginaw River were greater than those of individuals fed a control diet (Zwiernik et al., 2008). No differences were observed in mean organ masses of adult female mink fed diets that provided up to 51 ng $TEQ_{PCBs/PCDDs/PCDFs}$/kg feed (6.4 ng $TEQ_{PCBs/PCDDs/PCDFs}$/kg body wt/d) derived from fish collected from the Housatonic River relative to those of controls, but six-week-old female kits in the 51 ng $TEQ_{PCBs/PCDDs/PCDFs}$/kg feed (6.4 ng $TEQ_{PCBs/PCDDs/PCDFs}$/kg body wt/d) treatment group had greater mean relative brain, kidney, and liver masses, and 31-week-old male and female juveniles from the same treatment group had increased relative spleen masses compared to controls (Bursian et al., 2006b). Zwiernik et al. (2009) reported no effects on organ masses in mink that had been exposed from conception through 72 weeks of age to 24 and 240 ng TEQ_{TCDF}/kg feed (3.0 and 30 ng TEQ_{TCDF}/kg body wt/d) provided by TCDF.

Pathology

Other than mandibular and maxillary squamous epithelial proliferation (Bursian et al. 2012) hepatic vacuolation and mineralization of the kidney, liver, heart and thyroid gland were the only pathological effects noted in the present study. Hepatic vacuolation and renal mineralization occurred in juveniles in all treatment groups, including the control group, and thus, were not considered to be treatment related. These results are similar to those reported by Bursian et al. (2006b). In contrast, Heaton et al. (1995b) reported that adult female mink exposed to PCBs/PCDDs/PCDFs at dietary concentrations ranging from 17 to 66 ng $TEQ_{PCBs/PCDDs/PCDFs}$/kg feed (2.1 to 8.3 ng $TEQ_{PCBs/PCDDs/PCDFs}$/kg body wt/d) through dietary inclusion of fish collected from Saginaw Bay for a time period equivalent to that in the present study had enlarged and diffusely yellow livers. Histologically, the livers had various degrees of congestion, hepatocellular fatty change, and scattered aggregates of lymphocytes. Mineralization of the liver, thyroid gland, and heart in animals exposed to TCDF in the present study appeared to be related to dose. There are no reports in the literature of soft tissue mineralization induced by TCDD-like chemicals.

Hepatic and adipose TCDD/PeCDF/TCDF concentrations

Concentrations of TCDD, PeCDF and TCDF in liver and adipose increased with dose. Bioaccumulation factors suggested that TCDD and PeCDF bioaccumulated in both liver and adipose tissue, although to different degrees, and that TCDF was rapidly eliminated from the animal. Results from a toxicokinetic study of PeCDF and TCDF in mink (Zwiernik et al., 2008b, Moore et al., 2009) are similar to those reported here, in that PeCDF accumulated in the liver of the mink to a much greater extent than did TCDF. This suggested

hepatic sequestration of PeCDF, perhaps by binding of the congener to hepatic CYP1A2 protein, which has been shown to occur in rodents (Brewster et al., 1987, Brewster et al., 1988, DeVito et al., 1997, Diliberto et al., 1999). The lesser concentrations of TCDF in livers of mink suggested an efficient elimination and/or metabolism of the congener. The half-life of PeCDF was estimated to be approximately 8 d, whereas the half-life of TCDF was less than 0.5 d in mink (Fur Commision U.S.A., 2003). These values are less than those reported for rodents. The half-life of TCDF is approximately 2 d in mice (Brewster et al., 1987), and the half-life of PeCDF in the rat is more than 60 d (Brewster et al., 1987).

Conclusions

Results of the present study indicate that TEQ concentrations provided by TCDD, PeCDF or TCDF, which were expected to result in complete reproductive failure in mink based on studies using environmentally derived mixtures of TCDD-like chemicals with calculated sum of TEQ, had no significant effects on reproductive performance of adult female mink or growth and viability of their offspring through 27 weeks of age. Additionally, minimal and, in some cases, inconsistent effects were seen on more subtle individual health endpoints, including organ masses and morphology. Hepatic and adipose concentrations of the three congeners suggested that PeCDF is preferentially sequestered in the liver more so than in adipose tissue relative to TCDD and that TCDF is rapidly eliminated from the animal. Although the results of the present study are insufficient to calculate the relative potency of PeCDF and TCDF to TCDD for reproductive endpoints, histological data presented in Bursian et al. (2012) as well as comparisons with parallel studies suggest that the current TEF values may not accurately predict the toxic potency of

TCDD, PeCDF and TCDF as compared with PCB 126 or environmental contaminant mixtures composed largely of PCB 126.

ACKNOWLEDGEMENT

This research received financial support from an unrestricted grant from The Dow Chemical Company.

APPENDIX

SUPPLEMENTAL DATA

Table S1. Effects of TCDD, PeCDF, and TCDF on adult female mink absolute organ mass (g)[a,b].

Dietary treatment	Dose (ng/kg body wt/d)	*n*	Liver[c]	Thyroid gland	Thymus	Heart	Spleen
Control	0	9	41.17	0.063	0.52	6.72	1.95
			3.12	0.011	0.17	0.56	0.54
TCDD	2.1	8	51.86 A	0.088	0.95	8.33	2.27
			3.31	0.011	0.18	0.60	0.58
	4.6	9	50.08 A	0.094	0.78	6.56	3.25
			3.12	0.011	0.17	0.56	0.54
	6.0	8	47.96	0.070	0.69	7.91	2.19
			3.31	0.011	0.18	0.60	0.58
	8.4	9	48.23	0.074	0.50	7.67	3.04
			3.31	0.011	0.18	0.60	0.58
PeCDF	13	9	42.95	0.069	0.58	8.36 A	3.59 A
			3.12	0.011	0.17	0.56	0.54
	25	9	45.59	0.121 A	0.68	8.17	2.63
			3.12	0.011	0.17	0.56	0.54
	30	9	51.89 A	0.084	0.96	7.57	3.31
			3.12	0.011	0.17	0.56	0.54
	49	8	53.07 A	0.084	0.60	7.71	3.26
			3.31	0.012	0.18	0.60	0.58

Table S1. Cont'd.

TCDF	52	9	50.93 A	0.093	1.14	8.02	3.06
			3.12	0.011	0.17	0.56	0.54
	116	9	45.69	0.060	0.44	7.17	2.81
			3.12	0.011	0.17	0.56	0.54
	214	9	55.01 A	0.089	0.80	8.97	3.76 A
			3.12	0.011	0.17	0.56	0.54
	246	9	53.11 A	0.079	0.56	7.03	3.23
			2.96	0.011	0.16	0.53	0.52
Control vs Congener							
p-value TCDD			0.0190	0.1566	0.2731	0.1618	0.2303
p-value PeCDF			0.0420	0.0363	0.3208	0.0542	0.0439
p-value TCDF			0.0050	0.1754	0.2552	0.0897	0.0401

Table S1. Cont'd.

Dietary treatment	Dose (ng/kg bw/d)	*n*	Adrenal glands	Kidneys	Lymph Node	Brain	Reproductive Tract
Control	0	9	0.161	8.14	0.47	7.86	0.87
			0.030	0.34	0.11	0.21	0.18
TCDD	2.1	8	0.171	8.24	0.78 A	7.62	1.00
			0.031	0.37	0.11	0.23	0.17
	4.6	9	0.167	8.40	0.79 A	7.22	1.22
			0.030	0.34	0.11	0.21	0.16
	6.0	8	0.176	9.18	0.65	7.99	1.11
			0.031	0.37	0.11	0.23	0.17
	8.4	9	0.162	7.67	0.64	7.74	1.07
			0.031	0.39	0.11	0.23	0.18
PeCDF	13	9	0.160	7.77	0.61	8.06	1.00
			0.030	0.34	0.11	0.21	0.16
	25	9	0.202	8.00	0.69	7.71	1.32
			0.030	0.34	0.11	0.21	0.16
	30	9	0.209	8.28	0.81 A	7.86	1.29
			0.030	0.34	0.11	0.21	0.17
	49	8	0.062	8.72	0.76	8.03	1.24
			0.031	0.37	0.11	0.23	0.17

Table S1. Cont'd.

TCDF	52	9	0.270	8.46	0.93 A	7.76	1.34
			0.030	0.34	0.11	0.21	0.16
	116	9	0.174	8.33	0.61	7.61	1.05
			0.030	0.34	0.11	0.21	0.16
	214	9	0.190	9.03	0.72	7.87	1.13
			0.030	0.34	0.11	0.21	0.16
	246	9	0.180	7.90	0.61	7.74	1.20
			0.028	0.33	0.10	0.20	0.15
Control vs Congener							
p-value TCDD			0.8225	0.5526	0.0461	0.3641	0.1436
p-value PeCDF			0.5141	0.8917	0.0436	0.8252	1.0000
p-value TCDF			0.2069	0.4500	0.0405	0.6191	0.1171

[a]TCDD is 2,3,7,8-tetrachlorodibenzo-p-dioxin; PeCDF is 2,3,4,7,8-pentachlorodibenzofuran; and TCDF is 2,3,7,8-tetrachlorodibenzofuran.

[b]Data are presented as least squares mean with standard error beneath.

[c]Means that are significantly different than the control mean at $p < 0.05$ are designated with an A.

Table S2. Effects of TCDD, TCDF, and PeCDF on adult female mink relative organ mass[a] (% of body mass).

Dietary treatment	Dose (ng/kg body wt/d)	*n*	Body mass necropsy[b] (g)	Liver[c,d]	Thyroid gland	Thymus	Heart	Spleen
Control	0	9	788	5.25	0.008	0.06	0.86	0.25
			70	4.71-5.80	0.005-0.012	0.03-0.09	0.77-0.94	0.22-0.28
TCDD	2.1	8	931	5.65	0.009	0.09	0.90	0.24
			74	4.95-6.34	0.007-0.012	0.04-0.14	0.79-1.01	0.22-0.26
	4.6	9	968	5.29	0.011	0.07	0.73	0.33
			70	4.76-5.83	0.007-0.014	0.05-0.10	0.53-0.92	0.26-0.40
	6.0	8	949	5.13	0.007	0.07	0.84	0.23
			74	4.42-5.83	0.006-0.009	0.04-0.10	0.76-0.93	0.18-0.28
	8.4	9	877	5.61	0.008	0.06	0.89	0.34 A
			70	4.88-6.18	0.006-0.010	0.04-0.08	0.66-1.17	0.30-0.42
PeCDF	13	9	860	5.08	0.008	0.06	0.98	0.42
			70	4.52-5.64	0.007-0.010	0.04-0.09	0.81-1.16	0.04-0.80
	25	9	969	4.87	0.014	0.07	0.85	0.27
			70	4.03-5.70	0.004-0.025	0.04-0.09	0.73-0.97	0.23-0.31
	30	9	1028 A	5.18	0.009	0.09	0.77	0.32
			70	4.41-5.96	0.006-0.012	0.06-0.11	0.66-0.88	0.24-0.40
	49	8	1037 A	5.15	0.008	0.06	0.76	0.30
			74	4.64-5.66	0.007-0.010	0.05-0.07	0.64-0.88	0.22-0.39

Table S2. Cont'd.

TCDF	52	9	1052	4.96	0.009	0.10	0.78	0.30
			70	4.16-5.76	0.007-0.010	0.05-0.14	0.64-0.91	0.24-0.36
	116	9	841	5.58	0.008	0.05	0.88	0.35
			70	4.61-6.54	0.005-0.010	0.04-0.07	0.72-1.03	0.14-0.55
	214	9	947	5.99	0.010	0.08	0.96	0.41
			70	4.92-7.05	0.007-0.012	0.05-0.11	0.84-1.08	0.26-0.56
	246	9	877	6.16	0.010	0.06	0.82	0.34
			70	5.53-6.79	0.007-0.013	0.05-0.08	0.73-0.90	0.26-0.43
Control vs Congener								
p-value TCDD			0.0703	0.6617	0.2572	0.5972	0.2701	0.0004
p-value PeCDF			0.0196	0.8683	0.3180	0.3850	0.0479	0.6408
p-value TCDF			0.0728	0.1050	0.3669	0.2343	0.1547	0.2455

Table S2. Cont'd.

Dietary treatment	Dose (ng/kg body wt/d)	*n*	Body mass necropsy[b] (g)	Adrenal glands	Kidneys	Lymph Node	Brain	Reproductive Tract
Control	0	9	788	0.021	1.06	0.06	1.03	0.12
			70	0.016-0.027	0.92-1.20	0.04-0.07	0.87-1.19	0.10-0.13
TCDD	2.1	8	931	0.018	0.91	0.08	0.85	0.13
			74	0.014-0.023	0.76-1.05	0.06-0.10	0.70-1.00	0.11-0.15
	4.6	9	968	0.019	0.91	0.08	0.81	0.12
			70	0.013-0.025	0.74-1.09	0.06-0.10	0.57-1.05	0.10-0.14
	6.0	8	949	0.019	0.99	0.06	0.86	0.12
			74	0.014-0.024	0.83-1.15	0.04-0.09	0.71-1.01	0.10-0.13
	8.4	9	877	0.019	0.86	0.07	0.91	0.12
			70	0.013-0.024	0.73-1.00	0.05-0.08	0.75-1.03	0.10-0.14
PeCDF	13	9	860	0.020	0.92	0.07	0.96	0.11
			70	0.014-0.025	0.81-1.03	0.05-0.08	0.83-1.10	0.09-0.13
	25	9	969	0.022	0.86	0.07	0.83	0.13
			70	0.015-0.030	0.70-1.02	0.06-0.08	0.68-0.98	0.10-0.16
	30	9	1028 A	0.021	0.85	0.07	0.82	0.13
			70	0.018-0.025	0.70-1.00	0.06-0.09	0.64-1.00	0.02-0.14
	49	8	1037 A	0.017	0.87	0.07	0.81	0.11
			74	0.012-0.022	0.76-0.97	0.05-0.09	0.66-0.97	0.08-0.15

Table S2. Cont'd.

TCDF	52	9	1052	0.026	0.84	0.09	0.77	0.13
			70	0.005-0.047	0.71-0.97	0.07-0.11	0.65-0.89	0.09-0.16
	116	9	841	0.021	1.02	0.07	0.93	0.12
			70	0.017-0.026	0.83-1.21	0.06-0.08	0.82-1.04	0.10-0.14
	214	9	947	0.020	0.98	0.08	0.85	0.12
			70	0.017-0.023	0.82-1.15	0.05-0.10	0.75-0.95	0.10-0.13
	246	9	877	0.022	0.93	0.07	0.96	0.13
			70	0.016-0.028	0.81-1.06	0.06-0.08	0.76-1.15	0.10-0.16
Control vs Congener								
p-value TCDD			0.0703	0.8862	0.2381	0.2169	0.2269	0.7519
p-value PeCDF			0.0196	0.5203	0.0766	0.4772	0.0842	0.5409
p-value TCDF			0.0728	0.9907	0.1549	0.2012	0.0621	0.8898

[a]TCDD is 2,3,7,8-tetrachlorodibenzo-p-dioxin; PeCDF is 2,3,4,7,8-pentachlorodibenzofuran; and TCDF is 2,3,7,8-tetrachlorodibenzofuran.

[b]Body mass data are presented as least squares mean with standard error beneath.

[c]Relative organ mass data are presented as mean with 95% confidence interval beneath.

[d]Means that are significantly different than the control mean at $p < 0.05$ are designated with an A.

Table S3. Effects of TCDD, PeCDF, and TCDF on female mink kit absolute organ mass (g) at six weeks of age[a,b].

Dietary treatment	Dose (ng/kg bw/d)	*n*	Liver	Thyroid gland	Thymus	Heart	Spleen	Adrenal glands	Kidneys	Lymph Node	Brain
Control	0	5	16.70	0.044	0.491	2.41	0.97	0.108	3.33	0.230	8.33
			4.90	0.043	0.163	0.49	0.55	0.039	0.66	0.072	0.55
TCDD	2.1	0	---	---	---	---	---	---	---	---	---
	4.6	0	---	---	---	---	---	---	---	---	---
	6.0	6	16.16	0.032	0.457	1.94	1.82	0.070	3.03	0.224	7.60
			3.52	0.039	0.133	0.35	0.41	0.036	0.48	0.066	0.40
	8.4	1	11.03	0.011	0.114	1.35	0.90	0.084	2.72	0.119	7.45
PeCDF	13	4	18.43	0.125	0.616	2.49	1.53	0.174	3.31	0.415	8.44
			4.92	0.048	0.172	0.50	0.56	0.044	0.67	0.080	0.55
	25	0	---	---	---	---	---	---	---	---	---
	30	0	---	---	---	---	---	---	---	---	---
	49	1	2.76	0.028	0.065	0.62	0.21	0.088	1.13	0.057	5.13

Table S3. Cont'd.

TCDF	52	0	---	---	---	---	---	---	---	---	---
	116	2	12.10	0.026	0.123	1.46	1.60	0.049	1.96	0.161	7.29
			6.10	0.067	0.231	0.61	0.71	0.062	0.83	0.114	0.69
	214	1	9.43	0.031	0.229	1.11	0.73	0.069	1.87	0.246	8.26
	246	1	20.75	0.047	0.234	1.98	1.37	0.058	2.83	0.235	7.67
Control vs Congener											
p-value TCDD			0.4914	0.7620	0.3837	0.2476	0.7604	0.6435	0.5009	0.6345	0.3189
p-value PeCDF			0.3739	0.6653	0.5143	0.2589	0.8610	0.7427	0.2776	0.9684	0.1541
p-value TCDF			0.5257	0.8966	0.2528	0.1991	0.8712	0.4727	0.2299	0.8882	0.4278

[a]TCDD is 2,3,7,8-tetrachlorodibenzo-p-dioxin; PeCDF is 2,3,4,7,8 pentachlorodibenzofuran; and TCDF is 2,3,7,8-tetrachlorodibenzofuran.

[b]Data are presented as least squares mean with standard error beneath.

Table S4. Effects of TCDD, PeCDF, and TCDF on female mink kit relative organ mass (% of body mass) at six weeks of age[a].

Dietray treatment	Dose (ng/kg body wt/d)	*n*	Body mass necropsy[b] (g)	Liver[c,d]	Thyroid gland	Thymus	Heart	Spleen
Control	0	5	246	7.36	0.021	0.20	1.12	0.40
			63	6.03-8.70	0.014-0.028	0.06-0.34	0.97-1.27	0.15-0.66
TCDD	2.1	0	---	---	---	---	---	---
	4.6	0	---	---	---	---	---	---
	6.0	6	213	7.55	0.017	0.21	0.91 A	0.80 A
			37	5.87-9.22	0.004-0.030	0.12-0.29	0.80-1.02	0.57-1.03
	8.4	1	146	7.55	0.008	0.08	0.93	0.61 A
PeCDF	13	4	254	7.98	0.07	0.28	1.16	0.63
			63	6.74-9.21	0-0.265	0.08-0.05	0.83-1.49	0.32-0.94
	25	0	---	---	---	---	---	---
	30	0	---	---	---	---	---	---
	49	1	51	5.44	0.055	0.13	1.23	0.41

Table S4. Cont'd.

TCDF	52	0	---	---	---	---	---	---
	116	2	140	8.50	0.020	0.09	1.02	1.09 A
			56	0-17.33	0-0.115	0-0.55	0-2.42	0-4.31
	214	1	152	6.20	0.020	0.15	0.73	0.48
	246	1	188	11.03	0.025	0.12	1.05	0.73
Control vs Congener								
p-value TCDD			0.6420	0.9929	0.6457	0.3952	0.0509	0.0375
p-value PeCDF			0.3398	0.2004	0.7254	0.4616	0.8035	0.3325
p-value TCDF			0.5535	0.1845	0.9009	0.6678	0.2136	0.1745

Table S4. Cont'd.

Dietray treatment	Dose (ng/kg body wt/d)	*n*	Body mass necropsy[b] (g)	Adrenal glands	Kidneys	Lymph Node	Brain
Control	0	5	246	0.055	1.53	0.10	4.07
			63	0.013-0.096	1.37-1.69	0.05-0.15	2.73-5.41
TCDD	2.1	0	---	---	---	---	---
	4.6	0	---	---	---	---	---
	6.0	6	213	0.033	1.45	0.10	4.06
			37	0.027-0.039	1.25-1.64	0.08-0.13	2.47-5.66
	8.4	1	146	0.058	1.86	0.08	5.10
PeCDF	13	4	254	0.100	1.54	0.20	4.19
			63	0-0.275	1.08-2.01	0-0.44	2.11-6.27
	25	0	---	---	---	---	---
	30	0	---	---	---	---	---
	49	1	51	0.173	2.22	0.11	10.10 A

Table S4. Cont'd.

TCDF	52	0	---	---	---	---	---
	116	2	140	0.035	1.39	0.11	5.31
			56	0-0.035	1.16-1.62	0-0.32	0-12.80
	214	1	152	0.045	1.23	0.16	5.43
	246	1	188	0.031	1.50	0.12	4.08
Control vs Congener							
p-value TCDD			0.6420	0.2747	0.1677	0.8517	0.7158
p-value PeCDF			0.3398	0.4403	0.1132	0.4047	0.0558
p-value TCDF			0.5535	0.8143	0.3871	0.6064	0.4976

[a]TCDD is 2,3,7,8-tetrachlorodibenzo-p-dioxin; PeCDF is 2,3,4,7,8-pentachlorodibenzofuran, and TCDF is 2,3,7,8-tetrachlorodibenzofuran.

[b]Body mass data are presented as least squares mean with standard error beneath.

[c]Relative organ mass data are presented as mean with 95% confidence interval beneath.

[d]Means that are significantly different than the control mean at $p < 0.05$ are designated with an A.

Table S5. Effects of TCDD, PeCDF, and TCDF on male mink kit absolute organ mass (g) at six weeks of age[a,b].

Dietary treatment	Dose (ng/kg body wt/d)	*n*	Liver[c]	Thyroid gland	Thymus	Heart	Spleen	Adrenal glands	Kidneys	Lymph Node	Brain
Control	0	8	15.58	0.041	0.282	2.22	0.88	0.081	2.93	0.227	9.08
			2.93	0.007	0.120	0.35	0.30	0.012	0.62	0.053	0.44
TCDD	2.1	1	14.87	0.051	0.367	1.76	0.95	0.061	2.73	0.177	8.18
	4.6	2	23.45	0.038	0.603	2.53	2.22	0.107	4.01	0.361	8.57
			5.85	0.012	0.196	0.71	0.52	0.024	1.23	0.107	0.75
	6.0	2	15.42	0.053	0.485	1.84	1.11	0.095	3.57	0.247	8.84
			5.85	0.017	0.196	0.71	0.52	0.024	1.23	0.107	1.05
	8.4	8	13.78	0.039	0.283	1.70	1.26	0.067	2.80	0.204	8.21
			2.93	0.007	0.120	0.35	0.30	0.012	0.62	0.053	0.44
PeCDF	13	5	18.68	0.032	0.430	2.50	1.34	0.084	3.39	0.293	9.21
			3.70	0.008	0.136	0.45	0.35	0.015	0.78	0.067	0.51
	25	0	---	---	---	---	---	---	---	---	---
	30	3	22.18	0.033	0.561	2.78	2.66	0.076	3.69	0.340	9.63
			4.78	0.008	0.160	0.58	0.42	0.019	1.01	0.087	0.61
	49	0	---	---	---	---	---	---	---	---	---

Table S5. Cont'd.

TCDF	52	7	26.95 A	0.067	0.792	3.12	1.88	0.095	5.54	0.387	10.25
			3.13	0.007	0.121	0.38	0.31	0.013	0.66	0.057	0.46
	116	5	27.31 A	0.043	0.513	3.13	1.78	0.116	4.47	0.461	9.53
			3.70	0.008	0.136	0.45	0.35	0.015	0.78	0.067	0.51
	214	4	20.20	0.054	0.279	2.47	1.58	0.086	3.67	0.289	8.23
			4.14	0.008	0.138	0.50	0.37	0.017	0.87	0.075	0.53
	246	4	20.28	0.050	0.411	2.41	1.30	0.065	3.32	0.256	9.61
			4.14	0.008	0.138	0.50	0.37	0.017	0.87	0.075	0.53
Control vs Congener											
p-value TCDD			0.7631	0.7311	0.4439	0.6087	0.2742	0.9120	0.7188	0.7961	0.3519
p-value PeCDF			0.2756	0.3879	0.3034	0.4305	0.1005	0.9909	0.5099	0.2683	0.6262
p-value TCDF			0.0425	0.1156	0.1485	0.2140	0.0734	0.5403	0.1056	0.0846	0.7175

[a]TCDD is 2,3,7,8-tetrachlorodibenzo-p-dioxin; PeCDF is 2,3,4,7,8-pentachlorodibenzofuran; and TCDF is 2,3,7,8-tetrachlorodibenzofuran.

[b]Data are presented as least squares means with standard error beneath.

[c]Means that are significantly different than the control mean at $p < 0.05$ are designated with an A.

Table S6. Effects of TCDD, PeCDF, and TCDF on male mink kit relative organ mass (% of body mass) at six weeks of age[a].

Dietary treatment	Dose (ng/kg body wt/d)	*n*	Body mass necropsy[b] (g)	Liver[c]	Thyroid gland	Thymus	Heart	Spleen
Control	0	8	208	7.72	0.021	0.14	1.13	0.43
			25	6.73-8.71	0.017-0.025	0.10-0.18	0.99-1.28	0.29-0.56
TCDD	2.1	1	186	8.00	0.027	0.20	0.95	0.51
	4.6	2	253	9.46	0.015	0.21	1.02	0.88
			42	1.17-17.75	0-0.036	0-1.40	0.11-1.93	0.84-0.92
	6.0	2	174	9.23	0.022	0.26	1.16	0.63
			42	0-21.01	---	0-0.97	0-4.44	0.21-1.04
	8.4	8	191	7.26	0.017	0.15	0.96	0.65
			25	6.37-8.16	0.013-0.021	0.09-0.21	0.71-1.21	0.45-0.84
PeCDF	13	5	241	7.42	0.013	0.17	0.98	0.52
			27	6.24-8.59	0.006-0.020	0.13-0.22	0.75-1.21	0.27-0.76
	25	0	---	---	---	---	---	---
	30	3	279	7.88	0.012	0.20	1.01	0.77
			31	5.73-10.04	0.007-0.017	0.13-0.27	0.74-1.28	0.30-1.24
	49	0	---	---	---	---	---	---

Table S6. Cont'd.

TCDF	52	7	312	8.46	0.022	0.27	0.97	0.61
			33	7.39-9.54	0.019-0.025	0.09-0.46	0.76-1.18	0.45-0.79
	116	5	309	8.66	0.014	0.16	1.00	0.58
			39	6.93-10.39	0.011-0.016	0.08-0.23	0.83-1.18	0.45-0.71
	214	4	217	8.95	0.030	0.14	1.10	0.66
			43	5.80-12.10	0-0.070	0.05-0.23	0.44-1.76	0-1.38
	246	4	225	9.09	0.022	0.17	1.07	0.58
			43	8.03-10.14	0.012-0.032	0.07-0.27	0.96-1.18	0.55-0.62
Control vs Congener								
p-value TCDD			0.6869	0.1359	0.2330	0.4967	0.5789	0.1538
p-value PeCDF			0.2807	0.8102	0.0868	0.1524	0.5704	0.1348
p-value TCDF			0.0983	0.3892	0.2418	0.0938	0.6835	0.5371

Table S6. Cont'd.

Dietary treatment	Dose (ng/kg body wt/d)	*n*	Body mass necropsy (g)	Adrenal glands	Kidneys	Lymph Node	Brain
Control	0	8	208	0.042	1.48	0.11	4.66
			25	0.031-0.053	1.31-1.65	0.09-0.14	3.99-5.34
TCDD	2.1	1	186	0.032	1.47	0.10	4.40
	4.6	2	253	0.040	1.66	0.14	3.63
			42	0-0.155	0-5.12	0.00-0.28	0-14.68
	6.0	2	174	0.052	2.51	0.15	3.63
			42	0-0.130	0-17.16	0.03-0.26	---
	8.4	8	191	0.038	1.51	0.10	4.80
			25	0.032-0.044	1.38-1.64	0.07-0.14	3.61-5.99
PeCDF	13	5	241	0.034	1.34	0.12	3.80
			27	0.022-0.047	1.14-1.54	0.10-0.14	2.72-4.87
	25	0	---	---	---	---	---
	30	3	279	0.027	1.33	0.12	3.58
			31	0-0.060	1.15-1.50	0.05-0.18	1.74-5.43
	49	0	---	---	---	---	---

Table S6. Cont'd.

TCDF	52	7	312	0.031	1.72	0.12	3.63
			33	0.026-0.036	1.19-2.26	0.08-0.15	2.59-4.67
	116	5	309	0.038	1.48	0.14	3.24
			39	0.021-0.055	1.20-1.75	0.10-0.18	2.46-4.02
	214	4	217	0.046	1.67	0.14	4.06
			43	0-0.096	1.34-2.00	0.12-0.16	2.52-5.59
	246	4	225	0.029	1.46	0.11	4.51
			43	0.024-0.034	1.17-1.76	0.07-0.15	2.42-6.59
Control vs Congener							
p-value TCDD			0.6869	0.5331	0.2728	0.4901	0.7259
p-value PeCDF			0.2807	0.3063	0.4595	0.9486	0.4007
p-value TCDF			0.0983	0.4373	0.6485	0.3460	0.1939

[a]TCDD is 2,3,7,8-tetrachlorodibenzo-p-dioxin; PeCDF is 2,3,4,7,8-pentachlorodibenzofuran; and TCDF is 2,3,7,8-tetrachlorodibenzofuran.

[b]Body mass data are presented as least squares mean with standard error beneath.

[c]Relative organ mass data are presented as mean with 95% confidence interval beneath.

Table S7. Effects of TCDD, PeCDF, and TCDF on juvenile female mink absolute organ mass (g) at 27 weeks of age[a,b].

Dietary treatment	Dose (ng/kg body wt/d)	*n*	Liver	Thyroid glands	Thymus[c]	Heart	Spleen
Control	0	17	58.66	0.080	1.210	8.27	3.21
			2.45	0.021	0.084	0.49	0.19
TCDD	2.1	7	54.63	0.084	0.807 A	8.35	2.38
			3.80	0.033	0.134	0.78	0.31
	4.6	8	58.74	0.081	0.903 A	8.75	3.23
			3.53	0.031	0.123	0.71	0.29
	6.0	16	58.69	0.091	0.702 A	9.35	3.04
			2.56	0.022	0.092	0.54	0.21
	8.4	7	56.31	0.080	0.753 A	8.05	3.32
			3.67	0.033	0.126	0.72	0.30
PeCDF	13	13	58.04	0.086	1.060	8.64	2.53
			2.74	0.024	0.096	0.56	0.22
	25	7	56.00	0.083	0.581 A	8.84	2.64
			3.84	0.033	0.136	0.79	0.31
	30	8	61.42	0.206	0.758 A	7.93	3.38
			3.46	0.031	0.120	0.69	0.28
	49	1	56.37	0.076	0.582 A	7.39	3.40

Table S7. Cont'd.

TCDF	52	4	51.93	0.088	0.584 A	9.09	2.26
			4.68	0.044	0.155	0.88	0.38
	116	12	59.72	0.077	0.871 A	7.92	3.58
			2.97	0.025	0.106	0.62	0.24
	214	8	55.88	0.094	0.755 A	8.59	3.12
			3.40	0.031	0.115	0.66	0.28
	246	9	58.62	0.070	0.745 A	8.29	3.62
			3.32	0.029	0.117	0.68	0.27
Control vs Congener							
p-value TCDD			0.4490	0.8811	< 0.0001	0.9126	0.2911
p-value PeCDF			0.7644	0.3320	0.0002	0.6483	0.3936
p-value TCDF			0.4346	0.9445	< 0.0001	0.9519	0.7851

Table S7. Cont'd.

Dietary treatment	Dose (ng/kg body wt/d)	*n*	Adrenal glands	Kidneys	Lymph Node	Brain	Reproductive Tract
Control	0	17	0.293	8.20	2.10	8.65	1.82
			0.025	0.31	0.18	0.17	0.11
TCDD	2.1	7	0.353	7.99	1.13 A	8.54	1.41
			0.039	0.49	0.30	0.26	0.18
	4.6	8	0.287	7.89	1.44 A	8.75	1.67
			0.036	0.44	0.27	0.24	0.17
	6.0	16	0.292	7.95	1.19 A	8.40	1.39 A
			0.026	0.33	0.20	0.18	0.12
	8.4	7	0.366	7.85	1.50 A	8.21	1.54
			0.039	0.45	0.28	0.25	0.18
PeCDF	13	13	0.306	8.04	1.57 A	8.36	1.70
			0.028	0.35	0.21	0.19	0.13
	25	7	0.358	7.90	1.26 A	8.57	1.52
			0.039	0.49	0.30	0.27	0.18
	30	8	0.325	7.92	1.79	8.11	1.63
			0.036	0.43	0.26	0.24	0.17
	49	1	0.270	8.30	1.76	8.24	1.57

Table S7. Cont'd.

TCDF	52	4	0.281	7.88	1.18 A	9.14	1.33
			0.051	0.55	0.34	0.31	0.24
	116	12	0.355	8.72	1.45 A	8.21	1.63
			0.030	0.38	0.23	0.21	0.14
	214	8	0.306	8.17	1.37 A	8.28	1.32 A
			0.036	0.41	0.26	0.23	0.17
	246	9	0.271	7.28	1.47 A	8.21	1.61
			0.034	0.42	0.26	0.23	0.16
Control vs Congener							
p-value TCDD			0.3089	0.6358	0.0003	0.3103	0.0269
p-value PeCDF			0.5775	0.9506	0.0252	0.1970	0.2229
p-value TCDF			0.7611	0.9911	0.0008	0.3421	0.0198

[a]TCDD is 2,3,7,8-tetrachlorodibenzo-p-dioxin; PeCDF is 2,3,4,7,8-pentachlorodibenzofuran; and TCDF is 2,3,7,8-tetrachlorodibenzofuran.

[b]Data are presented as least squares mean with standard error beneath.

[c]Means that are significantly different than the control mean at $p < 0.05$ are designated with an A.

Table S8. Effects of TCDD, PeCDF, and TCDF on juvenile female mink relative organ mass (% of body mass) at 27 weeks of age[a].

Dietary treatment	Dose (ng/kg body wt/d)	*n*	Body mass necropsy[b] (g)	Liver[c,d]	Thyroid gland	Thymus	Heart	Spleen
Control	0	17	1164	5.13	0.007	0.11	0.71	0.28
			54	4.84-5.42	0.006-0.009	0.09-0.12	0.65-0.77	0.24-0.31
TCDD	2.1	7	999	5.44	0.008	0.08	0.83	0.24
			87	4.84-6.04	0.006-0.011	0.06-0.11	0.70-0.97	0.20-0.28
	4.6	8	990	5.66	0.008	0.09	0.85	0.31
			78	5.35-5.98	0.006-0.010	0.06-0.11	0.77-0.93	0.24-0.39
	6.0	16	1013	5.76	0.009	0.07 A	0.92 A	0.30
			60	5.26-6.26	0.006-0.011	0.06-0.08	0.87-0.96	0.26-0.33
	8.4	7	952	5.95	0.008	0.08	0.86	0.35
			79	5.16-6.73	0.007-0.010	0.06-0.10	0.72-0.99	0.32-0.38
PeCDF	13	13	1123	5.21	0.008	0.09	0.78	0.23
			63	4.82-5.60	0.007-0.009	0.08-0.11	0.72-0.84	0.02-0.26
	25	7	938	6.20	0.010	0.07	0.96 A	0.30
			90	4.26-8.15	0.004-0.015	0.04-0.09	0.74-1.18	0.17-0.42
	30	8	1043	5.87	0.019	0.07	0.76	0.32
			79	5.32-6.42	0-0.042	0.05-0.09	0.68-0.84	0.27-0.36
	49	1	967	5.83	0.008	0.06	0.76	0.35

Table S8. Cont'd.

TCDF	52	4	1025	5.05	0.008	0.06 A	0.89	0.22
			80	4.58-5.51	0.005-0.012	0.01-0.11	0.63-1.15	0.12-0.32
	116	12	1144	5.26	0.007	0.08	0.70	0.32
			58	4.95-5.57	0.005-0.008	0.06-0.09	0.62-0.78	0.27-0.36
	214	8	1019	5.49	0.009	0.07	0.84	0.31
			61	5.09-5.89	0.007-0.012	0.06-0.09	0.72-0.96	0.27-0.34
	246	9	1035	5.67	0.007	0.07	0.80	0.35
			64	5.18-6.16	0.005-0.009	0.06-0.09	0.70-0.89	0.30-0.40
Control vs Congener								
p-value TCDD			0.1609	0.0833	0.7000	0.0287	0.0203	0.0602
p-value PeCDF			0.2672	0.1689	0.2096	0.0784	0.0370	0.0754
p-value TCDF			0.2256	0.1712	0.2983	0.0167	0.0778	0.0248

Table S8. Cont'd.

Dietary treatment	Dose (ng/kg body wt/d)	*n*	Body mass necropsy[b] (g)	Adrenal glands	Kidneys	Lymph Node	Brain	Reproductive Tract
Control	0	17	1164	0.026	0.69	0.18	0.76	0.16
			54	0.021-0.030	0.66-0.73	1.14-0.22	0.70-0.83	0.14-0.18
TCDD	2.1	7	999	0.035	0.80	0.11	0.86	0.14
			87	0.024-0.047	0.72-0.89	0.08-0.14	0.76-0.96	0.11-0.17
	4.6	8	990	0.028	0.76	0.14	0.85	0.16
			78	0.020-0.036	0.71-0.82	0.11-0.17	0.79-0.91	0.14-0.19
	6.0	16	1013	0.029	0.79	0.11 A	0.85	0.13
			60	0.025-0.032	0.72-0.87	0.10-0.13	0.74-0.96	0.12-0.15
	8.4	7	952	0.038 A	0.83	0.16	0.88	0.16
			79	0.033-0.043	0.71-0.95	0.08-0.24	0.74-1.01	0.13-0.19
PeCDF	13	13	1123	0.027	0.73	0.14	0.76	0.15
			63	0.020-0.034	0.68-0.77	0.11-0.17	0.69-0.83	0.14-0.16
	25	7	938	0.039	0.87	0.14	0.95	0.16
			90	0.025-0.053	0.61-1.14	0.08-0.20	0.66-1.24	0.11-0.21
	30	8	1043	0.032	0.76	0.17	0.79	0.15
			79	0.022-0.042	0.71-0.80	0.13-0.20	0.64-0.94	0.10-0.20
	49	1	967	0.028	0.86	0.18	0.85	0.16

Table S8. Cont'd.

TCDF	52	4	1025	0.027	0.78	0.11	0.90	0.13
			80	0.018-0.037	0.52-1.04	0.07-0.16	1.67-1.13	0.06-0.20
	116	12	1144	0.031	0.77	0.13	0.73	0.14
			58	0.026-0.037	0.72-0.81	0.11-0.15	0.68-0.78	0.12-0.16
	214	8	1019	0.030	0.81	0.13	0.82	0.13
			61	0.021-0.039	0.74-0.88	0.09-0.17	0.74-0.89	0.11-0.15
	246	9	1035	0.027	0.71	0.14	0.80	0.16
			64	0.021-0.032	0.65-0.77	0.11-0.18	0.74-0.85	0.11-0.21
Control vs Congener								
p-value TCDD			0.1609	0.0528	0.0685	0.0549	0.3792	0.1205
p-value PeCDF			0.2672	0.1341	0.1457	0.4172	0.3862	0.9578
p-value TCDF			0.2256	0.5388	0.1443	0.1754	0.1443	0.5977

[a]TCDD is 2,3,7,8-tetrachlorodibenzo-p-dioxin; PeCDF is 2,3,4,7,8-pentachlorodibenzofuran; and TCDF is 2,3,7,8-tetrachlorodibenzofuran.

[b]Body mass data are presented as least squares mean with standard error beneath.

[c]Relative organ mass data are presented as mean with 95% confidence interval beneath.

[d]Means that are significantly different than the control mean at $p < 0.05$ are designated with an A.

Table S9. Effects of TCDD, TCDF, and PeCDF on juvenile male mink absolute organ mass (g) at 27 weeks of age[a,b].

Dietary treatment	Dose (ng/kg body wt/d)	*n*	Liver[c]	Thyroid gland	Thymus	Heart	Spleen
Control	0	12	76.85	0.094	0.768	12.61	2.86
			3.35	0.011	0.097	0.71	0.68
TCDD	2.1	7	69.54	0.089	0.718	10.95	2.43
			4.27	0.015	0.125	0.90	0.87
	4.6	6	71.30	0.086	0.668	11.47	2.92
			4.60	0.016	0.135	0.96	0.88
	6.0	6	62.43 A	0.074	0.476	11.41	1.90
			4.60	0.016	0.135	0.96	0.88
	8.4	9	70.04	0.065	0.788	10.52	3.19
			3.95	0.013	0.110	0.77	0.68
PeCDF	13	10	68.91	0.063	0.661	12.00	2.55
			3.52	0.012	0.104	0.73	0.63
	25	3	69.16	0.098	0.605	11.92	6.64
			6.29	0.022	0.188	1.29	0.92
	30	7	85.77	0.091	0.885	12.61	4.07
			4.18	0.015	0.124	0.86	0.70
	49	8	70.94	0.087	0.635	10.00	3.22
			4.03	0.014	0.117	0.85	0.86

Table S9. Cont'd.

TCDF	52	9	68.34	0.114	0.569	12.11	2.95
			3.75	0.013	0.110	0.78	0.64
	116	12	78.44	0.104	1.080	12.85	3.22
			3.25	0.011	0.095	0.68	0.62
	214	10	69.25	0.095	0.752	11.45	3.01
			3.60	0.012	0.105	0.76	0.75
	246	9	75.67	0.107	0.757	10.55	3.42
			3.69	0.013	0.109	0.76	0.63
Control vs Congener							
p-value TCDD			0.0405	0.2357	0.3424	0.0652	0.7210
p-value PeCDF			0.4424	0.4881	0.5204	0.2114	0.1363
p-value TCDF			0.3144	0.4179	0.8888	0.2258	0.7500

Table S9. Cont'd.

Dietary treatment	Dose (ng/kg body wt/d)	*n*	Adrenal glands	Kidneys	Lymph Node	Brain	Reproductive Tract
Control	0	12	0.32	9.81	1.56	11.02	0.89
			0.09	0.44	0.16	0.25	0.14
TCDD	2.1	7	0.30	10.09	1.46	10.11 A	0.89
			0.12	0.55	0.20	0.31	0.17
	4.6	6	0.27	10.66	1.35	10.67	0.98
			0.13	0.59	0.22	0.32	0.18
	6.0	6	0.26	8.69	0.73 A	10.22	0.60
			0.13	0.59	0.22	0.32	0.18
	8.4	9	0.29	9.30	1.15	9.73 A	0.94
			0.10	0.47	0.17	0.26	0.14
PeCDF	13	10	0.64	9.28	1.33	10.50	0.78
			0.10	0.44	0.16	0.24	0.13
	25	3	0.34	9.25	0.92	9.52 A	0.63
			0.18	0.76	0.29	0.40	0.21
	30	7	0.35	10.14	1.60	10.32	0.98
			0.12	0.52	0.19	0.28	0.15
	49	8	0.37	9.55	1.20	9.85 A	0.77
			0.11	0.52	0.19	0.29	0.17

Table S9. Cont'd.

TCDF	52	9	0.34	9.89	1.34	10.87	0.90
			0.10	0.47	0.18	0.26	0.14
	116	12	0.39	10.64	1.78	9.86 A	1.42
			0.09	0.41	0.15	0.23	0.13
	214	10	0.30	9.62	1.33	9.65 A	0.93
			0.10	0.47	0.17	0.26	0.15
	246	9	0.38	9.48	1.74	9.71 A	0.95
			0.10	0.46	0.17	0.25	0.14
Control vs Congener							
p-value TCDD			0.7016	0.7909	0.0358	0.0083	0.8211
p-value PeCDF			0.3517	0.5690	0.1028	0.0022	0.5412
p-value TCDF			0.7810	0.9063	0.8248	0.0018	0.3611

[a]TCDD is 2,3,7,8-tetrachlorodibenzo-p-dioxin; PeCDF is 2,3,4,7,8-pentachlorodibenzofuran; and TCDF is 2,3,7,8-tetrachlorodibenzofuran.

[b]Data are presented as least squares mean with standard error beneath.

[c]Means that are significantly different than the control mean at $p < 0.05$ are designated with an A.

Table S10. Effects of TCDD, PeCDF, and TCDF on juvenile male mink relative organ mass (% of body mass) at 27 weeks of age[a].

Dietary treatment	Dose (ng/kg body wt/d)	*n*	Body mass necropsy[b] (g)	Liver[c,d]	Thyroid gland	Thymus	Heart	Spleen
Control	0	12	1536	5.10	0.006	0.05	0.86	0.19
			79	4.79-5.42	0.005-0.008	0.03-0.07	0.73-0.99	0.16-0.21
TCDD	2.1	7	1355	5.19	0.007	0.05	0.81	0.18
			96	4.28-6.11	0.006-0.007	0.04-0.07	0.69-0.93	0.16-0.20
	4.6	6	1295	5.54	0.007	0.05	0.89	0.22
			103	4.73-6.36	0.004-0.009	0.02-0.08	0.72-1.05	0.20-0.25
	6.0	6	1072 A	5.99	0.007	0.04	1.09	0.18
			103	5.23-6.75	0.005-0.009	0.03-0.05	0.89-1.28	0.16-0.20
	8.4	9	1225	5.97	0.006	0.06	0.87	0.26 A
			81	5.50-6.45	0.004-0.007	0.05-0.07	0.79-0.95	0.21-0.31
PeCDF	13	10	1306	5.38	0.005	0.05	0.92	0.19
			71	4.62-6.15	0.003-0.006	0.04-0.06	0.83-1.00	0.17-0.22
	25	3	1093 A	6.67	0.009	0.05	1.17	0.78
			128	1.78-11.56	0.002-0.017	0.02-0.08	0.02-2.32	0-3.25
	30	7	1497	5.70	0.006	0.06	0.85	0.27
			85	5.35-6.06	0.004-0.008	0.04-0.08	0.67-1.02	0.23-0.32
	49	8	1058 A	6.83 A	0.008	0.06	0.98	0.31
			82	6.01-7.64	0.007-0.009	0.04-0.08	0.80-1.16	0.25-0.38

Table S10. Cont'd.

TCDF	52	9	1357	4.99	0.008	0.04	0.89	0.21
			90	4.50-5.48	0.004-0.012	0.03-0.05	0.78-0.99	0.14-0.28
	116	12	1574	4.98	0.007	0.07	0.82	0.20
			79	1.77-5.19	0.005-0.008	0.05-0.08	0.71-0.92	0.17-0.24
	214	10	1323	5.31	0.008	0.06	0.88	0.24
			90	4.87-5.75	0.005-0.011	0.04-0.07	0.79-0.98	0.19-0.28
	246	9	1302	5.85 A	0.008	0.06	0.82	0.27
			87	5.48-6.22	0.006-0.010	0.05-0.07	0.71-0.94	0.24-0.29
Control vs Congener								
p-value TCDD			0.0287	0.0757	0.5700	0.4088	0.1251	0.0068
p-value PeCDF			0.0963	0.0156	0.5088	0.0731	0.7055	0.0948
p-value TCDF			0.0021	0.0192	0.0157	0.7603	0.2945	0.1447

Table S10. Cont'd.

Dietary treatment	Dose (ng/kg body wt/d)	*n*	Body mass necropsy[b] (g)	Adrenal glands	Kidneys	Lymph Node	Brain	Reproductive Tract
Control	0	12	1536	0.021	0.66	0.10	0.75	0.06
			79	0.018-0.024	0.60-0.72	0.09-0.12	0.64-0.87	0.05-0.06
TCDD	2.1	7	1355	0.022	0.75	0.10	0.76	0.07
			96	0.019-0.025	0.07-0.84	0.07-0.14	0.65-0.86	0.06-0.07
	4.6	6	1295	0.021	0.83 A	0.10	0.84	0.07
			103	0.019-0.023	0.070-0.95	0.06-0.14	0.68-0.99	0.06-0.09
	6.0	6	1072 A	0.024	0.83	0.07	0.99	0.06
			103	0.021-0.028	0.74-0.92	0.05-0.09	0.82-1.17	0.05-0.06
	8.4	9	1225	0.024	0.76	0.10	0.83	0.08
			81	0.020-0.028	0.70-0.82	0.08-0.12	0.69-0.97	0.06-0.10
PeCDF	13	10	1306	0.046 A	0.72	0.10	0.83	0.06
			71	0.001-0.091	0.65-0.80	0.07-0.12	0.70-0.96	0.05-0.06
	25	3	1093 A	0.034	0.86 A	0.08	0.93	0.06
			128	0-0.079	0.56-1.17	0.08-0.09	0.09-1.78	0.05-0.07
	30	7	1497	0.024	0.68	0.11	0.69	0.07
			85	0.013-0.034	0.62-0.74	0.08-0.13	0.62-0.77	0.06-0.07
	49	8	1058 A	0.034 A	0.91 A	0.11	0.96	0.07 A
			82	0.027-0.041	0.82-0.10	0.08-0.14	0.80-1.13	0.06-0.09

Table S10. Cont'd.

TCDF	52	9	1357	0.024	0.73	0.09	0.82	0.06
			90	0.015-0.032	0.65-0.80	0.07-0.12	0.65-0.98	0.05-0.07
	116	12	1574	0.025	0.68	0.11	0.64	0.09
			79	0.020-0.030	0.65-0.70	0.09-0.14	0.56-0.71	0.06-0.12
	214	10	1323	0.023	0.75	0.10	0.77	0.07
			90	0.018-0.028	0.68-0.82	0.08-0.12	0.64-0.89	0.05-0.10
	246	9	1302	0.030	0.74	0.14	0.76	0.07
			87	0.023-0.036	0.67-0.80	0.11-0.16	0.70-0.82	0.06-0.09
Control vs Congener								
p-value TCDD			0.0287	0.3834	0.0457	0.1602	0.1805	0.3963
p-value PeCDF			0.0963	0.3271	0.1837	0.0938	0.1691	0.2387
p-value TCDF			0.0021	0.3046	0.0004	0.7383	0.0886	0.0314

[a]TCDD is 2,3,7,8-tetrachlorodibenzo-p-dioxin; PeCDF is 2,3,4,7,8-pentachlorodibenzofuran; and TCDF is 2,3,7,8-tetrachlorodibenzofuran.

[b]Body mass data are presented as least squares mean with standard error beneath.

[c]Relative organ mass data are presented as mean with 95% confidence interval beneath.

[d]Means that are significantly different than the control mean at $p < 0.05$ are designated with an A.

CHAPTER 4

CONCLUSIONS AND RECOMMENDATIONS FOLLOWING TWO LABORATORY FEEDING STUDIES EVALUATING THE EFFECTS OF TCDD AND TCDD-LIKE COMPOUNDS ON MINK (*MUSTELA VISON*)

CONCLUSIONS

Toxic equivalency factors (TEFs) are consensus values that reflect the relative toxicity of individual 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-like congeners to TCDD. Relative effect potency (REP) is determined for individual polychlorinated dibenzo-p-dioxin (PCDD), polychlorinated dibenzofuran (PCDF), and polychlorinated biphenyl (PCB) congeners for producing toxic or biological effects relative to a reference compound, usually TCDD (Van den Berg et al., 2006). These effect concentrations then provide Toxicity Reference Values (TRVs), which are used by risk managers to assess risk of harm for living organisms. A compound must meet certain criteria to be included in the TEF concept. These criteria include (1) a structural relationship to PCDDs and PCDFs; (2) ability to bind to the AhR receptor; (3) ability to elicit AhR-mediated biochemical toxic responses; and (4) persistent and accumulates in the food chain (Ahlborg et al., 1994; Van den Berg et al., 1998, 2006). Studies of TCDD-like chemicals are then conducted and/or evaluated to establish TEFs. However, uncertainties remain. Many of the TEFs are based on *in vitro* studies and thus do not take into account the potential differences in accumulation, disposition and metabolism in animals (Blankenship et al., 2008). Toxic equivalency factors based on biochemical effects such as enzyme induction in rodents may misrepresent an environmentally relevant endpoint if applied to reproduction in an environmentally relevant

and sensitive wildlife receptor. As a result, it may be necessary to consider species-specific TEFs for mink.

Results from the present study suggest that the TEFs for TCDF and PeCDF may not accurately reflect their relative toxicity in mink. The assigned TEF of 0.1 for 2,3,7,8-tetrachlorodibenzofuran (TCDF) suggests that TCDF elicits a relative toxic response that is 10% that of TCDD at a specific dose and is equivalent in toxicity to other TCDD-like compounds assigned a TEF of 0.1, specifically, 3,3', 4,4', 5-pentachlorobiphenyl (PCB 126). The TEF for 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) is 0.3, which suggests that at a specific dose, it is 30% as toxic as TCDD and three times as toxic as both TCDF or PCB 126. However, results from mink feeding studies described herein indicate that the effects of TCDF**,** PeCDF and TCDD on tissue morphology, reproduction and offspring viability and growth following exposure are less than what would be expected based on TCDD-normalized TEFs. Conversely, results of previous mink exposure studies completed at the same facility with similar methods using PCB 126-dominated PCB mixtures indicate that PCB 126 may be more toxic to mink than TCDF and PeCDF, and perhaps provides a more suitable reference compound for mink risk assessments than TCDD.

Taking into consideration the TEFs assigned to TCDF, PCB 126, PeCDF and TCDD, concentrations normalized to TEQs should elicit relative responses indicating that TCDD is more toxic than PeCDF, PeCDF is more toxic than TCDF and PCB 126, and TCDF and PCDF 126 are similar in toxicity. The present reproductive feeding study used doses of TCDF, PeCDF and TCDD that should have resulted in reproductive impairment based on TEFs, however, there were no adverse effects on reproduction or offspring viability at any of the doses. In contrast, PCB 126-dominated mixtures had effects on mink reproduction and

offspring survival and growth at TEQ doses at or below those used in the present study, indicating discrepancies in the TEF approach. In the case of mink, either the current TEF approach does not adequately reflect the actual toxicity of PCB 126 relative to other TCDD-like compounds, or the toxicity of PCB 126 should be standardized outside the TCDD-centric TEF approach.

The kinetic study described herein provides evidence that might in part explain this apparent discrepancy between dietary TEQ concentrations and the apparent lack of effects induced by TCDF and PeCDF. In the reported 180 d feeding study, PeCDF accumulated in liver of mink to a much greater extent than did TCDF when administered as a single congener or in combination with TCDF, which suggested an efficient metabolism and/or elimination of TCDF. In addition, enzyme induction resulting from exposure to the combination of the two furan congeners might not have been additive and perhaps was due primarily to the action of only one of the congeners. Based on liver concentration data indicating greater concentration of PeCDF compared to TCDF, it is possible that enzyme induction in those animals receiving a mixture of the two congeners was due primarily to PeCDF. Alternatively, TCDF might also have contributed to the increase in enzyme activities, but due to metabolism, its concentration in the liver was less than that of PeCDF. While exposure to PeCDF and TCDF in the 180 d study established EROD and MROD activity as a biomarker of exposure to TCDD-like compounds, the doses and time of exposure were not enough to address further questions of environmental relevance. For example, mink in the Tittabawassee River are exposed to TCDF and PeCDF (Zwiernik et al., 2008) *in utero,* during lactation and through the growth period to adulthood; therefore, it was

not possible to realistically extrapolate any effects from the 180 d feeding study to answer questions related to reproduction and offspring viability.

The second feeding study attempted to answer this question by assessing dose-and-time dependent effects of TCDD, PeCDF and TCDF exposure *in utero*, during lactation and throughout the growth period to 27 weeks of age. The highest dose of each congener (TCDD, PeCDF, TCDF) was expected to cause reproductive effects based on results of laboratory studies in which mink fed TEQ-normalized concentrations of PCBs (Heaton et al., 1995a,b, Tillitt et al., 1996, Bursian et al., 2006a,b,c, Beckett et al., 2008) at similar levels experienced decreased litter size and/or reduce offspring viability. However, dietary TEQ concentrations for TCDD, PeCDF and TCDF as high as 8.4 ng TEQ_{TCDD}/kg body wt/d, 49 ng TEQ_{PeCDF}/kg body wt/d and 246 ng TEQ_{TCDF}/kg body wt/d, respectively, had no significant effect on reproductive performance of mink or viability of their offspring, and therefore a Lowest Observable Adverse Effect Level (LOAEL) could not be defined for these specific environmentally relevant endpoints. The lack of an effect of TCDF on reproduction and survival and growth is consistent with results from another reproductive feeding study, where no significant effects on reproduction or survival endpoints were reported for dietary concentrations as high as 242 ng TEQ_{TCDF}/kg feed (Zwiernik et al., 2009). Hochstein et al. (2001) attempted a mink reproduction study utilizing TCDD at dietary concentrations as high as 1,400 ng TEQ_{TCDD}/kg feed, however, an effect on reproduction could not be clearly determined because of subnormal reproductive performance of the control group, which was attributed to the fact that the trial was conducted indoors. While results of Zwiernik et al. (2009) are consistent with the lack of reproductive effects from the TCDF reproductive study

described herein, another study completed at the same facility utilizing similar methods as the TCDF reproductive study were not.

Similar to the methods of the two TCDF studies discussed herein, feed containing PCB-contaminated fish collected from the Housatonic River was fed to mink at dietary concentrations of 50.4 ng $TEQ_{PCBs,\ PCDDs/Fs}$/kg for 147 to 164 d (Bursian et al., 2006a). This dietary TEQ concentration (90% TEQs contributed by non-ortho- and mono-ortho PCBs with PCB 126 being the predominant contributor) resulted in decreased survival of kits at 6 weeks of age and decreased body weight at 3 weeks. The TEF for PCB 126 is equal to that of TCDF while it is 3x less than PeCDF. However, when the Housatonic River study is compared to the present reproductive feeding study, results suggest that TCDF is less toxic than PCB 126 and PCB 126 is more toxic than PeCDF.

2,3,7,8-Tetrachlorodibenzofuran (TCDF) and 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) were fed to mink at concentrations expected to cause significant morphological, reproductive, and/or survival effects (Zwiernik et al., 2009, Moore et al., 2009, 2012) relative to its TEF of 0.1. A lack of adverse effects at dietary TEQ concentrations that exceed TRVs leads to the conclusion that the TEF for TCDF is not reflective of its toxicity. Other studies support this conclusion as TCDF is rapidly cleared from the mink (Zwiernik et al. 2008, Bursian et al., 2012). Results from these studies and similar studies performed at the same facility (Bursian et al., 2006b,c, 2012) also suggest that the current TEF values might not accurately predict the toxic potency of TCDF and PeCDF, while PCB 126 may be the most toxic TCDD-like compound to mink.

RECOMMENDATIONS

Evaluate TCDD-like compound interactions and the relative toxicity of TCDD-like compounds in mink

Since compound interactions may increase or decrease toxicity, additional studies evaluating environmentally relevant or site-specific mixtures may be necessary to understand TCDD-like compound interactions. The TEQ approach assumes additive toxicity, however, the administration of two environmentally relevant TCDD-like compounds to mink, which exceeded TRVs under controlled conditions, did not result in the expected toxic response. This presents challenges to risk managers, particularly where environmental exposures that include TCDF are assumed additive. Given the disparity, it may be appropriate to further evaluate interactions among environmentally relevant PCDDs, PCDFs, and PCBs in mink. This conclusion challenges the additive assumption of the TEQ method in the case of mink, while results from a mink reproductive feeding study following the sole administration of TCDF at TEQ normalized concentrations expected to elicit effects on reproduction and offspring viability presents another challenge to risk managers applying current TEFs for mink.

In order to definitively examine the TEFs assigned to TCDF and other TCDD-like compounds for a sensitive, environmentally relevant wildlife receptor, it is important to evaluate the relative toxicity of TCDD-like compounds solely and directly to TCDD as well as to PCB 126 under controlled conditions. Results from this study and other reproductive feeding studies performed at the same facility with similar methodology suggest that the TEF for PCB 126 is underestimated and should be reevaluated relative to TCDD or the toxicity of PCB 126 should be standardized outside the TCDD-centric TEF approach. It may be appropriate at this time to research the relative potency of TCDD-like compounds to TCDD and to PCB 126 via side-by-side sole administration of these compounds to mink to evaluate sensitive endpoints under controlled conditions including enzyme induction and jaw lesions (Bursian et al., 2012) as well as less sensitive endpoints of reproductive performance and offspring viability in order to establish species-specific TEFs. This information would provide risk managers with environmentally relevant TRVs for mink and lead to management actions, which represent ecologically relevant effects.

REFERENCES

REFERENCES

Ahlborg, U.G., Becking, G.C., Birnbaum, L.S., Brouwer, A., Derks, H.J.G.M., Feeley, M., Golor, G., Hanberg, A., Larsen, J.C., Liem, A.K.D., Safe S.H., Schlatter, C., Waern, F., Younes, M., Yrjänheikki, E. (1994). Toxic equivalency factors for dioxin-like PCBs: Report on a WHO-ECEH and IPCS consultation. Chemosphere. 28:1049-1067.

Aulerich, R.J. and Ringer, R.K. (1977). Current status of PCB toxicity to mink, and their effect on their reproduction. Arch. Environ. Contam. Toxicol. 6:279-292.

Aulerich, R.J., Powell, D.C. and Bursian, S.J. (1999). Handbook of biological data for mink. Department of Animal Science, Michigan State University, East Lansing, MI. 154 pp.

Basu, N., Scheuhammer, A.M., Bursian, S.J., Rouvinen-Watt, K., Elliott, J. and Chan H.M. (2007). Mink as a sentinel species in environmental health. Environ. Res. 103: 130-144.

Beckett, K.J., Millsap, S.D., Blankenship, A.L., Zwiernik, M.J., Giesy, J.P. and Bursian, S.J. (2005). Squamous epithelial lesion of the mandibles and maxillae of wild mink (*Mustela vison*) naturally exposed to polychlorinated biphenyls. Environ Toxicol Chem. 24:674-677.

Beckett, K.J., Yamini, B. and Bursian, S.J. (2008). The Effects of 3,3',4,4',5-pentachlorobiphenyl (PCB 126) on mink (*Mustela vison*) reproduction and kit survivability and growth. Arch Environ Contam Toxicol. 54:123-129.

Blankenship, A.L., Kay, D.P., Zwiernik, M.J., Holem, R.R., Newsted, J.L., Hecker, M. and Giesy, J.P. (2008). Toxicity reference values for mink exposed to 2,3,7,8-tetrachlodibenzo-*p*-dioxin. Ecotoxicol Environ Safety. 69:325-349.

Blankenship, A.L. and Giesy, J.P. (2002). Use of biomarkers of exposure and vertebrate tissue residues in the characterization of PCBs at contaminated sites: applications to birds and mammals. In: Environmental analysis of contaminated sites: Toxicological methods and approaches, Eds. Sunahra, G.I., Renoux, A.Y., Thellen, C., Caudet, C.L., and Pilon, A. pp. 153-180. New York, NY: Wiley.

Bleavins, M.R. and Aulerich, R.J. (1981). Feed consumption and food passage time in mink (*Mustela vison*) and European ferrets (*Mustela putorius furo*). Lab Anim Sci. 3:268-269.

Brewster, D.W. and Birnbaum, L.S. (1987). 2,3,4,7,8-pentachlorodibenzofuran (PCDF): Toxicokinetics and metabolism in the rat. Environ. Health Perspect. 75: 147-148.

Brewster, D.W. and Birnbaum, L.S. (1988). The acute toxicity of 2,3,4,7,8-pentachlorodibenzofuran (4-PeCDF) in the male Fisher rat. Fund. Appl. Toxicol. 11:236-249.

Brunström, B., Lund, B.O., Bergman, A., Asplund, L., Anthanassiadis, I., Athanasiadou, M., Jensen, S. and Orberg, J. (2001). Reproductive toxicity in mink (*Mustela vison*) chronically exposed to environmentally relevant polychlorinated biphenyl concentrations. Environ Toxicol Chem. 20:2318-2337.

Bursian, S.J., Sharma, C., Aulerich, R.J., Yamini, B., Mitchell, R.R., Orazio, C., Moore, D., Svirsky, S. and Tillitt, D.E. (2006a). Dietary exposure of mink (*Mustela vison*) to fish from the Housatonic River, Berkshire County, Massachusetts, USA: Effects on reproduction, kit growth, and survival. Environ Toxicol Chem. 25:1533-1540.

Bursian, S.J., Sharma, C., Aulerich, R.J., Yamini, B., Mitchell, R.R., Beckett, K.J., Orazio, C.E., Moore, D., Svirsky, S. and Tillitt D.E. (2006b). Dietary exposure of mink (*Mustela vison*) to fish from the Housatonic River, Berkshire County, Massachusetts, USA: Effects on organ weights and histology and hepatic concentrations of polychlorinated biphenyls and 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin toxic equivalence. Environ Toxicol Chem. 25:1541-1550.

Bursian, S.J., Beckett, K.J., Yamini, B., Martin, T.A., Kannan, K.L., Shields, K.L. and Mohr, F.C. (2006c). Assessment of effects in mink caused by consumption of carp collected from the Saginaw River, Michigan, USA. Arch Environ Contam Toxicol 50:614-623

Bursian, S.J., Moore, J.N., Newsted, J.L., Link, J.E., Fitzgerald, S.D., Bello, N., Bhat, V.S., Kay, D., Zhang, X., Wiseman, S., Budinsky, R.A., Giesy, J.P. and Zwiernik, M.J. (2012). Incidence of jaw lesions and activity and gene expression of hepatic P4501A enzymes in mink (*Mustela vison*) exposed to dietary 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, 2,3,7,8-tetrachlorodibenzofuran, and 2,3,4,7,8-pentachlorodibenzofuran. Environ Toxicol Chem. 31:2545-2556.

Denison, M. S. and Nagy, S.R. (2003). Activation of the aryl hydrocarbon receptor by structurally diverse exogenous and endogenous chemicals. Annual Review of Pharmacology and Toxicology. 43:309-334.

DeVito, M., Diliberto, J., Ross, D.G., Menache, M.G.and Birnmaum, L. (1997). Dose-response relationships for polyhalogenated dioxins and dibenzofurans following subchronic treatment in mice I. CYP1A1 and CYP1A2 enzyme activity in liver, lung, and skin. Toxico Appl Pharm. 147:267-280.

Diliberto, J.J., Burgin, D.E. and Birnbaum, L.S. (1999). Effects of CYP1A2 on disposition of 2,3,7,8-tetrachlorodibenzo-p-dioxin, 2,3,4,7,8-tetrachlorodibenzofuran, and 2,2’4,4’,5,5’-hexachlorobiphenyl in CYP1A2 knockout and parental (C57BL/6N and 129/Sv) strains of mice. Toxicol Applied Pharmacol. 159:52-64.

Erickson, M.D. (1997). Analytical Chemistry of PCBs. second ed. CRC Press. Boca Raton, FL, 688 pp.

Fur Commission U.S.A. (2003). Standard Guidelines for the Operation of Mink Farms in the United States. Fur Commission U.S.A., Coronado, CA, USA.

Giesy, J.P. and Kannan, K. (2002). Dioxin-like and non-dioxin like effects of polychlorinated biphenyls: Implications for risk assessment. Lakes Reserv. Res. Manag. 7:139-181.

Giesy, J.P., Ludwig, J.P. and Tillitt, D.E. (1994). Dioxins, dibenzofurans, PCBs and colonial, fish-eating water birds. In: Schecter, A. (Ed.), Dioxin and Heath. Plenum Press, New York, NY, pp. 254-307.

Giesy, J.P., Verbrugge, D.A., Othout, R.A., Bowerman, W.W., Mora, M.A., Jones, P.D., Newsted, J.L., Vandervoort, C., Heaton, S.N., Aulerich, R.J., Bursian, S.J., Ludwig, J.P., Dawson, G.A., Kubiak, T.J., Best, D.A. and Tillitt, D.E. (1994). Contaminants in fishes from great lakes-influenced sections and above dams of three Michigan Rivers. II: Implications for health of mink. Arch Environ Contam Toxicol. 27:213-223.

Hahn, M.E. (1998). The aryl hydrocarbon receptor: a comparative perspective. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol. 121:23-53.

Hamm, J.T., Ross, D.G., Richardson, V.M., Diliberto, J.J. and Birnbaum, L.S. (1998). Methoxyresorufin: an inappropriate substrate for CYP1A2 in the mouse. Biochem. Pharmacol. 56:1657-1660.

Heaton, S.N., Bursian, S.J., Giesy, J.P., Tillitt, D.E., Render, J.A., Jones, P.D., Verbrugge, D.A., Kubiak, T.J. and Aulerich, R.J. (1995a). Dietary exposure of mink to carp from Saginaw Bay, Michigan. 1. Effects on reproduction and survival, and the potential risks to wild mink populations. Arch Environ Contam Toxicol. 28:334–343.

Heaton, S.N., Bursian, S.J., Giesy, J.P., Tillitt, D.E., Render, J.A., Jones, P.D., Verbrugge, D.A., Kubiak, T.J. and Aulerich R.J. (1995b). Dietary exposure of mink to carp from Saginaw Bay, Michigan: 2. Hematology and liver pathology. Arch Environ Contam Toxicol. 29:411-417.

Hilsherova, K., Kannan, K., Nakata, H., Yamashita, N., Bradley, P., McCabe, J., Taylor, A.B. and Giesy, J.P. (2003). Polychlorinated dibenzo-p-dioxin and dibenzofuran concentration profiles in sediments and flood-plain soils of the Tittabawassee River, Michigan. Environ Sci Technol. 37:468-474.

Hochstein, J.R., Aulerich, R.J. and Bursian, S.J. (1988). Acute toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin to mink. Arch Environ Contam Toxicol. 17:33-37.

Hochstein, J.R., Bursian, S.J. and Aulerich, R.J. (1998). Effects of dietary exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in adult female mink. Arch Environ Contam Toxicol. 15:348-353.

Hochstein, J.R., Render, J.A., Bursian, S.J. and Aulerich, R.J. (2001). Chronic toxicity of dietary 2,3,7,8-tetrachlorodibenzo-*p*-dioxin to mink. Vet Human Toxicol. 43:134-139.

Käkelä, R., Jokinen, I., Käkelä, A. and Hyvärinen, H. (2001). Effects of gender, diet, exogenous melatonin and subchronic PCB exposure on plasma immunoglobulin G in mink. J Toxicol Environ Health A. 64: 427-446.

Kawajiri, K. and Fujii-Kuriyama, Y. (2007). Cytochrome P450 gene regulation and physiological functions mediated by the aryl hydrocarbon receptor. Arch Biochem Biophys. 464:207-212.

Kennedy, S.W. and Jones, S.P. (1994). Simultaneous measurement of cytochrome P4501A catalytic activity and total protein concentration with a fluorescence plate reader. Anal Biochem. 222:217-223.

Linscombe, G., Kinler, R. and Aulerich, R.J. (1982). Mink (*Mustela vison*). In Chapman JA, Feldhamer GA, eds. Wild Mammals of North America. Biology, Management and Economics. Johns Hopkins University Press, Baltimore, MD, USA, pp 629- 643.

Lubet, R.A., Syi, J.L., Nelson, J.O. and Nims, R.W. (1990). Induction of hepatic cytochrome P-450 mediated alkoxyresorufi O-deethylase activities in different species by prototype P450 inducers. Chem Biol Interact. 75:325-339.

Michigan Department of Environmental Quality (MDEQ). (2003). Final Report - Phase II Tittabawassee/Saginaw River Dioxin Flood Plain Sampling Study. Remediation and Redevelopment Division, Lansing, MI, USA.

Moore, J.N., Newsted, J.L., Hecker, M., Zwiernik, M.J., Fitzgerald, S.D., Kay, D.P., Zhang, X., Higley, E.B., Aylward, L.L., Beckett, K.J., Budinsky, R.A., Bursian, S.J. and Giesy, J.P. (2009). Hepatic P450 enzyme activity, tissue morphology and histology of mink (Mustela vison) exposed to polychlorinated dibenzofurans. Arch Environ Contam Toxicol. 57: 416-425.

Moore, J.N., Zwiernik, M.J., Newsted, J.L., Fitzgerald, S.D., Link, J.E., Bradley, P.W., Kay, D., Budinsky, R., Giesy, J.P. and Bursian, S.J. (2012). Effects of dietary exposure of mink (Mustela vison) to 2,3,7,8 – tetrachlorodibenzo-p-dioxin (TCDD), 2,3,4,7,8 – pentachlorodibenzofuran (PeCDF) and 2,3,7,8 – tetrachlorodibenzofuran (TCDF) on reproduction and offspring viability and growth. Environ Toxicol Chem. 31:360-369.

National Research Council. (1982). Nutrient Requirements of Mink and Foxes. Vol 7. Nutrient Requirements of Domestic Animals. National Academy of Sciences, Washington, DC.

Martin, P.A., Mayne, G., Bursian, S.J., Tomy, G.T., Palace, V.P., Pekarik, C. and Smits, J.E. (2007). Immunotoxicity of the commercial polybrominated diphenyl ether mixture DE-71 in ranch mink (*Mustela vison*). Environ Toxico Chem. 26:988-997.

Render, J.A., Aulerich, R.J., Bursian, S.J., Nachreiner, R.F. (2000a). 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., Hochstein, J.R., Aulerich, R.J. and Bursian, S.J. (2000b). Proliferation of periodontal squamous epithelium in mink fed 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Vet Human Toxicol. 42:85-86.

Render, J.A., Bursian, S.J., Rosenstein, D.S. and Aulerich, R.J. (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). Vet Human Toxicol. 43:22-26.

Safe, S. (1990). Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). Crit Rev Toxicol. 21: 51-88.

Safe, S. (1998). Development validation and problems with the toxic equivalency factor approach for risk assessment of dioxins and related compounds. J. Anim. Sci. 67:134-141.

Scientifur. (1987). Scientific Index I. I.G. Jorgensen (ed.). Scientific , Hilleroed, Denmark. 196 pp.

Scientifur. (1992). Scientific Index II. I.G. Jorgensen (ed.). Scientific, Tjele, Denmark. 164 pp.

Shipp, E.B., Restrum, J.C., Giesy, J.P., Bursian, S.J., Aulerich, R.J. and Helferich, W.G. (1998). Multigenerational study of the effects of consumption of PCB-contaminated carp from Saginaw Bay, Lake Huron on mink. 2. Liver PCB concentration and induction of hepatic cytochrome P-450 activity as a potential biomarker for PCB exposure. J Toxicol Environ Health. 54A:377-401.

Shump, A.U., Shump, Jr., K.A., Heidt, G.A., and Aulerich, R.J. (1976). A Bibliography of Mustelids, Part II: Mink. Department of Poultry Sciences, Michigan State University, East Lansing MI. 156 pp.

Smits, J.E.G., Wobeser, G.A. and Schiefer, B. (1995). Physiological, reproductive and pathological effects of dietary bleached pulp mill effluent on mink. Environ Toxicol Chem 14:2095-2105.

Suzuki, T., Nakagawa, Y., Tayama, K., Yaguchi, K. and Suga, T. (2001). Toxicity and effects of 2,6,-di-*tert*-butyl-4-methylphenyl *N*-methylcarbamate (terbutol) on hepatic cytochrome P450 in F344 rats. Arch Toxicol. 75:555-561.

Sundqvist, C. (1989). Mink Encyclopedia. Release 1.0, Department of Biology, Abo Akademi, Porthansgatam 3, Turku, Finland.

Tai, H.L., McReynolds, J.H., Goldstein, J.A., Eugster, H.P., Sengstag, C., Alworthy, W.L. and Olson, J.R. (1993). Cytochrome P4501A1 mediates metabolism of 2,3,7,8-tetrachlorodibenzofuran in the rat and human. Toxicol Appl. Pharmacol. 123: 34-42.

Taylor, A.B. and McCabe, J.M. (2002). Baseline chemical characterization of Saginaw Bay watershed sediments. Michigan Department of Environmental Quality, Lansing, MI, USA.

Telliard WA. 1994. Method 1613: Tetra- through octa-chlorinated dioxins and furans by isotope dilution HRGC/HRMS. U.S. Environmental Protection Agency, Washington, DC.

Tillitt, D.E., Gale, R.W., Meadows, J.C., Zajieck, J.L., Peterman, P.H., Heaton, S.N., Jones, P.D., Bursian, S.J., Kubiak, T.J., Giesy, J.P. and Aulerich, R.J. (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. Tech. 30:283-291.

Van den Berg, M., De Jongh, J., Poiger, H., and Olson, J.R. (1994). The toxicokinetics and metabolism of polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofuran (PCDFs) and their relevance for toxicity. Crit Rev Toxicol. 24:1-74.

Van den Berg, M., Birnbaum, L.S., Denison, M., De Vito, M., Farland, W., Feeley, M., Fiedler, H., Hakansson, H., Hanberg, A., Haws, L., Rose, M., Safe, S., Schrenk, D., Tohyama, C., Tritscher, A., Tuomisto, J., Tysklind, M., Walker, N. and Peterson, R.E. (2006). The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol Sci. 93:223-241

Weaver, R.J., Thompson, S., Smith, G., Dickins, M., Elcombe, C.R., Mayer, R.T. and Burke, D. (1994). A comparative study of constitutive and induced alkoxyresorufin O-dealkylation and individual cytochrome P450 forms in cynomolgus monkey (Macaca fascicularis), human, mouse, rat and hamster liver microsomes. Biochem Pharm. 47:763-773.

Zwiernik, M.J., Kay, D.P., Moore, J.N., Beckett, K.J., Khim, J.S., Newsted, J.L., Roark, S. and Giesy, J.P. (2008a). Exposure and effects assessment of resident mink exposed to polychlorinated dibenzofurans and other dioxin-like compounds in the Tittabawassee River Basin, Midland, MI, USA on wild mink (*Mustela vison*). Environ Toxicol Chem. 27: 2076-2087.

Zwiernik, M.J., Bursian, S.J., Aylward, L.L., Kay, D.P., Moore, J.N., Rowlands, C., Woodburn, K., Shotwell, M.S., Khim, J.S., Giesy, .J.P. and Budinsky, R.A. (2008b). Toxicokinetics of 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF in mink *(Mustela vison)* at ecologically relevant exposures. Toxicol Sci. 105: 34-43.

Zwiernik, M.J., Beckett, K.J., Bursian, S.J., Kay, D.P., Holem, R.R., Moore, J.N., Yamini, B. and Giesy, J.P. (2009). Chronic effects of polychlorinated dibenzofurans on mink in laboratory and field environments. Integrated Environmental Assessment and Management. 5:291-301.