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# Toxicokinetics of 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF in Mink *(Mustela vison)* at Ecologically Relevant Exposures

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**Wild mink (*Mustela vison*) living along the Tittabawassee River in central Michigan exhibit elevated hepatic and dietary polychlorinated dibenzofuran (PCDF) concentrations exceeding mink-specific, literature-reported toxicity reference values (TRVs) on a toxicity equivalents basis. However, no apparent effects on individuals or population are evident, suggesting that available TRVs may overpredict risk for the site-specific mix of congeners. To investigate this discrepancy, a 180-day spiked feed study was conducted to assess: (1) the dosages of key congeners necessary to achieve liver concentrations bracketing those observed in wild mink, (2) time to achieve steady-state concentrations, and (3) effect of coadministration of 2,3,7,8-tetrachlorodibenzofuran (TCDF) and 2,3,4,7,8-pentachlorodibenzofuran (4-PeCDF) on the toxicokinetics and distribution of each congener. Adipose and hepatic PCDF concentrations were measured at 0, 90, and 180 days. PCDF concentrations in mink scat were determined at several time points and indicated nearly complete absorption of both TCDF and 4-PeCDF from the diet. Elimination half-times of TCDF were < 15 h and were inversely proportional to dose, while those for 4-PeCDF were approximately 7–9 days with no clear dose dependency in the tested dose range. Coadministration of 4-PeCDF and TCDF accelerated clearance of TCDF compared to administration of TCDF alone. Clearance of 4-PeCDF was not affected by TCDF coadministration. Distribution of 4-PeCDF, but not TCDF, demonstrated increased hepatic sequestration with increasing dose. 4-PeCDF toxicokinetics were described using a previously published two-compartment model. Overall, the toxicokinetic information gathered here illustrates the impact of CYP1A1 induction on bioaccumulation and toxicity potential of TCDF and 4-PeCDF. This information may provide insight into why the current TRVs do not appear to correctly characterize the risk for these two congeners when they are the primary components of an environmental mixture.**

*Key Words:* **mink; polychlorinated dibenzodioxins and polychlorinated dibenzofurans (dioxins and furans); toxicokinetics; 2,3,7,8-tetrachlorodibenzofuran; 2,3,4,7,8-pentachlorodibenzofuran.**

Previous studies of individual polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxin (PCDD), and polychlorinated dibenzofuran (PCDF) congeners or their mixtures have demonstrated mink to be among the more sensitive species tested, with effects on reproduction, development, and morphological lesions of the jaw (Brunström *et al*., 2001; Bursian *et al*., 2006b,c; Heaton *et al*., 1995; Restum *et al*., 1998). Toxicity reference values (TRVs) based on World Health Organization (WHO) 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) toxic equivalency factors (TEFs) and toxic equivalency quotients (TEQs) (Van den Berg *et al*., 1998, 2006) have been derived from these studies.

In the Tittabawassee River floodplain downstream of Midland, MI, PCDF and TEQ concentrations in soil and mink dietary items exceed upstream reference locations by 2–4 orders of magnitude. Therefore, a multiple lines-of-evidence approach was used to evaluate the potential of PCDD/PCDFs to cause adverse effects in floodplain mink. During November and December of 2003 through 2005, 22 wild mink were trapped from areas with elevated soil and sediment concentrations of PCDD/PCDFs. Mink ($n = 26$) were also collected from a reference population upstream of Midland, MI, on the Tittabawassee River and two of its tributaries (Pine and Chippewa Rivers) where soil and sediment PCDD/PCDFs representing background concentrations have been measured (Hilscherova *et al*., 2003). Concentrations of PCDD/PCDFs in the dietary items and trapped mink from both populations were measured. In the floodplain, PCDD/PCDFs contributed as much as 91% of the total dietary TEQ and 72% of the liver TEQ (Zwiernik, 2008a). 2,3,7,8-Tetrachlorodibenzofuran (TCDF) and 2,3,4,7,8-pentachlorodibenzofuran (4-PeCDF) contributed 31 and 37% of the dietary TEQ, respectively. However, TEQ concentrations in mink liver were dominated by 4-PeCDF, which contributed 56% of TEQ, while TCDF accounted less than 1% of the hepatic TEQ. TCDD, the most prominent TEQ-contributing PCDD in floodplain soil and sediment, contributed 11% of the dietary and 6% of liver TEQ.

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The remaining 21 and 37% of dietary and liver TEQ, respectively, was contributed primarily by PCB 126 (3,3′,4,4′,5′-pentachlorobiphenyl). Overall liver TEQ concentrations in the exposed mink ranged from 58 to 1100 ng/kg on a weight wet (ww) basis (Zwiernik, 2008a). Mink from the three upstream reference locations exhibited median liver TEQ concentrations of 13 ng/kg (ww) similar to the approximate 20 ng/kg (ww) TEQ reported for wild mink from South Carolina, LA, and elsewhere (Tansy *et al*., 2003).

Hazard quotients (HQs) between < 1 and 10 were calculated for resident downstream Tittabawassee River mink based on a comparison of measured diet and liver concentrations to TRVs derived from a number of different environmental mixtures fed to mink and published in the literature (Zwiernik, 2008a). These HQ values suggest that Tittabawassee River floodplain mink could be adversely affected by exposure. However, the multiple lines-of-evidence results, including morphological, histological, population demographics, and mink abundance, indicate that these wild mink are not adversely affected (Zwiernik, 2008a).

The inconsistency between the apparent healthy population and the elevated HQ estimates may be due to a number of factors. First, the WHO TEF values and TEQ estimates are conservative and likely overestimate risk (Finley *et al*., 2003; Haws *et al*., 2006). Another factor could reflect the accuracy of TRVs derived from mink studies using dioxin-like mixtures that differ substantially from the TCDF- and 4-PeCDF–dominated mixture to which mink are exposed in the Tittabawassee River system. These two furans could differ from dioxins and PCBs with respect to coactivators, transcription factors, and chaperone proteins that regulate gene expression downstream of simple aryl hydrocarbon receptor (AhR) binding and activation, and as such, their toxicity might be poorly represented by standard TEF/TEQ models (Hankinson, 2005). Finally, uptake rates, metabolism, excretion, and disposition of TCDF and 4-PeCDF may differ relative to TCDD or PCBs (Diliberto *et al*. 1999; King *et al*., 1983; McKinley *et al*., 1993; NTP, 2006; Olson *et al*., 1994; Tai *et al*., 1993). For example, the dioxin-sensitive squamous epithelial lesions of the jaw, the most sensitive end point for TEQ-related effects previously reported, were not observed in mink treated with TCDF (Zwiernik, Beckett, Bursian, Kay, Holem, Moore, Yamini, and Giesy, unpublished data). TCDF's toxicokinetic disposition may explain the apparent lack of effects of TCDF in mink, or TCDF's toxic potency may not be accurately represented by TEQ-based TRVs available from the published literature (either qualitatively or quantitatively).

Since the density and age structure of mink in the Tittabawassee River are similar to the control population and indicative of a stable, lightly harvested system (Whitman, 2003) and since no jaw lesions were observed in the study population, site- and congener-specific toxicity studies, including a reproductive/developmental study on TCDF and 4-PeCDF, are being conducted in mink to develop more relevant TRVs. The current study was conducted to facilitate such studies to determine: (1) the spiked feed dosages necessary to achieve liver concentrations bracketing those observed in wild mink, (2) time to achieve steady-state concentrations in female mink at the time of mating, and (3) the effect of coadministration of TCDF and 4-PeCDF on the toxicokinetics and distribution of each of these congeners.

## MATERIALS AND METHODS

### *Test Substances*

TCDF and 4-PeCDF were purchased from Wellington Laboratories (Ontario, Canada). Analysis of TCDF and 4-PeCDF standards demonstrated 97.7 and 99.6% purity, respectively. 2,3,7,8-Substituted impurities were less than 0.01% on a TEQ basis calculated in both compounds (determined with international TEF values). Analysis of all samples, including standards purity, was conducted with United States Environmental Protection Agency Method 1613.

### *Animals and Care*

The study was conducted at the Michigan State University Experimental Fur Farm. Fifty-six first-year natural dark female mink were randomly assigned to the eight treatment groups and the control group (Table 1). Housing of animals complied with guidelines specified in the Standard Guidelines for the Operation of Mink Farms in the United States (Fur Commission USA, 1995). A standard dietary mix was used throughout the study. Specific ingredients, mix ratios, and nutritional data are presented elsewhere (Beckett *et al*., 2007). The base diet was used as the control diet with treatment diets differing only in the supplemental TCDF and 4-PeCDF added. Diets were prepared prior to the start of the study. The feed was frozen (− 7°C) in 2-l containers (1- to 2-day supply) and thawed in a walk-in cooler (4°C) as needed. Water was available *ad libitum*. Mink were maintained on their treatment diet throughout the course of the study.

### *Experimental Design and Analyses of Tissue and Scat*

Mink were exposed to three concentrations each of the compounds (TCDF and 4-PeCDF) and to a binary mixture of the two congeners through the diet. Each morning, 25 g of spiked feed was placed on the cage of each animal. After the 25 g of spiked feed was consumed, an additional 110–120 g of "clean" feed was given to each animal. It is estimated that approximately 100 g of the clean feed is actually consumed. This procedure ensured essentially complete ingestion of the spiked feed, eliminating the need to measure daily feed consumption in order to estimate doses (Table 1). Three animals from each of the TCDF and 4-PeCDF and the TCDF/4-PeCDF mixture dose groups were sampled on days 90 and 180. Livers were removed, weighed, and preserved for analysis for TCDF and 4-PeCDF. Adipose tissue was also collected for quantification of TCDF and 4-PeCDF. Control animals were sampled on days 0 and 180 and concentrations of PCDDs, PCDFs, and PCBs measured. Samples of scat were collected on days 2, 23, 45, 90, and 180.

Additional data collected during the course of this study included gross observations, histological examination of selected tissues, and measurement of CYP1A1 and CYP1A2 gene expression and enzyme activities. These data are presented elsewhere (Moore, Hecker, Zwiernik, Bursian, Newstead, Budinsky, Higley, Alward, Fitzgerald, and Giesy, unpublished data).

### *PCDF Analyses*

Concentrations of PCDDs and PCDFs in feed and hepatic and adipose tissues were determined. Concentrations of TEQ were calculated as the sum of the product of the concentration of each congener and its respective TEF (Van den Berg *et al*., 2006).

Two different extraction techniques were used depending on the sample matrix. For liver and adipose tissue, 5 g was added to 75 ml of hydrochloric acid and 100 ml of 5/95 (vol/vol) benzene/hexane solution and shaken for at

**TABLE 1**
**Study Design**

| Treatment | $n^a$ | Spiked feed concentration (ng/kg ww) | SD | Overall feed concentration[b] (ng/kg ww) | Daily dose (ng/kg/day)[c] | Daily dose TEQ (ng/kg/day) |
|---|---|---|---|---|---|---|
| Controls | 8 | | | | | |
| 4-PeCDF | | | | | | |
| Low | 6 | 110 | 3.0 | 22 | 2.1 | 0.62 |
| Mid | 6 | 390 | 22 | 77 | 7.3 | 2.2 |
| High | 6 | 1600 | 20 | 320 | 32 | 9.5 |
| TCDF | | | | | | |
| Low | 6 | 500 | 17 | 99 | 9.8 | 0.98 |
| Mid | 6 | 2000 | 140 | 400 | 38 | 3.8 |
| High | 6 | 9700 | 290 | 1900 | 200 | 20 |
| Mixture | 6 | | | | | |
| 2,3,4,7,8-PeCDF | | 490 | 17 | 98 | 9.2 | 2.8 |
| 2,3,7,8-TCDF | | 2200 | 78 | 440 | 41 | 4.1 |

[a]Total number of animals per dose group. Control animals were killed at days 0 and 180; three treated animals per dose group were sacrificed at 90 and at 180 days.

[b]Final dietary concentrations represent an approximate fivefold dilution based on adding the 25 g of spike feed to the 100 gof clean feed administered to mink every day.

[c]Average body weights by group ranged from approximately 1200 to 1300 g.

least 16 h. For mink feed, 5 g was added to 75 ml of hydrochloric acid and 100 ml of 5/95 (vol/vol) benzene/hexane solution and shaken for more than 16 h. For the mink scat, 10 g was Soxhlet Dean-Stark extracted for more than 16 h. The organic phase was removed and processed through a series of three classical liquid chromatography columns. The first column cleanup consisted from bottom to top: silica gel, caustic silica gel (33% NaOH/silica gel), silica gel, acid silica gel (44% $H_2SO_4$/silica gel), and silica gel. The second column consisted of 10% silver nitrate/silica by weight and the final column consisted of basic alumina. The cleanup extract was analyzed for PCDD/PCDFs by high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) using a Trace 2000 series gas chromatograph (Thermo Scientific Thermo Fisher Scientific, Waltham, MA) and a Finnigan MAT-95 double-focusing magnetic sector mass spectrometer (Thermo Electron Co., Bremen, Germany). The HRGC was equipped with a LEAP Technologies CTC A200SE autosampler (Carrboro, NC) and 60 m × 0.25 mm × 0.25 μm Varian 5-ms gas chromatography column. The HRMS was equipped with standard electron ionization ion source operating in positive ionization mode. The mass spectrometer data were obtained in the selected ion monitoring mode at resolution of 10,000 (10% valley).

*Uptake and Elimination Kinetics*

***First-order kinetic model.*** Despite the complexity of biological systems, simplifying assumptions including a rate-limiting step (Equation 1) with the slowest process generally involving a theoretical first-order diffusion process, allow use of a simple pseudo first-order kinetic model. A simple pseudo first-order kinetic model can be used to describe the dynamics of the uptake, metabolism, depuration, and disposition of compounds during controlled studies, under laboratory conditions, where the exposure is well characterized (Van den Berg *et al*., 1994).

$$C_{\text{food}} \xrightarrow{k_1} C_{\text{body}} \xrightarrow{k_2} . \tag{1}$$

The material balance for the dietary mink exposure was expressed by a differential equation describing the change in chemical concentration in mink as a function of time (Equation 2).

$$\partial\left(C_{\text{body}}\right)/\partial t = k_1 \times C_{\text{food}} - k_2 \times C_{\text{body}}, \tag{2}$$

where $C_{\text{body}}$ and $C_{\text{food}}$ are the specific chemical concentrations (ng chemical/g ww) in mink tissue and foodstuffs, respectively, $k_1$ is the first-order uptake rate constant (g food adsorbed/g mink/day), and $k_2$ (1/day) is the pseudo first-order clearance/metabolism (depuration) rate constant.

The product of the uptake rate ($k_1$) and concentration of compound in food, $C_{\text{food}}$, can also be expressed as the fraction of administered compound absorbed from diet ($f_{\text{abs}}$). That is, the assimilation or transfer efficiency (g food absorbed/g food consumed) was determined from the daily administered chemical dose, $D$ (ng chemical/day), and BW, the mink body weight expressed in kilograms (Equation 3).

$$\partial\left(C_{\text{body}}\right)/\partial t = D \times f_{\text{abs}}/\text{BW} - k_2 \times C_{\text{body}}. \tag{3}$$

This first-order model for whole-body elimination was applied to the data for each compound. Since body weights of each animal within each dose group were reasonably stable over the course of the 180-day administration period, a model of whole-body elimination could be applied without accounting explicitly for mink growth dilution. Integrating Equation 3 results in the analytical solution (Equation 4).

$$C_{\text{body}} = \frac{D * f_{\text{abs}}}{\text{BW} * k_2}\left(1 - \mathrm{e}^{-k_2 t}\right). \tag{4}$$

The value of $f_{\text{abs}}$ was assumed to be 0.97 based on analysis of scat concentration data (Zwiernik, 2008b). $C_{\text{body}}$ was estimated by assuming that all compound in the body resides in either liver or adipose tissues based on studies in mice and rats that show between 70 and 90% of the body burden is retained in liver and adipose tissue (Diliberto *et al*. 2001; Hurst *et al*., 2000). Measured liver and adipose tissue concentrations, measured liver mass, and estimated adipose tissue mass were used to estimate the total quantity of each test compound in the body and, thus, the body concentration. By substituting the estimated values for $f_{\text{abs}}$ and $C_{\text{body}}$ into Equation 4, the value for the depuration rate constant ($k_2$) was derived. For each compound, the model was applied to the body concentrations as estimated through the use of the 90- and 180-day tissue concentration data to estimate values for $k_2$. Estimated values for $k_2$ were assessed for consistency among dose groups.

The estimated depuration rate constants from dose groups receiving only TCDF or 4-PeCDF were compared to the estimated depuration rates for the two compounds from the mixture group to assess whether coadministration of the compounds affected the depuration rates of either or both of the individual congeners.

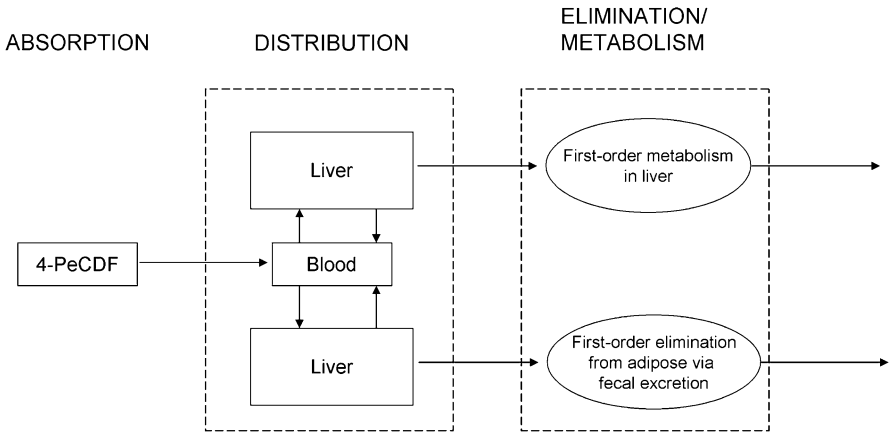


**FIG. 1.** Schematic of model structure for the modified model by Carrier *et al*. 1995a,b. (figure from Aylward *et al*. 2005).

***Two-compartment model.*** For 4-PeCDF, which demonstrated concentration-dependent distribution behavior, a previously developed two-compartment kinetic model that accounts for such behavior was also used to estimate rate constants and half-lives for depuration. The distribution among tissues and elimination kinetics of TCDD and other dioxin and furan compounds that exhibit hepatic sequestration in laboratory rodents have been modeled and described previously (Aylward *et al*., 2005; Carrier *et al*., 1995a,b; Emond *et al*., 2005; Wang *et al*., 1997). Although these models differ somewhat in structural details, each incorporates physiological processes that control distribution and depuration of these compounds. These models also make several simplifying assumptions, which include the following:

(1) The compounds are lipophilic (i.e., log $K_{ow} > 5$), so that in the absence of other factors, the compounds will partition into adipose/lipid tissues throughout the body, with adipose tissue being the largest reservoir of compound and distribution to other tissues occurring in proportion to their lipid content;

(2) The compounds induce hepatic CYP1A2 protein and activity. This induction can be modeled as a saturable Michaelis-Menten function, which is a special case of the Hill model with shape parameter set to 1 (see additional details below); and

(3) The compounds bind to the CYP1A2 protein avidly, so induction of hepatic CYP1A2 results in an unequal distribution of compound between adipose and lipid tissue.

For this evaluation, the modified model by Carrier *et al*. (1995a,b) described in detail by Aylward *et al*. (2005) is used and is described briefly here (Figure 1).

In the model by Carrier *et al*., the fraction of the total body mass of compound in the liver ($f_h$) is proportional to the magnitude of induction of hepatic CYP1A2. Specifically, $f_h$ is a saturable function of the body concentration ($C_{body}$) of compound with a minimum and maximum value ($f_{h\min}$ and $f_{h\max}$), each between 0 and 1 (Equation 5).

$$f_h = f_{h\,\min} + \frac{(f_{h\,\max} - f_{h\,\min}) * C_{body}}{k_{half} + C_{body}}, \quad (5)$$

where $k_{half}$ is the body concentration at which CYP1A2 protein is half-maximally induced.

The concentrations in liver and adipose tissue measured for each 4-PeCDF dose group at day 90 were used to estimate $C_{body}$ and $f_h$ for each animal by assuming that all compound in the body is distributed in either liver or adipose tissue. The resulting paired values of $C_{body}$ and $f_h$ were used to estimate the remaining parameters in the function for $f_h$ (Equation 5). Because all the compounds were assumed to reside in either liver or adipose, the fraction of compound residing in adipose tissue at any given $C_{body}$, $f_a$, can be calculated as $1 - f_h$.

This concentration-dependent distribution function (Equation 5) was coupled with a simple two-compartment model of depuration in which a first-order loss process operated from each compartment (Figure 1). The hepatic elimination likely represents metabolism or biliary excretion of unmetabolized compound; the adipose elimination mechanism represents a passive diffusion across the intestinal lumen into the relatively "clean" contents of the large intestine (Moser and McLachlan, 2002). Using the distribution function (Equation 5), the first-order elimination rates from hepatic and adipose tissues ($k_e$ and $k_a$, respectively, from Figure 1) were estimated from a best fit to the tissue concentration data collected after 90 days of exposure. The complete input and parameter set for the model is provided in Table 2.

*Data Analyses*

All data are expressed as a mean ± 1 SD. Prior to conducting statistical comparisons, data were tested for normality using the Shapiro-Wilks test and probability plots (SPSS, Chicago, IL). If necessary, data were log transformed to approximate normality. Differences among exposure groups were tested using a two-way ANOVA followed by the Dunnett test. Due to the nature of the mixture experiment (only two groups), these data were subjected to a one-way ANOVA followed by the Student *t*-test. Statistical significance was accepted when $p < 0.05$.

## RESULTS

### Treated Feed and Test Substance Intake

Instrumental analysis of the basal mink diet identified trace amounts of PCBs, PCDDs, and PCDFs in the concentrations of < 0.1, < 0.2, and < 0.4 ng/kg TEQ ww, respectively. The administered concentrations of TCDF, 4-PeCDF, and the TCDF/4-PeCDF mixture and the estimated daily doses for each treatment are given in Table 1. Concentrations in feed ranged from 22 to 320 ng 4-PeCDFs/kg ww, based on the dilution of the 25-g spiked allotment into a total 125 g of feed provided to each mink daily. This dietary concentration range effectively bracketed the concentration estimated for the 95th centile diet ingested by local wild mink (i.e., 71 ng 4-PeCDF/kg ww [Zwiernik, 2008a]). The concentration of TCDF in spiked food ranged from 99 to 1900 ng/kg ww after accounting for dilution of 25 g by the addition of 100 additional grams of

**TABLE 2**
**Parameters and Inputs for Two-Compartment Model for 4-PeCDF**

| Parameter | Value | Units | Comments |
|---|---|---|---|
| Parameters for model | | | |
| $f_{h\min}$ | 0.215 | Unitless | Best fit from distribution data |
| $f_{h\max}$ | 0.826 | Unitless | Best fit from distribution data |
| $k_{\text{half}}$ | 151 | ng/kg | Best fit from distribution data |
| $k_e$ | 0.048 | 1/day | Optimized to tissue concentration data |
| $w_a$ Adipose weight fraction | 0.070 | Unitless | Constant, for adipose tissue (not total lipid) |
| $w_h$ Liver body weight fraction | Average for group | Unitless | Average of measured liver ww |
| $k_a$ Adipose clearance factor | 0.129 | 1/day | Optimized to tissue concentration data |
| Model inputs | | | |
| Daily feed dose 1 | 2.7 | ng | Given 7 days/week |
| Daily feed dose 2 | 9.63 | ng | Given 7 days/week |
| Daily feed dose 3 | 39.7 | ng | Given 7 days/week |
| Mixture daily feed dose | 12.2 | ng | Given 7 days/week |
| Estimated absorption fraction | 0.966 | Unitless | Based on scat data analysis |
| Body weight | Average for group | g | Fit to average of body weight over first 90 days |
| Time of administration | 180 | days | |
| Initial $C_{\text{body}}$ | 0.0255 | ng/kg | Estimated based on controls |

clean feed. These TCDF spiked feed concentrations bracketed the 95th centile value observed to occur in the diet of wild mink captured from the Tittabawassee River (i.e., 175 ng TCDF/kg ww [Zwiernik, 2008a]). The TCDF/4-PeCDF mixture treatment consisted of 98 ng/kg ww of 4-PeCDF and 440 ng/kg ww of TCDF or roughly three- and fivefold greater concentrations than estimated to occur in the median diet of wild mink from the Tittabawassee River. The overall feed concentration of TEQ in the mixture treatment (73 ng TEQ/kg ww) was approximately 2.4-fold greater than that of the median total dietary TEQ estimated for the wild mink based on collection of prey species (including other PCDF, TCDF, and PCB congeners).

## Morphological Effects

No statistically significant ($p < 0.05$) dose effects were observed for any morphological parameter measured in this study. The parameters examined included body weight, liver weight, brain weight, liver to brain weight ratio, and body length with and without tail. Body weights were stable over the time course of the experiment in all groups. More information on these end points will be published elsewhere.

## Tissue Concentrations

Concentrations of TEQ in liver of control animals were less than 1.2 ng/kg TEQ ww (31% PCDD, 11% PCDFs, and 58% PCBs). Mean concentrations of TEQ in adipose of control animals were 2.7 ng TEQ/kg ww (81% PCDD and 19% PCDFs; PCBs were not measured). Mean concentrations of 4-PeCDF and TCDF in liver and adipose are reported by dose group for each of the time points (0, 90, or 180 days), respectively (Figures 2 and 3). Concentrations in liver and adipose were proportional to dose, and tissue concentrations were statistically significant among doses ($p < 0.05$). Exposure to the mixture treatment resulted in concentrations that were not statistically different from the mid-dose used for the single congener group. Concentrations of 4-PeCDF in tissues were constant between 90 and 180 days with no statistically significant differences among time points within treatments. This result suggests that steady-state conditions for 4-PeCDF were achieved by exposure day 90 and that steady-state conditions were maintained throughout the remainder of the 180-day experiment. Concentrations of TCDF were slightly higher at 180 days compared to the 90 days in both liver and adipose tissue. The apparent increase was not statistically significant for liver tissue but was statistically significant for adipose tissue ($p < 0.05$).

The distribution of retained compounds between liver and adipose is presented as the ratio of concentrations in liver to that in adipose (distribution ratio) for both TCDF and 4-PeCDF (Figure 4). For TCDF, the distribution ratios were essentially constant among dose groups and between time points, and the distribution was approximately proportional to the lipid content of the tissues. However, for 4-PeCDF, the distribution ratio was dose dependent and increased from less than 1 to greater than 3 from the least to greatest doses, respectively. This pattern is similar to that observed in laboratory studies with rodents, in which dose-dependent changes in distribution patterns for 4-PeCDF are observed due to induction of hepatic CYP1A2 protein (NTP, 2006). CYP1A2 protein in rodent liver binds 4-PeCDF avidly and results in hepatic sequestration (Diliberto *et al*. 1999); the current liver/fat data for 4-PeCDF suggest that a similar sequestration phenomenon may be

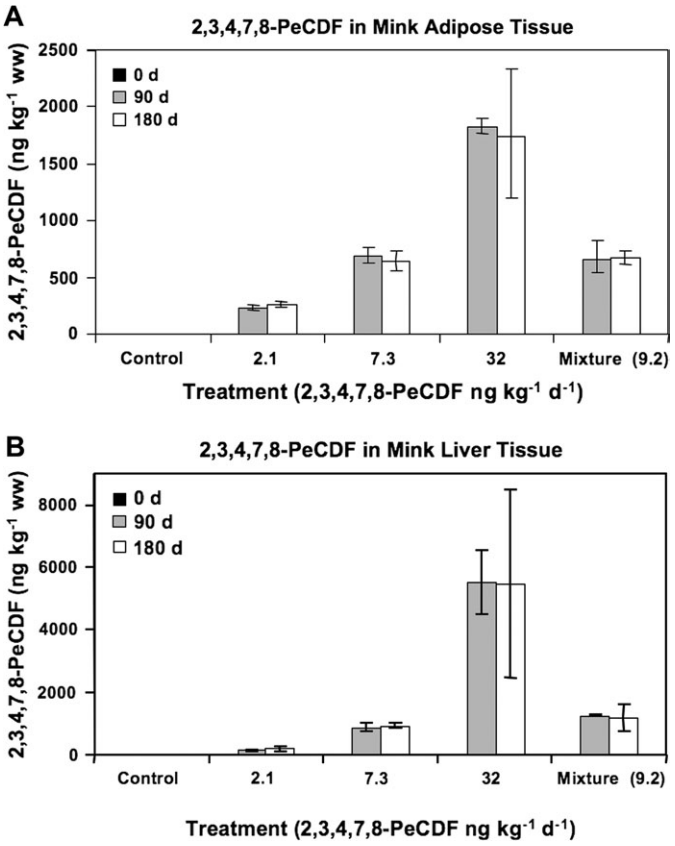


**FIG. 2.** Concentrations of 4-PeCDF in adipose and liver.

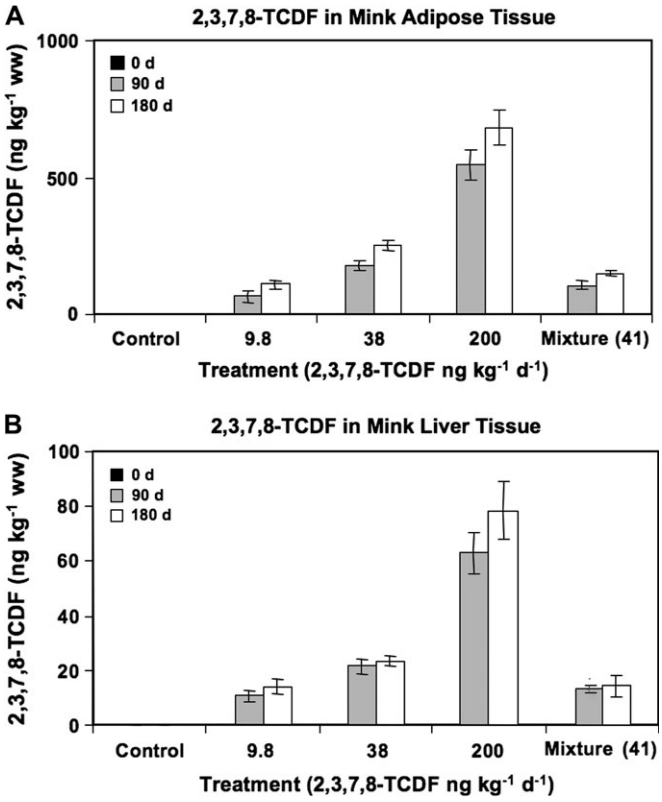


**FIG. 3.** Concentrations of TCDF in adipose and liver.

occurring in mink but to a lesser extent than observed in mice and rats.

Because liver and adipose are the main repositories of dioxin-like compounds (Diliberto *et al*. 2001; Hurst *et al*., 2000), the total quantity of retained compound in the body (and, therefore, the overall body concentration) can be estimated by multiplying the tissue concentration times the mass of each tissue. Livers were weighed when the mink were killed. The mass of adipose was estimated to be approximately 7% of body weight (Aulerich *et al*. 1999). The ratio of the total body concentration to the administered daily dose rate is an indicator of the overall rate of elimination: a greater ratio of body concentration to daily dose indicates a slow rate of elimination (i.e., greater bioaccumulation), while a lesser ratio indicates more rapid overall elimination.

Ratios of body concentration to dose were determined for TCDF and 4-PeCDF (Figure 5). For TCDF, the ratio was found to be inversely proportional to dose, with values consistently less than 1.0, and increasing slightly from the 90- to 180 days of exposure for each dose group. The observed pattern of an inverse body concentration/dose-response is consistent with inducible metabolism of TCDF, which has been observed in laboratory studies of rodents (Tai *et al*., 1993). In contrast, the body concentration/dose ratios for 4-PeCDF were constant over both time and dose. This result indicates that there was no dose-dependent influence on whole-body clearance. Finally, ratios of body concentration to administered dose rate for 4-PeCDF were greater (generally more than 10-fold) than those for TCDF (generally less than 1). This implies that TCDF was eliminated and/or metabolized more than 10-fold faster than was 4-PeCDF.

The ratio between the concentrations in the liver to that in the diet suggests that, for both furan congeners, bioaccumulation in liver is dose dependent (Table 3). For 4-PeCDF, bioaccumulation factors (BAFs) ranged from 9.5 at the least dose to 17 at the greatest dose. However, as discussed above, the ratio of whole-body concentration of 4-PeCDF to that in the diet was constant among dose groups (Figure 5). The greater 4-PeCDF BAF at greater doses was due to sequestration in the liver (Figure 4).

TCDF was accumulated in liver to a lesser extent, with a liver:diet BAF of 0.14 at the least dose and 0.041 at the greatest tested dose. The presence of 4-PeCDF in the mixture diet greatly reduced the accumulation of TCDF in liver tissue via CYP1A1-induced metabolism of TCDF (to be reported elsewhere), with a BAF of 0.032 in the mixture relative to 0.06 for the mid-dose of the single congener diet mix, even though the TCDF doses were similar. Conversely, the presence of TCDF had no effect on the accumulation of 4-PeCDF, with

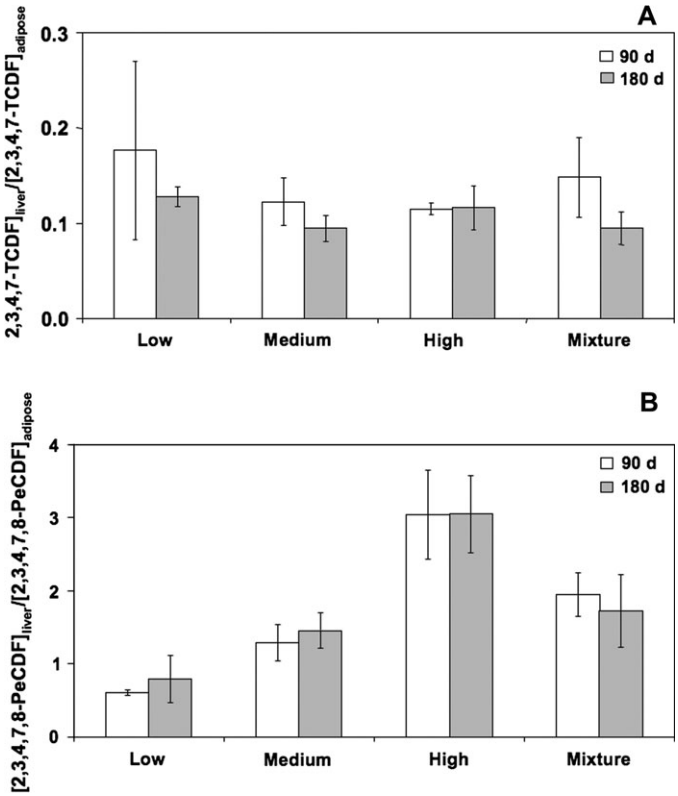


**FIG. 4.** Ratio of liver to adipose tissue concentrations for TCDF and 4-PeCDF.

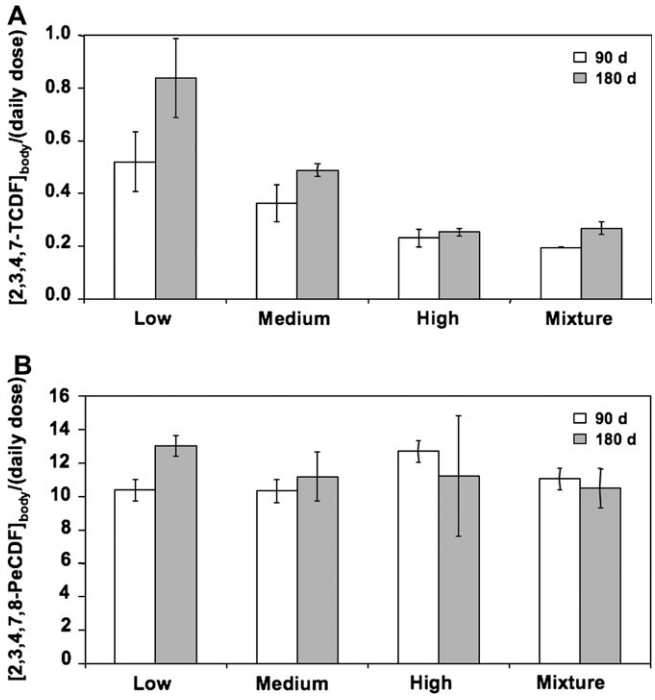


**FIG. 5.** Ratio of body concentration (ng/kg ww) to daily dose (ng/kg ww) after 90 or 180 days for TCDF and 4-PeCDF.

a BAF of 12 observed for both the mixture and mid-dose single congener treatments. The effect of 4-PeCDF on TCDF accumulation and the absence of an impact on 4-PeCDF accumulation suggest that 4-PeCDF facilitated the clearance of TCDF or altered its tissue distribution characteristics.

## Concentrations in Scat

Concentrations of PCDF in scat of the mink were determined at several time points. Based on concentrations measured in the scat, nearly all the test compounds were absorbed from the diet. The results suggest that TCDF and 4-PeCDF concentrations in scat were significantly correlated with the concentrations in both liver and adipose. These relationships and their predictive capacity are more fully discussed elsewhere (Zwiernik, 2008b).

## Modeling of Uptake and Elimination Kinetics

### *First-Order Kinetic Model*

The estimated whole-body first-order elimination rates among dose groups for TCDF appeared to be dose dependent, with more rapid elimination observed at greater doses (Table 4). These findings are consistent with induction of CYP1A1 as measured by ethoxyresorufin-O-deethylase activity at 180 days (Table 5). These results are also consistent with those observed in rats where the metabolism of TCDF was accelerated by induction of CYP1A1 (Tai *et al*., 1993). The elimination half-times estimated for TCDF in all dose group concentrations were less than 12 h. The first-order model fit to the full set of tissue concentration data from 90 to 180 days sacrifices slightly overpredicted body concentrations at 90 days and underpredicted concentrations at 180 days, consistent with the observation of increased concentrations in adipose tissue at 180 days compared to 90 days.

**TABLE 3**
**BAF Estimates for Liver:Diet Based on the Ratio of Wet Weight Concentrations in Liver at 180 days to Overall Dietary Concentration (Table 1)**

| Treatment | 4-PeCDF liver:diet BAF | TCDF liver:diet BAF |
|---|---|---|
| Low | 9.5 | 0.14 |
| Mid | 12 | 0.060 |
| High | 17 | 0.041 |
| Mixture | 12 | 0.032 |
| Saginaw River feeding study 10% carp (Tillitt *et al*., 1996) | 43 | 1 |
| Saginaw River feeding study 20% carp (Tillitt *et al*., 1996) | 53 | 0.5 |
| Saginaw River feeding study 40% carp (Tillitt *et al*., 1996) | 35 | 0.25 |
| Tittabawassee River field study (Zwiernik, 2008) | 11 | 0.14 |

**TABLE 4**
**Estimated Average First-Order Elimination Rate Constants for 2,3,7,8-TCDF and 4-PeCDF by Dose Group. Rates Were Estimated Using Tissue Concentration Data from Both the 90- and 180-day Time Points. $N = 6$ Except Where Noted.**

| Dose group | First-order rate constant, 1/day Mean (SD) | Estimated half-life, days Mean |
|---|---|---|
| 2,3,7,8-TCDF | | |
| Low | 1.6 (0.6) | 0.43 |
| Mid | 2.6 (0.7) | 0.27 |
| High | 4.1 (0.6) | 0.17 |
| Mixture ($n = 5$) | 4.3 (0.7) | 0.16 |
| 2,3,4,7,8-PeCDF | | |
| Low | 0.086 (0.012) | 8.1 |
| Mid | 0.095 (0.008) | 7.3 |
| High | 0.087 (0.019) | 8.0 |
| Mixture | 0.094 (0.008) | 7.4 |

The results of the mixture study indicate more rapid elimination than would have been predicted based on the data for TCDF administered alone at the same dose. This is consistent with increased metabolism by CYP1A1 due to induction of this enzyme by coadministered 4-PeCDF (Table 5). The coadministration results in a greater induction of CYP1A1 and a concomitantly more rapid metabolism and subsequent depuration of TCDF. This result suggests that the metabolism of TCDF in the diet of wild mink would also be enhanced by coexposure to other AhR-active compounds that induce CYP1A1 enzyme activity. This would be expected to result in lower retention of TCDF. Because of the relatively rapid elimination rates observed for TCDF, the predicted tissue concentrations appear to achieve a rapid steady-state across nearly the entire time period of the study. However, the tissue concentrations did increase slightly over time for TCDF. Because the distribution between liver and adipose tissues was constant across doses and time points for TCDF, the whole-body model (first-order kinetic model, Equation 4) can be used to predict tissue concentrations associated with a variety of dosing regimens. However, due to the dependence of elimination rate on dose, the usefulness of these models is limited to the observed dose range.

**TABLE 5**
**EROD and MROD* Activities at 180 days**

| Congener and dosage (ng/kg/day) | EROD pmol/min/mg (mean ± SD) | MROD pmole/min/mg (mean ± SD) |
|---|---|---|
| Control | 430 ± 69 | 115 ± 15.8 |
| 4-PeCDF | | |
| 2.1 | 495 ± 67 | 90 ± 1.9 |
| 7.3 | 538 ± 19 | 131 ± 3.5 |
| 32 | 644 ± 99 | 150 ± 28 |
| TCDF | | |
| 9.8 | 351 ± 24 | 59 ± 3.9 |
| 38 | 572 ± 33 | 91 ± 6.5 |
| 200 | 431 ± 250 | 113 ± 19 |
| Mixture | 576 ± 49 | 148 ± 7.2 |

* methoxyresorufin-*O*-deethylase

The first-order model (Equation 4) was also used to estimate depuration rate constants for 4-PeCDF. The estimated first-order elimination rates for 4-PeCDF were consistent among dose groups at approximately 0.09 per day. This corresponds to a whole-body half-time of approximately 7.3–8 days (Table 4). There were no clear patterns with dose group or time of exposure. Because of the complex relationship between dose rate and disposition between liver and fat of 4-PeCDF, the first-order model could not accurately predict the observed pattern of distribution of compound between liver and adipose tissue or the concentration of compound in these tissues at other time points or from other dose levels. There is no physiologically based understanding of the distribution and accumulation of the compound incorporated in the first-order model for 4-PeCDF, so its application to predicting tissue concentrations under other dosing regimens is extremely limited.

The first-order elimination half-lives for TCDF and 4-PeCDF are dependent upon the assumption of 97% absorption from diet. If the absorption from diet was actually 90%, the estimated half-lives would increase by approximately 6%.

### Two-Compartment Model

Parameters for the modified model by Carrier *et al.* (Aylward *et al.*, 2005) for 4-PeCDF as derived from the 90-day study data are presented in Table 2. The measured adipose and liver tissue concentrations at 180 days are presented in Figure 6 along with the tissue concentrations derived from the model. The model provides a good fit to all the body and tissue concentration data at all three doses and for the mixture using a single set of parameters and allows the prediction of tissue concentrations at further time points for the given doses as well as tissue concentrations that would be expected from different dose rates or dosing regimens (Figure 6).

## DISCUSSION

Mink reside at the top of the riparian food web (Alexander, 1977) which results in a relatively greater exposure and bioaccumulative potential for mink exposed to elevated PCDD/PCDF congeners and other bioaccumulative chemicals in their diet. In addition, mink are reportedly more sensitive to the toxic effects of dioxins and dioxin-like compounds than most other animals (Hochstein *et al.*, 1998; Tillitt *et al.*, 1996). Exposure of mink to dioxins and dioxin-like compounds has been shown to lead to reproductive and developmental effects such as decreased litter size, mortality, jaw lesions, reduced body weight, and decreased litter size (Beckett *et al.*, 2005; Restum *et al.*, 1998). TRV for mink based on total TEQ developed with food sources

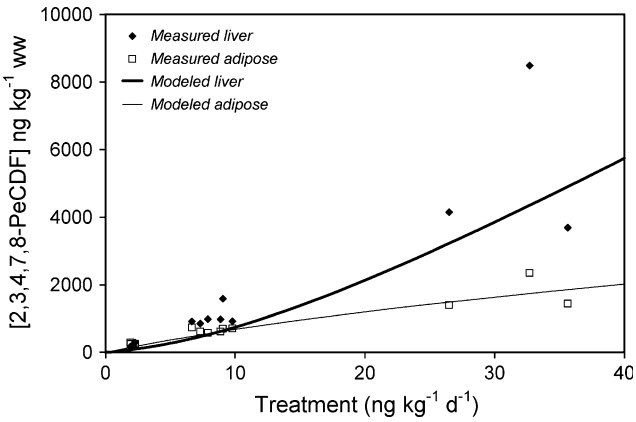


**FIG. 6.** Measured liver and adipose tissue concentrations of 4-PeCDF at 180 days and modeled tissue concentrations using the model by Carrier *et al.* 1995a,b. with parameters estimated based on the 90-day tissue concentration data. Measured concentrations and estimated dose rates are presented for individual animals. The model predicts the increasing distribution of 4-PeCDF to liver at increasing dose rates.

from specific locations, that is, the Housatonic, Hudson, and Saginaw Rivers differ by site and, therefore, may not be directly applicable to assessing risk to mink in other locations (Brunström *et al.*, 2001; Bursian *et al.*, 2006a,c; Heaton *et al.*, 1995; Millsap *et al.*, 2004). This phenomenon is possibly due to the confounding exposure to co-contaminants in the food supplies from different locations and to potential differences in toxicity to mink among TEQ-contributing compounds. The presence of co-contaminants confounds our ability to assign direct causality to one particular contaminant.

The overall liver:diet BAFs estimated for Tittabawassee River wild mink of 11 for 4-PeCDF and 0.14 for TCDF are in excellent agreement with the values observed in this controlled laboratory study, suggesting in wild mink that 4-PeCDF either enhanced clearance of TCDF or altered its tissue distribution (Zwiernik, 2008a). BAFs calculated from the laboratory study where mink fed a diet containing varying proportions of Saginaw River carp were in the range of fourfold greater than those found in the study reported here (Table 3).

These results for mink add to our understanding of species differences in toxicokinetics for these two furan congeners. The estimated whole-body half-times for depuration for TCDF and 4-PeCDF observed for mink (< 0.5 and 7–9 days, respectively) are significantly shorter than those observed for rats exposed to the same compounds. The half-time for depuration of TCDF from rats has been estimated to be approximately 2 days (Birnbaum *et al.*, 1980). Half-time for depuration of 4-PeCDF from rats was estimated to be more than 60 days (Brewster and Birnbaum, 1987). This more rapid depuration from mink is contrary to what would be expected based on general trends of half-life being directly proportional to body weight that has usually been observed for dioxin-like compounds.

Distribution of TCDF and 4-PeCDF between liver and adipose was also significantly different from that observed in rats exposed to similar doses. The liver to adipose ratios for TCDF were found to be greater than 2 in rats (DeVito *et al.*, 1998), while in mink, in this study, the ratio was approximately 0.1. This result indicates that less hepatic accumulation was occurring in mink that occurs in rats. Similarly, the liver: adipose ratios for 4-PeCDF in rats were greater than 10 (DeVito *et al.*, 1998) at administered dose rates that resulted in liver:adipose ratios of only approximately 3.0 in mink in this study.

A consequence of the rapid clearance of TCDF from mink is the finding in the wild mink liver of a relative absence of TCDF (only 0.4% of the liver TEQ on a wet weight basis) when 30% of the dietary TEQ was provided by this congener (Zwiernik, 2008a). The relative absence of TCDF has also been observed in deer liver from deer collected along the Tittabawassee River floodplain. In these deer, 4-PeCDF accounted for most of the tissue TEQ (Dow, unpublished data). Results from this and other studies demonstrate that TCDF is readily eliminated due to induction of the hepatic mixed function monooxygenase enzymes such as CYP1A1. This induction may be due to TCDF itself or to other congeners in the mixture that are less readily metabolized (Brewster and Birnbaum, 1987; McKinley *et al.*, 1993; Olson *et al.*, 1994; Tai *et al.*, 1993). The ability of CYP1A1 induction to enhance metabolic clearance of TCDF and result in lower TCDF liver concentrations was also observed in a soil bioavailability study conducted in rats with a floodplain soil taken from the Tittabawassee River (Budinsky *et al.*, unpublished data). The rapid clearance and resulting low retention of TCDF following intake of diet in which the proportion of the TEQ contributed by TCDF was 30% suggest that risk to mink based on a TEQ dietary (intake basis) may exaggerate the potential risk of this specific mixture of compounds. Mixture interactions and pharmacokinetic factors have not been explicitly considered in the current TEF/TEQ scheme, and the rapid clearance of TCDF in mink suggests that in this context, TCDF does not fulfill the explicit criteria of persistence and bioaccumulation for inclusion of congeners in the TEF scheme (Van den Berg *et al.*, 2006).

In summary, daily dietary dosages that resulted in steady-state tissue concentrations of TCDF and 4-PeCDF in mink in a controlled laboratory study that were similar to those observed in wild mink on the Tittabawassee River (MI) were studied and kinetics for uptake and depuration determined. The toxicokinetic data developed here will support any future toxicity studies to develop site-specific toxicity data for wild mink under environmentally relevant exposure conditions. The tissue distribution data and pharmacokinetic model parameters reveal significant differences between TCDF and 4-PeCDF in mink, with TCDF undergoing enhanced clearance with increasing dosages of TCDF or in combination with 4-PeCDF. Alternatively, 4-PeCDF exhibited selective uptake and sequestration in the liver consistent with its ability to induce and bind to CYP1A2 protein (Diliberto *et al.*, 1999). Mink effectively absorbed both TCDF and 4-PeCDF (97%) from the diet. This is greater than the

approximate 70–90% fraction of the dose absorbed or bioavailability reported for TCDD absorption from corn oil in rats and mice (Diliberto *et al*., 2001; Rose *et al*., 1976). The notable differences between mink and other animals with respect to tissue distribution and pharmacokinetic parameters, combined with the interesting apparent discrepancy between administered dose and tissue dose for TCDF, will support future risk assessment efforts for characterizing risk, if any, to wild mink living along the Tittabawassee River downstream of Midland, MI.

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Animal use: All aspects of this study that involve the use of animals were conducted in the most humane manner. The protocols for this study were approved by the Michigan State University Institutional Animal Care and Use Committee (AUF # is 12/05-165-00).

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