

## Nondestructive Scat Sampling in Assessment of Mink (*Mustela vison*) Exposed to Polychlorinated Dibenzofurans (PCDFs)

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**Abstract** The mink (*Mustela vison*) is often utilized as a sentinel species for ecological assessments at sites where contaminants of concern include dioxins and dioxin-like compounds. Utilizing mink scat as a nondestructive tool to determine internal exposure to dioxin-like compounds may allow for rapid, accurate estimates of exposure without the need to capture mink or their prey. To determine the relationships between concentrations of polychlorinated dibenzofurans (PCDFs) in tissues (liver and adipose) and those in scat, mink were fed PCDFs in scat during a controlled laboratory study for 180 days. Mink were fed a

control diet, diets with three doses of 2,3,4,7,8-pentachlorodibenzofuran (2,3,4,7,8-PeCDF) or 2,3,7,8-tetrachlorodibenzofuran (2,3,7,8-TCDF), and a diet with an environmentally relevant mixture of the two congeners. Concentrations of PCDFs in liver and adipose were measured after 0, 90, and 180 days of exposure. Concentrations of the two PCDF congeners in mink scat were determined after 2, 23, 45, 90, and 180 days of exposure. Concentrations of both PCDF congeners in scat were significantly correlated with those in liver and adipose tissue ( $r^2 = 0.94–0.97$ ,  $p < 0.01$ ). This indicates that measurements of concentrations of both PCDFs in scat can be used to predict concentrations of PCDFs in liver and adipose. Assimilation and elimination characteristics of 2,3,4,7,8-PeCDF or 2,3,7,8-TCDF and a mixture of the two congeners by mink could be predicted from concentrations of these congeners in scat. Overall, concentrations of PCDFs in mink scat can be used as a rapid and inexpensive nondestructive method to predict concentrations of PCDFs in mink when certain assumptions are met.

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The mink (*Mustela vison*), as a fish-eating mammal, occupying a high trophic status in aquatic ecosystems, is recognized as a sentinel species for ecological assessment of various environmental pollutants including both metals and organic compounds, such as polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) (Basu et al. 2007). Previous laboratory studies have assessed exposure and/or effects for dioxin-like chemicals including PCDDs and PCDFs through dietary exposure of mink (Heaton et al. 1995; Tillitt et al. 1996; Hochstein et al. 1998; Bursian et al. 2006a–c), but application of these types of studies usually requires subsequent

chemical analyses of mink or their diet. Also, several recent studies have employed field investigations as part of a multiple lines of evidence approach to reduce uncertainty in ecological assessments. Field investigations and bio-monitoring are expensive, labor-intensive, and require trapping significant numbers of wild animals (Millsap et al. 2004; Neigh et al. 2006; Zwiernik et al. 2008a, b). The taking of live mink requires approval from agencies and is unpopular with the public. For these reasons we investigated the potential to use scat to estimate exposure of resident populations of mink.

In addition to the generally recognized and well-defined benefits of using sentinel species (Basu et al. 2007), the use of simple, nondestructive sampling schemes can provide useful information for ecological assessments and several such approaches have been tested. For example, excrement, boluses, and feathers of nestling songbirds have been successfully utilized as indicators of exposure to metals (Dauwe et al. 2000).

Mink reside at the top of the aquatic and riparian food web (Alexander 1977), which results in a relatively greater exposure to classical dioxin-like compounds including coplanar PCBs (Martin et al. 2006) and PCDD/DF congeners (Tansy et al. 2003; Blankenship et al. 2007) than organisms lower in the food web. While studies of exposure to single congeners and/or mixtures of PCDDs/DFs and PCBs have been conducted (reviewed by Blankenship et al. 2007), relatively little is known about the kinetics of uptake and depuration and tissue disposition of PCDDs/DFs in mink (Millsap et al. 2004; Zwiernik et al. 2008a, b). While many studies of bioaccumulation and effects have been conducted with mink and mink have been employed as a sentinel species, the relationship between concentrations of PCDDs/DFs in mink tissues and those in scat had never been examined. Studies of organophosphorus insecticides in rat saliva (Timchalk et al. 2007) and polychlorinated biphenyls (PCBs) in otter scat (Gutleb and Kranz 1998) and spraints (van den Brink and Jansman 2006) have shown that fecal matter is relatively easy to obtain and can be useful in predicting body residues of target compounds.

The study on which we report here determined the potential for use of scat to predict concentrations of PCDDs/DFs in liver and adipose tissue of mink and their diet. Mink were fed either 2,3,4,7,8-pentachlorodibenzofuran (2,3,4,7,8-PeCDF) or 2,3,7,8-tetrachlorodibenzofuran (2,3,7,8-TCDF) or a mixture of the two congeners. These two congeners were selected because they are the predominant congeners contributing to the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TEQ) in the Tittabawassee River and downstream of Midland, Michigan, USA (Hilscherova et al. 2003). Concentrations of 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF were measured in adipose and liver after 90 and 180 days and in scat after 2,

23, 45, 90, and 180 days of exposure, and then predictive relationships were developed. This study was also designed to determine the effects of single congeners and mixtures of those congeners to mink. Detailed results for the responses of mink to these congeners are reported elsewhere (Zwiernik et al. 2008a, b).

## Materials and Methods

### Test Substances

2,3,4,7,8-PeCDF and 2,3,7,8-TCDF were purchased from Wellington Laboratories (Guelph, Ontario, Canada). Analysis of 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF standards demonstrated 99.6% and 97.7% purity, respectively. 2,3,7,8-Substituted impurities were less than 0.01% on a TEQ basis calculated for each of the compounds (determined with WHO 2006 mammalian TEFs (Van den Berg et al. 2006). Analysis of all samples, including standards, was conducted with U.S. EPA Method 1613.

### Experimental Design

Accumulation of 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF from the diet by mink was determined over an exposure duration of 3 or 6 months. The study design included a total of four treatments: (1) control without test chemical exposure, (2) exposure to single congener 2,3,4,7,8-PeCDF, (3) exposure to single congener 2,3,7,8-TCDF, and (4) exposure to a mixture of the two congeners (Table 1). The base diet was used as the control diet, with treatment diets differing only in the amount of 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF added. Treatment groups included three doses of each individual congener and a single treatment dose of the mixture (Table 2). A 25-g portion of diet containing the entire daily PCDF dose was placed on the cage of each mink every morning. After this feed was consumed, to ensure complete ingestion of the treatment dose, an additional larger portion of clean diet was given to each mink in the afternoon and the mink were allowed to eat until satiated. The mink consumed approximately 100 g of the clean diet each day. This procedure ensured essentially complete ingestion of the spiked feed and eliminated the need to measure daily feed consumption in order to estimate doses.

### Tissue and Scat Sampling

A total of 50 mink were distributed among the treatments with the number of replicates ranging from two to three (Table 1). Dosing began on January 31, 2006 (day 0).

**Table 1** Study design showing exposure treatments for mink and sampling schemes for tissue and scat

Condition	Treatment								
	Control (no dose)			Single-congener dose			Mixture dose (PeCDF+TCDF)		
				PeCDF		TCDF			
<b>Exposure</b>									
No. of treatments	1			3		3		1	
Dose level <sup>a</sup>	–			Low/mid/high		Low/mid/high		Mid	
No. of animals used	8			18		18		5	
<b>Tissue sampling</b>									
Collection times	0 d	90 d	180 d	90 d	180 d	90 d	180 d	90 d	180 d
Adipose samples ( <i>n</i> )	3	2	3	9	9	9	9	2	3
Liver samples ( <i>n</i> )	3	2	3	9	9	9	9	2	3
<b>Scat sampling</b>									
Collection times	2, 23, 45, 90 (pooled sample) and 180 d (individual samples)								
No. of pooled samples	4			12		12		4	
No. of individual samples	3			9		9		3	

Note. d, day

<sup>a</sup> Details for daily dose concentrations of 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF in each treatment provided in Table 2

**Table 2** Concentrations of 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF in spiked feed (initial 25 g), overall feed concentrations (total 125 g), and estimated daily doses in each treatment

Treatment	Dose level	Spiked feed concentration (ng kg <sup>-1</sup> ww)	Overall feed concentration (ng kg <sup>-1</sup> ww)	Daily dose (ng d <sup>-1</sup> )	Daily dose (ng TEQ d <sup>-1</sup> ) <sup>a</sup>
<b>PeCDF</b>					
Single-congener dose	Low	110	22	2.7	0.8
	Mid	390	78	9.6	2.9
	High	1600	320	40	12
Mixture dose	Mid	490	98	12	3.6
<b>TCDF</b>					
Single-congener dose	Low	500	100	12	1.2
	Mid	2000	400	49	4.9
	High	9700	1900	240	24
Mixture dose	Mid	2200	440	55	5.5

Note. ww, wet weight; d, days

<sup>a</sup> Daily dose converted to 2,3,7,8-TCDD equivalents (TEQs) using the WHO 2006 mammalian toxic equivalency factors (TEFs) of 0.3 for 2,3,4,7,8-PeCDF and 0.1 for 2,3,7,8-TCDF (van den Berg et al. 2006)

Control animals were sampled on day 0 and 180. Three animals from each of the 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF and the 2,3,4,7,8-PeCDF/2,3,7,8-TCDF mixture treatment groups were sampled after 90 and 180 days of exposure. Livers and adipose were removed, weighed, and preserved for quantification of 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF. Body weights were measured every 30 days, beginning 1 day prior to the commencement of dosing, and also when the mink were euthanized for sampling. Scat was collected on days 2, 23, 45, 90 (three mink in each treatment, ca. 50 g per mink, samples pooled then analyzed), and 180 (three mink in each treatment, ca. 50 g per mink, samples analyzed individually). Scat was collected from individual mink in order to measure the mass of scat per individual mink and then estimate assimilation efficiency on a mass basis.

### Sample Extraction and Residue Analysis

Two different extraction techniques were used, depending on the sample matrix. For liver and adipose tissue and mink feed, 5 g of the sample was added to 75 mL of hydrochloric acid and 100 mL of 5/95 (v/v) benzene/hexane solution and shaken for at least 16 h. For the mink scat, 10 g of sample was extracted using a Soxhlet Dean-Stark apparatus 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<sub>2</sub>SO<sub>4</sub>/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 processed extract was analyzed for PCDFs by HRGC/HRMS (high-resolution gas chromatography/high-resolution mass spectrometry) with a Trace 2000 series gas chromatograph (Thermo Scientific Thermo Fisher Scientific, Waltham, MA, USA) 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, USA) and 60 m × 0.25 mm × 0.25 μm Varian 5 ms GC column. The HRMS was equipped with a standard electron ionization (EI) ion source operating in positive ionization mode. The mass spectrometer data were obtained in the selected ion monitoring (SIM) mode at a resolution of 10,000 (10% valley).

## Results and Discussion

### Spiked Feed and Daily Dose

Measured concentrations of 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF in the spiked feed were used to calculate daily doses for each treatment (Table 2). Overall the daily concentration of 2,3,4,7,8-PeCDF in the diet ranged from 22 to 320 ng kg<sup>-1</sup> wet weight (ww) based on the mink's consumption of 25 g of dosed feed plus 100 g of clean feed. This dietary concentration range effectively brackets the concentration estimated for the 95th centile of the diet of wild mink inhabiting the Tittabawassee River (71 ng kg<sup>-1</sup> ww) (Zwiernik et al. 2008a). The overall daily concentration of 2,3,7,8-TCDF in the diet ranged from 100 to 1900 ng kg<sup>-1</sup> ww, and this also brackets the value estimated to occur in the 95th centile of the diet of mink inhabiting the Tittabawassee River (180 ng kg<sup>-1</sup> ww). The mixture treatment consisted of 98 ng kg<sup>-1</sup> ww of 2,3,4,7,8-PeCDF and 440 ng kg<sup>-1</sup> ww of 2,3,7,8-TCDF, or slightly above the 95th centile and roughly three- and five-fold greater, respectively, than estimated to occur in the median diet exposures of wild mink from the Tittabawassee River.

### Concentrations of PCDFs in Tissues

Mean concentrations of 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF in mink liver, adipose, and scat are reported by treatment and dose group for each of the time points ( $t = 0$ , 90 or 180 days), respectively (Table 3). Concentrations of 2,3,4,7,8-PeCDF in liver and adipose were relatively constant between the 90- and the 180-day time points for each dose group, with no statistically significant differences between the two times, within treatments. This suggests that steady state was achieved prior to 90 days for 2,3,4,7,8-PeCDF. Concentrations of 2,3,7,8-TCDF also did

not show great variations between the two time points except for adipose tissue in the low-dose single-congener treatment (Table 3).

Tissue concentrations were directly proportional to dose for both 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF when mink were exposed to these congeners individually. Concentrations were statistically different ( $p < 0.05$ ) among doses for both liver and adipose. Exposure to the mixture treatment (12 ng 2,3,4,7,8-PeCDF day<sup>-1</sup> and 55 ng 2,3,7,8-TCDF day<sup>-1</sup>) resulted in differences in tissue concentrations that were not statistically significant from those in the corresponding mid doses (9.6 ng 2,3,4,7,8-PeCDF day<sup>-1</sup> and 49 ng 2,3,7,8-TCDF day<sup>-1</sup>) of the single-congener groups. Thus, additive exposure of the two congeners did not affect the accumulation kinetics of either congener in mink.

### Concentrations of PCDFs in Scat

When exposed to 2,3,4,7,8-PeCDF or 2,3,7,8-TCDF individually, concentrations of these two congeners in scat were directly proportional to those in the diet. Also, there was no statistically significant difference in concentrations between 90 and 180 days of exposure for either congener in liver, adipose, or scat. These results suggest that measuring concentrations of PCDFs in a representative sample of scat could be a useful indicator of the degree of exposure for these compounds in the environment when mink are at steady state with the concentrations of these two congeners in their diet.

While concentrations of 2,3,4,7,8-PeCDF in scat were generally 100-fold less than corresponding concentrations in liver and adipose, concentrations of 2,3,7,8-TCDF in scat were similar to or even greater than the corresponding concentrations in liver and adipose (Table 3). This result could be due to different accumulation characteristics for 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF. Approximately >10% and < 0.1% of the total ingested dose of 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF, respectively, were accumulated into mink (Table 4).

### Bioaccumulation

The ratio between concentrations in liver relative to those in the diet suggests that bioaccumulation in liver is dose-dependent for both PCDF congeners. For 2,3,4,7,8-PeCDF, bioaccumulation factors (BAFs) ranged from 9.5 at the least dietary concentration (22 ng 2,3,4,7,8-PeCDF kg<sup>-1</sup>) to 17 at the greatest dietary concentration (320 ng 2,3,4,7,8-PeCDF kg<sup>-1</sup>). Bioaccumulation of 2,3,7,8-TCDF was less, with the liver-to-diet BAF of 0.14 at the least

**Table 3** Mean ( $\pm$ SD) concentrations (ng kg<sup>-1</sup> ww) of 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF in adipose, liver, and scat samples collected from mink exposed after 0, 90, and 180 days in each treatment

Treatment	PeCDF			TCDF		
	Adipose	Liver	Scat <sup>a</sup>	Adipose	Liver	Scat <sup>a</sup>
<b>Control</b>						
No dose	0 ng PeCDF d <sup>-1</sup>			0 ng TCDF d <sup>-1</sup>		
<i>t</i> = 0 d	1.2 $\pm$ 0.35	0.29 $\pm$ 0.01	0.17	0.42 $\pm$ 0.12	0.12 $\pm$ 0.01	0.06
<i>t</i> = 90 d	0.60 $\pm$ 0.26	0.40 $\pm$ 0.01	0.20	0.15 $\pm$ 0.03	0.14 $\pm$ 0.01	0.07
<i>t</i> = 180 d	0.70 $\pm$ 0.06	0.53 $\pm$ 0.12	0.11 $\pm$ 0.01	0.20 $\pm$ 0.03	0.18 $\pm$ 0.04	0.12 $\pm$ 0.04
<b>Single-congener</b>						
Low dose	2.7 ng PeCDF d <sup>-1</sup>			12 ng TCDF d <sup>-1</sup>		
<i>t</i> = 90 d	230 $\pm$ 26	140 $\pm$ 11	4.3	67 $\pm$ 19	11 $\pm$ 1.9	13
<i>t</i> = 180 d	260 $\pm$ 21	210 $\pm$ 74	4.2 $\pm$ 0.70	110 $\pm$ 14	14 $\pm$ 2.4	12 $\pm$ 3.1
Mid dose	9.6 ng PeCDF d <sup>-1</sup>			49 ng TCDF d <sup>-1</sup>		
<i>t</i> = 90 d	690 $\pm$ 66	880 $\pm$ 110	17	180 $\pm$ 17	22 $\pm$ 2.4	64
<i>t</i> = 180 d	650 $\pm$ 81	920 $\pm$ 70	19 $\pm$ 7.3	250 $\pm$ 18	24 $\pm$ 1.7	46 $\pm$ 15
High dose	40 ng PeCDF d <sup>-1</sup>			240 ng TCDF d <sup>-1</sup>		
<i>t</i> = 90 d	1800 $\pm$ 64	5500 $\pm$ 910	53	550 $\pm$ 49	63 $\pm$ 6.8	180
<i>t</i> = 180 d	1700 $\pm$ 540	5400 $\pm$ 2600	39 $\pm$ 16	680 $\pm$ 57	79 $\pm$ 9.2	250 $\pm$ 76
<b>Mixture</b>						
Mid dose	12 ng PeCDF d <sup>-1</sup>			55 ng TCDF d <sup>-1</sup>		
<i>t</i> = 90 d	660 $\pm$ 120	1300 $\pm$ 36	18	110 $\pm$ 9.7	13 $\pm$ 0.03	51
<i>t</i> = 180 d	670 $\pm$ 52	1200 $\pm$ 370	25 $\pm$ 13	150 $\pm$ 8.3	14 $\pm$ 3.2	93 $\pm$ 57

Note. d, days

<sup>a</sup> Concentrations of target compounds in scat at 0 and 90 days measured from pooled samples ( $n = 1$ ; see Table 1)

concentration in the diet (100 ng 2,3,7,8-TCDF kg<sup>-1</sup>) and 0.041 at the highest dietary concentration (1900 ng 2,3,7,8-TCDF kg<sup>-1</sup>). The presence of 2,3,4,7,8-PeCDF in the diet resulted in less accumulation of 2,3,7,8-TCDF in liver, with a BAF of 0.32 in the mixture relative to 0.060 for the single mid dose, even though the 2,3,7,8-TCDF dietary concentrations in the mixture (440 ng 2,3,7,8-TCDF kg<sup>-1</sup>) and single-congener treatment (400 ng 2,3,7,8-TCDF kg<sup>-1</sup>) were not significantly different ( $p < 0.05$ ). Conversely, the presence of 2,3,7,8-TCDF had no effect on the bioaccumulation of 2,3,4,7,8-PeCDF. The BAF for 2,3,4,7,8-PeCDF observed in both the mixture and the mid-dose treatments was 12. The BAF values of 11 for 2,3,4,7,8-PeCDF and 0.14 for 2,3,7,8-TCDF previously estimated for wild mink (Zwiernik et al. 2008b) are in good agreement with the values observed in this controlled laboratory study.

The different bioaccumulation characteristics in mink tissue between 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF were also evident from the ratio of concentrations in liver to those in adipose. For example, the ratios of 2,3,7,8-TCDF concentrations in liver to those in adipose were essentially constant (about 0.1) among dose groups and between time points, and the distribution was approximately proportional to the lipid content of the tissues. However, for 2,3,4,7,8-

PeCDF, the distribution between liver and adipose was dose-dependent. At the lower doses, concentrations in adipose exceeded those in liver, while at higher doses concentrations in liver exceeded those in adipose. This pattern is similar to that observed in laboratory studies with rodents in which dose-dependent changes in distribution patterns were related to induction of hepatic CYP1A2 protein. CYP1A2 in liver binds 2,3,4,7,8-PeCDF avidly and results in hepatic sequestration of this congener (Diliberto et al. 1999).

Because liver and adipose are the main repositories of dioxin-like compounds in the body, the total mass of retained compound in the body was estimated by summing the mass of retained compound in those two tissues. The mass in each tissue was calculated by multiplying the concentration in the tissue by the mass of corresponding tissue. The relative proportion of the total mass of retained compound in the body to the administered daily dose on a mass basis (given as percentage accumulation in Table 4) might be an indicator of the overall rate of elimination: a greater percentage accumulation indicates a slower rate of elimination, while a lesser percentage accumulation may indicate more rapid overall elimination. In general, bioaccumulation of 2,3,4,7,8-PeCDF relative to the administered daily dose was greater (generally more than 100-fold) than

**Table 4** Percentage accumulation (in adipose, liver, and whole body) of 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF exposed to mink at the 90- and 180-day sacrifice time points in each treatment

Treatment		% accumulation <sup>b</sup>							
		Dose (ng/d) <sup>a</sup>		In adipose		In liver		In body	
				90 d	180 d	90 d	180 d	90 d	180 d
PeCDF									
Single-congener dose	Low (2.7)	7.3	8.9	3.0	4.0	10	13		
	Mid (9.6)	5.5	6.0	4.3	4.8	10	11		
	High (40)	4.4	3.7	8.0	7.1	12	11		
Mixture dose	Mid (12)	4.9	4.8	6.0	5.1	11	10		
Mean		5.5	5.9	5.3	5.2	11	11		
TCDF									
Single-congener dose	Low (12)	0.07	0.10	0.04	0.06	0.12	0.16		
	Mid (49)	0.04	0.04	0.02	0.03	0.06	0.07		
	High (240)	0.02	0.03	0.01	0.01	0.04	0.04		
Mixture dose	Mid (55)	0.02	0.02	0.01	0.01	0.04	0.04		
Mean		0.04	0.05	0.02	0.03	0.06	0.08		

Note. d, days

<sup>a</sup> Daily feed dose

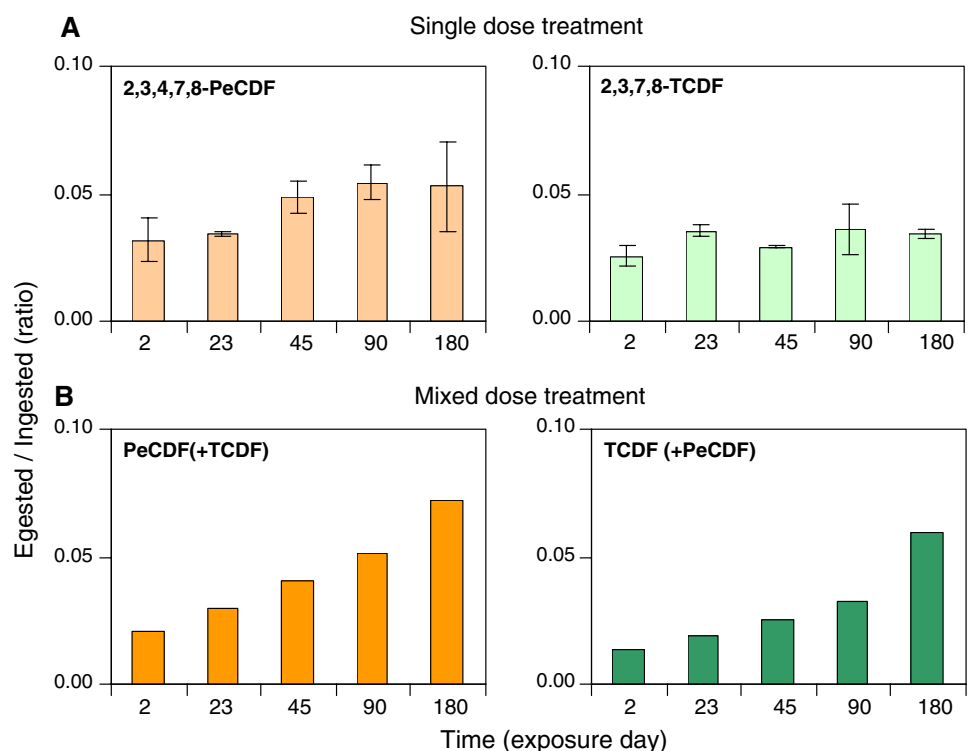
<sup>b</sup> Based on the total quantity of retained compounds (concentration by measured tissue weight) in the adipose and live tissue relative to daily feed dose, on mass basis

that of 2,3,7,8-TCDF, in both adipose and liver tissue. Bioaccumulation of 2,3,7,8-TCDF was inversely proportional to dose, but increased somewhat from 90 to 180 days of exposure for each dose group. The observed pattern of lesser retention for higher doses is consistent with inducible metabolism of 2,3,7,8-TCDF, which has been observed for this compound in laboratory studies of rodents (Tai et al. 1993). In contrast, the bioaccumulation of 2,3,4,7,8-PeCDF was relatively stable over both time and dose level.

#### Assimilation and Elimination Characteristics

The different proportions of accumulation of the congeners relative to dose indicates that 2,3,7,8-TCDF was eliminated and/or catabolized more than 100-fold faster than was 2,3,4,7,8-PeCDF. To better understand the bioaccumulation of target compounds in mink, rates of elimination (egested/ingested ratio) of PCDFs were calculated from concentrations in scat. Assuming that excretion in scat was

**Fig. 1** Elimination of target compounds, expressed as the ratio of the quantity egested to the quantity ingested, for (A) single-congener and (B) mixture treatment of 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF at 2, 23, 45, 90, and 180 days of exposure

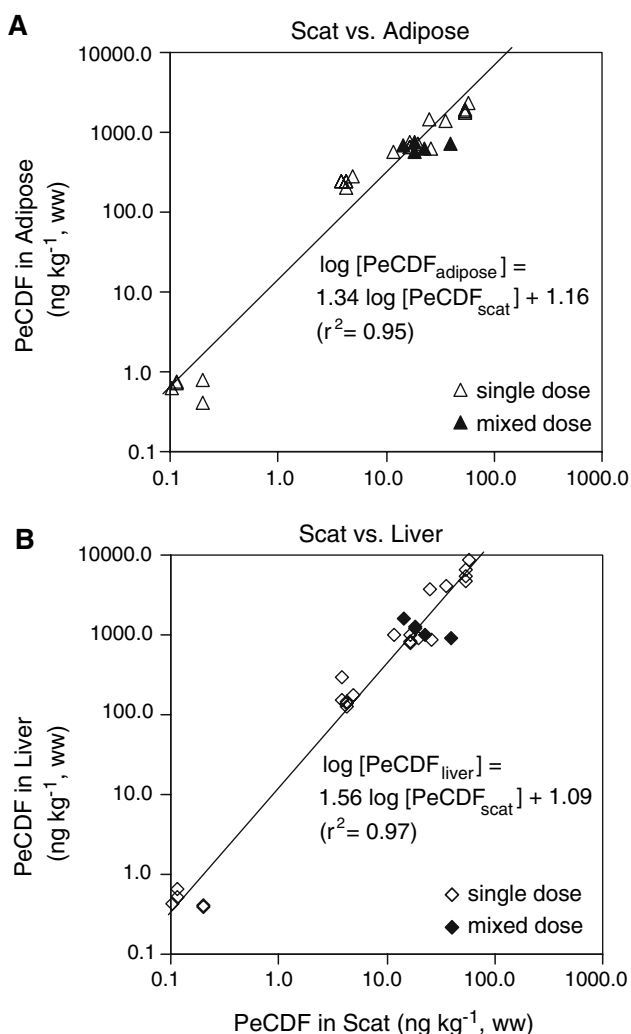


the only pathway for the elimination of the two congeners, the rate of elimination could be calculated from the quantity of residue egested per day by the quantity of ingested per day (expressed as ratio). Since scat was collected from mink from each treatment on days 2, 23, 45, 90, and 180 of the experiment, the rate of elimination could be obtained at five time points during apparent steady state (Fig. 1).

In general, the rate of elimination of the two PCDF congeners increased over time in the single-dose treatments of 2,3,4,7,8-PeCDF or 2,3,7,8-TCDF and reached steady state at 90 or 180 days of exposure. However, in the mixed treatment, the elimination of 2,3,4,7,8-PeCDF did not reach steady state even after 180 days of exposure. This indicates slower elimination of this congener in the mixture treatment.

The mean rates of elimination through scat at steady state (180 days) were found to be 5.8% and 4.1% for

2,3,4,7,8-PeCDF and 2,3,7,8-TCDF, respectively. This indicates that mink effectively absorbed most of the ingested 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF (94% and 96%, respectively) from the diet and relatively little was eliminated through scat. Based on the bioaccumulation of 2,3,4,7,8-PeCDF at 11% and of 2,3,7,8-TCDF at 0.1% (Table 4) and the similar elimination of both compounds at 94% and 96% (Fig. 1), both congeners were catabolized significantly by the mink, although 2,3,7,8-TCDF was catabolized more rapidly than 2,3,4,7,8-PeCDF. Additional quantification for the catabolized products of PCDFs would help to address the different characteristics of 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF in terms of assimilation/elimination process. Overall, the fact that there was relatively little variation in accumulation (in tissue) and elimination (in scat) regardless of dose supports the conclusion that concentrations of PCDFs in the tissues and diet can be predicted from concentrations measured in scat.



**Fig. 2** Concentrations of 2,3,4,7,8-PeCDF in (A) adipose and (B) liver tissue relative to those in scat

### Predictive Relationships

In order to develop predictive relationships, concentrations of 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF in adipose and liver tissue were compared to concentrations in scat for all treatments at 90 and 180 days. No statistically significant differences (ANOVA;  $p < 0.05$ ) were found between single-congener (mid-dose treatment) and mixture data, or among single-congener treatments at 90 or 180 days, so these data were combined for final regression analyses.

Concentrations of 2,3,4,7,8-PeCDF in adipose or liver were accurately predicted from concentrations in scat, described by Eqs. 1 and 2 (Fig. 2):

$$\log [\text{PeCDF}_{\text{adipose}}] = 1.34 \log [\text{PeCDF}_{\text{scat}}] + 1.16 \quad (r^2 = 0.95, p < 0.01) \quad (1)$$

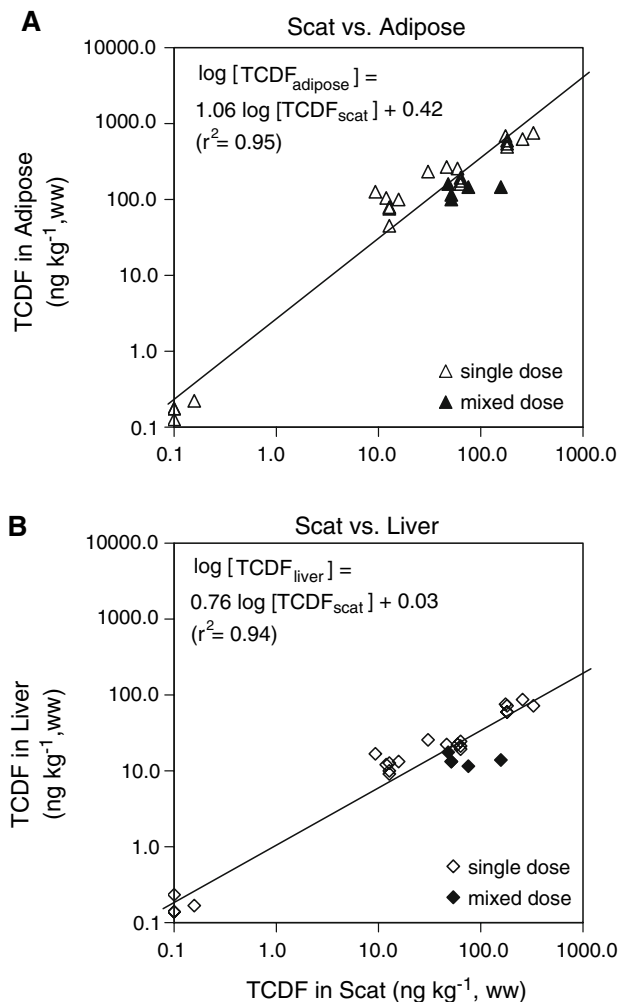
$$\log [\text{PeCDF}_{\text{liver}}] = 1.56 \log [\text{PeCDF}_{\text{scat}}] + 1.09 \quad (r^2 = 0.97, p < 0.01) \quad (2)$$

Concentrations of 2,3,7,8-TCDF in adipose or liver were accurately predicted from concentrations in scat, described by Eqs. 3 and 4 (Fig. 3):

$$\log [\text{TCDF}_{\text{adipose}}] = 1.06 \log [\text{TCDF}_{\text{scat}}] + 0.42 \quad (r^2 = 0.95, p < 0.01) \quad (3)$$

$$\log [\text{TCDF}_{\text{liver}}] = 0.76 \log [\text{TCDF}_{\text{scat}}] + 0.03 \quad (r^2 = 0.94, p < 0.01) \quad (4)$$

Overall, the strong log-log relationship between concentrations of PCDFs in tissue and scat after 90 or 180 days of exposure suggested the proportional accumulation of PCDFs in mink, followed by elimination through scat, in a dose-dependent manner. These results strongly support the potential use of scat concentrations to estimate and predict



**Fig. 3** Concentrations of 2,3,7,8-TCDF in (A) adipose and (B) liver tissue relative to those in scat

the body concentrations of chronically exposed mink to PCDFs and related dioxin-like compounds. Potential use of scat sampling for exposure assessment of mink would be a good addition to current research efforts to develop non-destructive biomonitoring schemes such as use of body fluids (Timchalk et al. 2007) and fecal matter (van den Brink and Jansman 2006; Gutleb and Kranz 1998).

## Conclusion

Concentrations of 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF in scat and mink tissues reached an apparent steady state in a relatively short exposure period. Bioaccumulation factors for single congeners and mixtures were in agreement with estimates derived from observations of wild mink in the Tittabawassee River. The assimilation efficiency was found to be 94% and 96% from scat data for 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF, respectively. There are strong log-log

correlations between concentrations of 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF in mink liver or adipose and scat. Overall, the results of these laboratory studies suggest that under circumstances of consistent long-term exposure, where a steady-state relationship can be assumed, scat can be a useful tool to predict the exposure of mink to PCDFs.

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