RANGE FINDING STUDY WITH ECOLOGICALLY RELEVANT DIETARY CONCENTRATIONS OF 2,3,7,8-TCDF AND 2,3,4,7,8-PeCDF IN MINK (*MUSTELA VISON*)

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Abstract
Wild mink (*Mustela vison*) living along the Tittabawassee River floodplain in central Michigan exhibit elevated hepatic 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) toxicity equivalents (TEQ) exceeding mink-specific Toxicity Reference Values (TRVs). These TRV values have been derived from studies of TCDD or complex mixtures of polychlorinated dibenzodioxins and furans (PCDDs and PCDFs) and polychlorinated biphenyls (PCBs) and do not reflect the potential toxicity from exposure where two different 2,3,7,8-chlorinated dibenzofuran congeners predominate in the soil, sediment, and food web. Despite tissue TEQ concentrations exceeding these TRV values, no apparent dioxin-related effects on population health have been observed. In order to conduct future experimental studies on ranch mink, a range-finding study was conducted to assess: 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; and 3) the impact of co-administration of 2,3,7,8-tetrachlorodibenzofuran (TCDF) and 2,3,4,7,8-pentachlorodibenzofuran (4-PeCDF) on the pharmacokinetics and distribution of each. In addition, the animals were evaluated for signs of toxicity. The elimination of TCDF and 4-PeCDF was more rapid than in rats, and both congeners appeared to be less potent than predicted based on the TEQ-based TRVs for sensitive endpoints in mink.

Methods
Fifty first-year natural dark female mink were randomly assigned to one of six single congener TCDF or 4-PeCDF treatment groups, or the mixture treatment group (Table 1). Each morning, 25 grams of spiked feed was placed on the cage of each animal with additional "clean" feed given to each animal later in the day so that approximately 120-125 grams of total feed was consumed. TCDF and 4-PeCDF concentrations in adipose and hepatic tissues (measured with Hr-GC/MS) along with hepatic CYP1A1 and 1A2 activities were measured at 0, 90, and 180 days. Three animals from each dose group were killed on day 90 or 180. Control animals were killed on day 0 or 180 and measured for background levels of PCDDs, PCDFs and PCBs Gross and histopathological examinations were done at the end of 180 days. Scat samples were collected on days 2, 23, 45, 90 and 180. All TEQ values were derived with 2005 WHO TEF values [1].

Results
Both compounds approached steady-state concentrations in the tissues of the tested animals in less than 90 days, although TCDF tissue concentrations increased slightly between day 90 and 180. Whole body half-lives for TCDF elimination were <1 day and decreased with increasing dose, while those for 4-PeCDF were between 7 and 11 days (depending on assumptions regarding oral absorption efficiency) with no clear dose-dependency in the tested dose range. Co-administration of TCDF with 4-PeCDF resulted in a doubling of the clearance rate for TCDF compared to administration of TCDF alone at the same dose, but clearance of 4-PeCDF was not affected by co-administration with TCDF. Bioaccumulation factors relating liver to dietary concentrations for the two test compounds are presented in Table 2. TCDF did not demonstrate any potential to bioaccumulate, with BAFs far below 1 at all tested doses.

Table 1: Study design.

Treatment	n[a]	Spiked feed concentration ng kg^{-1} ww (SD)	Daily dose (ng TEQ d^{-1})[b]
Controls	8		
2,3,4,7,8-PeCDF			
Low	6	110 (3.0)	0.81
Mid	6	390 (22)	2.9
High	6	1600 (20)	12
2,3,7,8-TCDF			
Low	6	500 (17)	1.2
Mid	6	2000 (140)	4.9
High	6	9700 (290)	24
Mixture			
2,3,4,7,8-PeCDF	6	490 (17)	3.7
2,3,7,8-TCDF		2200 (78)	5.5

[a] Total number of animals per dose group.
[b] Average bodyweights ranged from approximately 1100 to 1300 g.

Table 2: Estimated bioaccumulation factors (BAF) relating liver to dietary concentrations. BAF values assume total feed consumption of 125 g per day (25 g spiked feed and 100 g clean feed).

Treatment	2,3,4,7,8-PeCDF	2,3,7,8-TCDF
Low	9.5	0.14
Mid	12	0.060
High	17	0.041
Mixture	12	0.032

TCDF increased EROD activity over time and was directly proportional to concentrations in the liver. Induction of both EROD and MROD enzyme activities appeared to have been both dose and time dependent. EROD induction due to exposure to 4-PeCDF was dose-dependent after 90 days of exposure, but dose-independent after 180 days of exposure. 4-PeCDF-induced MROD activity appeared to be largely dose-independent at all but the lowest dose when mink were exposed for 90 days.

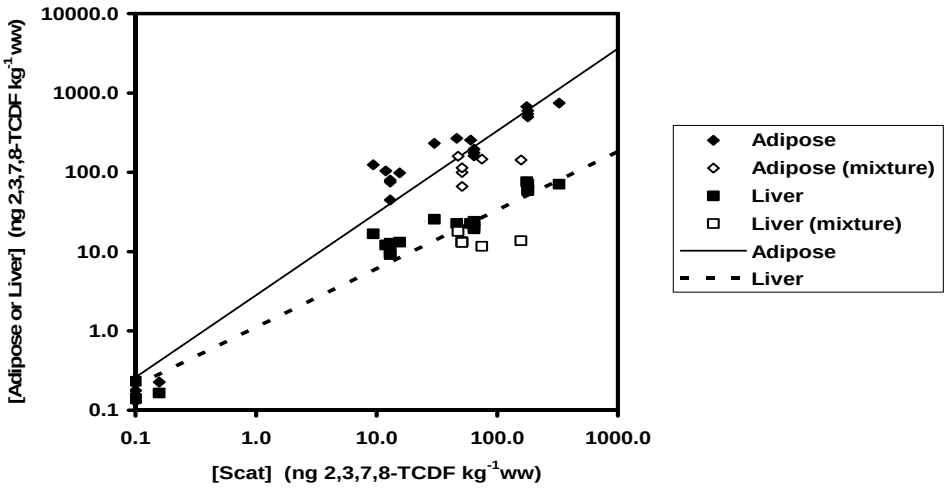
Scat concentrations closely correlated with adipose and hepatic tissue concentrations for both compounds (Figures 1a and b) and could be used as a non-destructive method to monitor internal exposures during chronic studies.

No statistically significant ($p<0.05$) dose-related 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. This suggests that the treatment doses did not adversely affect nutritional status or organ weights. One mink (mixture, 90 day) was in poor condition due to chronic kidney infection and was not included in this analysis. All of the mink exhibited "good" to "very good" nutritional status with no external lesions or abnormalities, and no reported conditions concerning pleural surfaces, lungs, gastrointestinal tract, heart, or abdominal cavity.

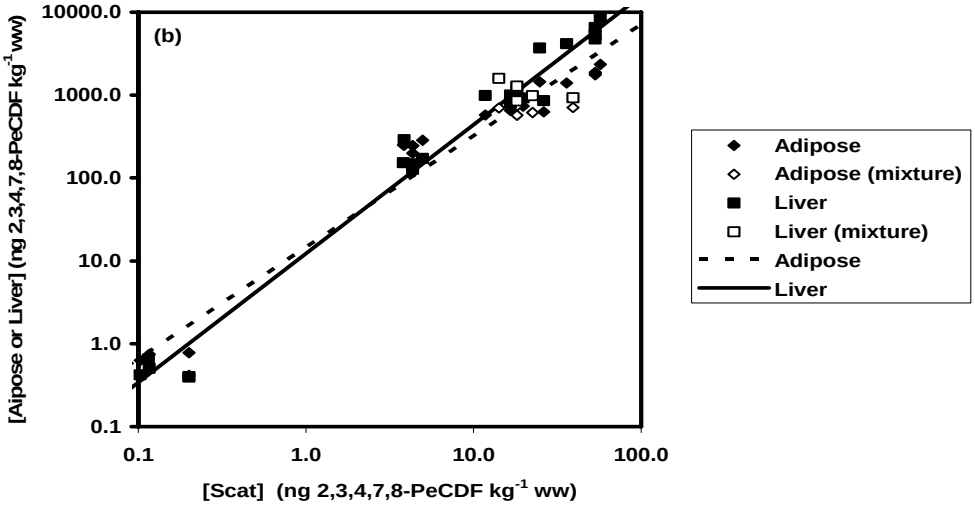
Proliferation of both mandibular and maxillary squamous epithelial cells are indicators of exposure to some dioxin-like compounds because proliferation occurs at a dose less than those expected to cause adverse population-level ecological effects. Thus, jaw lesions are considered biomarkers of exposure rather than indicators of ecologically relevant effects.[2-4] None of the mink in this study exhibited gross oral lesions involving the gingiva and all of the mink had straight white teeth. Three mink (one control mink, 180 day, and two high dose 4-PeCDF mink) had identifiable jaw lesions when evaluated histologically. For the control mink, gingivitis and gum recession were determined to be the cause of inflammation and bone degradation, leading to apparent periodontitis. As this animal was a control, and the lesions did not indicate a pre-neoplastic process,

the periodontitis was not treatment related or induced by chemical exposure. The latter two mink were both in the high 4-PeCDF treatment group. For one of the 4-PeCDF high dose mink, there was evidence of mild squamous epithelial proliferation in the mandible. No loss of teeth was observed, and thus the effect was rated as mild. There did not appear to be any indication of pre-neoplastic processes occurring in any other organs or systems that were reviewed histologically. For the other 4-PeCDF high dose mink that exhibited a jaw lesion, it was determined that no pre-neoplastic tissue was present within the jaw, and therefore, there was no indication of pre-neoplastic processes occurring within the jaw tissue or any of the organs or systems that were reviewed histologically. The overall health and condition of this mink, but for the slight squamous epithelial proliferation of the mandible, was therefore considered to be normal and bone tissue deterioration not due to exposure to 4-PeCDF.

2,3,7,8-TCDF Tissue Concentrations vs Scat Concentrations



2,3,4,7,8-PeCDF Tissue Concentrations vs Scat Concentrations



Figures 1a and b: Relationship between measured concentrations of TCDF and 4-PeCDF in mink scat to liver and adipose tissue concentrations.

Discussion

Pharmacokinetic information gained from this study will be used to set dietary exposure for future mink toxicity studies. Mink exhibited faster clearance than observed in rats, especially for 4-PeCDF. [5] Another finding, consistent with the known CYP1A1-mediated metabolism of TCDF, was the accelerated clearance of TCDF in the mixture group where 4-PeCDF induction of CYP1A1 was a factor. [6] Hence, on an administered dosage basis the current TEF value for TCDF may over-estimate the potential for AH receptor effects in wild mink since TCDF exhibits very rapid clearance, particularly in the presence of other dioxin or furan compounds that induce CYP1A1. Scat-to-tissue concentration relationships observed here may allow for an accurate surrogate for assessing internal mink exposures to these congeners without the need to capture and destroy wild mink for ecological assessment. Finally, the toxicity data suggest that mink are not as sensitive to TCDF and 4-PeCDF as they are to TCDD on an administered TEQ dose basis,[7] and that the current TRVs for dioxin-like effects will overestimate the risk to mink exposed to these two congeners in the wild.

Acknowledgements

This study was funded and supported by The Dow Chemical Company.

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