EFFECTS OF POLYCHLORINATED DIBENZOFURANS ON MINK

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Introduction

Mink are the preferred receptor species in ecological risk assessments where polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs) and other dioxin-like compounds are the contaminants of concern (COC). This is because mink, as apical carnivores, consume a great amount of food relative to their body mass, and are among the mammals that are more sensitive to aryl hydrocarbon receptor (AhR)-mediated effects. As such, mink are often predicted to have the greatest potential for adverse effects in multi-species risk calculations for sites with a substantial aquatic habitat[1]. Thus, remedial criteria are often derived for mink in situations where risks are predicted to occur due to AhR-active compounds[2]. Hence it is important that exposure concentrations at which adverse effects are predicted to occur be as accurate as possible to appropriately protect wildlife from adverse effects due to chemical exposure but also to protect from habitat destruction due to remediation based on misunderstanding of critical effect concentrations.

To simplify the risk assessment process, it has been assumed that the effects mediated through the AhR are the critical responses and that the relative potency of each congener in a mixture can be determined. Toxic equivalency factors (TEFs) provide the framework for potency normalization whereby the concentration of one or more AhR-active compound can be multiplied by the appropriate TEF and then added to describe the sum toxicity of an environmental mixture in terms of total 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalent (TEQ)[3-5]. TEF values have been derived specifically to be protective estimates of toxicity. If TEFs are accurate, and assumptions of additive effects are met, then the normalized dioxin-like potency (TEQ) should predict the toxic effects of AhR-active compounds regardless of origin (individual congener or environmental mixture).

Considerable toxicological information is available on the effects of PCBs and PCDDs on mink, but limited toxicological information is available for PCDFs. This report compares the toxic effects reported for laboratory and field studies with both mixed and single congener exposures and demonstrates that exposure concentrations at which adverse effects occur cannot be determined reliably for complex mixtures in which PCDFs dominate the total calculated TEQ values, thereby suggesting that the values of the mammalian-specific TEFs suggested by the WHO may overestimate the toxic potency of PCDFs to mink.

Materials and methods

The three primary studies discussed herein include a three-year field study during which indicators of individual health including hematological and morphological parameters were determined for mink chronically exposed to a mixture of PCDFs and PCDDs under field conditions, a laboratory chronic exposure study in which mink were exposed to 2,3,7,8-tetrachlorodibenzofuran (2,3,7,8-TCDF) through diet, and a laboratory evaluation of the toxicokinetics of 2,3,7,8-TCDF and 2,3,4,7,8-pentachlorodibenzofuran (4-PeCDF).

Tittabawassee River Field Study[6]

Forty-eight wild mink, 22 from the study area and 26 from reference areas, were collected throughout the Tittabawassee River, Midland, Michigan, USA drainage basin during the winters of 2003-2005. Gross and histological examinations were made. Concentrations of seventeen individual 2,3,7,8-substituted PCDF and

PCDD congeners and twelve individual PCB congeners were measured in the dietary items and livers of mink collected from the Tittabawassee River. Concentrations of TCDD equivalents ($TEQ_{2006\text{-}WHO\text{-}mammal}$) were calculated as the sum of the products of the concentrations of congeners multiplied by their respective $TEF_{2006\text{-}WHO\text{-}mammal}$ given by the World Health Organization (WHO)[3]. Estimates of the daily dose were created from site-specific dietary composition and measured dietary item contaminant concentrations.

Laboratory chronic exposure to 2,3,7,8-TCDF[7]

This laboratory study was designed to determine the toxic effects threshold for mink exposed to 2,3,7,8-TCDF through the diet. Methods were previously established for determining the effects of chemicals on mink by evaluating ecologically relevant parameters of survival, health, and reproduction[8]. Adults and kits were examined for sub-lethal effects including kit growth, organ masses, and tissue histology. Thirty randomly selected 10-m old adult (P_0) pastel female mink were fed diets containing 0.0 (Control), $2.4x10^2$, or $2.4x10^3$ ng 2,3,7,8-TCDF/kg feed on a wet-weight (ww) basis (0, 26, and 240 ng TEQ/kg, respectively) [9]. Dietary exposure was started 3 wk prior to the initiation of breeding. Necropsies were conducted on all P_0 and a randomly selected subset of F_1 mink. Jaws were examined histologically for the presence of squamous epithelial cell proliferation as described in Beckett et al.[10].

Laboratory toxicokinetic evaluation of 2,3,7,8-TCDF and 4-PeCDF[11]

A controlled laboratory feeding study was performed to determine the toxicokinetics of 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF using mink as a mammalian model. Mink were exposed to three concentrations each of the compounds and to a binary mixture of the two congeners through the diet (Table 1). Three animals from each of the 2,3,7,8-TCDF and 4-PeCDF and the 2,3,7,8-TCDF/4-PeCDF mixture dose groups were sampled on day 90 and 180. Livers were removed, weighed, and preserved for analysis for 2,3,7,8-TCDF and 4-PeCDF. Adipose tissue and scat was also collected for quantification of TCDF and 4-PeCDF. Additional data collected during the course of this study included gross observations, histological examination of select tissues, measurement of CYP1A1 and CYP1A2 enzyme activities.

Results and discussion

A mink hazard assessment based on concentrations of furans, dioxins, and PCBs in site-specific dietary items from the Tittabawassee River, and toxicity reference values (TRVs) derived from mixtures of other Ah-R active compounds resulted in values of hazard quotients (HQ) that were greater than 1.0, which suggested potential adverse effects for mink[6]. However, there were no statistically significant differences in any of the measured parameters between mink exposed to a median estimated dietary dose of 31 ng $TEQ_{2006\text{-}WHO\text{-}mammal}$ /kg ww, and mink from an upstream reference area where they had a median dietary exposure of 0.68 ng $TEQ_{2006\text{-}WHO\text{-}mammal}$/kg ww. Surveys of the conditions of individual mink, and the mink population, including track surveys, trapping and age distributions and sex ratios indicated that the mink population was not being adversely impacted. The contributing compounds to the Tittabawassee River mink dietary exposure of 31 ng $TEQ_{2006\text{-}WHO\text{-}mammal}$/kg, ww included 75% due to PCDFs with a majority of that originating from TCDF (31%) and 4-PeCDF (37%)[6]. Similarly, chronic exposure of mink to TCDF concentrations as great as $2.4x10^3$ng TCDF/kg ww feed ($2.4x10^2$ ng$TEQ_{2006\text{-}WHO\text{-}mammal}$/kg ww feed) exhibited transient decreases in body masses of kits relative to the controls as the only statistically significant effect observed.

In both studies, concentrations of $TEQ_{2006\text{-}WHO\text{-}mammal}$ to which the mink were exposed exceeded those at which adverse effects, based on studies with PCDD or PCB congeners, would have been expected. Yet in both instances where PCDF congeners were the sole or predominant source of the $TEQ_{2006\text{-}WHO\text{-}mammal}$, predicted adverse effects were not observed. The reason for this is unknown, however, the results of the laboratory study of the toxicokinetics of 2,3,7,8-TCDF and 4-PeCDF in mink demonstrated that 2,3,7,8-TCDF is quickly metabolized relative to TCDD and 4-PeCDF[11](Table 1). Thus, the apparent discrepancy between predicted and observed relative potency for 2,3,7,8-TCDF and mixtures containing 2,3,7,8-TCDF as compared to TCDD- and PCB 126-containing mixtures may be in part due to dissimilar metabolic transformation and elimination.

Part of the reason for a relatively wide range of values for $TRV_{TEQ\text{-}mink}$, some of which would predict effects that

were not observed in the field study, are uncertainties associated with the relative potencies of individual components which would differentially affect mixtures of varying composition. The uncertainty associated with the utilization of a $TRV_{TEQ\text{-}mink}$ based on exposure to dissimilar compounds, although each is AhR active, can be highlighted by comparing dose responses for similar measurement endpoints across studies where different congeners or AhR-active mixtures were utilized. The most direct comparison of relative potencies of two AhR-active congeners relevant to the field study can be made by comparing the data collected from the 2,3,7,8-TCDF study reported herein to a parallel study, conducted at the same facility (MSU Experimental Fur Farm) and using the same methodologies, of 3,3',4,4',5-pentachlorobiphenyl (PCB 126)[9]. Mink were exposed to concentrations of PCB 126 that were equivalent on both a mass or $TEQ_{2006\text{-}WHO\text{-}mammal}$/kg feed basis to the dietary concentrations used in the 2,3,7,8-TCDF laboratory study (0, 26 or $2.4x10^2$ ng $TEQ_{2006\text{-}WHO\text{-}mammal}$/kg ww). Exposure of adult, female mink to $2.4x10^2$ ng $TEQ_{2006\text{-}WHO\text{-}mammal}$/kg ww feed of PCB 126 resulted in complete reproductive failure. However, when adult female mink were exposed to the same dose ($2.4\ x10^2$ ng $TEQ_{2006\text{-}WHO\text{-}mammal}$/kg ww) of 2,3,7,8-TCDF they had a whelping rate (80%), which was not different from that of the controls ($p<0.5$). Furthermore for the lesser dietary concentration ($2.4\ x10^1$ ng $TEQ_{2006\text{-}WHO\text{-}mammal}$/kg ww feed), kits in the PCB126 study displayed an 80% incidence of mandibular and maxillary squamous epithelial cell proliferation or jaw lesions (K. Beckett) while no jaw lesions were identified in the 2,3,7,8-TCDF study even at a 10-fold greater exposure[10]. These comparisons demonstrate that there is a difference between the toxic potency for these two compounds for both reproductive and the more sensitive jaw lesion endpoints in mink.

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

Taken together, the results of these studies suggest that the values of the mammalian-specific TEFs suggested by the WHO overestimate the toxic potency of PCDFs to mink. Therefore, hazard cannot be accurately predicted by making comparisons to TRVs derived from exposure studies conducted with PCBs or PCDDs in situations where mink are exposed to TEQ mixtures dominated by PCDFs.

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Table 1. Estimated average first-order elimination rate constants, based on data from both 90- and 180-d time points, for 2,3,7,8-TCDF and 4-PeCDF by dose group. N=6 except where noted.

Daily dose TEQ (ng kg^{-1} d^{-1})	First order rate constant, d^{-1} Mean (S.D.)	Estimated half-life, d Mean
2,3,7,8-TCDF		
0.98	1.6 (0.6)	0.43
3.8	2.6 (0.7)	0.27
20	4.1 (0.6)	0.17
Mixture: 4.1 TCDF and 2.8 PeCDF (n=5)	4.3 (0.7)	0.16
2,3,4,7,8-PeCDF		
0.62	0.086 (0.012)	8.1
2.2	0.095 (0.008)	7.3
9.5	0.087 (0.019)	8.0
Mixture: 2.8 PeCDF and 4.1 TCDF (n=5)	0.094 (0.008)	7.4

Table 2. Effect levels for mink dietary exposure to dioxin-like compounds in ng $TEQ_{2006\text{-}WHO\text{-}mammal}$/kg diet, ww.

Study	Sum TEQs	PCB 126 TEQs	2,3,7,8 TCDF TEQs	% Jaw lesions	Dioxin TEQs	Furan TEQs	Non-ortho PCB TEQs
Housatonic River fish lab study[12]	50	41	0.3	100% (6/6)	0.9	1.9	44
PCB 126 lab study[9]	24	24	0	80% (12/15)	0	0	24
Saginaw River fish lab study[13]	57	19	2.1	75% (6/8)	20	14	20
Saginaw River fish lab study[13]	36	11	0.9	57% (4/7)	14	8.8	12
Housatonic River fish lab study[12]	12	9.8	0.1	33% (2/6)	0.3	0.5	10
Saginaw River fish lab study[13]	22	7.2	0.7	0% (0/8)	11	3.0	7.2
Housatonic River fish lab study[12]	6.8	5.4	0.1	17% (1/6)	0.3	0.3	5.8
Housatonic River fish lab study[12]	4.3	3.2	0.1	0% (0/6)	0.3	0.3	3.4
Tittabawassee River wild mink[6]	31	2.4	8.8	0% (0/22)	4.4	22	2.5
2,3,7,8-TCDF lab study[7]	240	0	240	0% (0/8)	0	240	0