Depletion of Selected Polychlorodibenzodioxins and Polychlorodibenzofurans in Farmed Trout Exposed to Contaminated Feeds

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Introduction

Farmed fish can bioaccumulate persistent toxic substances when fed on animal-based fat feeds.[1] This fact has recently prompted a re-evaluation of the overall toxicological risk associated with contamination levels recorded in farmed *vs.* wild salmons.[2] The bioaccumulation of polychlorinated dibenzodioxins (PCDDs) and dibenzofurans (PCDFs) in farmed trout has recently been described;[3,4] nevertheless, poor information is available about their depletion under controlled conditions. In this paper, the results of a 90-day depletion study in groups of trout exposed to three different levels of feed contamination for 30 days are reported. As a follow-up of a PCB depletion study,[5,6] the present paper aims at giving indications for risk management in fish farming practices, to prevent an unacceptable contamination of the produce intended for human consumption.

Materials and methods

Fish treatment: an all-vegetal fish feed formula — to lower PCDD and PCDF contribution from animal constituents — was designed, prepared, and contaminated with vegetal oil containing selected PCDD and PCDF congeners at the levels (C_F) reported in Table 1.[5]

Trout of 70-g body weight were grouped in four tanks (200 fish each), fed on the blank feed for 30 days, and then exposed for 30 more days to the aforesaid low, medium, and high contamination feeds. During the following 90-day clearance period, all groups were fed again on the blank feed. 10-Fish samples were collected on Days 0, 15, 30, 45, 60, 75, and 90 from each pool. Water and light conditions were under control; growth rate, feed intake, and animal welfare were monitored.[6]

Analysis: *solvents and chemicals were high quality grade, suitable for residue analysis, as assayed in the laboratory. All laboratory glassware, tools, and utensils were checked for analytical integrity. Natural and* ^{13}*C-labeled PCDD and PCDF congener standards were certified.*

Several feed samples were analyzed as previously described.[5] Fish muscle (20 g) was omogenized and combined with excess anhydrous Na_2SO_4. During homogenization, the ^{13}C-labeled congeners were added as internal standards for congener-specific measurements with high

resolution gas chromatography coupled with high resolution mass spectrometry (HRGC-HRMS), the latter used in the single ion monitoring (SIM) mode.

Table 1. PCDD and PCDF congener levels analytically determined in all-vegetal trout feeds used for the chemobiokinetic study. Fortification levels (C_F) are identified as low, medium, and high; unfortified feed was utilized as a blank. C_F and SD values are expressed in pg/g, whole weight.

Congeners	*Low*	*Medium*	*High*	*Blank*
2,3,7,8-T_4CDD	0.81 ± 0.13	2.24 ± 0.25	6.34 ± 0.51	<0.07
1,2,3,7,8-P_5CDD	2.19 ± 0.24	6.24 ± 0.50	19.7 ± 1.2	<0.2
O_8CDD	1.75 ± 0.21	6.51 ± 0.59	16.5 ± 1.2	1.10
2,3,7,8-T_4CDF	1.21 ± 0.13	4.14 ± 0.41	13.2 ± 1.1	<0.06
1,2,3,7,8-P_5CDF	1.70 ± 0.19	4.91 ± 0.49	13.8 ± 1.1	<0.07
O_8CDF	1.70 ± 0.20	4.95 ± 0.59	13.3 ± 1.2	≈0.2

Extraction was mostly performed with a Soxhlet apparatus. Extracts were purified with chromatographic filtrations through a column of Extrelut impregnated with 96% H_2SO_4 followed by an automated cleanup with a Power-Prep™ unit: in this unit, three sequential chromatographic steps take place on columns packed with silica gel, alumina, and carbon. Final results were corrected for recovery and estimated according to the medium bound approach. Mean estimated uncertainty on single measurements, $\approx \left| \pm 10\ \% \right|$.

Empirical chemobiokinetic modelling: a one-compartment first-order kinetic model was adopted to describe the diminishing trends of the PCDD and PCDF congeners in fish muscle.[6] The canonical *Eqn. 1*, $C = C_0\ exp(-k\ t)$, was used to fit the data as a first approach. The diminishing trends previously observed for PCBs were assumed to depend on the combined effect of clearance and dilution, the latter associated with body mass increase.[6] Therefore, *Eqn. 1* was modified into *Eqn. 2*, $C = C_0\ [exp(-k\ t)\ (m\ t + 1)^{-1}]$, to distinguish between clearance ($exp(-k\ t)$) and dilution ($(m\ t + 1)^{-1}$) contributions to contaminant diminishing. *Eqn. 2* presumes that the lipid fraction, housing the highly lipophilic contaminants dealt with, maintain a constant ratio with fish weight.

The dilution function was determined separately by fitting the weight variation over time with linear *Eqn. 3*, $W = W_0\ (m\ t + 1)$, that yielded highly significant regressions for all groups of fish.[6] The mean estimates of m for the low, medium, and high PCDD and PCDF fortification levels were respectively 0.393, 0.370, and 0.418 $month^{-1}$.

Results and discussion

Self-explanatory Tables 2 and 3 summarize the mean estimates of C_0 and k obtained by fitting *Eqns. 1* and *2*: as already seen in the PCB study,[6] for a given data set the patterns of the two regression curves are substantially undistinguishable (in all cases: $R_1^2 \approx R_2^2$ and $P_R < 0.01$, where R is the correlation coefficient). Figure 1 shows *Eqn. 2* regression curves against each pertinent seven-data (mean values): in the pictures, the paired clearance functions are the upper curves. Bioaccumulation ($C_0 \times C_F^{-1}$) appears to be maximum for 2,3,7,8-T_4CDF, intermediate for 2,3,7,8-T_4CDD, and minimum for 1,2,3,7,8-P_5CDD and -P_5CDF. In addition, for a given congener,

bioaccumulation decreases with increasing concentration in feed. No significant bioaccumulation was observed for O_8CDD and O_8CDF, as expected.[7]

Fittings appear to be good for all exposure groups (in all cases: $F_{2,5} \geq 111$ and $P_F < 0.001$), although a visible point scattering is present in some of the exposure groups: an influence of the small number (five) of specimens analyzed per time point cannot be excluded.[6]

Table 2. PCDD and PCDF depletion kinetics in farmed trout muscle. C_0, k, and their standard errors were estimated from regressions with empirical model $C = C_0\ exp(-k\ t)$ (*Eqn. 1*). Regressions, carried out on results from 35-(*t*, C)-sample sets, were highly significant.

Congener level	***2,3,7,8-T_4CDD***	***1,2,3,7,8-P_5CDD***	***2,3,7,8-T_4CDF***	***1,2,3,7,8-P_5CDF***
Estimates of initial congener concentration C_0 (pg g^{-1}, lipid base)				
Low	1.38 ± 0.06	2.82 ± 0.10	3.91 ± 0.15	2.13 ± 0.17
Medium	3.50 ± 0.31	7.35 ± 0.43	9.23 ± 0.40	4.48 ± 0.19
High	8.20 ± 0.29	17.7 ± 1.1	19.9 ± 1.5	10.8 ± 0.9
Estimates of time constant k ($month^{-1}$)				
Low	0.426 ± 0.036	0.335 ± 0.028	0.206 ± 0.026	0.411 ± 0.069
Medium	0.359 ± 0.073	0.403 ± 0.051	0.304 ± 0.034	0.446 ± 0.038
High	0.290 ± 0.027	0.446 ± 0.054	0.358 ± 0.061	0.447 ± 0.074

From *Eqn. 1* regression parameters (Table 2), the time points ("half-lives") when congener levels are halved in trout muscle can be estimated ($HL = ln(2)\ k^{-1}$): as for PCBs,[6] HLs seem to be fairly similar, on average spanning between 1.6 and 3.4 months. In agreement with the aforesaid comments on bioaccumulation, 2,3,7,8-T_4CDF in general exhibits the slowest diminishing.

The paired mean clearance HLs of the different congeners in trout muscle are estimated from *Eqn. 2* regression parameters (Table 3). They range between 3.9 and 11 months for 1,2,3,7,8-P_5CDD and -P_5CDF, whereas 2,3,7,8-T_4CDD and -T_4CDF clearance appears to be eventually slower when not absent (HL undefined for 2,3,7,8-T_4CDD at high exposure) or "reversed" (not a loss of 2,3,7,8-T_4CDF at low exposure, but a concentration increase): the meaning of these results requires further investigation and possibly the use of a more complex chemobiokinetic model.

Lastly, in the case of PCBs it was observed that, relative to dilution, clearance slowed down with increasing of exposure: it eventually became irrelevant in contributing to the diminishing trend.[6] No such a conclusion can be drawn for the PCDD and PCDF congeners investigated, whose clearance HLs do not show any evident regular patterns.

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Table 3. PCDD and PCDF depletion kinetics in farmed trout muscle. C_0, k, and their standard errors were estimated from regressions with empirical model $C = C_0 [exp(-k\,t)\,(m\,t + 1)^{-1}]$ (*Eqn. 2*), with the m values defined in text. Regressions, carried out on results from 35-(*t*, C)-sample sets, were highly significant.

Congener level	***2,3,7,8-T_4CDD***	***1,2,3,7,8-P_5CDD***	***2,3,7,8-T_4CDF***	***1,2,3,7,8-P_5CDF***
Estimates of initial congener concentration C_0 (pg g^{-1}, lipid base)				
Low	1.41 ± 0.05	2.90 ± 0.09	4.04 ± 0.09	2.18 ± 0.14
Medium	3.57 ± 0.33	7.49 ± 0.39	9.46 ± 0.38	4.57 ± 0.18
High	8.46 ± 0.22	18.1 ± 1.0	20.5 ± 1.2	11.1 ± 0.7
Estimates of time constant k ($month^{-1}$)				
Low	0.147 ± 0.029	0.061 ± 0.024	–0.062 ± 0.016	0.131 ± 0.056
Medium	0.096 ± 0.076	0.136 ± 0.045	0.042 ± 0.030	0.178 ± 0.035
High	0.006 ± 0.019	0.153 ± 0.050	0.070 ± 0.048	0.152 ± 0.060

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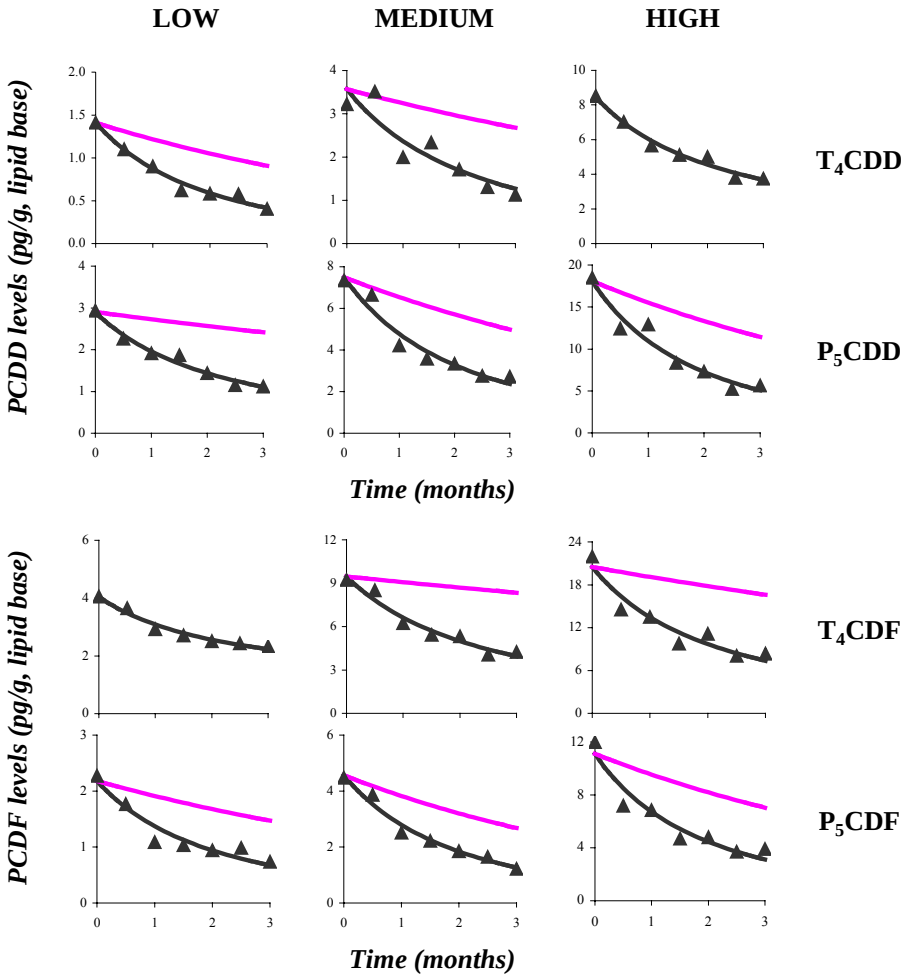

Figure 1. *Eqn. 1* or *2* non-linear regression curves of PCDD and PCDF diminishing with time in trout muscle are visible through the data points. In each box, the upper curve describes the clearance effect alone based on *Eqn. 2* (cfr. Table 3). Time points are the means of determinations on five-sample subsets. Measurements were corrected for interferences as accounted for by the control group (*Blank*).