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ACCUMULATION OF 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN BY RAINBOW TROUT (*ONCHORHYNCHUS MYKISS*) AT ENVIRONMENTALLY RELEVANT DIETARY CONCENTRATIONS

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Abstract—Rainbow trout were fed a diet containing 1.8, 18, or 90 pg/g ^{3}H-2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) for up to 320 d. Concentrations of TCDD were determined in muscle, liver, and ovaries at 100, 150, 200, and 250 d. Concentrations of TCDD reached an apparent steady-state concentration in liver after 100 d of exposure, whereas concentrations in other tissues continued to increase until 150 d of exposure. The greatest portion of the total mass of TCDD was present in the muscle tissue with lesser proportions in other organs. As the ovaries developed before spawning, an increase occurred in the total mass of TCDD present in this tissue. The assimilation rate of TCDD during the initial 100 d of the exposure was determined to be between 10 and 30%. This is somewhat less than estimates derived based on both uptake and elimination constants determined during shorter exposures. Biomagnification factors (BMFs) were estimated for all tissues and exposure concentrations, and at all exposure periods. Lipid-normalized BMFs for muscle ranged from 0.38 to 1.51, which is consistent with the value of 1.0 predicted from fugacity theory. Uptake and depuration rate constants were determined and used to predict individual organ TCDD concentrations. Comparison with observed values indicated that the model could be used to predict tissue concentrations from the known concentrations of TCDD in food. This model will allow more refined risk assessments by predicting TCDD concentrations in sensitive tissues such as developing eggs.

Keywords—Dioxins Fish Accumulation Kinetics Chronic exposure

INTRODUCTION

Fish are often exposed to relatively small concentrations of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and similar compounds but can accumulate these compounds to concentrations that can have adverse effects on the fish or on predators that consume them [1]. In ecological risk assessment, ability to predict tissue-specific concentrations of PCDDs and PCDFs (PCDD/Fs) that could be accumulated during long-term exposures is important. The primary vector for accumulation of PCDD/Fs by fish is through dietary intake [2–6]. Previous studies of PCDD/F accumulation in fish have used short-term exposures to relatively large doses. In addition, the typical routes of exposure in these studies were intraperitoneal injection [7,8], single-dose gavage, or inappropriately large concentrations in water [9]. Differences in exposure regimes between field and laboratory studies make the prediction of field effects from laboratory studies difficult. This is due to differences in toxicokinetics between high- and low-dose exposures and between short-term and chronic exposures. Finally, previous studies investigated accumulation of some specific dioxin congeners whose chemical and physical characteristics are similar to, in some respects, but also distinct from, the most toxic dioxin congeners [10,11].

Only a few studies are available on the dietary uptake of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in laboratory-exposed fish [12,13]. These experiments have typically used small or juvenile fish [12] or have used large initial doses to provide a loading phase to rapidly increase tissue concentrations. In some studies of smaller fish (<0.1 g), evidence exists that dietary uptake is of lesser importance than water-borne uptake [14], whereas the opposite seems to be true for larger cold-water species [3]. In addition, many of the studies do not address the long-term distribution of the chemicals in the fish but usually monitor the distribution after a few days or weeks [15]. Finally, understanding the tissue distribution of dioxinlike chemicals is also important because this can affect the elimination of the chemicals and the exposure of the developing young, which are the most sensitive life stages of the organism [16].

To reflect field conditions more accurately, a long-term dietary exposure to environmentally relevant concentrations of TCDD was conducted. In this paper we report the results of a study that measured the accumulation of TCDD by adult rainbow trout exposed for 320 d under laboratory conditions. Data are presented on the accumulation, disposition, and depuration of TCDD in rainbow trout. The biochemical, histopathologic, and reproductive effects measured in this study are discussed elsewhere [17; and in an article to be published in the future, J. Giesy et al., unpublished data].

MATERIALS AND METHODS

A detailed description of fish culture and exposure procedures is provided elsewhere [17]. Adult (age class II; 350 g), female rainbow trout (*Oncorhynchus mykiss*) of the spring-spawning Shasta strain were collected from rearing ponds at the Stoney Creek Trout Farm (Grant, MI, USA). Fish were

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Table 1. Concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and radiometric doses in food[a] (moist wt)

Dose group	Control	pg TCDD/g diet 1.8	18	90
TCDD in food (pg/g)	<0.2	5.4	54	270
Specific activity (dpm/pg TCDD)	NA	541	57.0	10.5
Radiometric dose (dpm/g food)	NA	974	1,026	949

[a] dpm = disintegrations per minute; NA = not applicable.

sorted by sex and held at the Michigan State University Aquaculture facility (East Lansing, MI, USA) until the study began in March 1991. Fish were acclimated for 60 d in the exposure tanks prior to the commencement of exposure.

Tritium-labeled TCDD was synthesized and purified at the Pesticide Research Center, Michigan State University. The radiochemical purity (>99.9%) and specific activity were confirmed by gas chromatography–mass spectrometry and liquid scintillation counting. Concentrations of TCDD in food were confirmed by gas chromatography–mass spectrometry (Table 1). Food was spiked with both ^{3}H-labeled TCDD and nonlabeled TCDD so that, although the TCDD dose varied, the total TCDD specific activity measured as disintegrations per minute per picogram of TCDD was also varied so that the radiometric dose remained constant. In this way fish were exposed to the same dose of radiation over the course of the study (Table 1). Control fish were not exposed to any form of ^{3}H. Calculated and observed doses of radioactivity were determined not to be detrimental to the health of the fish based on accepted exposure criteria [18].

Fish were exposed in 1,700-L flow-through tanks. The flow rate was 71.5 L/h, which resulted in approximately two turnovers of the water per day. Temperature was maintained at 12°C and photoperiod was adjusted weekly to match ambient conditions. Tanks were located in a negative-pressure facility with three levels of containment for water and one for air. Control fish were held in the same facility but in an adjacent room to prevent TCDD contamination between tanks. All groups of fish were maintained in the same water source.

Control and treated fish were fed Silver Cup Fish Feed (Murray Elevators, Murray, UT, USA) with or without tritium-labeled TCDD for up to 320 d. Food spiked with TCDD at 5.4, 54, or 270 pg TCDD/g was fed on every third day. This resulted in average dietary concentrations of 0, 1.8, 18, and 90 pg TCDD/g moist weight of food [17]. The quantity of food fed was adjusted throughout the experiment to maintain a constant ration of 1.5% of body weight per day.

The experiment was initiated with 35 females in each exposure group (three TCDD exposure concentrations and one control). Two to four fish from each exposure group were evaluated for clinical pathology alterations and gross lesions and tissue 2,3,7,8-TCDD concentrations after 100, 150, and 200 d of exposure. Fish were also collected at various time points during spawning from day 255 to day 320, and data from these fish were grouped together as a 250+-d treatment group. Fish were anesthetized by submersion in tricaine methane sulfonate (MS-222, Sigma Chemical, St. Louis, MO, USA). Blood was collected by venipuncture of the caudal vein and placed on ice. Anesthetized fish were killed by concussion followed by cervical spinal cord transection. Liver and ovaries were removed and weighed. Tissues were stored at −20°C until analysis. Lipid and TCDD were extracted from food and fish tissues using previously described methods [19]. Briefly, samples were homogenized with anhydrous sodium sulfate before organic solvent extraction. A fixed proportion of the extract was removed for gravimetric lipid determination and the remainder of the extract was used for ^{3}H-TCDD determination using liquid scintillation counting. Portions of selected extracts were analyzed by independent laboratories using high-resolution gas chromatography–mass spectrometry to confirm TCDD concentrations (results not shown).

RESULTS AND DISCUSSION

This is one of only a few studies that have investigated the dietary accumulation of a specific dioxin congener at realistic environmental concentrations over an extended time period. Other studies have shown that water can be a major pathway for accumulation of PCDD/F congeners [14]. However, these experiments were conducted with small (<0.1 g), warm-water fish. Mass balance studies, both in the laboratory and field, indicate that in larger predatory species, the primary vector of accumulation is via diet [2,3]. These observations make it necessary to investigate the accumulation and effects of PCDD/F congeners during long-term exposures at ecologically relevant concentrations. In our study, fish were kept under standard aquaculture conditions and were fed environmentally relevant concentrations of TCDD based on data obtained for forage fish from the North American Great Lakes [1,2,20]. Because no effect was found on growth or reproductive status of the fish in this study, the accumulation characteristics observed for these fish should be representative of those that would be expected to occur in the environment.

Fish health

Exposure to dietary TCDD caused some adverse effects in the trout. Detailed descriptions of the results of these effects are given elsewhere [17; J. Giesy, unpublished data]; however, because some of these results could affect interpretation, we present some of the effect results here. Some mortality was observed in all groups; however, mortality was least in the control group (1 of 35 fish). After 300 d of exposure, mortality in the 1.8-pg TCDD/g food exposure group was significantly greater than in the control group (4 of 35 fish). Mortality increased in the 18- and 90-pg TCDD/g food groups after 100 d, and reached 10 of 35 fish in each of these groups after 300 d. This indicates that the no-observed-effect concentration for mortality in adult rainbow trout is less than 1.8 pg TCDD/g wet weight in the diet. This is less than previously reported no-observed-effect concentrations based on shorter exposure periods or exposures based on waterborne or intraperitoneally administered doses [7,9,21]. No significant differences in growth were observed among the exposure groups (Table 2). This was partly due to the considerable variability observed in the growth of the fish, particularly at the longer exposure times.

Several physiologic parameters were monitored to evaluate the health of the fish, to ensure that adverse effects were not affecting accumulation, and to assess the breeding condition of the fish. Condition factor (the ratio of weight to length, [(length/weight^3)100]) was not significantly different among exposure groups or times. However, condition factor tended to be greater at longer exposure times (Table 2). Liver somatic index (liver weight as a proportion of total body weight) was not significantly different among exposure groups (Table 2), whereas ovary somatic index (ovary weight as a proportion of total body weight) was greater at longer exposure times.

Table 2. Fish weight and length, and organ weights (mean with standard deviation) after dietary exposure to ^{3}H-2,3,7,8-tetrachlorodibenzo-*p*-dioxin

Exposure (d)	Concn. (pg/g)	*n*	Weight (g)	Length (cm)	Liver weight (g)	Ovary weight (g)	Condition factor	Liver somatic index	Ovary somatic index
50	0	3	364 (48)	32.5 (1.9)	4.93 (0.34)	1.88 (0.69)	1.06 (0.12)	1.38 (0.25)	0.539 (0.24)
	1.8	4	428 (52)	33.6 (1.3)	5.38 (1.1)	2.83 (1.9)	1.12 (0.02)	1.25 (0.15)	0.667 (0.41)
	18	4	401 (168)	32.0 (4.1)	4.4 (1.1)	3.18 (1.8)	1.16 (0.08)	1.17 (0.28)	0.766 (0.32)
	90	4	411 (57)	32.5 (1.9)	5.83 (0.72)	2.08 (1.1)	1.20 (0.09)	1.43 (0.19)	0.535 (0.30)
100	0	4	505 (64)	36.1 (0.85)	6.08 (1.1)	5.15 (3.0)	1.07 (0.10)	1.20 (0.06)	1.01 (0.61)
	1.8	4	337 (99)	31.5 (2.9)	4.56 (1.1)	4.25 (3.5)	1.05 (0.12)	1.42 (0.40)	1.33 (0.97)
	18	4	402 (53)	32.9 (0.85)	5.28 (0.64)	7.23 (4.3)	1.13 (0.07)	1.32 (0.07)	1.86 (1.2)
	90	3	573 (169)	35.9 (2.7)	8.47 (3.4)	10.9 (7.0)	1.21 (0.1)	1.47 (0.50)	1.81 (0.73)
150	0	4	555 (109)	36.4 (2.7)	6.99 (1.8)	10.3 (7.4)	1.15 (0.06)	1.27 (0.34)	1.75 (1.01)
	1.8	4	468 (97)	33.3 (1.9)	6.79 (1.4)	17.4 (16)	1.26 (0.15)	1.46 (0.24)	3.55 (2.8)
	18	3	615 (175)	36.4 (3.6)	8.31 (2.7)	25.7 (12)	1.25 (0.10)	1.35 (0.29)	4.28 (1.8)
	90	4	639 (77)	37.3 (2.1)	9.25 (2.7)	21.1 (22)	1.23 (0.09)	1.49 (0.57)	3.60 (3.9)
200	0	2	759 (249)	38.8 (5.5)	11.1 (4.5)	69.5 (60)	1.29 (0.18)	1.37 (0.14)	10.9 (9.6)
	1.8	6	653 (205)	37.9 (3.6)	8.15 (3.0)	35.4 (32.4)	1.26 (0.096)	1.25 (0.17)	5.84 (6.1)
	18	3	809 (238)	40.3 (3.2)	10.1 (2.2)	1.3 (0.42)	1.21 (0.06)	1.27 (0.23)	0.10 (0.09)
	90	4	588 (103)	36.0 (2.3)	8.38 (3.0)	39.0 (32.4)	1.25 (0.03)	1.41 (0.35)	7.44 (6.6)

The ovary somatic index was unusually small in the 18-pg TCDD/g exposure group after 200 d of exposure. All three fish sampled from this group at this time exhibited essentially no ovarian development. Although this lack of development was observed in some fish from all exposure groups throughout the study, such a lack of development was never observed in 100% of the individuals sampled at any specific time or dose. The reason for this lack of ovarian development in certain individuals is unknown. Therefore, the small ovarian somatic index observed for the 18-pg/g exposure group after 200 d is a result of inconsistent ovarian development and small sample size.

Accumulation of TCDD

The TCDD was accumulated in the tissues of exposed trout in a dose-dependent manner (Table 3 and Fig. 1). Because uptake was determined by quantifying the accumulated ^{3}H, no data are available for nonexposed controls. Concentrations in the dorsal muscle from fish exposed to 1.8, 18, or 90 pg TCDD/g ranged from 0.2 to 0.5 pg/g, 1.4 to 4 pg/g, and 7 to 56 pg/g wet weight, respectively. Similar dose-dependent accumulation was observed in other tissues.

Concentrations of TCDD in liver seemed to reach a steady state after 50 to 100 d of exposure. Concentrations of TCDD seemed to reach a similar steady state in ovary and adipose tissue after 100 to 150 d of exposure. The TCDD concentrations in adipose tissue were the least variable, which would be expected from the lipophilic properties of TCDD. A uniform decrease in concentrations of TCDD in the liver of all exposure groups at 150 d of exposure was not associated with similar decreases in the concentrations of TCDD in ovary, adipose tissue, or muscle. Concentrations of TCDD on a wet-weight basis remained relatively constant from 100 d to more than 250+ d of exposure. Changes in organ weights over the same period resulted in an increase in the total mass of TCDD in the ovary during egg development, whereas the total mass of TCDD in the liver remained relatively constant (Table 3). Concentrations and total masses of TCDD in muscle were the most

Table 3. Concentrations (pg/g wet wt; mean with standard deviation) of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in various trout tissues after dietary exposure to ^{3}H-2,3,7,8-TCDD

Dietary exposure (d)	Concentration (pg/g)	Fillet lipid (%)	Adipose TCDD (pg/g)	Liver TCDD (pg/g)	Ovary TCDD (pg/g)	Fillet TCDD (pg/g)	Fillet TCDD (pg/g lipid)
50	0	2.54 (0.86)	—	—	—	—	—
	1.8	4.65 (1.27)	0.80 (0.35)	0.26 (0.06)	0.48 (0.16)	0.21 (0.08)	4.45
	18	6.66 (6.10)	8.73 (5.66)	1.65 (0.57)	4.3 (0.63)	1.45 (1.0)	2.17
	90	4.47 (2.34)	60.0 (17.0)	—	15.1 (5.02)	8.25 (3.5)	184.4
100	0	2.20 (0.98)	—	—	—	—	—
	1.8	5.26 (1.76)	1.75 (0.66)	0.31 (0.05)	0.65 (0.32)	0.39 (0.06)	7.47
	18	7.11 (0.75)	15.4 (6.54)	2.88 (0.72)	7.94 (1.6)	3.07 (0.64)	106.7
	90	6.95 (3.37)	84.4 (28.5)	12.9 (2.75)	36.8 (3.6)	21.8 (25.6)	313.8
150	0	3.04 (3.02)	—	—	—	—	—
	1.8	3.24 (1.19)	2.32 (1.06)	0.22 (0.08)	0.11 (0.06)	0.21 (0.08)	6.32
	18	5.43 (2.02)	17.3 (6.94)	1.72 (0.56)	7.9 (0.56)	2.69 (0.53)	49.4
	90	5.71 (1.36)	89.7 (21.3)	9.93 (0.52)	34.6 (2.1)	56.8 (29.0)	993.5
200	0	0.51 (0.07)	—	—	—	—	—
	1.8	3.08 (1.59)	1.99 (0.66)	0.29 (0.00)	1.15 (0.22)	0.44 (0.08)	14.1
	18	4.78 (3.82)	15.8 (9.22)	2.85 (0.64)	5.16 (—)	4.03 (1.1)	84.2
	90	1.95 (0.33)	92.1 (14.4)	16.2 (2.2)	47.1 (12.7)	7.64 (1.06)	392.4

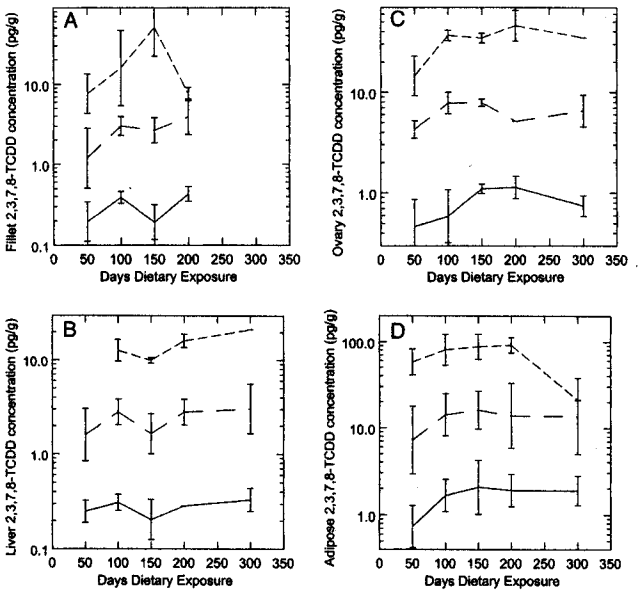

Fig. 1. Tissue concentrations (pg 2,3,7,8-tetrachlorodibenzo-*p*-dioxin [TCDD]/g wet weight) of TCDD in trout after exposure to ^{3}H-2,3,7,8-TCDD. Tissues analyzed were (**A**) fillet, (**B**) liver, (**C**) ovary, and (**D**) adipose tissue. Exposure concentrations were 1.8 pg TCDD/g wet weight food (solid line), 18 pg TCDD/g wet weight food (dashed line), and 90 pg TCDD/g wet weight food (dotted line).

variable of the tissues studied. This is, in part, due to changes in the lipid content of the muscle (Table 3). Concentrations of TCDD were relatively stable in high lipid content tissue (adipose). The TCDD concentrations were more variable in tissues such as muscle, which would be expected to demonstrate more variable lipid content. The greatest total mass of TCDD was present in the muscle. This is due to the relatively great amount of tissue present as muscle despite the relatively low lipid concentration of the tissue. Only in the later stage of the exposure, when the ovaries were becoming fully developed, was a significant amount of the body burden found in the ovaries.

The data obtained for the concentrations of TCDD in various tissues were used to determine rate constants for the accumulation and depuration of TCDD. The change in the concentration of TCDD in the fish is

$$\frac{dc_{\text{fish}}}{dt} = k_1 \cdot c_{\text{food}} - k_2 \cdot c_{\text{fish}} \quad (1)$$

where k_1 and k_2 are the uptake and depuration rate constants, respectively; and c_{food} and c_{fish} are the TCDD concentrations in food and fish tissue, respectively. During the initial phase of the exposure, c_{fish} is minimal and k_2 is negligible. Therefore, the total net influx can be used to estimate k_1 from the known values for c_{food} (Table 4 and Eqn. 2). For this study accumulation seemed to be constant up to at least day 100; therefore, initial flux was estimated as the degree of uptake observed at 50 d

$$k_1 = \frac{\text{initial flux}}{c_{\text{food}}} \quad (2)$$

It also follows that at steady state, uptake and depuration rates must be approximately equal to maintain a constant tissue concentration

$$c_{\text{fish}_{ss}} = \frac{k_1}{k_2} \cdot c_{\text{food}} \quad (3)$$

where $c_{\text{fish}_{ss}}$ is the concentration in fish tissue at steady state; k_1 and k_2 are the uptake and depuration rate constants; and c_{food} is the concentration in the food. By rearranging Equation 3, k_2 can be estimated

$$k_2 = \frac{\left(\frac{c_{\text{food}}}{c_{ss}}\right)}{k_1} \quad (4)$$

Using these relationships k_1 and k_2 were calculated for TCDD from c_{ss} and c_{food} (Table 4). For this analysis c_{ss} was

Table 4. Uptake rate constants (k_1s) and depuration rate constants (k_2s) for ^{3}H-2,3,7,8-tetrachlorodibenzo-*p*-dioxin in different tissues. Rate constants were derived as described in the text[a]

Tissue	Dose (pg/g)	Initial net flux (pg/g/d)	k_1 (d^{-1})	k_2 (d^{-1})	c_{ss} (pg/g)
Muscle	1.8	0.0042	0.0023	0.0120	0.35
	18	0.029	0.0016	0.0089	3.25
	90	0.17	0.0018	[b]	
Mean (SD)			0.0019 (0.0004)	0.01 (0.002)	
Liver	1.8	0.0052	0.0029	0.019	0.27
	18	0.033	0.0018	0.013	2.48
	90	—	—	—	13.0
Mean (SD)			0.0023 (0.0008)	0.016 (0.004)	
Adipose tissue	1.8	0.016	0.0089	0.0079	2.02
	18	0.17	0.01	0.011	16.2
	90	1.2	0.013	0.013	88.7
Mean (SD)			0.0106 (0.0024)	0.0107 (0.0028)	
Egg	1.8	0.0096	0.0053	0.011	0.9
	18	0.086	0.0048	0.012	7.0
	90	0.30	0.0034	0.0077	39.5
Mean (SD)			0.0045 (0.001)	0.010 (0.0023)	
Overall mean (SD)			0.0051 (0.0039)	0.0116 (0.0033)	

[a] c_{ss} = steady-state concentrations, determined as the average of the respective tissue concentrations at 100, 150, and 200 d; SD = standard deviation.

[b] Steady state apparently was not reached; c_{ss} could not be determined and therefore k_2 could not be determined.

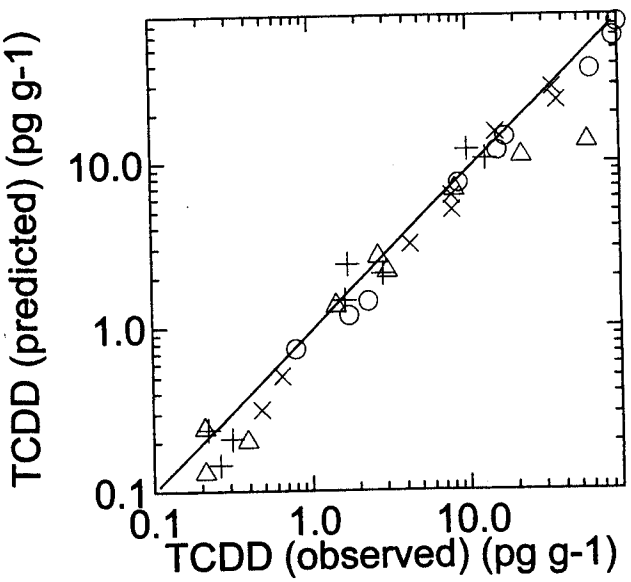

Fig. 2. Observed versus predicted ^{3}H–2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) concentrations in various tissues of rainbow trout. △ = muscle; ○ = adipose tissue; × = egg; + = liver.

calculated as the average TCDD concentration in the respective tissue at the 100-, 150-, and 200-d time points. Estimates of k_1 ranged from 0.0016/d to 0.0133/d for the various tissues examined with an overall mean value of 0.00507/d (standard deviation = 0.0039). It should be remembered that the overall k_1 value would be expected to vary among tissues so that although the coefficient of variation (CV) for the overall k_1 was 77%, CVs for individual tissues only ranged from 21 to 35%. The k_2 values for individual tissues showed CVs in the same range as for k_1 (20–26%). However, in contrast to the k_1 CVs, the overall CV for the mean k_2 for all tissues was considerably less (28%) than that for k_1 (77%).

Using the estimated k_1 and k_2 values, tissue concentrations were predicted based on c_{food}

$$c_{a_t} = c_{food}\left(\frac{k_1}{k_2}\right)(1 - e^{(-k_d t)}) \quad (5)$$

where c_{a_t} is the predicted concentration in tissue a at time t and all other parameters are as described above. Using the tissue-specific rate constants and Equation 5, we predicted tissue concentrations and compared them to the experimental values (Fig. 2). Residuals were calculated for the differences between observed and predicted tissue concentrations. The average residual for the predicted versus the observed concentration as a function of the observed concentrations was 27.1%, with the greatest deviation being 78% for the muscle concentration measured at 150 d.

Although several studies have determined values for k_1 in fish, the majority of studies have used waterborne exposures [22]. Therefore, comparing these values to k_1 values determined in this study is not possible. In other studies that have determined uptake rates in fish from dietary exposure, the uptake is typically expressed as a function of feeding rate (g food/d) and an assimilation factor. Therefore, the most direct comparison of the results to those of the current study are by comparing assimilation efficiencies. For this comparison, experiments in which chemicals were administered as a single oral or intraperitoneal dose [23] were not considered relevant because the key factor in assimilation of TCDD is the transfer from food across the intestinal mucosa into the tissues of the fish. Studies using intraperitoneal injection do not consider this factor nor is a single oral bolus likely to reflect the actual assimilation conditions at environmentally relevant concentrations. Assimilation efficiencies as great as 43 to 75% of the administered dose have been reported for juvenile rainbow trout and whitefish [12]. Similar assimilation efficiencies of 41 to 44% have been reported for 2,3,4,7,8-pentachlorodibenzofuran. However, as these authors pointed out, the assimilation of dioxinlike compounds depends greatly on chemical structure and can vary with efficiencies ranging from 2 to 51% for various dioxinlike compounds [24]. The apparent assimilation efficiencies determined in our study were relatively constant and ranged between 8 and 11% in the early phases of the exposure (Table 5). Assimilation efficiency could not be calculated for the later stages of the experiment because an apparent steady state had been achieved and elimination of TCDD had become a significant process.

Reports of elimination constants (k_2) are more frequent than for k_1 because values derived from waterborne exposure experiments also provide data relevant to the estimation of k_2. In addition, estimates of k_2 are relatively insensitive to the high exposure concentrations used in many waterborne exposure experiments. Rainbow trout exposed to waterborne TCDD at a concentration of 320,000 pg/L exhibited a k_2 value of 0.012/d [25], whereas carp and fathead minnows exposed to between 49 and 62 pg/L had k_2 values ranging from 0.01 to 0.013 [4]. Juvenile rainbow trout exposed to between 38 and 789 pg/L TCDD had k_2 values ranging from 0.047 to 0.015/d [9]. Elimination rate constants ranging from 0.0075 to 0.022 d have also been reported for rainbow trout and whitefish exposed to

Table 5. Apparent uptake rates (pg/d) and assimilation efficiencies (%) for trout after exposure to ^{3}H-2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)[a]

Days exposed	Dose (pg/g)	Estimated daily dose (pg/d)	Total body TCDD mass (pg)	Increase in total mass (pg/kg/d)	Assimilation efficiency (%)
50	1.8	11.65	66.7	3.12	11.5
	18	116.5	506	25.24	8.7
	90	582.5	3,370	164.0	11.6
100	1.8	15.41	140	4.35	9.5
	18	144.4	1,311	40.05	11.2
	90	739.8	14,120	375.2	29.1
150	1.8	12.64	118	–0.94	–3.5
	18	166.1	1,055	8.33	–3.1
	90	862.7	35,996	684.7	50.7

[a] Total mass of TCDD was estimated from specific organ weights and concentrations and assuming that the remainder of the fish weight was represented by fillet.

Table 6. Tissue-specific biomagnification factors (BMFs) for the accumulation of ^{3}H-2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in trout after dietary exposure to ^{3}H-2,3,7,8-TCDD[a]

Exposure time	Exposure concentration (ng/g)	Adipose	Liver	Ovary	Muscle	Lipid (muscle) BMF
50	1.8	0.44	0.14	0.26	0.12	0.34
	18	0.49	0.092	0.24	0.081	0.17
	90	0.67	—	0.167	0.092	0.28
100	1.8	0.97	0.17	0.36	0.22	0.57
	18	0.86	0.16	0.44	0.17	0.81
	90	0.94	0.14	0.41	0.24	0.48
150	1.8	1.27	0.12	0.06	0.12	0.48
	18	0.96	0.096	0.44	0.15	0.38
	90	1.0	0.11	0.38	0.63	1.5
200	1.8	1.1	0.16	0.64	0.24	1.07
	18	0.88	0.16	0.29	0.22	0.64
	90	1.0	0.18	0.52	0.09	0.60

[a] Concentrations on a wet wt basis.

40 to 413 pg TCDD/g food [12]. Muir et al. [24] reported k_2 values of 0.008 to 0.126/d for the structurally related compound 2,3,4,7,8-pentachlorodibenzofuran [24]. The k_2 values determined in the current study (Table 4) are in good agreement with those described above. The tissue-specific rate constants determined in this study have an additional advantage in that they are able to predict concentrations in specific tissues and can be used to predict TCDD exposure in specific target organs such as liver or eggs.

The group feeding of the adult fish in this study meant that the kinetics and assimilation efficiency of TCDD uptake for individual fish could not be determined. However, from the average feeding rate, fish body weight and total mass of TCDD present in fish apparent uptake rates and assimilation efficiencies could be estimated (Table 5). Rates of TCDD accumulation and assimilation efficiencies were only determined over the first 100 d of exposure, before steady-state conditions were reached. After this time, if the accumulation kinetics are first order, the elimination of TCDD from the fish becomes significant and the apparent uptake rate is no longer an adequate indicator of the actual uptake rate. The instantaneous net flux of TCDD into fish varied in a dose-dependent manner and ranged from 1.3 pg TCDD/d in fish exposed to 1.8 pg TCDD/g food up to 67.6 pg TCDD/d for fish exposed to 90 pg TCDD/g food for the first 50 d of exposure. Theoretically, these values for net flux would be underestimates because some flux out of the fish would occur as soon as some TCDD was accumulated. Thus, the rate constants for uptake would be slight underestimates. Because no pure depuration phase exists from which to calculate the first-order depuration rate constants, this parameter was estimated from the uptake rate constant (k_1) and the observed apparent steady-state concentrations. Thus, the estimate of the depuration rate constant (k_2) would also be slightly underestimated. However, by comparing the observed net uptake flux to the net flux, corrected for first-order depuration rate (flux out) at each point by using the assumed depuration rate constant, the error can be demonstrated to be less than 10%.

Biomagnification

Biomagnification factors (BMFs) were calculated based on dietary exposure concentrations and measured tissue concentrations on a wet-weight basis (BMF_{ww}) (Table 6). The BMF values were generally least after 50- and 100-d exposures but thereafter remained relatively constant. After 200 d, the BMF values for adipose tissue, liver, ovary, and muscle were 0.99, 0.17, 0.48, and 0.18, respectively. The tissues with the greatest lipid content, adipose tissue and ovary, exhibited the greatest BMF. Lipid-normalized BMFs were also calculated for fillets based on a dietary lipid content of 13.7% (results not shown). Lipid-normalized BMF values (BMF_{lipid}) generally increased until day 100, presumably because steady-state conditions had not yet been achieved. Lipid-normalized BMF values rarely exceeded 1 and were generally between 0.5 and 1.0. This is consistent with fugacity theory that predicts the lipid-normalized BMF to be 1.0 [26–28]. The TCDD is somewhat different than other nonpolar, diaromatic hydrocarbons because it is often found in tissues with lesser lipid contents. The TCDD can bind to proteins as well as partition into lipids. Thus, one might expect some deviation from the expected BMF_{lipid}, where values less than 1.0 might indicate that a steady-state condition was not reached. Compounds with large K_{ow} values, such as TCDD, have been predicted to require very long times to reach steady state [29].

Significantly, the lipid-normalized BMF for TCDD in this experiment is close to one. This observation indicates that in situations where equilibrium conditions are likely to apply, the lipid-normalized TCDD concentrations can be used to estimate fish tissue concentrations from concentrations measured in dietary items. This will greatly improve many risk assessments that are currently based on water concentrations of dioxinlike chemicals. The use of large bioconcentration factor (BCF) values and their associated uncertainty factors hinders the accurate prediction of effects of these chemicals. For example, BCF values for TCDD determined in one study ranged from 39,000 to 86,000 based on the assumption that equilibrium was attained in a 28-d flow-through exposure [9]. The same authors also noted that the BCF could be as great as 1,000,000 based on the theoretical solubility of TCDD in water. Other studies have reported measured BCF values ranging from 300 to 14,000 and summarized literature values range from 15,000 to 500,000 [14]. In addition to the great degree of variability introduced by the use of such BCFs, measurement of dioxinlike chemicals in water at the small concentrations present in the environment is technically difficult. As a result, many risk assessments must be based on proxy values derived from the method detection limits for the most biologically active dioxinlike chemicals in water samples. Finally, the propensity of these chemicals to accumulate in lipophilic matrices as opposed to aqueous matrices means that the use of BCF values is not relevant to the major route of exposure.

Tissue distribution

The least variable concentrations of TCDD were measured in adipose tissue, a tissue with relatively great lipid content. In contrast, concentrations of TCDD in other tissues examined were more variable. Although lipid contents were not determined in all tissues, the variability can be clearly seen for muscle tissue, which in fish exposed to the greatest dose varied from 1.95 to 6.95%. The least muscle lipid content for this group was observed at 200 d and coincided with a decrease in both muscle TCDD concentration and the total TCDD mass in muscle. The reason for this decrease in muscle lipid is unknown but it coincided with an increase in ovarian somatic index and, more specifically, with an increase in the total mass

of TCDD in this tissue. This suggests a possible remobilization of lipids and TCDD into the developing ovaries.

Tissue distributions of TCDD remained relatively consistent among doses and exposure times. Concentrations were greatest in adipose tissue, approximately one half of that concentration in ovary, and again one half of the ovary concentration in liver and fillet; concentrations of TCDD in liver and fillet were similar. Although the concentration of TCDD in the ovary remained constant, the total mass of TCDD in this organ increased as the ovaries developed. The relative tissue distribution of TCDD in this experiment was similar to previously reported results.

CONCLUSION

This study has determined that the rate constants, assimilation efficiencies, and BMFs determined in a range of other studies are generally similar to those developed during this long-term, low-level exposure to TCDD. This is of considerable significance because it indicates that even at small environmental concentrations the physicochemical characteristics of dioxinlike chemicals can result in adverse effects in salmonid fish. That the prediction of effects at low concentrations from laboratory experiments at relatively great concentrations would lead to inaccurate estimates of risk has always been of concern. The results of this study indicate that the factors previously determined are relevant to low-level exposures and should result in adequate assessments of risk. In the additional papers written on this study we provide data on effects observed in adult fish and the reproductive success of those fish. These additional data will provide means of further assessing the risks posed by low-concentration exposures to dioxinlike compounds.

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