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Chronic Oral Toxicity of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) to Rainbow Trout

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ABSTRACT

Rainbow trout (*Salmo gairdneri*) consumed diets containing 2.3 parts per trillion (10^{-12}, pptr), 2.3 parts per billion (10^{-9}, ppb), and 2.3 ppm TCDD 6 days each week for 105 days, resulting in an average intake of, respectively, 3.2×10^{-8}, 3.6×10^{-5}, or 2.1×10^{-2} μg/TCDD/g fish, freeze-dry weight per day. Consumption of food containing 2.3 pptr or 2.3 ppb TCDD caused no mortality, no reduction in food consumption or growth, and no fin erosion. Consumption of diets containing 2.3 ppm TCDD caused an average mortality of 50% and 88% in 61 and 71 days, respectively. Feeding activity and growth were also reduced, and fin erosion and liver pathology increased. These data indicate that the "no-effect" level for survival, growth, feeding activity, and fin erosion in rainbow trout receiving TCDD orally is between 2.3 ppm and 2.3 ppb.

The herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) is an important tool in brush control operations on the forest and rangelands of the United States. The presence of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), as a highly toxic contaminant, has, however, raised serious concern over the use of the herbicide.

The principal route of 2,4,5-T (and associated TCDD) entry to streams is direct application or drift to the water surface during forest spray operations. Miller et al. (1973) estimate maximum TCDD concentrations in forest streams immediately after aerial application of 2,4,5-T would be less than 0.01 parts per trillion (pptr). The herbicide could also enter streams from runoff during periods of intense precipitation, but this rarely happens on forest lands because the infiltration capacity of the forest floor greatly exceeds precipitation rate. Leaching through the soil is slow and capable of moving only small amounts of herbicide short distances (Norris and Moore 1971). TCDD is immobile in soil and does not leach to streams (Kearney et al. 1973). TCDD can also enter streams on the leaves of sprayed vegetation. Fish may be exposed to TCDD through consumption of stream organisms which have accumulated TCDD from their environment.

The purpose of this study was to determine the toxicity of TCDD to rainbow trout (*Salmo gairdneri*) and the possible implications of this type of exposure. Observations included survival, growth, feeding behavior, disease, and internal organ pathology. Initial findings from this study were reported by Miller et al. (1973). The completed study is reported here.

METHODS

Young rainbow trout received TCDD in dried fish food at concentrations of 2.3 parts per trillion (pptr, 10^{-12}), 2.3 parts per billion (ppb, 10^{-9}), and 2.3 ppm to an intake level of, respectively, 3.2×10^{-8} μg, 3.6×10^{-5} μg, or 2.1×10^{-2} μg TCDD/g of fish, freeze-dry weight per day, 6 days each week. Control fish received food free of TCDD. The TCDD, to 49% saturation in acetone, was added to the oil base of a casein-dextrose-herring oil fish food, slightly modified from Lee et al. (1967). Acetone was removed from the oil by vacuum evaporation, leaving an average of 0.3% acetone in the dry fish food.

Two hundred young rainbow trout, selected for uniformity of size (mean standard length = 7.78 ± 0.97 cm), were randomly placed in 20 aquaria, each of 18-liter capacity. The aquaria were assigned at ran-

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TABLE 1.—*Growth rate and total growth of fish fed different doses of TCDD in the diet.*

TCDD[a] exposure level μg/g/day	TCDD concentration[b] in food	Days after initial exposure	Mean growth rate[c]		Mean total growth[c]	
			mm²/mm²/day[d]	g/g/day[e]	mm²/fish[f]	g/fish
0.0	0.0	30	0.007	0.012	297Γ	0.78
		105	0.005	0.008	707Φ	1.96
3.2×10^{-8}	2.3 pptr	30	0.007	0.011	307Γ	0.75
		105	0.004	0.008	691Φ	1.90
3.6×10^{-5}	2.3 ppb	30	0.007	0.012	308Γ	0.78
		105	0.005	0.009	790Φ	2.08
2.1×10^{-2}	2.3 ppm	30[g]	−0.0002	−0.0002	−10	−0.01

[a] Dose is expressed in μg TCDD/g fish, freeze-dry weight/day, dose delivered 6 days a week.
[b] Concentration in food is pptr—part per trillion, 10^{-3} μg/kg; ppb—parts per billion, μg/kg; ppm—parts per million, mg/kg.
[c] Mean values for 50 fish.
[d] Mean size growth rate is $(mm_2^2 - mm_1^2)/mm_2^2$ per day where mm_1^2 is side view area at the beginning of the growth period and mm_2^2 is side view area at the end of the growth period.
[e] Mean weight growth rate is $(g_2 - g_1)/g_2$ per day where g_1 is dry weight at the beginning of the growth period and g_2 is dry weight at the end of the growth period. Dry weights are calculated from the regression equation $Y = -0.18 + 0.000988X + 0.000000426X^2$, where Y is freeze-dry weight in g and X is side view area (depth × length) in mm².
[f] Values for a given exposure period with a symbol in common are not significantly different ($P > 0.05$).
[g] All but two of these fish were dead at 71 days. The remaining two were sacrificed for histopathological examination.

dom among one control and three treatments in five replications.

Each aquarium received fresh water at the rate of 9 liters/h. Water temperature was maintained at 15 C. Outflow from the aquaria was passed through four 76- × 25-cm charcoal filters to remove TCDD. Fluorescent lights provided a 14.5-h light and 9.5-h dark daily cycle. Fish were acclimatized to the flowing-water aquaria and TCDD-free food for 3 weeks before the experiment began.

Food containing the daily dose of TCDD was given 6 days a week at 0900 h. Fish routinely consumed all TCDD-contaminated food. At 1500 h daily, food without TCDD was given *ad libitum* and all excess food was removed. All fish were photographed (full side view) weekly to determine indices of fish size (the product of fish length and depth). The advantages of this technique are outlined by Hawkes (1975). Test feeding continued for 105 days. At the end of the experiment, all surviving fish were photographed and freeze-dried, and a regression equation was generated relating dry weight to side view area. For fish between 750- and 4,100-mm² side view area, the equation is:

$$Y = -0.18 + 0.000988X + 0.000000426X^2$$

where, Y is fish freeze-dry weight in g, and X is the index of side view area in mm² ($r^2 = 0.94$). Effects of TCDD on growth were tested by analysis of variance on the indices of fish side view area. In addition, growth rate and total growth were calculated for both side view area and freeze-dry weight of individual fish.

After 71 days, liver tissues from two fish which died during the preceding 24 h and two surviving (but nearly moribund) fish (all fed food containing 2.3 ppm TCDD), were examined histologically. After 105 days, livers from two control fish and one test fish fed food with 2.3 ppb TCDD were similarly examined. A gas chromatographic-mass spectrophotometric analysis for TCDD was conducted on one sample of each food and on one fish selected at random from each of the diet regimes. Cleanup and analysis procedures outlined by Dow Chemical Company[2] were followed.

RESULTS AND DISCUSSION

Feeding activity declined after 10 days in test fish fed the highest dose of TCDD (2.3 ppm), and continued to decline through about 24 days. Total food consumption stabilized at approximately 8% of initial amounts; consumption of uncontaminated food in this group ceased while consumption of food with TCDD dropped to near 19% of initial amounts. No effect on feeding activity was noted from fish diets with 2.3 ppb TCDD, 2.3 pptr TCDD, or from control foods.

[2] Unpublished report. Analytical method determination of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in fish and soil. Dow Paper ML-AM 73-97. Dow Chemical Company, Midland, Michigan.

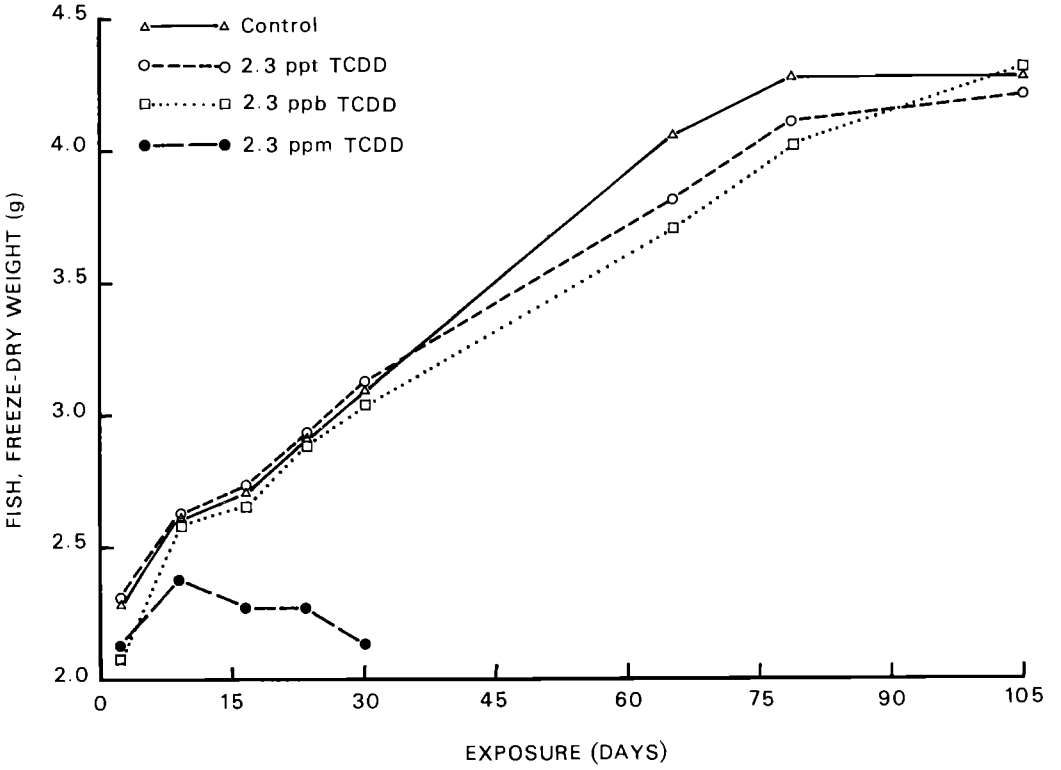

FIGURE 1.—*Mean freeze-dry weight of rainbow trout receiving TCDD in food daily.*

Weight loss began on the 7th day in test fish receiving food with 2.3 ppm TCDD (Fig. 1). By the 30th day, the difference in growth between fish receiving this dose and all fish receiving lesser quantities of TCDD was highly significant ($P < 0.01$). Analysis of fish receiving 2.3 ppm TCDD in food was not continued beyond 30 days because they showed reduced growth and gave no indication of recovery. There were no significant differences ($P > 0.05$) in growth after 30 or 105 days of exposure among fish receiving food with 2.3 ppb TCDD, 2.3 pptr TCDD, or controls (Table 1).

Fin erosion was apparent by 14 days in fish fed food with 2.3 ppm TCDD. Fin erosion continued as long as the fish lived. Some fish developed a large fungal growth on the caudal fin shortly before death. Fin erosion and fungal growths were not observed in fish receiving lesser doses of TCDD during the entire 105 days of the experiment. Miller et al. (1973) and Norris and Miller (1974) first noticed fin erosion at 30 days in coho salmon (*Oncorhynchus kisutch*) and at 10 days in guppies (*Poecilia reticulatus*) exposed to 50- to 100-pptr TCDD in water.

Histological examinations of livers from four fish receiving 2.3 ppm TCDD in the diet revealed definite degenerative changes. Many parenchymal cells had vacuoles containing globular material in place of the glycogen normally present in healthy cells. Only a single fish receiving the 2.3-ppb-TCDD diet was examined. It exhibited a similar pathology but only in one lobe of the

TABLE 2.—*Analysis of food and fish tissues for TCDD.*

TCDD mixed into food ppm	TCDD analysis of food[a] ppm	TCDD analysis of fish tissues[b] ppm
0.0	ND[c]	475×10^{-6}
2.3×10^{-6}	ND	63×10^{-6}
2.3×10^{-3}	3.29×10^{-3}	1.573×10^{-3}
2.3	1.70	1.38

[a] Analysis of a single sample of fish food for each dietary level.
[b] Analysis of a single fish from each exposure level.
[c] ND: concentration below limits of detection (10×10^{-6} ppm).

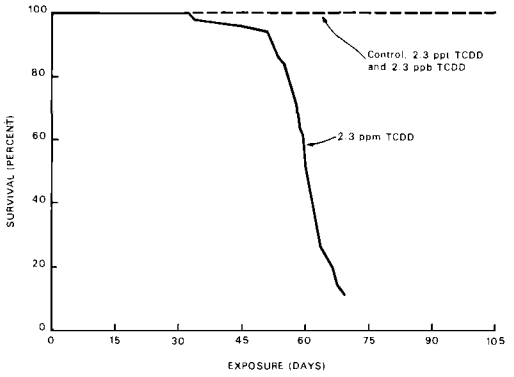

FIGURE 2.—*Mean survival of rainbow trout receiving TCDD in food daily.*

liver. Examination of more fish receiving this dose is necessary before conclusions regarding exposure and liver pathology can be reached. Fish consuming the 2.3-pptr-TCDD diet were not examined.

The first death occurred on the 33rd day among fish receiving the 2.3-ppm-TCDD food. By the 61st day, 50% of these fish were dead, and all but two were dead by the 71st day (Fig. 2). None of the fish held at the other test regimes had died at termination of the experiment (105 days of exposure).

Several months after toxicity testing was completed, an opportunity developed to determine TCDD levels in the fish food and in a few fish. The fish used for this determination had been killed, wrapped in aluminum foil, and frozen at the conclusion of the test. The residue levels in Table 2 represent analyses of only a single sample of each dietary level and a single fish selected at random from each exposure level.

Some contamination of control fish and fish exposed to 2.3 pptr TCDD was apparent. The fish fed diets containing 2.3 ppb or 2.3 ppm TCDD had whole body residues that indicated TCDD was not concentrating in body tissues despite exposures varying from 65 to 105 days. Our analyses provide a first estimate of possible body burdens in fish receiving known amounts of TCDD in their food. A complete pharmacokinetic study is needed before definitive conclusions can be reached concerning the tissue residue dynamics of TCDD in fish.

The concentration of TCDD in or on organic material entering streams could range up to 8 pptr TCDD, extrapolated from Getzendaner and Hummel[3] and Isensee and Jones (1971). This is well below the 2.3 ppb TCDD found in this study to cause slight response (liver pathology) in young rainbow trout. The amount of TCDD that might enter streams can be estimated from litter input values and data on TCDD persistence on vegetation from Getzendaner and Hummel[3] and Isensee and Jones (1971). Studies on two different streams (unsprayed) showed the entrance of 620 and 1,095 g of detritus/m^2 per year (Fisher 1971; Vannote 1970). Entry values might range from 0.0006 μg TCDD/m^2 to 0.0085 μg TCDD/m^2 of stream bottom per year. We lack information on residue levels which may occur in stream organisms that feed on leaves and are prey for fish.

Our study indicates that high concentrations of TCDD in food causes decreased feeding, growth reduction, fin erosion, changes in liver tissue, and mortality in young rainbow trout. The oral threshold level for all responses (except possibly for changes in liver tissue) for exposure periods up to 105 days was between 2.3 ppb and 2.3 ppm TCDD in food. Additional chronic toxicity testing is needed in which growth, survival, and reproduction during a complete life cycle are measured. A determination is also needed of the fate of TCDD entering streams on vegetation.

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