The Toxicity of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) in Guppies (*Poecilia reticulatus* Peters)

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2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a contaminant of the commercially produced herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). TCDD is immobile and only slowly degraded in soils, not readily taken up by plants, subject to photodecomposition, and low in water solubility (0.2 ppb)[1/] (ISENSEE and JONES 1971, KEARNEY et al. 1971, 1972). It is highly toxic and teratogenic in mammals (COURTNEY et al. 1970, COURTNEY and MOORE 1971). The effects of TCDD on aquatic organisms have not been previously reported.

2,4,5-T is an extremely important tool for reforestation of brush-dominated forest sites. During 1969, 358,800 pounds of 2,4,5-T were applied to forest and rangelands managed by U.S. Department of Agriculture and U.S. Department of Interior (MacLEAD 1971). 2,4,5-T probably presents minimum hazard to organisms in the forest environment, but the effect of TCDD must also be considered (NORRIS 1971). Small quantities of 2,4,5-T are found for short periods in streams after aerial application on nearby forest land. TCDD is presumably also present because most of the herbicide in the stream results from drift or direct application of spray formulation to the stream surface (NORRIS 1967). We are determining the toxic characteristics of TCDD in aquatic organisms and report here some of these characteristics in guppies, *Poecilia reticulatus* (Peters).

MATERIALS AND METHODS

The TCDD used in these tests was part of lot 851:142-22 from the Dow Chemical Company, Midland, Michigan.[2/] Based on mass spectral analysis, the chemical was 98 percent 2,3,7,8-tetrachlorodibenzo-p-dioxin and the balance was 1.3 percent trichloro- and 0.7 percent pentachlorodibenzo-p-dioxin.

Stock solutions (166.6 ppm) were made in chloroform and subsequent dilutions in acetone.

Guppies of various sizes (9 to 40 mm) were placed in 1-gallon glass jars (20 to 22 fish per jar) and received one of four treatments (three replications). The fish were exposed to 0, 0.1,

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[1/] ppb - parts per billion.

[2/] Mention of a company does not imply endorsement by the U.S. Department of Agriculture.

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1.0, or 10.0 ppb TCDD in 3 liters of well water. WILSON (1968) reported the composition of the well water. All jars received 1.2 ml acetone and were maintained at 20 C for the duration of the experiment. After 120 hours exposure, fish were transferred to clean jars with well water containing no dioxin. Water was changed at 14-day intervals. Dead fish were removed each day and their length recorded (DOUDOROFF et al. 1951). Fish were fed live tubifex worms *ad lib* daily except during the exposure period.

RESULTS

The effect of 120-hour exposure to 0.1, 1.0, or 10.0 ppb TCDD on guppies was irreversible, and all guppies eventually died. Some of the treated guppies died during the 5-day exposure period, but most fish died after they were placed in clean water. All treated fish were dead 37 days after the beginning of the exposure period, and in general, the smaller fish died first. There was no mortality among control fish (Table 1).

TABLE 1

CUMULATIVE GUPPY DEATH AFTER 120-HOUR EXPOSURE TO TCDD

Days since start of exposure period	Mean cumulative death (percent)[1] TCDD (ppb)[2]			
	0	0.1	1.0	10.0
5	0	8	11	18
10	0	24	38	32
15	0	34	65	42
20	0	45	80	56
25	0	61	96	83
30	0	72	100[3]	97
35	0	97		100
37	0	100		
Days to 50 percent mortality		21.7	11.6	18.2

[1] Mean of three replications.

[2] Parts per billion.

[3] Last fish died in this treatment on 28th day.

The data were expressed as survival time in days and body length in mm for each fish and subjected to analysis of covariance. Survival time was significantly different at the 5 percent probability level for fish exposed to different concentrations of TCDD ($F_{2,5} = 11.818$). The analysis showed the effect of body length on survival of fish exposed to TCDD was significant at the 1 percent

level ($F_{1,5}$ = 65.896) (Figure 1). Using the regression equation in Figure 1, we adjusted the mean survival time of fish exposed to each concentration of TCDD for the effect of body length on survival (Table 2).

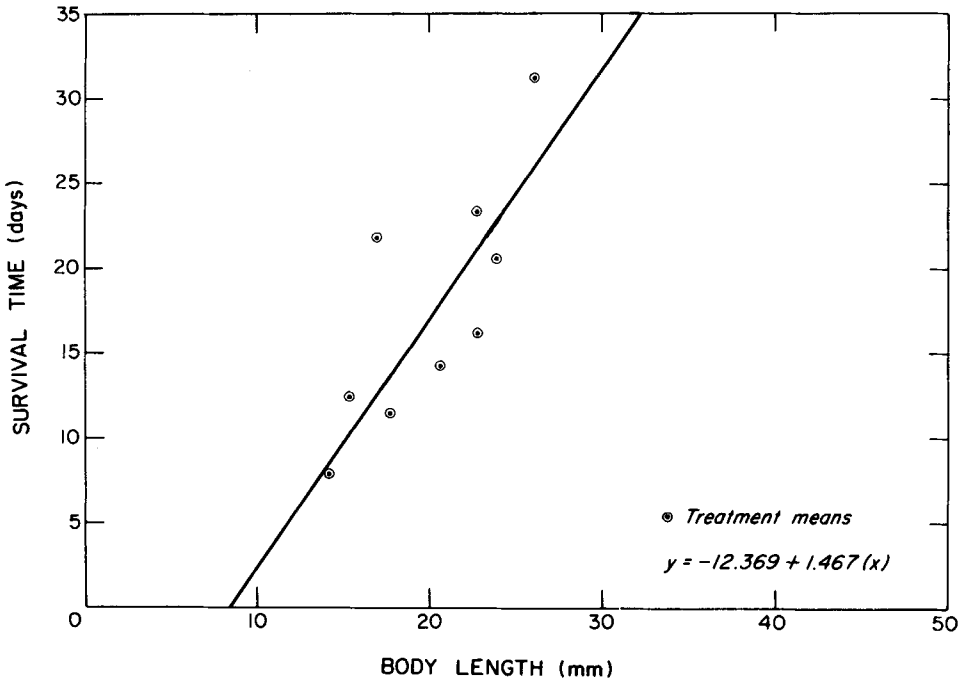

Figure 1. Effect of body length on survival time of guppies exposed to 0.1, 1.0, or 10.0 ppb dioxin in water for 120 hours.

TABLE 2

GUPPY SURVIVAL TIME AFTER 120-HOUR EXPOSURE TO TCDD

Mean[1/]	TCDD (ppb)[2/]		
	0.1	1.0	10.0
Fish length (mm)	20.1	20.7	20.6
Survival time (days)	21.3	14.1	17.2
Survival time adjusted for fish length (days)	21.9	13.7	17.0

[1/] Mean of 3 replications.

[2/] Parts per billion.

DISCUSSION

Our results show smaller fish are considerably more sensitive to TCDD than larger fish. Similar results have been reported for several other toxicants. Many organisms increase their tolerance to environmental stresses with increasing age and body mass. Toxicant uptake, storage, and detoxication patterns probably change with fish age as lipid levels increase and the ratio of gill surface area to body mass decreases (COPE 1971, MURPHY and MURPHY 1971).

We are uncertain about the reason for the apparent decrease in toxicity between 1.0 and 10.0 ppb TCDD, but suggest it may be the result of working beyond the normal limits of TCDD solubility. Fish in the 1.0-ppb treatment may have been exposed to supersaturated levels of TCDD, possibly mediated by interaction with fish skin mucus in the water. The solubility of DDT in water is enhanced in the presence of some large organic molecules (WERSHAW et al. 1969).

Approximately 1 week after initial exposure to TCDD, guppies exhibited a declining interest in feeding and swimming. All treated fish surviving more than 10 days showed fin necrosis which became more pronounced with time. Necrosis of the maxillary cartilage appeared in two large females surviving more than 25 days after exposure to 0.1 and 10.0 ppb TCDD. None of these behavioral or pathological signs of distress were observed in control fish. The site of action of TCDD in guppies is not known; however, experiments with other animals suggest the liver may be involved (BUU-HOI et al. 1972a, 1972b, CUNNINGHAM and WILLIAMS 1972, PIPER and ROSE 1971). The delayed mortality we observed with guppies is consistent with the hypothesis that TCDD induces liver dysfunction.

Static water bioassays are not particularly satisfactory for determining chemical toxicity in terms of field exposures (JUNTUNEN and NORRIS 1972). They can, however, provide information on the toxic characteristics of a particular chemical and on the relative toxicity among chemicals. Most static water tests are terminated after 96 hours of exposure. The degree of TCDD toxicity in guppies was hardly apparent in our tests even after 120 hours of exposure. We suggest all tests of chemical toxicity to aquatic organisms provide for observation of delayed mortality.

The concentrations of TCDD used in our tests were much higher than those likely to be encountered in forest streams. Concentrations of 2,4,5-T in excess of 0.1 ppm have not been found in Northwest forest streams (NORRIS 1967). Current production grade 2,4,5-T contains less than 0.1 ppm TCDD; therefore, streamwater containing 0.1 ppm 2,4,5-T should contain no more than 0.01 part per trillion TCDD. Experiments are now in progress to determine the threshold-response level of salmon fingerlings and guppies to TCDD in water.

SUMMARY

Exposure of guppies to 0.1, 1.0, or 10.0 ppb TCDD for 120 hours caused complete mortality in the next 32, 21, and 30 days, respectively. Duration of survival was significantly and positively correlated with body length. The concentrations of TCDD used in these tests were considerably greater than expected to occur in forest streams after aerial application of 2,4,5-T. The threshold-response level for TCDD in fish has not been reported.

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