Toxicity of 2, 3, 7, 8-Tetrachlorodibenzo-p-Dioxin in Larval and Adult Forms of *Rana catesbeiana*

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2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a contaminant of preparations of the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). It also occurs as a contaminant of some preparations of polychlorinated phenols. TCDD is one of the most toxic synthetic compounds known. Published oral LD_{50} values in mammalian species show some inter-species variation. However, in general, TCDD is highly toxic to all mammalian species so far examined (SCHWETZ et al 1973). TCDD also exhibits marked toxicity to guppies (NORRIS and MILLER 1974), young salmon and young rainbow trout (MILLER et al 1973).

The effect of TCDD on an amphibian species has not yet been reported. In this study we have examined the toxicity of TCDD in both the larval and adult forms of the American bullfrog (Rana catesbeiana).

METHODS

Rana catesbeiana tadpoles[a], Taylor and Kollros stages I-VII, (TAYLOR and KOLLROS 1946) were injected intraperitoneally with TCDD (>98% pure, Dow Chemical Company) dissolved in olive oil. The dosages administered were 0,25,50,100,200 and 1000 µg/kg body weight. Each treatment group, containing 15 tadpoles, was maintained in a separate 8 in. by 12 in. plastic container filled to a depth of approximately 2 inches with dechlorinated tap water. The water was changed every three days and the tadpoles were fed uncooked spinach.

Adult Rana catesbeiana of mixed sex, 150-250 g, were randomly assigned to five treatment groups of five animals each. TCDD was administered i.p. in olive oil

[a] All animals were purchased from Mogul Ed Company, Oshkosh, Wisconsin.

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to give dosages of 0,50,100,250 and 500 µg/kg. The adult bullfrogs were kept in large plastic cages through which fresh water was running continuously. The animals were fed live crickets ad libitum.

RESULTS AND DISCUSSION

In the experiment with Rana catesbeiana tadpoles, there was no mortality attributable to TCDD at any of the dosages examined (Table 1). A mortality rate of approximately 20% was seen in all groups including controls.

TABLE 1

SURVIVAL OF Rana catesbeiana TADPOLES AFTER I.P. INJECTION OF TCDD

Dose TCDD (µg/kg)	% Survival on Day 50 Post-injection
0	80
25	87
50	73
100	93
200	80
1000	80

Those deaths which did occur may be attributed to the injection procedure or to infection by polyhedral cytoplasmic deoxyribovirus (tadpole edema virus). All surviving tadpoles were observed to successfully complete metamorphosis with no observable morphological abnormalities. In addition, histopathological examination of the liver, heart, kidney, lung and reproductive organs of these animals shortly after the completion of metamorphosis revealed no lesions other than occasional parasites.

In light of these results, it was decided to examine the toxicity of TCDD in adult bullfrogs. There are biochemical and physiological differences in the larval as compared to the adult forms of Rana catesbeiana. It was reasoned, therefore, if TCDD was toxic to the adult bullfrog but not the larval form, that this animal might be a good model for studying the mechanism of toxicity of TCDD.

Adult bullfrogs were administered TCDD as described in METHODS and observed for a period of 35 days. During this period, there was no mortality in any treatment group. The only observable difference was somewhat lessened food intake during the initial part of the experiment in the frogs receiving 500 µg/kg TCDD. However, at the termination of the experiment, the food consumption of the high dose animals was not different from that of the controls. Histopathological examination of liver, kidney, lung, heart, and gonads of these animals revealed no significant lesions at any of the doses of TCDD administered.

The results of this study are of considerable interest since TCDD has been reported to be extremely toxic to various fish species in static water exposure tests (NORRIS and MILLER 1974; MILLER et al 1973). In these latter studies TCDD concentrations as low as 0.054 ppt in the surrounding water caused significantly increased mortality in young salmon. Lesions including fin necrosis and increased susceptibility to fungal infection were also observed. It is possible that the lack of effect of TCDD in larval and adult bullfrogs as compared to fish may be the result of the route of exposure rather than species differences. However, it would appear that i.p. administration of TCDD would be as toxic or more so than absorption from the water.

TCDD has been reported to be a potent teratogen in rats and mice (NEUBERT et al 1973), therefore it is of interest that TCDD appears to have no effect on amphibian metamorphosis, a somewhat analogous developmental process.

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SUMMARY

Varying doses of TCDD ranging from 25 to 1000 µg/kg were administered to the larval and adult forms of the American bullfrog, Rana catesbeiana. Doses of TCDD as high as 1 mg/kg failed to have any significant effect upon survival or completion of metamorphosis in tadpole and doses of up to 500 µg/kg had no effect on survival of adult frogs. Histopathological examination of various tissues from the metamorphosed tadpoles and adult frogs failed to show any abnormalities.

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