

Insecticide-induced feminization of frogs: effect of exposure of common hourglass tree frog to three insecticides

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Many pesticides are known endocrine disruptors and act as gonadotoxins especially in males with demasculinization and feminization effects. Here we investigated the effects of three insecticides: Profenofos, Diazinon and Carbosulfan on the development of gonads of males and females of common hourglass tree frog, *Polypedates cruciger* under laboratory conditions. Egg masses of *P. cruciger* were collected from natural habitats and brought to the laboratory. Upon hatching, 20 tadpoles at Gosner stage 25 were placed in glass tanks and were exposed to an ecologically relevant concentration series of the three insecticides. Each chemical trial had four treatments and dechlorinated tap water as a control. The survival was recorded weekly and the tadpoles were raised until metamorphosis (Gosner stage 31). The metamorphs that survived were reared until Gosner stage 42, euthanized using MS-222, fixed in Bouin's and the gonads were sectioned, slide mounted and stained with hematoxylin and eosin. Survival of tadpoles in all insecticide-exposed trials was significantly lower compared to the control (Chi square test; $p < 0.001$). Among the three insecticides, Carbosulfan exposed frogs had the highest reduction in survival in all the concentrations. Gonads of a total of 62 males and 43 females were sectioned. None of the females or males in the control group and the lowest concentration of the Diazinon (0.375 ppb) exposed group had any abnormalities in their gonads. However, the males exposed to different concentrations of the three chemicals had developed testicular oocytes at varying percentages. The number of oocytes per testis varied with the insecticide and the concentration. Among the three insecticides, Carbosulfan had a more profound feminization effect on male frogs than other chemicals. Feminization of male gonads is the development of oocytes in the testes or complete ovarian differentiation of genetic males leading to decreases in the frequency of morphologic males.

Financial assistance is given by the National Science Foundation (Grant no. RG/2014/EB/02)