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The effect of Gramoxone (main ingredient gramoxone) on growth and survival of larvae of the endemic frog, *Polypedates cruciger*

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Long-term (52-day) renewal experiments were conducted to assess the effects of gramoxone (with active ingredient paraquat), a widely used herbicide, on the growth and survival of larvae of the common hourglass frog, *Polypedates cruciger.* Two egg masses were collected and left to hatch under laboratory conditions. Hatchlings, corresponding to Gosner stage 20 (gill stage), were reared in glass tanks filled with tap water (20 per tank), exposed to 4, 10 and 40 ppb of gramoxone and monitored for eight weeks. Treatments and untreated controls were maintained in triplicate. Water was changed and pesticide concentrations were renewed every five days. Mortality was noted daily and body length measurements were taken at approximately weekly intervals. A significant positive dose-dependent trend was evident with exposure to higher doses resulting in greater levels of larval mortality (Pearson's correlation: $r = 0.99$, $p<0.001$). Our observations also showed that long-term exposure to 40 ppb gramoxone caused a significant elevation in larval mortality (65 %) as compared to the control (8.33 %) (one-way ANOVA and post hoc Tukey test: $F_{3,8} = 19.67$, $p<0.0001$). No significant reductions in survival were noted at doses of 4 and 10 ppb. Considering mortality levels at the different doses and using Regression Analysis the LD50 value was determined. After 52 days of repeated exposures, the tadpoles had a LD50 value of 32 ppb. Furthermore, survival rates in all treatments varied throughout the experiment with the highest mortality occurring between 11-21 days after exposure.

Despite the profound effects observed with mortality, gramoxone did not seem to affect the growth rates of the tadpoles. Although statistical analysis of the final body length measurements showed that there was significant differential growth among tadpoles across the various treatments (one-way ANOVA; $F = 10.46$, $p<0.001$), the post hoc Tukey tests revealed that this difference existed only between the controls and the lowest dose of 4 ppb. Detailed examination of the results indicated that growth at the highest dose was impaired during the initial phase of the exposure but this trend was seen to reverse with the few survivors showing an abnormally high growth rate during the latter stages. Due to the adverse effects on mortality observed in this study, we conclude that gramoxone, at concentrations above 40 ppb, may pose a serious threat to the survival of natural populations of this endemic amphibian species in agricultural landscapes.

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