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Relative toxicity of phenoxy herbicides on *Lebistes* (*Poecilia*) *reticulatus* (Peters)

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Abstract. Phenoxy herbicides 2,4-D, its sodium and ethyl amine salts and ethyl esters, commonly used in aquatic weed eradication programmes, were tested by acute and static bioassay investigations on a cyprinid fish *Lebistes* (*Poecilia*) *reticulatus* under laboratory conditions. The toxicities of these different formulations are compared and reported in relative toxicity ratings. 'Safe application rate' and 'safe application factor' equations were also calculated. Median lethal concentrations at various time intervals are also reported along with respective fiducial limits and regression equations. Comparative regressions are drawn for three time intervals, 24, 48 and 96 hr.

Keywords. Herbicide; 2,4-D; bioassay; relative toxicity; LC_{50}; *Lebistes* (*Poecilia*) *reticulatus*.

1. Introduction

Toxic effects of herbicides are now common in fish and other freshwater organisms (Kamler *et al* 1974; Klekowski *et al* 1977; Kaniewska-Prus 1977; Spehar *et al* 1980; Rao and Dad 1979; Vardia and Durve 1981a, b). Phenoxy herbicides have also attracted attention. They formed the first organic compounds used for selective weed control in an aquatic environment and were sold in many formulations *viz.*, 2,4-dichlorophenoxyacetic acid (2,4-D), sodium salt of 2,4-dichlorophenoxyacetic acid (2,4-D-Na or Phen-D), dimethylamine salt of 2,4-dichlorophenoxyacetic acid (2,4-D-DMA or phenamine) and diethylester of 2,4-dichlorophenoxyacetic acid (2,4-D-DEE or phenest). These chemicals represent varied chemical structures *viz.*, ester, amine, acid and sodium salt.

In the present study acute bioassay investigations were used to assess the relative toxicity of different herbicides. In relative toxicity studies, the test chemicals are compared with a standard by using the same test species and calculating the toxicity values of these chemicals. This procedure is followed for determining relative toxicity of 4 formulations of phenoxy herbicides *viz.*, 2,4-D-acid, ester, amine and sodium salts with the test fish *Lebistes* (*Poecilia*) *reticulatus* (Peters).

2. Materials and methods

The acute and static bioassay experiments were conducted by the methods of APHA (1980). The fish, *Lebistes* (*Poecilia*) *reticulatus* ($1{\cdot}7 \pm 0{\cdot}3$ cm in length and $0{\cdot}10 \pm 0{\cdot}04$ g in weight) were obtained from the departmental ponds and acclimated to laboratory regime on a diet of 1 : 1 mixture of wheat-bran and oil-cake. The diluent (dechlorinated tapwater) was analysed for its physical and chemical characteristics *i.e.*, dissolved

oxygen–$6 \pm 1{\cdot}1\ mg\,l^{-1}$, carbonate alkalinity–nil, bicarbonate alkalinity–$160 \pm 12{\cdot}3\ mg\,l^{-1}$, pH $8{\cdot}1 \pm 0{\cdot}3$, conductivity $745 \pm 10\ \mu mhos\,cm^{-1}$, total hardness–$240 \pm 20\ mg\,l^{-1}$ and temperature–$27{\cdot}1 \pm 1{\cdot}5°C$.

Thirty to fifty fishes were used for each concentration tested in 3–5 replicates. The minimum possible quantity ($< 2\ ml\,l^{-1}$) of ethanol (rectified spirit) was used as a carrier agent due to its high solubilizing powers and negligible toxicity. The fish mortalities were noted and LC_{50}, LC_{84} and LC_{20} were calculated by probit analysis (Finney 1981). LC_{100} and LC_0 were calculated using probit values of 99·9 and 0·1 respectively. 95% fiducial limits, heterogenity factor (x^2) and slope function were also calculated by the methods of Finney (1981). The mean relative toxicities of four formulations were calculated on the basis of 96 hr LC_{50} values by dividing them with 2,4-D-Na (Finney 1981). The relative toxicity of 2,4-D-Na was assumed to be one. The safe application factor equation and rates were calculated by the methods of Ghosh and Konar (1976).

The experimental herbicides, Phen-D is 80% active ingredients (*a.i.*) (2,4-D-Na), 2,4-D-acid 100% *a.i.* (2,4-D-acid), Phenest 340 g acid equivalents (*a.e.*) l^{-1} (2,4-D-DEE) and phenamin 720 g *a.i.* l^{-1} (2,4-D-DEA). Phen-D and acid used were fine powders while phenest and phenamine were liquid formulations. 2,4-D-acid was applied with spirit as carrier agent while sodium salt was dissolved in water. Ester and amine were, however, used directly without a carrier agent.

3. Results and discussion

Toxicity of herbicides to fish varies with formulation and even within the same formulation, due to different manufacturing techniques and batch lots. However, with any formulation, the application of lethal concentration in water led to erratic swimming, difficulty with respiration and convulsions caused by nervous non-coordination. Further, the fish exhibited jerky movements, mucus secretion, surfacing, slow and at times backward swimming, barrel-rolling or spiralling reflex, paralysis, irregular opercular beats and ultimate death. The relative toxicity data with different phenoxy herbicides indicate that esters are highly toxic though greater in use because of desirable physical properties, control of droplet size, limited solubility in water, good spread on contact with vegetation and less persistence in the environment. The relative toxicity comes to be 339·2 for diethylamine ester. Further increase in length of carbon chain decreases the toxic action of esters. Meehan *et al* (1974) observed isopropyl and butyl esters to be more toxic than octyl ones. Esters were most toxic to phytoplankton than parent acids (Butler 1965). Highest toxicity of esters were also discussed by Hughes and Davis (1963), Davis and Hughes (1963) and Folmer (1976). Ninety six hr LC_{50} in 2,4-D-DEE varies from 4·7 to 3·7 $mg\,l^{-1}$ (table 1). Safe application factor as reported by Lhoste and Roth (1946) for 2,4-D-DEE was $0{\cdot}3\ mg\,l^{-1}$ while it is $0{\cdot}015\ mg\,l^{-1}$ in our investigations.

The next in series of relative toxicity is 2,4-D-acid which is ranked moderately toxic with a relative value at 169·7 and LC_{50} ranging from 10·2 to $8{\cdot}4\ mg\,l^{-1}$. The safe application rate for this technical grade 2,4-D acid comes to $0{\cdot}17\ mg\,l^{-1}$. This confirms the earlier finding that parent acids are more toxic than their amines. The amine, 2,4-D-DEA is median toxic with LC_{50} ranging from 448·7 to $375{\cdot}8\ mg\,l^{-1}$. Various formulations of 2,4-D-amine are in use. The formulations diethylamine and sodium salt

Table 1. Toxicity of four formulations of 2,4-D showing their various lethal concentrations at different exposures along with regression equations, Safe application factor equation (SAFE), Safe application rates (SAR) and relative toxicity on *Lebistes reticulatus*.

Herbicide formulation	Exposure duration (hr)	LC_{50} (fiducial limits in parentheses)	LC_{84} (fiducial limits in parentheses)	LC_{20} (fiducial limits in parentheses)	Regression equation $Y = a + bx$	Relative toxicity	SAFE (ppm)	SAR
2,4-D-DEE	24	4·851 (5·9–4·0)	10·02 (12·2–8·2)	2·625 (3·2–2·1)	2·8357 + 3·156x	—	—	—
	48	4·48 (5·0–4·0)	9·521 (11·6–7·8)	2·379 (2·9–1·9)	3·02135 + 3·0379x	—	—	—
	96	4·18 (4·5–3·7)	8·662 (10·7–6·9)	2·270 (2·7–1·9)	3·0312 + 3·167x	339·2	0·003616	0·015
2,4-D-Acid	24	10·21 (11·9–9·3)	14·25 (16·5–12·8)	7·703 (8·8–6·6)	−1·933 + 0·8699x	—	—	—
	48	9·616 (10·5–8·8)	14·29 (16·9–12·1)	6·88 (7·8–6·0)	−0·6881 + 5·786x	—	—	—
	96	8·356 (11·0–6·3)	14·76 (19·3–11·3)	5·168 (6·9–3·0)	1·2854 + 4·028x	169·7	0·0203	0·1695
2,4-D-DEA	24	448·7 (449–403)	645·1 (742–560)	292·2 (355–241)	−9·1599 + 5·3394x	—	—	—
	48	394·9 (438–356)	544·1 (623–475)	270·3 (322–227)	−10·6877 + 6·0421x	—	—	—
	96	375·8 (398–334)	533·4 (615–462)	248·4 (309–199)	−9·2398 + 5·5301x	3·77	0·0585	21·9763
2,4-D-Na	24	2971 (3428–2575)	4903 (6101–3940)	1644 (2017–1129)	−8·43813 + 3·8694x	—	—	—
	48	2348 (2742–2011)	3416 (3886–3002)	1509 (2017–1129)	−12·4393 + 5·1726x	—	—	—
	96	1418 (1805–1006)	2423 (2920–2001)	744·6 (1577–3612)	−6·44 + 3·6315x	1	0·0133	18.9128

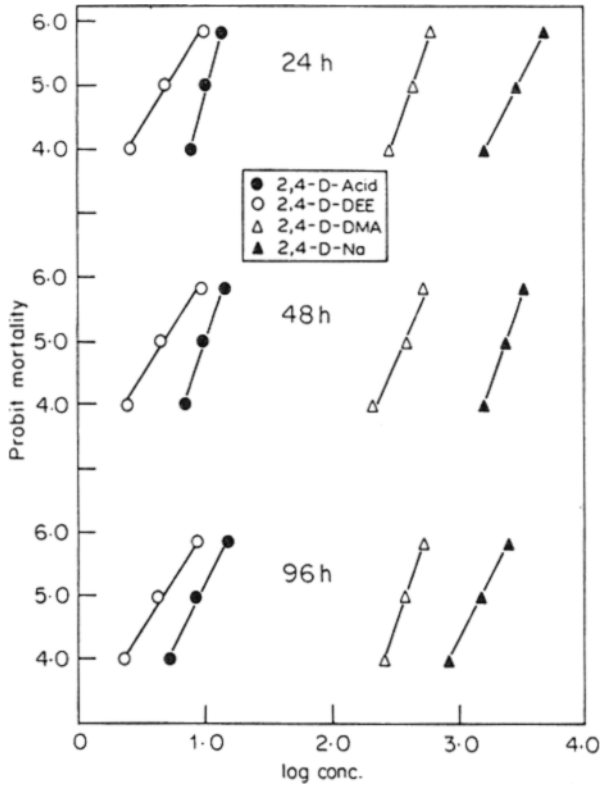

Figure 1. Probit regression lines for 24, 48 and 96 hour exposure toxicity of 2,4-D acid 2,4-D-DEE, 2,4-D-DMA, 2,4-D-Na to the fish *Lebistes reticulatus*.

are quite akin in their safe application rates. The latter is least toxic and is numbered 1 in relative toxicity. Its LC_{50} values are 2971 to 1418 mg l^{-1} for 24 and 96 hr respectively (table 1). Safe application rate is 19 mg l^{-1} which is very close to that of amines. Probit regression lines showing toxicity relations amongst various 2,4-D compositions at different exposures are shown in figure 1.

Relative toxicity has been described by Holden (1964) for many herbicides when applied at agricultural rates *viz.*, 2,4-D = 1, MCPA = 1·5, 2,4,5-T = 0·5, Paraquat = 1/12, Simazine 1/27, Diquat = 1/40, Dalapon = 1/46. Pravada (1973) categorised herbicides as most toxic, median toxic, moderately toxic and least toxic.

Induction of abortion in a viviparous fish like *Lebistes* (*Poecilia*) by herbicidal toxicity appeared symptomatic. In the present study, this occurred in every carrying female at all the dose levels. The progeny was premature and hence did not survive.

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