

THE EFFECT OF TWO PESTICIDES DICHLORVOS AND PARAQUAT ON *HETEROBRANCHUS LONGIFILIS* (Valenciennes)

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(Received 22 July 2002; Revision accepted 9 September 2003)

ABSTRACT

Ninety-six (96) - hour bioassays were carried out to determine the toxicity of two pesticides, Dichlorvos and Paraquat on juveniles of *Heterobranchus longifilis*. Various concentrations in (mg/l) of 1.20, 1.40, 1.60, 1.80, 2.00 and a control were tested for Dichlorvos; while 4.30, 6.40, 8.50, 10.60, 14.90, 17.00 and a control for Paraquat. 96 hrs LC₅₀ at 95% confidence limit in mg/l were 1.32 for Dichlorvos and 7.67 for Paraquat. *Heterobranchus longifilis* was more susceptible to Dichlorvos. Manifestation and survival times of the fish for each toxicant were not size dependent, rather they were concentration dependent, decreasing with increasing concentrations. Fading of colour of fishes, mucous secretion from gills and mouth, as well as, the expansion and swelling of the ventral surfaces of the opercular region were observed. Skin peeling was noticed in specimens treated with Paraquat. Quick burst of erratic swimming and general loss of equilibrium, darting movement and overturning, time increased with increase in concentrations. The data obtained suggest that both pesticides are environmentally unfriendly and should be used with utmost restraint, especially in the aquatic environment where they could be hazardous to both aquatic life and man.

KEY WORDS: Dichlorvos, Paraquat, Manifestation, Mortality, *Heterobranchus longifilis*

INTRODUCTION

Agriculture is a basic industry for production of food supply. Its activities are the sources of several types of pollutants. These include pesticides (including herbicides, insecticides, fungicides, rodenticides, growth regulators, defoliants and others), mineral, nutrients, salts and radionuclides from fertilizers. Some of these are minor and of little toxicology significance, where as, others are of major importance (Sheets, 1980).

A large number of chemicals are possible materials for accidentally or intentionally release into the environment. Their importance as potential pollutants will depend upon their rate of release, persistence in the environment; or in organisms and toxicity. The accumulation of pesticides in systems and organisms is primarily a function of water and lipid solubility, sedimentation and binding to inorganic or organic substances (Sheets, 1980). The toxicity of many industrial chemicals is partially known in humans, largely because of potential exposure of labour force, but the effects especially the chronic ones are poorly known on the vast majority of organisms both terrestrial and aquatic (Guthrie and Perry, 1980).

Pesticides are chemicals designed to destroy undesirable plants and/or aquatic pests. Once in the aquatic environment, they have considerable impact on animal life and large amount of these in water can have catastrophic

effects (Muirhead - Thompson, 1980).

The pesticides of concern are Dichlorvos which is an insecticide that acts via the gas phase due to its relatively high vapour pressure (BMZ, 1995). It inhibits acetylcholinesterase and hence impairs the central nervous system. It also inhibits the respiratory system, (Wirth, 1981) but does not represent a genetic risk. Dichlorvos being an organophosphate insecticide has residual problems similar to those of halogenated insecticides which are persistent in the environment, bio-magnifying in organisms along the food chain. The other pesticide of interest is Paraquat (dichloride) which is a non-selective contact herbicide used in particular cultivation of fruits and vines to destroy weeds directly before cultivating useful plants. Paraquat is toxic and damages the liver, kidney and lungs. It also has a caustic effect on the skin, mucous membrane and conjunctiva (BMZ, 1995). If Paraquat is not subject to sorption, it is rapidly degraded by micro-organisms and becomes only a low risk of ground-water pollution. There is also no accumulation by way of food chain.

However, *Heterobranchus longifilis* is of high commercial importance and readily available in the fresh water fisheries of Nigeria, hence it was used for this study, to investigate the LC₅₀ of these pesticides- Dichlorvos and Paraquat.

MATERIALS AND METHODS

One hundred and sixty specimens of *Heterobranchus longifilis* were procured from the University of Calabar fish farm. The fishes were collected using scoop net and immediately transported at dawn to the laboratory in plastic buckets containing pond water. The standard lengths of the fishes were measured using a measuring board, while the weights were recorded according to the method described by Lagler (1978), using a top loading mettler balance. 160 fishes of approximately uniform lengths in (cm) which are 9.69 ± 0.23 and weight in (g) 8.41 ± 0.35 were used at each concentration to avoid errors due to weight effect in the interpretation of results.

The fish were acclimatized to a laboratory condition for two weeks in a 96cm x 49cm glass tank filled to an 8cm mark with habitat water. Water parameters such as temperature (25.0

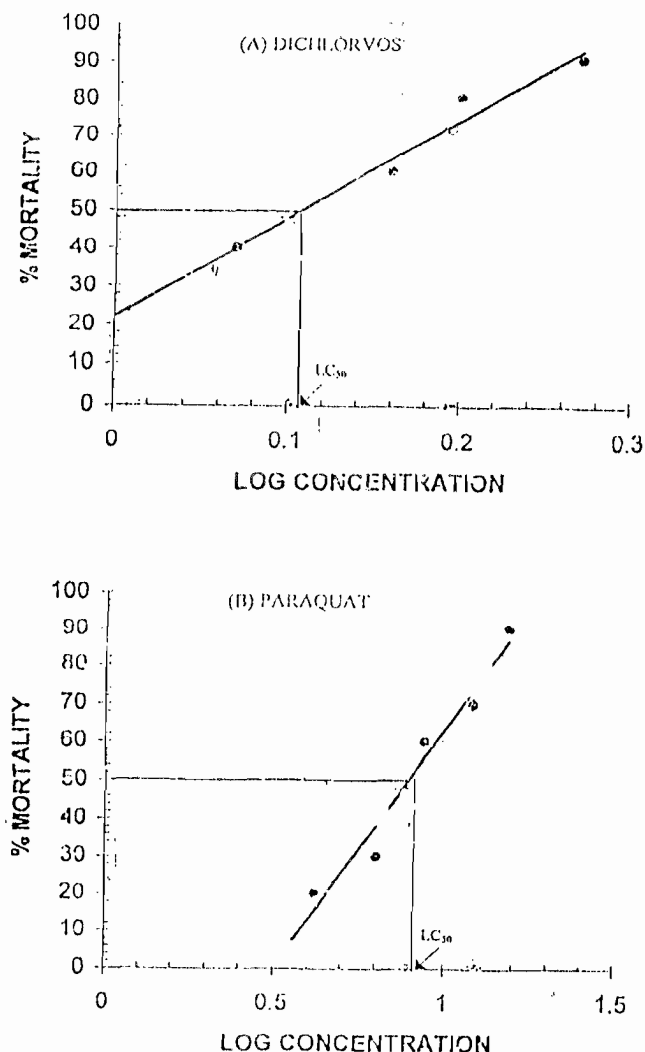


FIG. 1: Percentage mortality of *H. longifilis* against concentrations of (a) Dichlorvos and (b) Paraquat (96 hr LC₅₀).

-26.5°C) and a mean pH of 7.6 ± 0.5 were closely monitored. These values ranged within tolerance limits of warm water fish species (Boyd, 1979).

Fish were fed with a pelleted diet made up of muscle of other fishes, clams, earthworms, maggots and crump of bread and cake during acclimation. Acclimation water was changed at intervals of 3 – 4 days for two weeks to avoid contamination by faecal matter and other metabolites (Jones, 1964). Dissolved oxygen was maintained at near saturation level (5.8 mg/l) by aerating with a mechanical pump aerator. Water analysis were carried out using standard methods of analysis for determination of oxygen and pH (Boyd, 1979).

Preliminary tests were conducted for both chemicals for 24hrs to determine the concentration range to be used for actual test. A wide range of concentrations were tested including some which killed all the organisms within 24hrs, while some did not kill any of the fish within the same period, and others showed partial mortality. Following this, appropriate and closely spaced concentrations were selected for both Dichlorvos and Paraquat. This was done since the experiment was not designed for chronic exposure.

Short-term static toxicity test (Rao and Dad, 1979) was used to determine the 96 hr LC₅₀ of Dichlorvos and Paraquat on *Heterobranchus longifilis*. A series of five spaced concentrations and a control were tested in each case. Tests were conducted in test tanks which were of same size with holding tanks containing 47 litres of test substances. 10 fish per tank were used for each test concentration including the control. Fish were randomly assigned to test vessels, each of which was gently aerated to maintain dissolved oxygen level above saturation level. Temperature and pH were monitored regularly. Observation of the state of the fish in both the test and control tanks were made at fixed intervals of 1, 3, 6, 12, 18, 24, 48, 60, 72, 84, 96 hrs and a record of the number of fish alive, overturned and death in each concentration was noted. Dead fishes were removed as soon as death occurred. Fish was certified dead when opercular movement ceased, and it failed to respond to mechanical stimulation (Rao, and Dad, 1979).

Gross external changes in fish as well as behaviour in the different concentrations of the test chemicals were carefully noted.

RESULTS

A graph of cumulative percentage mortality against time (in log units) for each

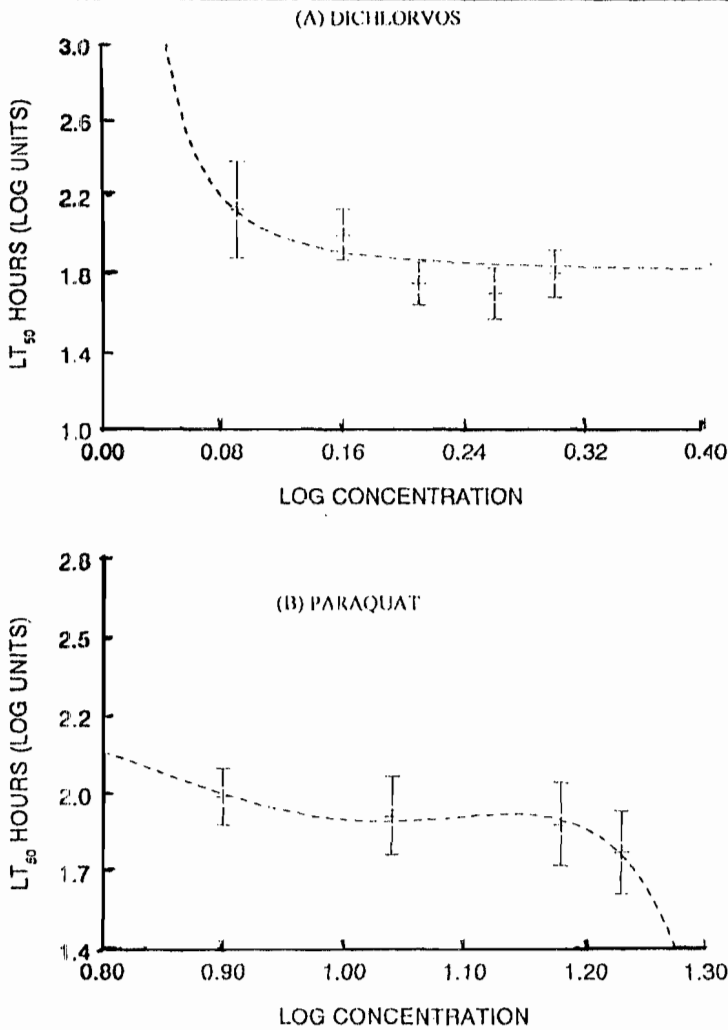


FIG 2: Median survival times of *H. longifilis* and their 95% confidence limits against concentrations of (a) Dichlorvos and (b) Paraquat (Toxicity curves)

concentration was plotted. Lines of best fit were drawn for the pesticides to show LC₅₀ for 96 hours (Fig 1). Similarly graphs of percentage survival against concentrations (in log units) were plotted (Fig. 2). The LC₅₀ was determined by monographic method at 95% confidence limits (UNEP, 1989).

Manifestation time, the time interval between the initial exposure of fish to poison and the appearance of fish reaction to it was observed to decrease with increase in concentration of both toxicants (fig. 3). A linear pattern was obtained or Dichlorvos while that of Paraquat tended to be curvilinear tending towards an asymptote at 17.0 mg/l concentration. This value was the concentration that showed the highest survival time of 72hrs of this fish in Paraquat as compared to 2.0mg/l in 71hrs in Dichlorvos. This showed that Dichlorvos has more adverse effect on the fish than Paraquat.

The effect of the toxicants was demonstrated by behavioural response of the fish in different concentrations of the toxicants. The behavioural responses included a quick burst of erratic swimming, followed by movements of quiescent behaviour, darting, swimming, upside down and general loss of equilibrium. Fish exposed to the toxic solution also frequently darted to water-air inter-phase of the test tanks to gulp air and then dive down to the bottom. The frequencies of these behaviours were observed to increase with increased concentrations.

The percentage survival time of the fish was not size dependent rather it was concentration dependent (Table 1).

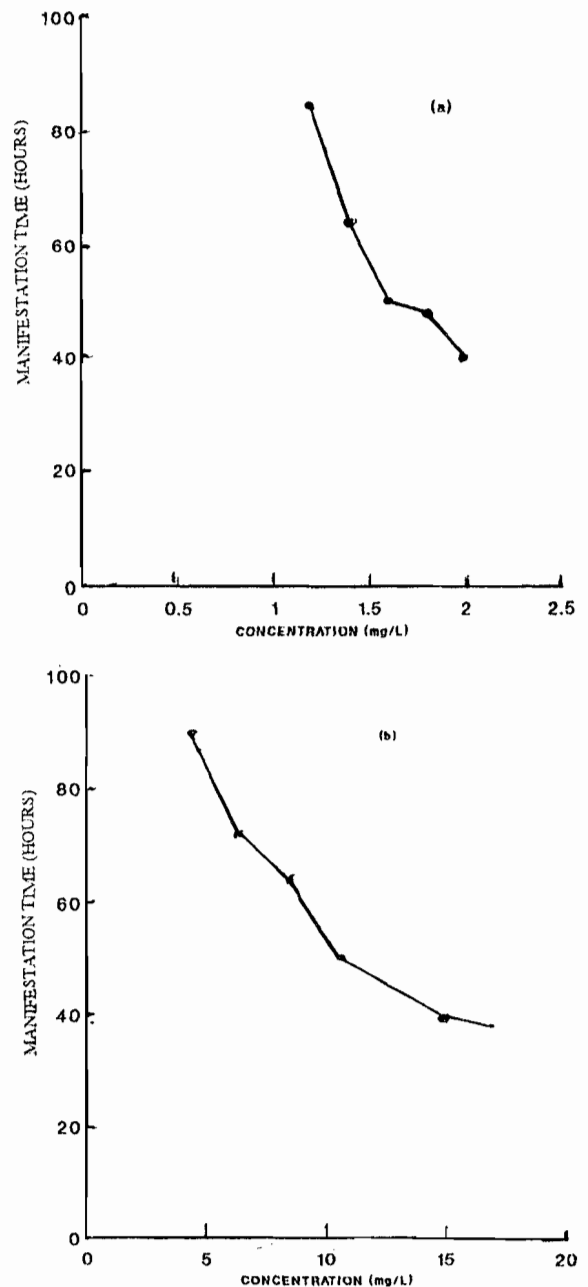


FIG. 3: Manifestation Time (hours) of *H. Longifilis* Against Concentrations for (a) Dichlorvos and (b) Paraquat.

TABLE 1: PERCENTAGE SURVIVAL TIME OF FISH AT DIFFERENT CONCENTRATIONS OF DICHLORVOS AND PARAQUAT.

PESTICIDE	Concentration mg/l	Mean standard length (cm)	Mean standard weight (g)	Percentage survival
DICHLORVOS	1.2	9.74 ± 0.53	8.72 ± 0.47	60
	1.4	9.09 ± 0.41	7.88 ± 0.50	40
	1.6	9.20 ± 0.02	7.90 ± 0.50	20
	1.8	9.38 ± 0.14	8.90 ± 0.26	10
	2.0	9.14 ± 0.33	8.92 ± 0.48	0
PARAQUAT	4.3	10.60 ± 0.06	8.76 ± 0.42	80
	6.4	10.40 ± 0.20	8.54 ± 0.02	70
	8.5	9.60 ± 0.17	8.80 ± 0.15	40
	10.6	8.88 ± 0.45	7.46 ± 0.57	30
	14.9	9.64 ± 0.14	7.90 ± 0.50	10
	17.0	10.90 ± 0.07	8.90 ± 0.46	0

The 96hr LC₅₀ of 7.67 mg/l obtained for Paraquat was much higher than the 1.32mg/l obtained for Dichlorvos which showed to be more active in small amounts (Table 2).

Other effects like fading of colour of fishes in test tanks, mucous secretion from gills and mouths of toxicated fishes, spreading of fins, gaping of mouth, as well as, the expansion and swelling of the ventral surface of the opercular regions were observed. Toxicated fishes in tanks treated with Paraquat showed skin peeling.

No mortality was recorded in the controls of both pesticides. 96hr LC₅₀ values with 95% confidence limits for *Heterobranchus longifilis* exposed to various concentrations of Dichlorvos and Paraquat are listed in Table 2.,

DISCUSSION

Following the exposure of fish to the toxicants, the initial excitation of fast, erratic and unco-ordinated swimming was observed among test fish. This was usual with all fish reactions to toxicants (Jones, 1964). These reactions were followed by increase in the rate of opercular beats. The opercular beats reached their maximum at about the time the fish were overturning. Essiet (1980) cited by Eni (1985) opined that increase in operculation could be correlated with reduced dissolved oxygen level in the test solutions. This explanation seems unlikely to apply to this study due to the short time span between introduction of fish into the test solution and the manifestation of increased

TABLE 2: 96HR LC₅₀ VALUES WITH 95% CONFIDENCE LIMITS FOR *HETEROBRANCHUS LONGIFILIS* EXPOSED TO VARIOUS CONCENTRATIONS OF DICHLORVOS AND PARAQUAT.

Fish Used	Toxicant	Calculated 96hr LC ₅₀ (Mg/l)	95% Confidence Limits (Survival)	
			Upper	Lower
<i>Heterobranchus longifilis</i>	Dichlorvos	1.32	1.78	1.15
<i>Heterobranchus longifilis</i>	Paraquat	7.67	7.67	5.79

operculation. The explanation by Hughes (1976) appears to be tenable in this study. The author reported that sapotoxins act by altering the surfaces which prevent fish from breathing properly. The decrease in manifestation time with increased toxicant concentration has been a general pattern (Eni, 1985).

The lethargy and general weakness which followed the out-burst of activity could probably have been due to the suppression of breathing by toxicants leading to tissue hypoxia, indicated by decrease in operculation. This resulted in the accumulation of poisons, which finally led to the disruption of nerve and muscle tissue functions (Perry and Conway, 1977). This effect was more pronounced in a fishes exposed to higher concentrations of Dichlorvos, and could be due to the fact that the contact insecticide inhibits acetylcholinesterase and consequently impairs the normal functioning of the central nervous system (Wirth, 1981).

Following the toxicity tests, there was fading colouration of the skin of the fish especially in the Paraquat tests. Fading in skin colour of fish has also been reported elsewhere (Eni, 1985). Paraquat is toxic and damages kidneys, liver and lungs. Besides, in aqueous form, it is a non-selective contact herbicide, which has caustic effect on skin, mucous membrane and conjunctiva (BMZ, 1995).

The observed increase in mucous secretion by the gills, mouth and expansion of ventral surface of opercular regions of fish exposed to higher concentrations of both toxicants could be due to irritations caused by these toxicants. Jones (1964) also reported these observation and attributed the symptoms to asphyxiation.

Similarly changes have also been reported by other authors and appear to be typical in fish exposed to pesticides (Walsh *et al.*, 1975); (Cruz *et al.*, 1983).

The results of this study show that Dichlorvos is more toxic. The 96hours LC₅₀ with 95% confidence limits for both pesticides were considered significantly different since their confidence limits did not overlap (APHA, 1980). The observed difference in tolerance or survival of the test fish in both toxicants may not be attributed to biological variations since the same species of fish and of similar size (length and weight) were exposed to the same experimental conditions. Perhaps these observed difference could be traced to the concentration of the active principles of the respective toxicants and the mode of action of both toxicants which differ (BMZ, 1995). The results finally show that the two pesticides are environmentally active, so users of the chemicals should exercise restraint

in their use, particularly on water bodies and farmlands which are based near aquatic sources or where intensive irrigation is practiced.

It is therefore recommended that the levels of these toxicants should not exceed 10% of their 96hr LC₅₀. This 10% corresponds to the Maximum Allowable Toxicant Concentration (APHA, 1980) which is used in environmental quality standards for chemicals and effluents. This is necessary because chronic exposures not only affect fecundities of fish, it also affects their growth as well as their palatability.

REFERENCES

- APHA, 1980. American Public Health Association. Methods for the Examination of Water and Waste Water. American Water Works Association. Water Pollution Control Federation 6th ed. American Public Health Association, Washington D.C. 347pp.
- B.M. Z., 1995. Bundesministerium für wirtschaftliche Zusammenarbeit und Entwicklung. Environmental Handbook: Documented on Monitoring and Evaluating Environmental Impact. German Federal Ministry for Economic Cooperation and Development, 80pp.
- Boyd, C. E., 1979. Water Quality in Warm Water Fish Ponds, Alabama Agricultural Experiment Station, Auburn University, Auburn. 359pp.
- Cruz, E. E., Cruz, M. C. D. and Sunaz, N. A., 1988. Hematological and Histopathological Changes in *Oreochromis mossambicus* After Exposure to The Molluscicides Aquations and Brestan. Puffin, *et al* (eds.) In The 2nd Int. Symp. On Tilapia in Aquaculture (ICLARM Conf. Proc. 15: 623p.
- Eni, G. E., 1985. Some Observation on Ichthyotoxic Effects on *Tephrosia vogeli* (Hooker) on *Tilapia zilli* (Gervais) and *Clarias gariepinus* (Burchell). B.Sc. Thesis, University of Calabar, Calabar. 78pp.
- Essiet, S. N., 1980. Effects of Varying Concentration of Specific Water Pollutants on the Survival of Selected Cichlids. B.Sc. Thesis, University of Calabar, Calabar. 42pp.
- Gutherie, F. E. and Perry, J. J., 1980. Introduction to Environmental Toxicology. Elsevier New York. 484pp.
- Hughes, G. M. 1976. Polluted Fish Respiratory Physiology. In Effects of Pollutants on Aquatic Organisms. Vol. 2, edited by Lockwood, A.P.M. Cambridge University Press, Cambridge. 179pp.
- Jones, J. R., 1964. Fish and River Pollution. Butterworths, London, 29pp.

- Lagler, K. F., 1978. Capture, Sampling and Examination. In: *Methods for the Assessment of Fish Production in Freshwater*. (3rd edition) edited by Bagenal, T. B. Blackwell Scientific Publication, London, 47pp.
- Muirhead-Thompson, R. C., 1980. Resistance of Vectors of Diseases to Pesticides. *Pub. Health Rep.* 60: 753 – 774.
- Perry, J. W. and Conway, M. W., 1977. Retronone induced Blood Respiratory Changes in the Green Sunfish *Lepomis cyanellus*. *J. Comp. Biochem. Physiol.* 56: 123-126.
- Rao, K. S. and Dad, N. K., 1979. Studies of Herbicide: Toxicity in some Freshwater Fish and Ectoprocta. *Journal of Fish Biology.* 14: 517-522.
- Sheets, T. J., 1980. Agricultural Pollutants. In *Introduction to Environmental Toxicity*, edited by Guthrie, F. E and Perry, J. J. Elsevier, New York 2433pp.
- UNEP, 1989. United Nations Environmental Programme. *Testing the Acute Lethal Toxicity of Pollutants to Marine Fish and Invertebrates. Reference Methods For Marine Pollution Studies.* Kansas University Press, Kansas. No. 43 295 pp.
- Walsh, A. H. and Ribelin, W. E., 1975. *The Pathology of Fishes*, edited by Ribelin, W. E and Migaki, G. University of Wisconsin Press, Madison: 541pp.
- Wirth, J., 1981. *Environmental Handbook on the Composition of Pesticides and Their Effects on Aquatic Life and Man.* 635pp.