EFFECT OF "NUVAN" ON SOME BIOCHEMICAL AND PHYSIOLOGICAL PARAMETERS OF *LIZA PARSIA* (HAMILTON AND BUCHANAN)

DISSERTATION SUBMITTED BY
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C E R T I F I C A T E

This is to certify that this Dissertation is a bonafide record of work carried out by Shri. **Bikash Chandra Mohapatra** under my supervision and that no part thereof has been presented before for any other degree.

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CONTENTS

P R E F A C E

Pesticides are synthetic chemicals widely used for protecting crops from pests. Though their short-term benefits are undeniable they are considered as hazardous because of their interference with the environment. Pesticides wherever applied, ultimately find their way into water bodies affecting aquatic fauna especially the fish.

Acute toxicity test is only a first step in monitoring the effect of pollutants. Responses of fish to chronic stress are usually predicted from water quality standards (e.g., LC50 tests), life cycle toxicity tests or changes in single or a few biological variables. Such approaches may be generally acceptable for acute toxicity studies or for screening the effects of contaminants on a short-term basis, but they are less appropriate for providing information on integrated responses of fish to chronic environmental stress.

Pesticides interfere with carbohydrate, protein and lipid metabolism, ion transport, nerve conduction and the energy production at biomolecular levels due to uncoupling of oxidative phosphorylation. The effects are suggested to be mediated by adsorption into blood through respiratory surfaces, and then their distribution in different tissues resulting in numerous damaging interactions to the organism. The long staying effect of pesticides also causes serious damages to various organs in fishes. The degree of damage to the organs helps in determining the toxicity of pesticides. The

liver and kidney are the vital organs which easily get affected by the pollutants.

Besides contributing to the capture fisheries, mullets form one of the most highly cultured group of fishes. Infact, it is opined that the significance of mullet resources lies not so much in the existing capture fisheries, but in their potential as cultivable fishes for extensive and intensive fish farming. Among the mullets, along with Mugil cephalus, owing to its resistance to environmental changes Liza parsia gained considerable importance as a candidate species for aquaculture. On account of this and due to its economic value Liza parsia was selected as the test species in the present study.

The guideline in this work is to evaluate the acute toxicity range of "Nuvan", find out alterations if any in the biochemical composition in blood and muscle, and physiological study including histological disorders in kidney and liver tissue.

I wish to express my deep sense of gratitude to my supervising teacher, Dr.A. Noble, Principal Scientist, CMFRI, Cochin for his constant advice, guidance and wholehearted support through out the course of the study and in the preparation of the manuscript. I express my thanks to Dr. P.S.B.R. James, Director, CMFRI for the facilities provided. I am deeply indebted to Dr. A.D. Diwan, S-3 without whose help my work would not have been complete. I wish to express my sincere thanks to

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INTRODUCTION

The pesticides which drain into water bodies through rain, agricultural run-off, discharge of industrial effluents and dust storms over cultivated land and industrial areas cause degradation and disaster to the aquatic ecosystem. But considering the usefulness of the pesticides and since no other chemicals are found suitable to replace them, we cannot presently suggest a ban on the use of pesticides. Pesticides or the biocidal agricultural chemicals that include insecticides, acaricides, nematocides, rodenticides, herbicides and fungicides have got vast applicability in agriculture and forestry. Of these, insecticides which include organochlorines, organophosphates, thiocyanates and carbamates are the most widely used.

Organochlorine persists in the environment and accumulates in different tissues. Its use as pesticides in agriculture has given rise to criticism in recent years prompting to prefer organophosphates by most of the agriculturists. The water soluble organophosphate insecticide "Nuvan" is widely used in the Kolleru region of Andhra Pradesh for control of ectoparasites such as Lernea, Argulus, etc. (Muthu et al. 1988). But the long range effects of this practice are not known. The chemical "Nuvan Fish 500 EC" has been granted a U.K. Government product licence for use as medicine in salmon farming to treat against the sea lice (Anon. 1989). The use of these chemicals in salmon farming appears to have deleterious effects on marine invertebrates species (Egidius and Moester 1987). Stephanie Pain (1989) links the epidemic of eye disease in salmon of the wild to the use of "Nuvan 500 EC" in farms.

In general, levels of chemical pollutants are higher in coastal than offshore areas and still higher in those areas associated with intense human activities.

The backwaters and estuaries serve as nurseries for many organisms including several commercially important species of fishes and prawns. *Liza parsia* (Hamilton & Buchanan) a brackishwater species of economic importance inhabiting both the coasts of India (FAO 1984) spends majority of its life stages in estuarine condition. During this period it is subjected to the toxicity of several pollutants discharged into the environment; accidentally or directly. The level of toxic effects on fishes have been experimentally identified for organophosphate chemicals by Rao(1974), Anees (1975), Sailatha *et al.* (1981), Verma *et al.*(1982), Pal (1983) and Ravikumar and Gupta (1988).

Responses by individual organisms to changes in the environment, including pollutants can be measured as physiological and or biochemical events (Bayne 1986, Conner and Huggett 1988). The former may lack specificity but relate directly to the fitness of the individual. The later provides specificity and increased sensitivity but may be difficult to relate directly to traits of phenotypic fitness (Uthe *et al.* 1980). Together they are useful in assessments of environmental impact, and current toxicological research provides a cogent integrated frame-work for quantifying pollution damage.

Acute toxicity is rate in natural environment. Sometimes it occurs due to accidents of direct application of pesticides. Voluminous literature, on the other hand, is available on the acute toxicity of organophosphates

to fishes (Matton and LaHam 1969, Symons 1973, Shakoori et al. 1976, Konar 1977, Shaffi 1980, Qureshi et al. 1983, Rashatwar and Ilyas 1984, Pal and Konar 1985) and decapods (Eisler 1969).

In the long run, the sub-lethal concentrations may prove more deleterious than the lethal concentrations, because subtle and small effects on the fish may alter their behaviour, feeding habits, position in the school, reproductive success, etc. Subtle effects at the organ or cellular level may alter the metabolism of the fish and hence its ability to withstand stress. Even if the fish is not directly affected, any effect on its food organisms may result in a starved population of fish. The effects of sub-lethal exposure to organophosphates in relation to growth, behavioural, biochemical, histological and physiological alterations in the body have been studied for fish (Bull and McInerney 1974, Thomas and Murty 1976, Konar 1977, Mukhopadhyay and Dehadrai 1980, Ramalingam and Ramalingam 1982, Awasthi et al. 1984, Desai et al. 1984, Basha et al. 1984, Kumar and Alam Ansari 1986, Bashamohideen et al. 1987, Khillare and Wagh 1988), decapods (Shukla and Shukla 1985) and Lamellidens marginalis (Ahamed et al. 1978).

Recently much attention has been paid to evaluate the hazards of pesticides on the physiology of the non-target organisms both at lethal and more often, at sub-lethal levels. The symptoms generally involve respiratory distress, increase in the glycolytic rate, changes in oxidative metabolism, ionic concentration, enzyme activity, endocrine activity, osmoregulation, etc. As such respiratory changes are used as good indicators of stress

on fishes (Dalela et al. 1980a, and Murty 1986). Several studies have been done on respiratory metabolic disturbance, opercular movement, respiration rate, etc. of fish due to pollution stress. Some of the recent Indian contributions on this are Lingaraj and Venugopalan (1978), Singh and Singh (1979), Rao et al. (1980), Ramalingam and Srinivasa Rao (1982), Bakthavathsalam and Srinivasa Reddy (1983 and 1985), Patel and Saxena (1983), Basha et al.(1984), Jawale (1985), Pal and Konar (1985), Bashamohideen et al.(1987) and Singh et al. (1987).

Protein represents an enormous group of complex nitrogenous compounds having high molecular weight found in all living cells. Wherever they occur, they play vital role in the biological functions and serve as building blocks for cellular and organic structures. The plasma proteins help in the maintenance of acid-base balance and osmotic pressure of the body fluids. Plasma protein, being a source of nutrition to tissue proteins, establishes a dynamic equilibrium with the proteins of the tissues. In condition of protein deficiency tissue proteins are broken down to maintain the plasma protein level (Shanmugam 1977). There are reports on the changes induced by pollutants on protein content of serum (Chitra and Ramana Rao 1980, Saxena and Mani 1985, Rai 1987), muscle tissue (Panigrahi and Mishra 1980, Ramalingam and Ramalingam 1982, Verma and Tonk 1983, Sashikala et al. 1985, Sastry et al. 1987, and Parveen et al. 1987), liver and kidney (Dubale and Shah 1981a, Dubale and Awasthi 1982, Verma and Tonk 1983, Awasthi et al. 1984, Kumar and Alam Ansari 1986, and Parveen et al.1987). Mustafa (1977) has studied the effect of maturation on the

muscle protein content of the fish. Ahamad et al.(1978) have studied the effect of malathion,on the mantle protein content of Lamellidens marginalis.

The magnitude of change in total free amino acids of fish has been studied by several workers in relation to different concentrations of chemicals and exposure periods in blood (Mukhopadhyay and Dehadrai 1980, Dabrowska and Walasow 1986), muscle tissue (Sashikala et al. 1985) and liver (Kumar and Alam Ansari 1986).

Phosphatase comprises of a large group of hydrolysing enzymes involved in the process of digestion and intermediary metabolism. They are present in the blood as alkaline phosphatase and acid phosphatase. The alkaline phosphatase has an optimum pH of about 9 formed mainly in the bones and in the liver, responsible for membrane transport. The acid phosphatase, a lysosomal enzyme has an optimum pH of 4.9 present in plasma in small amounts and also in liver and red cells. During stress the creatine phosphatase in the skeletal muscle breaks down resulting in the release of phosphoric acid and the glycogen present in the tissues gets oxidised to lactic acid (Shanmugam 1977, Sastry and Siddiqui 1983, Govindan 1985, and Sastry et al. 1987). Both the acids mentioned above constitute to a rapid lowering of the pH value of the tissue fluids thus augmenting the process of acid phosphatase formation in the blood. There are several reports by many investigators on the changes induced by pesticides on alkaline phosphatase and acid phosphatase contents of serum (Dalela et al. 1980b , Sharma et al. 1982, Gupta and Dhillon 1983, and Verma et al. 1984), liver

and kidney (Thomas and Murthy 1976, Sastry and Gupta 1978b,c, Sastry and Agrawal 1979, Sastry 1979, Sastry and Malik 1979, Shaffi 1980, Dubale and Shah 1981a, Dubale and Awasthi 1982, Kumar and Alam Ansari 1986 and Sharma and Maya 1987), brain (Sastry and Sharma 1980 and 1981, and Shaffi 1980), intestine (Lata and Sriwastwa 1983, and Arora and Kalshrestha 1985), and muscle (Shaffi 1980) of fish. However, reports on the enzyme contents of decapods are a few (Shukla and Shukla 1985 and Omkar 1986) only.

Ribonucleic acid is present largely in the cytoplasm of actively growing cells which are engaged in the production and secretion of protein. The concentration of RNA increases with increase in protein synthesis or vice versa. DNA is present always in the nuclei of cells and is confined to the chromosomes. A very small amount is present in mitochondria, which are outside the nuclei. Altered DNA and RNA content in muscle of fish exposed to various pollutants have been noted by Mustafa (1977), Dubale and Shah (1981a), Kabeer Ahamad et al. (1981), Denizeau and Marion (1984), Stueber and Zahn (1985), and Kumar and Alam Ansari (1986). Recently the RNA-DNA ratio is used as a sensitive index of toxicant stress in relation to growth (Bulow 1970, Dagg and Littlepage 1972, Kearns and Atchinson 1979 and Barron and Adelman 1984).

Several surveys and experimental studies have identified histological disorders in liver and kidney associated with pollutant exposure in fish which serve as indicator of pollution (Mukharjee and Bhattacharya 1975,

Bass et al. 1977, Konar 1977, Sastry and Gupta 1978a,b, Sastry and Malik 1979, Goel and Garg 1980, Dubale and Shah 1981a,b, Kumar and Pant 1981, Ramalingam and Reddy 1981, Sultan and Khan 1981, Akhilendra Naidu et al. 1983, Bakthavathsalam et al. 1984 and 1987, Desai et al. 1984, Rashatwar and Ilyas 1984, Radhaih et al. 1986, Razani et al. 1986, Gupta and Dalela 1987, Mukhopadhyay et al. 1987, Ram and Satyanesan 1987 and Bhatnagar et al. 1987).

The present investigation was undertaken to study the effect of "Nuvan" on the physiology, biochemistry and histology of Liza parsia.

MATERIALS AND METHODS

Acute toxicity tests were conducted to determine the LC50 values of "Nuvan" to Liza parsia.

Collection of test animals:

Liza parsia of 85-120 mm sizes and 6.50-13.25g weight were collected by cast nets from brackishwater canals of Puduvypeen area. The salinity, dissolved oxygen and pH of the collection site were determined to indicate the water quality into which the animals should be transferred on arrival at laboratory.

Acclimatization of the test animals in laboratory condition:

The live animals were acclimatized to laboratory condition by maintaining them in plastic pools of 2 tonne capacity containing water of salinity 10.0±1‰, pH 6.0±0.5 and temperature 27.5±1.5°C. To avoid fungal attack of test animals the medium was treated with 11 mg of malachite green per 100 litres of water. The organisms were fed once in a day. The faecal matter and other waste materials were daily siphoned off, and to reduce the ammonia content in water the biological filter was used. Once in two days the medium in the tank was changed. Electrically operated aerators were used for aeration. The test animals were acclimatized for about 2 weeks prior to the experiments.

Test medium:

The commercial grade "Nuvan" of Ciba-Geigy having the composition of "Dichlorvos 76% m/m, Emulsifier 10.6% m/m, solvent 13.4% m/m" was used for the preparation of stock solution. The desired concentration of test media were obtained by diluting the stock solution in distilled water.

Test containers:

Fibre glass tanks of 40 litre capacity were used as test containers. Each of the test containers was provided facilities for drainage of water from bottom and continuous aeration. Each container was covered with velon screen netting to prevent the animals from jumping out.

Selection of test concentrations:

A range-finding bioassay was conducted after APHA-AWWA-WPCF (1975) and Reish and Oshida (1987) with the test organisms exposed to a range of concentrations, in logarithmic scale such as 0.01, 0.1, 1.0, 10 and 100 ppm. Four animals were released to 32 litre of test solution per test container without feeding and water was changed during the tenure of the experiment. At intervals of 24, 48, 72 and 96 hr the percentage of mortality was recorded. As no mortality was observed at 0.1 ppm and 75% mortality at 1 ppm during 96 hr, the concentrations between 0.1 and 1.2 ppm were selected for bioassay procedure.

Bioassay procedure: [Plate I. a,b]

Static bioassay method (Reish and Oshida 1987) was used in which the organisms were kept in same test solution for the entire experimental

Plate I.a: Photograph showing experimental set-up for bioassay.

Plate I.b: A test container with experimental animals after 48 hours of exposure to 0.2 ppm "Nuvan" during bioassay.

Plate I.a

Plate I.b

period. Each bioassay consisting of a series of six test concentrations and a control were used. Each concentration was run in duplicate(APHA-*AWWA-WPCF 1975). To avoid contamination, the controls were maintained* away from bioassay tanks. As suggested in the method, the test animals were not fed during the experiment. The percentage of survival at the end of every 24, 48, 72 and 96 hr was accounted. Dead animals were removed from the experiment immediately.

The data obtained from experiments were processed by "probit analysis" (Finny 1952, Reish and Oshida 1987) for determination of LC50 value in computer. The percentage mortality verses log concentrations were plotted in probability papers to get the LC50 values graphically(Seegert et al. 1979). The slope function, 95% confidence limit and 95% fiducial limits (upper limit and lower limit) were calculated using the following formulae from "response curves" for different exposure times (Reish and Oshida 1987)

$$\text{Slope (S)} = \frac{\frac{LC84}{LC50} + \frac{LC50}{LC16}}{2}$$

$$95\% \text{ confidence limit } (f_{LC50}) = S\sqrt{\frac{2.77}{N}}$$

(Where N = total number of organisms tested at those exposure concentrations whose expected results were between 16% and 84%, and "2.77" is a constant)

95% fiducial limits are:

$$\text{Upper limit} = \text{LC50} \times f_{\text{LC50}}$$

$$\text{Lower limit} = \frac{\text{LC50}}{f_{\text{LC50}}}$$

The lethal concentrations were plotted against time in hours in nomograph paper to get the "toxicity curve" and corresponding 95% fiducial limits were shown for each LC50 values on graph paper.

Acute exposure studies:

The test animals were exposed to lethal concentration of 0.48 ppm (i.e., 96 hr LC50 concentration in the bioassay) at pH 5.5, salinity 10.0± 1‰ and temperature 27.5 ± 1.5°C in three tanks. Simultaneously controls were maintained. The test solutions were not renewed and animals both in experiments and control tanks were not fed. To each test container 10 animals were released. At the end of 24, 48, 72 and 96 hr exposure from each tank one specimen was taken for oxygen consumption estimation and subsequently sacrificed for biochemical analysis. Animals exposed for 96 hr were used for histological studies also.

Acute exposure studies on lethal concentrations of 1.01 (24 hr LC50), 0.75 (48 hr LC50), 0.55 (72 hr LC50) ppm for 24, 48 and 72 hours respectively against controls were conducted with 8 animals each released to ensure at least 3 animals after the exposure period for physiological, biochemical and histological studies.

Chronic exposure studies:

Three sub-lethal concentrations (1/5th, 1/10th and 1/15th of 96 hr LC50) were selected for chronic exposure studies and 3 sets of each concentration and control were maintained. Total 12 tanks of 40 litre capacity each were maintained with 4 animals in each. The animals were fed with pellet feed once a day and the test media were kept well aerated. Half of the test media from each test container was replaced every two days through the drainage pipe provided at the bottom of container. After 15 days of exposure the test organisms were removed from one control tank and one tank of each concentration. They were put in respirometer for oxygen consumption estimation and later sacrified for biochemical and histological study. The same procedure was repeated after 30 and 45 days with other animals.

Physiological study:

The oxygen consumption of fishes subjected to lethal and sub-lethal concentrations of "Nuvan" were studied (Grothe and Eaton 1975) by using 4 litre rectangular glass jar and liquid paraffin to seal the exchange of gas between the water of respirometer and atmosphere. In respirometer, water of same salinity was used to avoid stress on the experimental animals. Utmost care was taken to avoid stress while handling the fishes and to recover them from the effects of handling if any, they were kept in respirometer for sometime (Fry 1967). Dissolved oxygen in water samples (125 ml each) collected just before and after the experiment from respirometer were estimated by using unmodified Winkler technique (APHA, 1955). After

each experiment the individual weight of fishes was recorded. The oxygen consumption was calculated based on following formula:

$$(\text{Initial } DO_2\text{-Final } DO_2) \times \frac{\text{Water vol. in ml}}{1000 \text{ ml}} \times \frac{1000 \text{ gm}}{\text{Weight in gm of fish}} \times \frac{60 \text{ minutes}}{\text{time in minutes}}$$

The values were expressed in "mg/Kg body wt/Hour."

Biochemical studies:

1. Extraction

In biochemical estimation, test animals subjected to lethal and sub-lethal concentrations were wiped free of water. Blood was removed by directly puncturing the heart with a hypodermic needle attached to a 2 ml syringe, and transferred to a 15 ml glass centrifuge tube corked air tight so as to avoid any kind of haemolysis. It was than allowed to clot at room temperature for 40-60 minutes. After clotting, it was centrifuged at 3000 rpm for 15 minutes following Mahobia (1987). The clear serum was transferred by micropipettes to 5 ml bottles and frozen in sealed condition at 0°C till chemical analysis was performed (NewComb 1974).

The belly muscle tissue was dissected out and kept in deep freezer for the biochemical analysis (Sastry and Siddiqui 1983).

2. Analysis

The methods used in the analysis of biochemical parameters were as follows:

I. The protein content of muscle tissue and blood serum was estimated by the Biuret method (Gornall et al. 1949). Bovine serum crystals dissolved in 1N NaOH was used as the standard solution in the preparation of standard graph.

II. The total free amino acids of muscle tissue and blood serum were estimated with ninhydrin (Yemm and Cocking 1955) using glycineand glutamic acid mixture as the standard.

III. DNA and RNA in muscle tissue were estimated as per the scheme given by Dagg and Littlepage (1972). Nucleic acids were extracted using cold perchloric acid solution. The optical density of DNA and RNA were measured by using double beam spectrophotometer at 595 and 665 nm respectively. Purified and high polymerised Calf-thymus DNA and purified yeast RNA obtained from SIGMA were used as standards for DNA and RNA estimation respectively.

IV. The alkaline and acid phosphatases activity were determined following the procedure of Barret (1972). P-nitrophenyl phosphate (PNP) was used as substrate and in the experiment the enzymatic reaction was stopped by 10% cold trichloroacetic acid. Potassium dihydrogen orthophosphate (KH_2PO_4)was used as standard.

Standard deviation, percentage coefficient of variation for samples of same treatment, and percentage variation of parameters in exposed animals from control were calculated. Using student 't' test the significance

of difference between two sample mean was tested for acute exposure biochemical parameters. The 'F' test was worked out for testing the significance among the samples of chronic exposure.

Histological studies:

The liver and kidney of test animals exposed to lethal and sub-lethal concentrations were used for histological studies. The tissues were fixed in Bouin's fluid for about 24 hours and then processed by routine histological techniques. Sections of 4-5 μ were stained with haematoxylene and eosine and mounted in DPX. Photomicrographs of sections were taken using an Olympus Universal Research Microscope.

R E S U L T S

1. Acute toxicity studies:

The results of acute toxicity studies expressed in terms of LC50 values for 24, 48, 72 and 96 hours obtained in computer analysis are given in Table 1A-1D and Fig.1A-1D. The LC50 values are found to be 1.01 ppm, 0.75 ppm, 0.55 ppm and 0.48 ppm for 24, 48, 72 and 96 hours respectively showing gradual decrease with increase of time. The 95% fiducial limits of LC50, LC16, LC84 of each response curve for different exposure periods in hours given in Table 1E also show decreasing order. The 95% confidence limit and slope function shown against each LC50 value in Table 1E have overall decreasing trend except for a slight increase in 48 hr LC50 visible clearly from acute toxicity curve in Fig. 1E.

However, the graphical values of all LC50 were tallying well with the results obtained by computer analyses. Among different concentrations of LC50(Table 1E) the values showed variations. All these results were obtained at salinity 10.0 ± 1‰, temperature 27.5 ± 1.5°C, and pH 5.5 of test solution.

2. Biochemical studies:

2.1. Studies on muscle

2.1.1. Protein content

2.1.1.1. On acute exposures

From the Table 2A and Fig. 2A it is seen that there is gradual

TABLE - 1A [Computer analysis]

Test for 24 hour lethal concentration

(Salinity 10.0 ± 1‰, Temperature 27.5 ± 1.5°C, pH 5.5)

Concentration (ppm)	Log concentration	Animals released	Mortality in number	Percentage mortality	Response	Emperical probit
0.2	-0.69897	16	0	0	0	-
0.4	-0.39794	16	2	12.50	0.13	3.87
0.6	-0.22185	16	4	25.00	0.25	4.33
0.8	-0.09691	16	6	37.50	0.38	4.69
1.0	0	16	7	43.75	0.44	4.85
1.2	+0.07918	16	10	62.50	0.63	5.33

't' value = 3.182; $(Chi)^2$ = 7.815; 24 hr LC50 = 1.014725 ppm; ± Standard deviation.

TABLE - 1B [Computer analysis]

Test for 48 hour lethal concentration

(Salinity 10.0 ± 1‰ , Temperature 27.5 ± 1.5°C, pH 5.5)

Concentration (ppm)	Log concentration	Animals released	Mortality in number	Percentage mortality	Response	Emperical probit
0.2	-0.69897	16	2	12.50	0.13	3.87
0.4	-0.39794	16	4	25.00	0.25	4.33
0.6	-0.22185	16	5	31.25	0.31	4.50
0.8	-0.09691	16	9	56.25	0.56	5.15
1.0	0	16	10	62.50	0.63	5.33
1.2	+0.07918	16	11	68.75	0.69	5.50

't' value = 2.776; $(Chi)^2$ = 9.488; 48 hr LC50 = 0.7501091 ppm; ± Standard deviation.

TABLE - 1C [Computer analysis]

Test for 72 hour lethal concentration

(Salinity 10.0 ± 1% , Temperature 27.5 ± 1.5°C, pH 5.5)

Concentration (ppm)	Log Concentration	Animals released	Mortality in number	Percentage mortality	Response	Emperical probit
0.2	-0.69897	16	2	12.50	0.13	3.87
0.4	-0.39794	16	5	31.25	0.31	4.50
0.6	-0.22185	16	7	43.75	0.44	4.85
0.8	-0.09691	16	11	68.75	0.69	5.50
1.0	0	16	13	81.25	0.81	5.88
1.2	+0.07918	16	14	87.50	0.88	6.18

't' value = 2.776; $(Chi)^2$ = 9.488; 72 hr LC50 = 0.554255 ppm; ± Standard deviation.

TABLE - 1D [Computer analysis]

Test for 96 hour lethal concentration

(Salinity 10.0 ± 1‰ , Temperature 27.5 ± 1.5°C, pH 5.5)

Concentration (ppm)	Log Concentration	Animals released	Mortality in number	Percentage mortality	Response	Emperical probit
0.2	-0.69897	16	2	12.5	0.13	3.87
0.4	-0.39794	16	6	37.5	0.38	4.69
0.6	-0.22185	16	9	56.25	0.56	5.15
0.8	-0.09691	16	13	81.25	0.81	5.88
1.0	0	16	16	100	1.0	-
1.2	+0.07918	16	16	100	1.0	-

't' value = 4.303; $(Chi)^2$ = 5.991; 96 hr LC50 = 0.482347 ppm; ± Standard deviation.

Fig. 1A: Response curve for "24 hr LC50"

LC84 = Anti Log (0.337) = 2.175 ppm.

LC50 = Anti Log (0.006) = 1.015 ppm.

LC16 = Anti Log (-0.330) = 0.468 ppm.

Fig. 1B: Response curve for "48 hr LC50"

LC84 = Anti Log (0.290) = 1.950 ppm.

LC50 = Anti Log (-0.125) = 0.750 ppm.

LC16 = Anti Log (-0.530) = 0.295 ppm.

Fig. 1C: Response curve for "72 hr LC50"

LC84 = Anti Log (0.030) = 1.072 ppm.

LC50 = Anti Log (-0.256) = 0.554 ppm.

LC16 = Anti Log (-0.550) = 0.282 ppm.

Fig. 1 D: Response curve for "96 hr LC50"

LC84 = Anti Log (-0.070) = 0.851 ppm.

LC50 = Anti Log (-0.317) = 0.482 ppm.

LC16 = Anti Log (-0.565) = 0.272 ppm.

Fig. 1 A

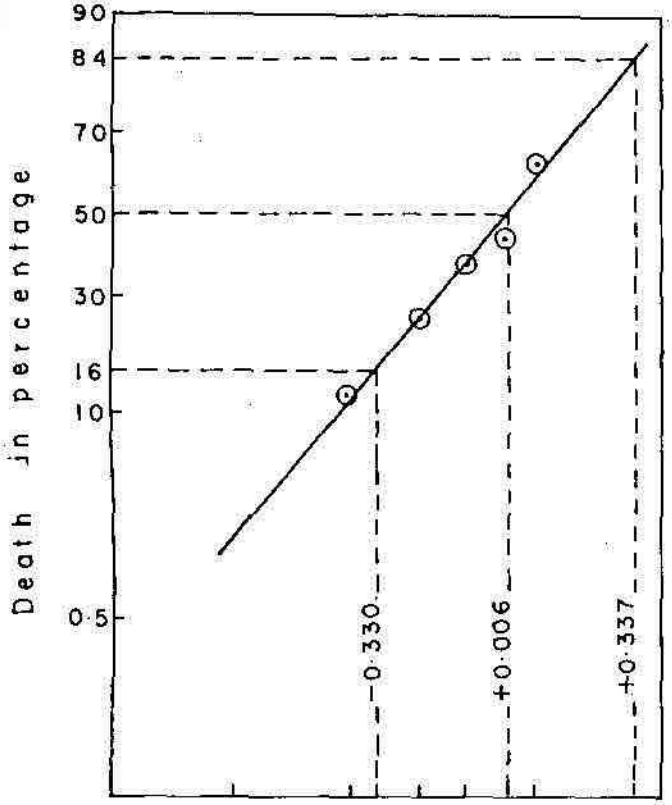
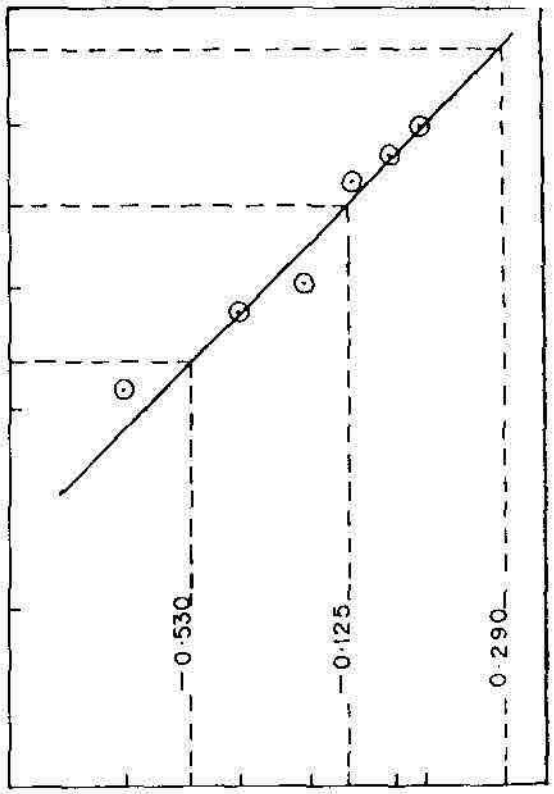


Fig. 1 B



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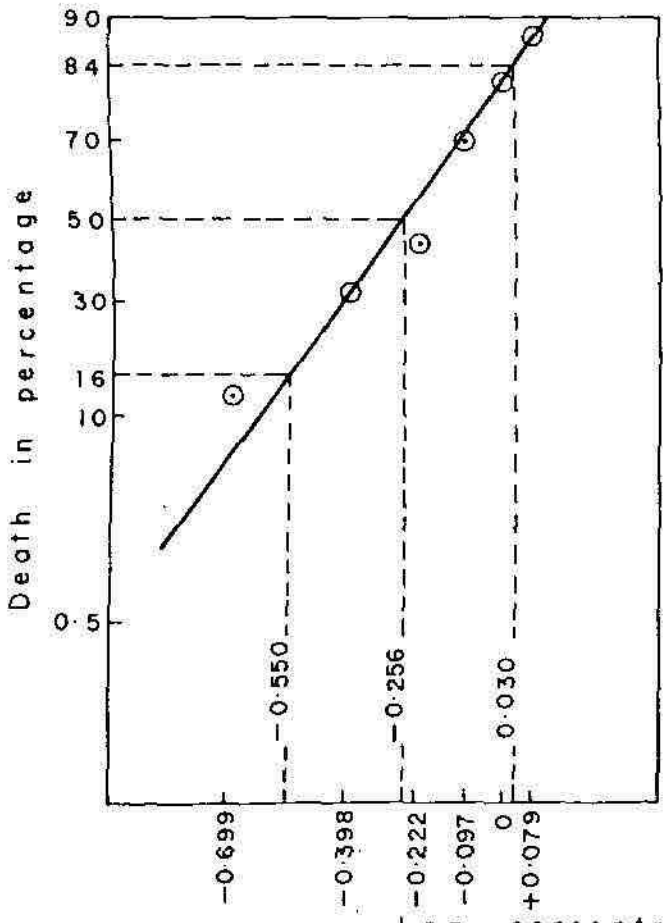


Fig.1 D

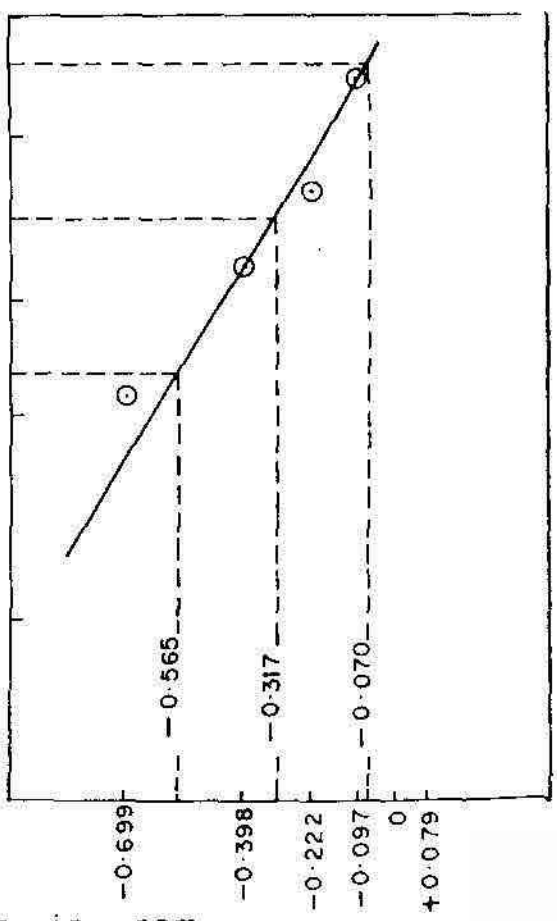


TABLE - 1E

Acute toxicity values of "Nuvan" to Liza parsia

(pH 5.5; salinity 10.0 ± 1‰ ; temperature 27.5 ± 1.5°C of test solution)

Exposure period (Hours)	LC50(ppm)	95% fiducial limits		LC16(ppm)	LC 84(ppm)	Slope function	95% confidence limit
		Upper limit (ppm)	Lower limit (ppm)				
24	1.015	1.324	0.778	0.468	2.175	2.157	1.305
48	0.750	1.005	0.560	0.295	1.950	2.571	1.340
72	0.554	0.698	0.440	0.282	1.072	1.950	1.260
96	0.482	0.606	0.384	0.272	0.851	1.768	1.256

± Standard deviation.

Fig. 1. E.

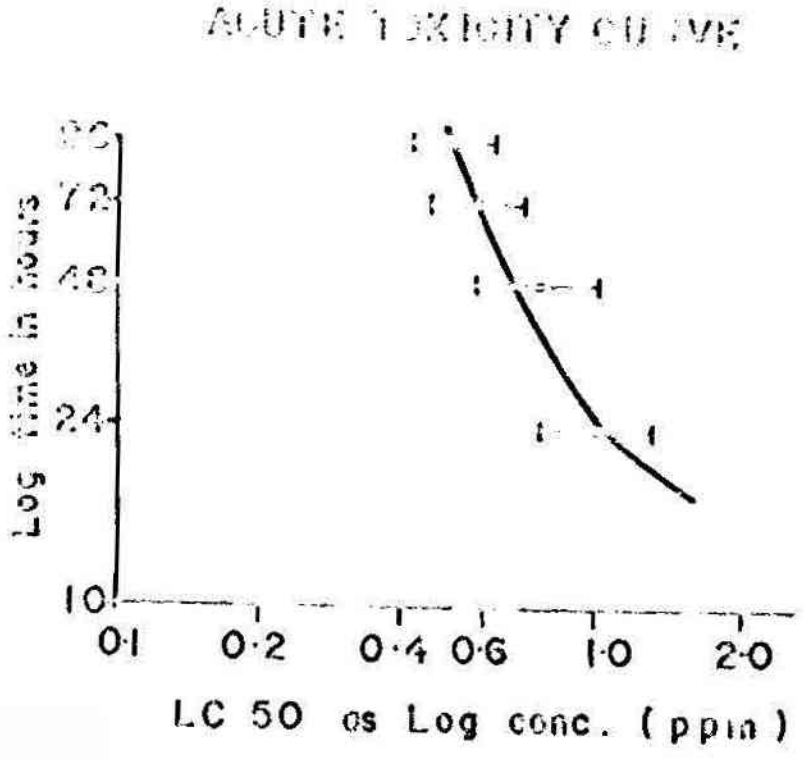

Log time in hours
72
48
24
10
0·1
0·2
0·4
0·6
1·0
2·0
LC 50 as Log conc. (ppm)

TABLE - 2A

Variation in muscle tissue protein of Liza parsia exposed to "96 hr LC50" of "Nuvan".

(Salinity 10.0 ± 1%, Temperature 27.5 ± 1.5°C, pH 5.5)

Exposure period (Hours)	CONTROL		EXPOSURE			
	Protein (mg/100 mg wet weight)	% coefficient of variation	"Nuvan" Conc. (ppm)	Protein (mg/100 mg wet weight)	% coefficient of variation	% variation from control
0	29.216 ± 2.912	9.965	0	29.216 ± 2.912	9.965	0
24	29.216 ± 2.727	9.334	0.48	29.961 ± 1.158	4.295	-7.715
48	28.824 ± 1.907	6.616	0.48	24.902 ± 2.531	10.162	-13.607
72	28.333 ± 1.457	5.141	0.48	22.059 ± 2.363	10.710	-22.144
96	27.745 ± 0.675	2.433	0.48	17.353 ± 1.080	6.224	-37.455

± Standard deviation.

Fig. 2A: Variation in muscle tissue protein of Liza parsia exposed to "96 hr LC50" of "Nuvan".

Fig. 2B: Variation in muscle tissue total free amino acids of Liza parsia exposed to "96 hr LC50" of "Nuvan"

Fig. 2C: Variation in muscle tissue RNA of Liza parsia exposed to "96 hr LC50" of "Nuvan".

Fig. 2D: Variation in DNA of Liza parsia muscle tissue exposed to "96 hr LC50" of "Nuvan".

Fig. 2 A

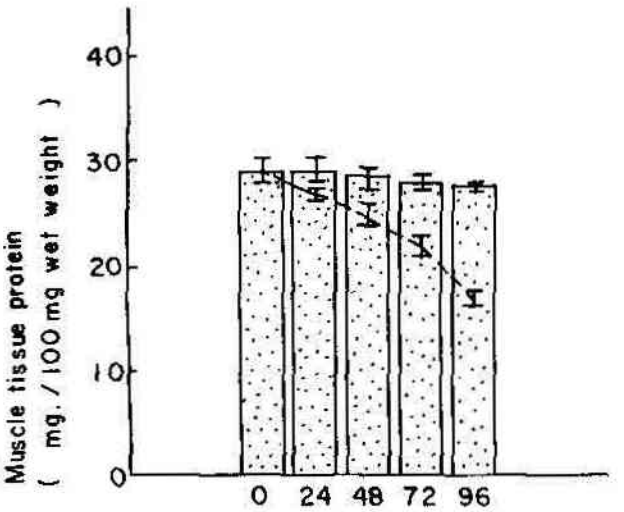


Fig. 2 C

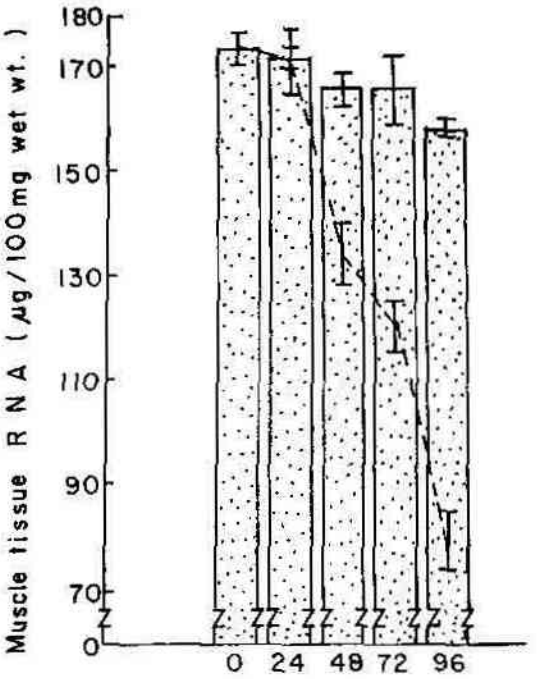


Fig. 2 B

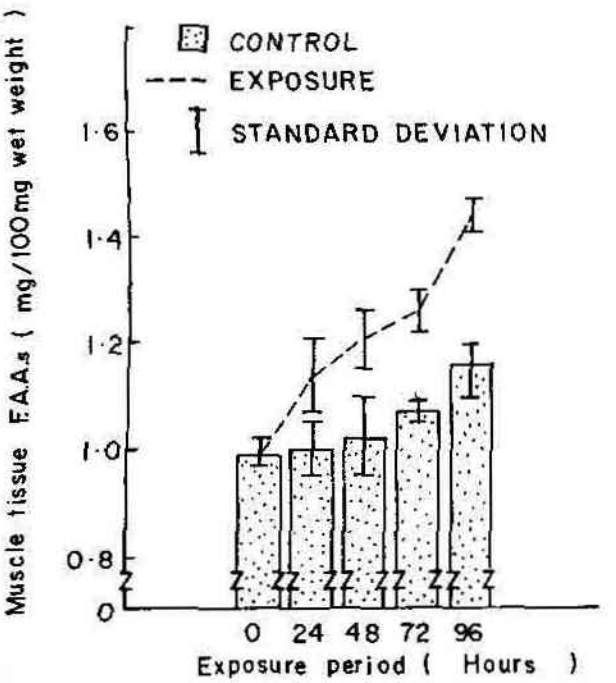
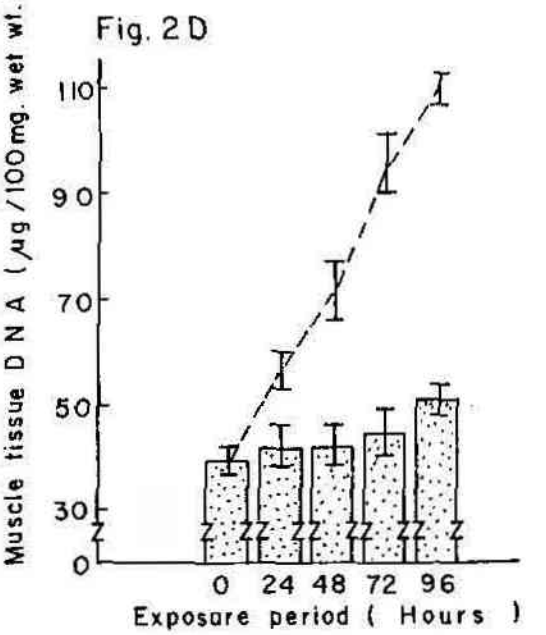


Fig. 2 D



decrease in the muscle protein content in fishes exposed to 24, 48, 72 and 96 hours in 96 hr LC50 experiment. In student 't' test means significantly differed at 5% level between protein contents of control and exposed animals. The calculated 't' value was 3.706 against tabulated value of 1.701.

From Table 3A and Fig. 3A it is observed that there is decrease in the protein content in fishes exposed to 1.01 ppm (24 hr LC50), 0.75 ppm (48 hr LC50), 0.55 ppm (72 hr LC50) and 0.48 ppm (96 hr LC50) for 24, 48, 72 and 96 hours respectively. The decrease was maximum for exposure periods of 96, 72 and 48 hours and minimum for 24 hours against control value.

2.1.1.2. On sub-lethal exposures

Decrease in protein content of muscle tissue was observed following sub-lethal exposures (Table 4A and Fig. 4A) for 15, 30 and 45 days at 1/15th, 1/10th and 1/5th of 96 hr LC50. The decrease was maximum for higher concentration and longer exposure period than lower concentration and shorter exposure period. Anova analysis showed significant differences between the treatments and exposure periods at 1% F-Value.

2.1.2. Total free amino acids content

2.1.2.1. On acute exposures:

There was gradual elevation in muscle total free amino acids (Table 2B and Fig. 2B) following acute exposure to 0.48 ppm for 24, 48, 72 and

TABLE - 3A

Variation in muscle tissue protein of Liza parsia after acute exposures to "Nuvan"

(Salinity 10.0 ± 1‰ , Temperature 27.5 ± 1.5°C, pH 5.5)

Exposure period (Hours)	CONTROL		EXPOSURE			
	Protein (mg/100mg wet weight)	% coefficient of variation	"Nuvan" Conc. (ppm)	Protein (mg/100mg wet weight)	% coefficient of variation	% variation from control
0	29.216 ± 2.912	9.965	0	29.216 ± 2.912	9.965	0
24	28.824 ± 1.907	6.526	1.01	20.882 ± 1.299	6.220	-27.553
48	29.215 ± 2.727	9.334	0.75	18.529 ± 2.192	11.827	-36.577
72	28.333 ± 1.457	6.616	0.55	17.843 ± 2.912	16.317	-37.024
96	27.745 ± 0.675	2.433	0.48	17.353 ± 1.080	6.224	-37.455

± Standard deviation.

Fig. 2I: Variation in oxygen consumption of Liza parsia exposed to "96 hr LC50" of "Nuvan".

Fig. 3A: Percentage variation in muscle tissue protein of Liza parsia after acute exposures to "Nuvan".

Fig. 3B: Percentage variation in muscle tissue total free amino acids of Liza parsia exposed to acute exposures of "Nuvan".

Fig. 2 I

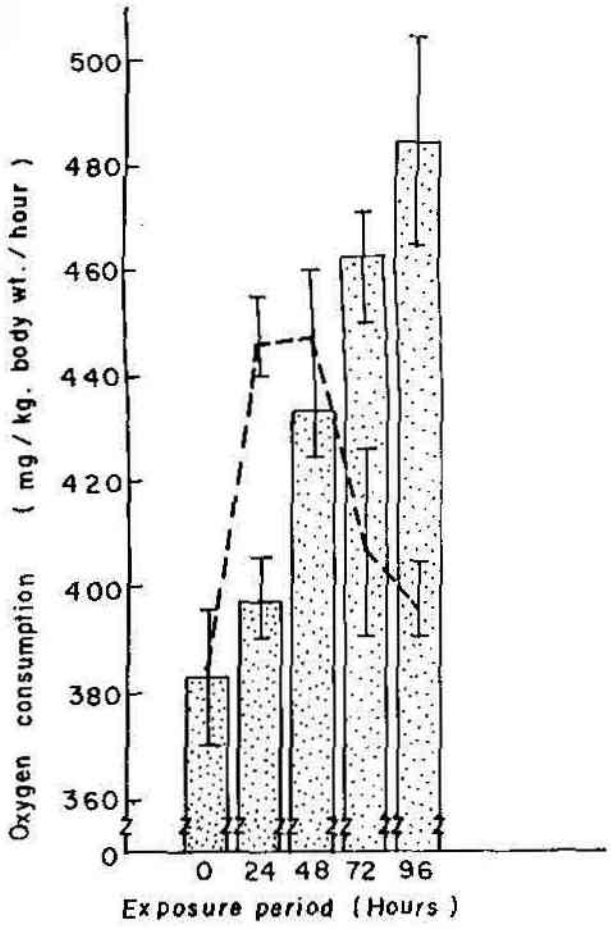
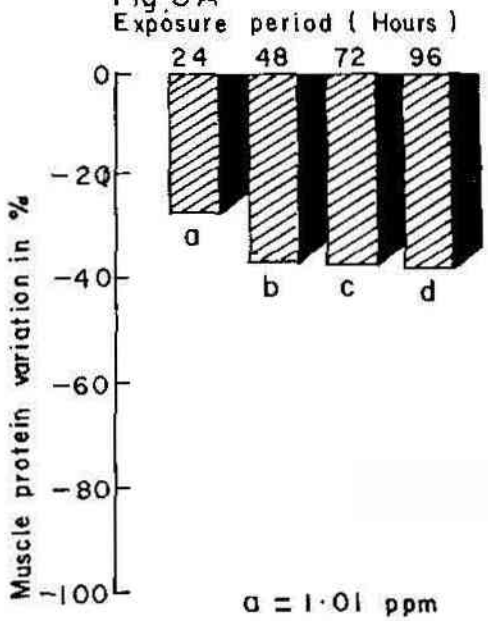


Fig. 3 A



a = 1·01 ppm
b = 0·75 ,,
c = 0·55 ,,
d = 0·48 ,,

Fig. 3 B

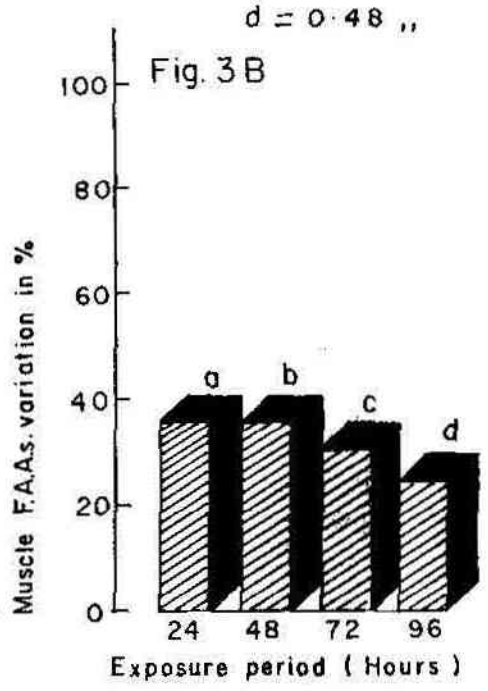


TABLE - 4A

Protein content (mg/100mg wet weight) in body muscle tissue of Liza parsia after sub-lethal exposures to "Nuvan"

(Salinity 10.0 ± 1‰ , Temperature 27.5 ± 1.5°C, pH 5.75 ± 0.25)

Exposure period (Days)	Protein in control	Sub-lethal concentrations					
		1/15th 96 hr LC50		1/10th 96 hr LC50		1/5th 96 hr LC50	
		Protein content	% variation from control	Protein content	% variation from control	Protein content	% variation from control
15	27.255 ± 1.329	23.333 ± 1.698	-14.39	21.667 ± 2.002	-20.50	20.686 ± 0.741	-24.10
30	26.961 ± 0.849	21.078 ± 0.741	-21.82	17.451 ± 2.091	-35.27	17.941 ± 0.883	-33.46
45	24.019 ± 2.128	16.667 ± 1.224	-30.61	15.686 ± 1.480	-34.69	14.706 ± 1.019	-38.77

± Standard deviation.

Fig. 4A: Percentage variation in protein content of muscle tissue on chronic exposures to "Nuvan".

Fig. 4B: Percentage variation in total free amino acids of muscle tissue on chronic exposures to "Nuvan".

Fig. 4C: Percentage variation in RNA content of muscle tissue on chronic exposures to "Nuvan".

Fig. 4 A

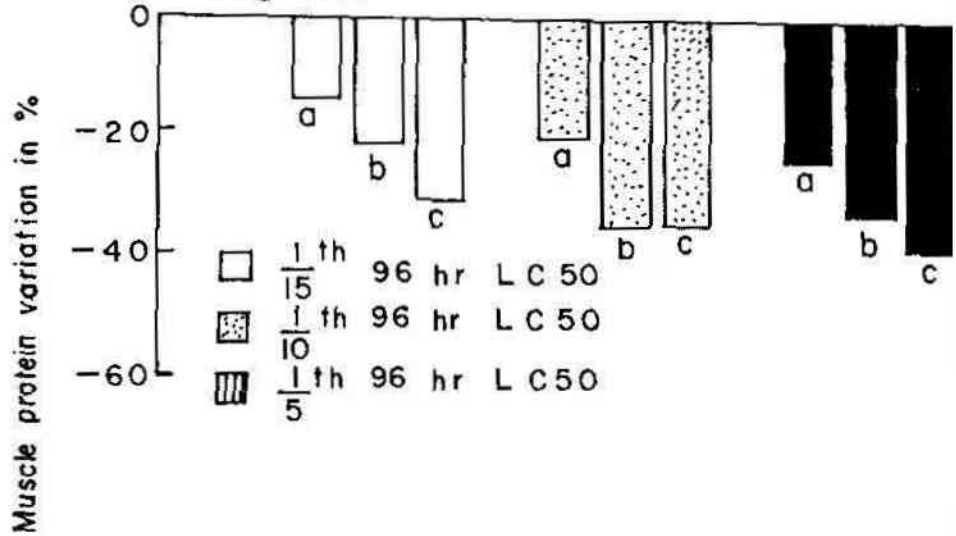


Fig. 4 B

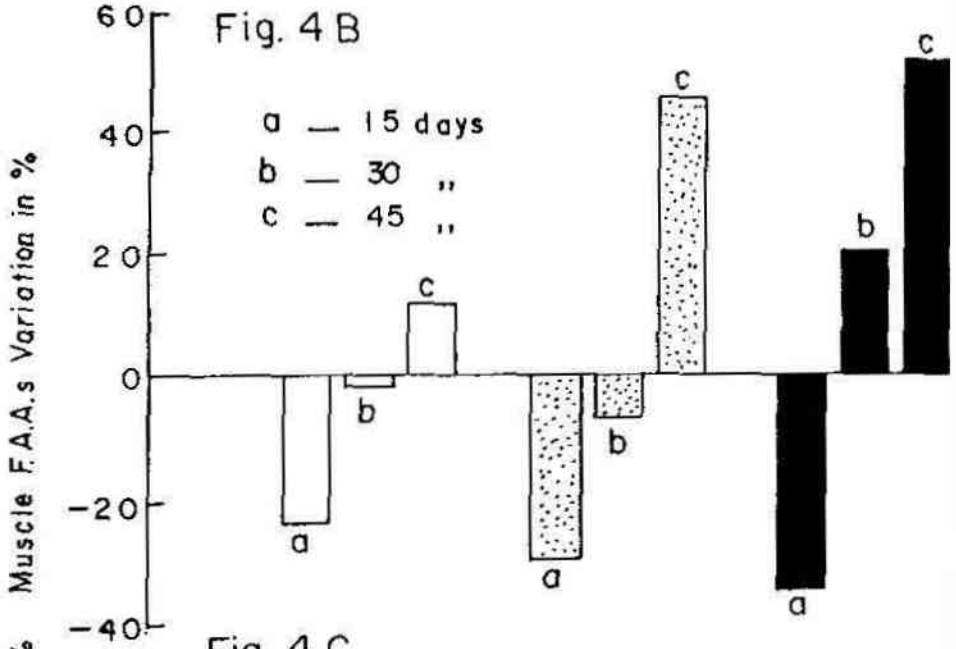


Fig. 4 C

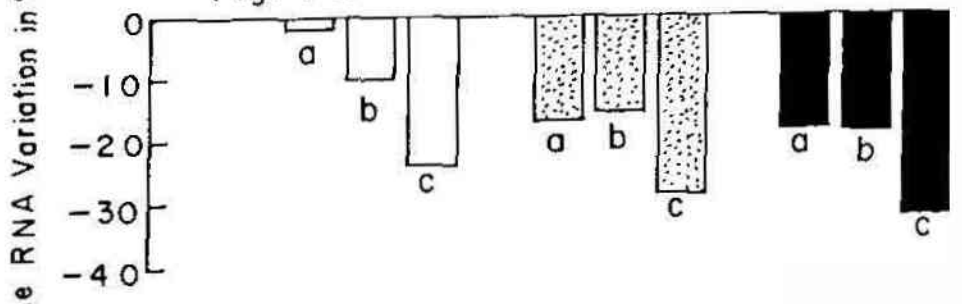


TABLE - 2B

Variation in muscle tissue total free amino acids of Liza parsia exposed to "96 hr LC50" of "Nuvan"

(Salinity 10.0 ± 1‰, Temperature 27.5 ± 1.5°C, pH 5.5)

Exposure period (Hours)	CONTROL		EXPOSURE			
	Total free amino-acids (mg/100mg wet weight)	% coefficient of variation	"Nuvan" Conc. (ppm)	Total free amino-acids (mg/100mg wet weight)	% coefficient of variation	% variation from control
0	0.986 ± 0.050	5.020	0	0.986 ± 0.050	5.020	0
24	0.997 ± 0.101	10.080	0.48	1.138 ± 0.114	10.018	+14.142
48	1.027 ± 0.153	14.848	0.48	1.217 ± 0.113	9.244	+18.500
72	1.076 ± 0.042	3.903	0.48	1.262 ± 0.081	6.418	+17.286
96	1.159 ± 0.104	8.930	0.48	1.443 ± 0.057	3.950	+24.503

± Standard deviation.

96 hours. Using student 't' test it was seen that means are differing significantly at 5% level between control and exposure values. The calculated 't' value (3.242) was seen higher than that of tabulated value (1.701).

Fishes exposed to lethal concentrations of 1.01, 0.75, 0.55 and 0.48 ppm for 24, 48, 72 and 96 hours respectively had elevated total free amino acids in the muscle tissue (Table 3B and Fig. 3B). The increase was maximum (35.707%) in muscle of fishes exposed to 1.01 ppm for 24 hours and minimum (24.5%) in the muscle of fishes exposed to 0.48 ppm for 96 hours. In control the total free amino acids showed a gradual increase but less significant than treatment.

2.1.2.2. On sub-lethal exposures

Table 4B and Fig. 4B show the variations in the muscle total free amino acids in fishes exposed to sub-lethal concentrations of "Nuvan". The decrease was observed for all the 3 sub-lethal concentrations (i.e.,1/15th, 1/10th and 1/5th 96 hr LC50) at 15th day and also for 1/15th 96 hr LC50 at 30th day exposure. But elevation was noted for all the 3 sub-lethal concentrations at 45th day and for 1/10th 96 hr LC50 and 1/5th 96 hr LC50 at 30th day. Slight increase in control value was seen with increased exposure periods. Anova indicated that the variations in total free amino acids between 15th day and 45th day was significant at 5% level.

TABLE - 3B

Variation in muscle tissue total free amino acids of Liza parsia exposed to acute exposures of "Nuvan".

(Salinity 10.0 ± 1‰ , Temperature 27.5 ± 1.5°C, pH 5.5)

Exposure period (Hours)	CONTROL		EXPOSURE			
	Total free amino acids (mg/100mg wet weight)	% of coefficient of variation	"Nuvan" Conc. (ppm)	Total free amino acids(mg/100mg wet weight)	% coefficient of variation	% variation from control
0	0.986 ± 0.050	5.020	0	0.986 ± 0.500	5.020	0
24	0.996 ± 0.101	10.080	1.01	1.353 ± 0.092	6.763	+35.707
48	1.027 ± 0.153	14.848	0.75	1.390 ± 0.030	2.158	+35.346
72	1.076 ± 0.042	3.903	0.55	1.401 ± 0.099	7.066	+30.204
96	1.159 ± 0.104	8.930	0.48	1.443 ± 0.057	3.950	+24.503

± Standard deviation.

TABLE - 4B

Total free amino acids (mg/100mg wet weight) of muscle tissue of Liza parsia after sub-lethal exposures to "Nuvan"

(Salinity 10.0 ± 1‰ , Temperature 27.5 ± 1.5°C, pH 5.75 ± 0.25)

Exposure period (Days)	Total free amino acids in control	Sub-lethal concentration					
		1/15th 95 hr LC50		1/10th 96 hr LC50		1/5th 96 hr LC50	
		Total free amino acids	% variation from control	Total free amino acids	% variation from control	Total free amino acids	% variation from control
15	0.981 ± 0.016	0.742 ± 0.025	-24.363	0.686 ± 0.018	-30.071	0.631 ± 0.020	-35.678
30	1.013 ± 0.032	0.995 ± 0.037	-1.777	1.079 ± 0.040	+6.515	1.219 ± 0.019	+20.336
45	1.042 ± 0.032	1.167 ± 0.032	+11.996	1.521 ± 0.025	+45.969	1.588 ± 0.019	+52.399

± Standard deviation.

2.1.3. RNA content

2.1.3.1. On acute exposures

Table 2C and Fig. 2C indicate the gradual decrease in muscle tissue RNA in fishes exposed to 0.48 ppm (96 hr LC50) for 24, 48, 72 and 96 hours. The maximum decrease in RNA content (-50.467%) was observed at 96 hours of exposure. From student 't' test it was seen that the means are significantly differing between control and exposed RNA values at 5% level. The calculated 't' value was 3.214 against tabulated 't' value 1.701.

The RNA content in all groups of fishes exposed to 4 lethal concentrations (1.01 ppm, 0.75 ppm, 0.55 ppm and 0.48 ppm for 24, 48, 72 and 96 hours respectively) were depleted (Table 3C and Fig. 3C) against control value. The maximum depletion in RNA touched the value of -50.467% in 0.48 ppm for 96 hours against -34.483% in 1.01 ppm for 24 hours.

2.1.3.2. On sub-lethal exposures

In chronic exposures to "Nuvan" the RNA content in muscle tissue was depleted against control value (Table 4C and Fig. 4C). The higher concentrations and longer exposure periods showed the increased trend of depletion. Anova analysis showed significant differences between the treatments and exposure periods at 1% F-Value.

2.1.4. DNA content

2.1.4.1. On acute exposures

The DNA in muscle tissue increased gradually in fishes exposed

TABLE - 2C

of

Variation in muscle tissue RNA / *Liza parsia* exposed to "96 hr LC50" of "Nuvan"

(Salinity 10.0 ± 1‰, Temperature 27.5 ± 1.5°C, pH 5.5)

Exposure period (Hours)	CONTROL		EXPOSURE			
	RNA (µg/100mg wet weight)	% Coefficient of variation	"Nuvan" Conc. (ppm)	RNA (µg/100mg wet weight)	% Coefficient of variation	% variation from control
0	173.333 ± 5.444	3.140	0	173.333 ± 5.444	3.140	0
24	171.852 ± 3.143	1.829	0.48	171.852 ± 12.572	7.315	0
48	165.926 ± 6.285	3.788	0.48	133.333 ± 11.159	8.369	-19.643
72	165.926 ± 12.701	7.654	0.48	121.481 ± 8.315	6.844	-26.786
96	158.519 ± 3.143	1.983	0.48	78.519 ± 9.132	11.630	-50.467

± Standard deviation.

TABLE - 3C

Variation in muscle tissue RNA of Liza parsia after acute exposures to "Nuvan"

(Salinity 10.0 ± 1% , Temperature 27.5 ± 1.5°C, pH 5.5)

Exposure period (Hours)	CONTROL		EXPOSURE			
	RNA (µg /100mg wet weight)	% coefficient of variation	"Nuvan" Conc. (ppm)	RNA (µg /100mg wet weight)	% coefficient of variation	% variation from control
0	173.333 ± 5.444	3.140	0	173.333 ± 5.444	3.130	0
24	171.852 ± 3.143	1.829	1.01	112.593 ± 17.390	15.445	-34.483
48	165.926 ± 6.285	3.788	0.75	105.185 ± 15.129	14.383	-36.607
72	165.926 ± 12.701	7.654	0.55	87.407 ± 11.331	12.963	-47.322
96	158.519 ± 3.143	1.983	0.48	78.519 ± 9.132	11.630	-50.467

± Standard deviation.

Fig. 3C: Percentage variation in muscle tissue RNA of Liza parsia after acute exposures to "Nuvan".

Fig. 3D: Percentage variation in muscle tissue DNA of Liza parsia after acute exposures to "Nuvan".

Fig. 3E: Percentage variation in blood serum protein of Liza parsia after acute exposures to "Nuvan".

Fig. 3F: Percentage variation in blood serum total free amino acids of Liza parsia after acute exposures to "Nuvan".

Fig. 3C

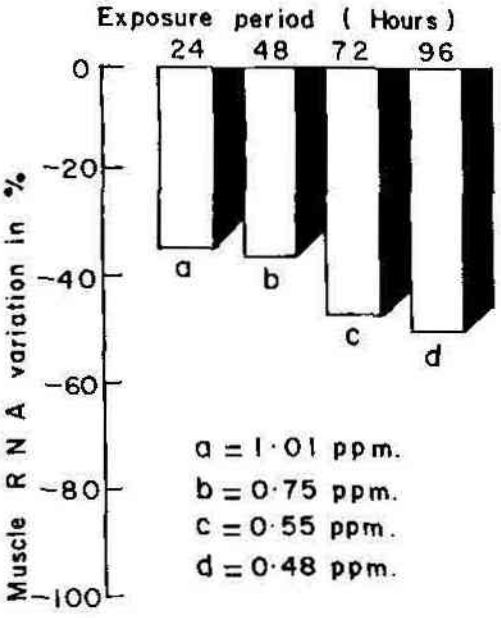


Fig. 3E

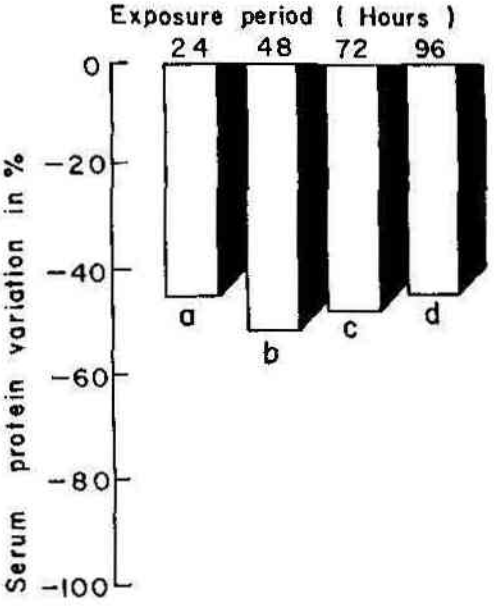


Fig. 3D

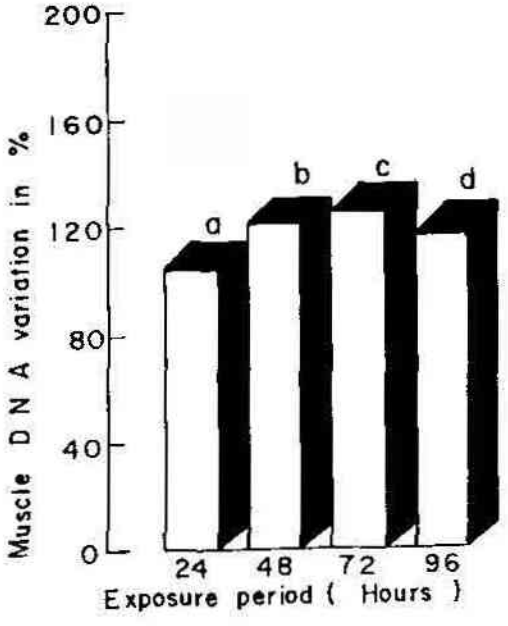


Fig. 3F

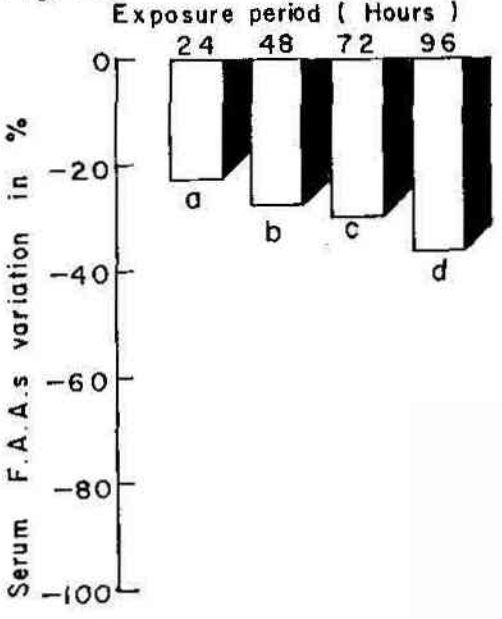


TABLE - 4C

RNA (μ /100mg wet weight) in muscle tissue of Liza parsia after sub-lethal exposures to "Nuvan"

(Salinity 10.0 ± 1‰ , Temperature 27.5 ± 1.5°C, pH 5.75 ± 0.25)

Exposure period (Days)	RNA in control	Sub-lethal concentrations					
		1/15th 96 hr LC50		1/10th 96 hr LC50		1/5th 96 hr LC50	
		RNA content	% variation from control	RNA content	% variation from control	RNA content	% variation from con-rom
15	165.926 ± 10.264	162.963 ± 18.574	-1.79	137.778 ± 19.373	-16.96	134.815 ± 6.789	-18.75
30	158.518 ± 6.970	142.222 ± 8.889	-10.28	133.333 ± 8.889	-15.89	127.407 ± 13.415	-19.63
45	158.518 ± 9.252	120.000 ± 7.698	-24.30	112.592 ± 11.189	-28.97	105.185 ± 6.789	-33.64

± Standard deviation ,

to 0.48 ppm (96 hr LC50) for 24, 48, 72 and 96 hours as shown in Table 2D and Fig. 2D. The enhancement was maximum (117.648%) for 96 hours against minimum (35.717%) for 24 hours exposure period. Student 't' test showed the significant difference between DNA content of control and exposure animals at 5% level (where t-cal = 4.210 and t-tab = 1.701).

RNA-DNA ratio of muscle tissue of fish after acute exposure to 96 hr LC50 is shown in Table 2J. Both in control and exposure the ratios are seen in decreasing order, but it is more pronounced in exposed animals.

Table 3D and Fig. 3D indicate the elevation of DNA content in muscle tissue of fishes exposed to 24 hr LC50, 48 hr LC50, 72 hr LC50 and 96 hr LC50 of "Nuvan" for 24, 48, 72 and 96 hours respectively. The maximum elevation (126.665%) was observed in fishes exposed to 0.55 ppm for 72 hours against minimum value (107.147%) in 1.01 ppm for 24 hours. Table 3D also shows slight elevation of DNA content in control.

RNA-DNA ratio of muscle tissue of fish after acute exposures to "Nuvan" is shown in Fig. 3J. In all the exposures the ratios were dedcreasing steeply than in control.

2.1.4.2. On sub-lethal exposures

The elevation in DNA content of muscle tissue following exposures to 1/15th 96 hr LC50, 1/10th 96 hr LC50 and 1/5th 96 hr LC50 for 15,30 and 45 days are given in Table 4D and Fig. 4D. At higher concentration and longer exposure period the elevation was seen maximum than the

TABLE - 2D

Variation in DNA of Liza parsia muscle tissue exposed to "96 hr LC50" of "Nuvan"

(Salinity 10.0 ± 1‰ , Temperature 27.5 ± 1.5°C, pH 5.5)

Exposure period (Hours)	CONTROL		EXPOSURE			
	DNA (µg/100mg wet weight)	% coefficient of variation	Nuvan Conc. (ppm)	DNA (µg /100mg wet weight)	% coefficient of variation	% variation from control
0	38.690 ± 6.314	16.318	0	38.690 ± 6.314	16.318	0
24	41.666 ± 8.418	20.204	0.48	56.548 ± 6.314	11.166	+ 35.717
48	41.666 ± 7.754	18.609	0.48	71.429 ± 10.935	15.309	+ 71.432
72	44.643 ± 9.282	20.792	0.48	95.238 ± 12.627	13.258	+113.332
96	50.595 ± 6.314	12.479	0.48	110.119 ± 6.314	5.734	+117.648

± Standard deviation.

TABLE - 2J

RNA-DNA ratio of muscle tissue of Liza parsia after acute exposure to "96 hr LC50" of "Nuvan"

(Salinity 10 ± 1‰ , Temperature 27.5 ± 1.5°C, pH 5.5)

Exposure period (Hours)	Nuvan concentration (ppm)	RNA-DNA ratio in control	RNA-DNA ratio in exposure
0	0	4.48	4.48
24	0.48	4.12	3.04
48	0.48	3.98	1.87
72	0.48	3.72	1.28
96	0.48	3.13	0.71

TABLE - 3D

Variation in muscle tissue DNA of Liza parsia after acute exposures to "Nuvan"

(Salinity 10.0 ± 1‰ , Temperature 27.5 ± 1.5°C, pH 5.5)

Exposure period (Hours)	CONTROL		EXPOSURE			
	DNA (µg/100mg wet weight)	% coefficient of variation	"Nuvan" Conc. (ppm)	DNA (µg/100mg wet weight)	% coefficient of variation	% variation from control
0	38.690 ± 6.314	16.318	0	38.690 ± 6.314	16.318	0
24	41.666 ± 8.418	20.204	1.01	86.310 ± 6.314	7.315	+107.147
48	41.666 ± 7.754	18.609	0.75	92.261 ± 6.314	6.844	+121.430
72	44.643 ± 9.282	20.792	0.55	101.190 ± 16.704	16.508	+126.665
96	50.595 ± 6.314	12.479	0.48	110.119 ± 6.314	5.734	+117.648

± Standard deviation.

TABLE - 33

RNA-DNA ratio of muscle tissue of Liza parsia after acture exposures to "Nuvan"

(Salinity 10 ± 1‰ , Temperature 27.5 ± 1.5°C, pH 5.5)

Exposure period (Hours)	"Nuvan" concentration (ppm)	RNA-DNA ratio in control	RNA-DNA ratio in control
0	0	4.48	4.48
24	1.01	4.12	1.30
48	0.75	3.98	1.14
72	0.55	3.72	0.86
96	0.48	3.13	0.71

TABLE - 4D

DNA (µg/100mg wet weight) in muscle tissue of Liza parsia after sub-lethal exposures to "Nuvan"

(Salinity 10.0 ± 1‰ , Temperature 27.5 ± 1.5 °C, pH 5.75 ± 0.25)

Exposure period (Days)	DNA in control	Sub-lethal concentrations					
		1/15th 96 hr LC50		1/10th 96 hr LC50		1/5th 96 hr LC50	
		DNA content	% variation from control	DNA content	% variation from control	DNA content	% variation from control
15	41.667 ± 5.155	50.595 ± 13.639	+21.43	50.595 ± 5.155	+21.43	56.548 ± 13.638	+35.71
30	41.667 ± 13.639	56.548 ± 10.340	+35.71	59.524 ± 5.155	+42.86	65.476 ± 5.155	+57.14
45	44.643 ± 8.929	68.452 ± 10.340	+53.27	89.286 ± 15.464	+100	98.857 ± 14.939	+121.44

± Standard deviation.

Fig. 4D: Percentage variation in DNA content of muscle tissue on chronic exposures to "Nuvan"

Fig. 4E: Percentage variation in protein content of blood serum on chronic exposures to "Nuvan".

Fig. 4F: Percentage variation in total free amino acids of blood serum on chronic exposures to "Nuvan".

Fig. 4 D

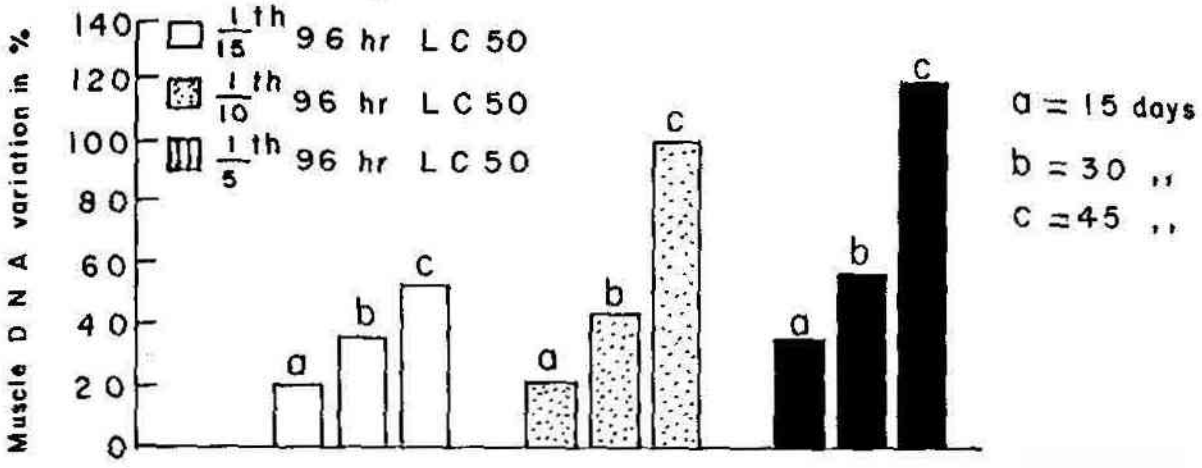


Fig. 4E

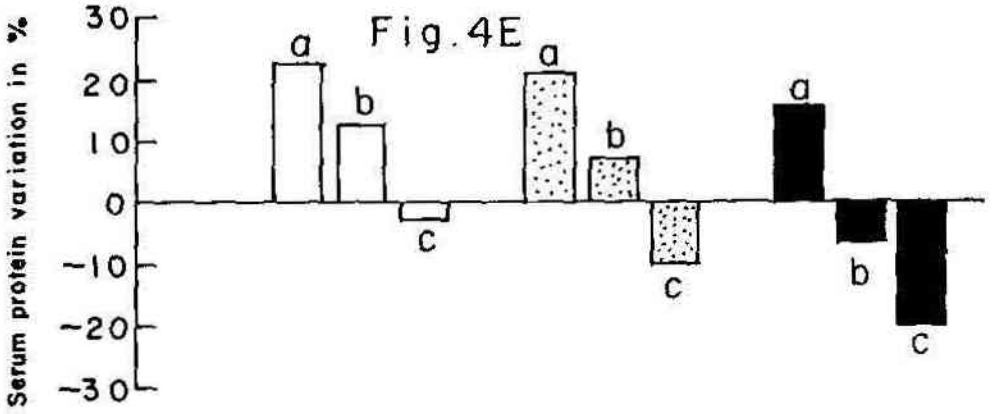
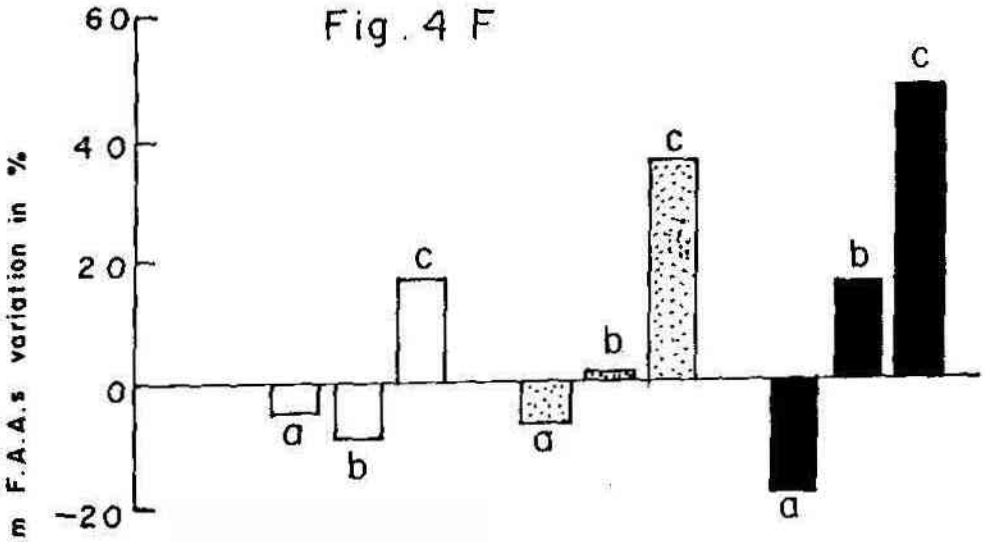


Fig. 4 F



lower concentration and shorter exposure period. Anova indicated the significant difference between control and 1/10th 96 hr LC50, control and 1/5th 96 hr LC50, 15 days and 45 days, 30 days and 45 days at 5% F-value.

RNA-DNA ratio of muscle tissue of fishes after sub-lethal exposures to "Nuvan" is shown in Table 4J. In all the concentrations, the ratio decreased over the control value but it was more at higher concentrations and longer exposure periods.

2.2. Studies on blood serum

2.2.1. Protein content

2.2.1.1. On acute exposures

From Table 2E and Fig. 2E it is seen that there is gradual elevation in protein content in fishes exposed to 0.48 ppm (96 hr LC50) for 24, 48 and 72 hours followed by a sudden depletion at 96 hours (-44.554%). From student 't' test it was observed that the calculated 't' value (0.478) is less than that of tabulated value (1.701) for protein contents of control and treated animals at 5% level.

There was depletion in blood serum protein (Table 3E and Fig. 3E) following acute exposure to 24 hr LC50, 48 hr LC50, 72 hr LC50 and 96 hr LC50 for 24, 48, 72 and 96 hours respectively and it ranged between -44.554% in fishes exposed to 0.48 ppm for 96 hours to -51.261% in fishes exposed to 0.75 ppm for 48 hours. Changes in control values was less significant than treatment.

TABLE 4J

RNA-DNA ratio of muscle tissue of Liza parsia after sub-lethal exposures to "Nuvan"

(Salinity 10.0 ± 1‰ , Temperature 27.5 ± 1.5°C, pH 5.75 ± 0.25)

Exposure period (Days)	RNA-DNA ratio in control	Sub-lethal concentrations		
		1/15th 96 hr LC50	1/10th 96 hr LC50	1/5th 96 hr LC50
		RNA-DNA ratio	RNA-DNA ratio	RNA-DNA ratio
15	3.98	3.22	2.72	2.38
30	3.80	2.52	2.24	1.95
45	3.55	1.75	1.26	1.06

TABLE - 2E

Variation in blood serum protein of Liza parsia exposed to "96 hr LC50" of "Nuvan"

(Salinity 10.0 ± 1‰ , Temperature 27.5 ± 1.5°C, pH 5.5)

Exposure period (Hours)	CONTROL		EXPOSURE			
	Protein (mg/ml)	% coefficient of variation	"Nuvan" Conc. (ppm)	Protein (mg/ml)	% coefficient of variation	% variation from control
0	59.804 ± 7.499	12.538	0	59.804 ± 7.499	12.538	0
24	60.294 ± 5.403	8.961	0.48	66.667 ± 5.502	8.253	+10.570
48	58.333 ± 7.277	12.474	0.48	78.431 ± 9.066	11.559	+34.454
72	52.941 ± 6.239	11.754	0.48	60.784 ± 6.506	10.703	+14.815
96	49.510 ± 5.789	11.692	0.48	27.451 ± 5.546	20.203	-44.554

± Standard deviation .

Fig. 2E: Variation in blood serum protein of Liza parsia exposed to "96 hr LC50" of "Nuvan".

Fig. 2F: Variation in blood serum total free amino acids of Liza parsia exposed to "96 hr LC50" of "Nuvan".

Fig. 2G: Variation in blood serum acid phosphatase (ACP) of Liza parsia exposed to "96 hr LC50" of "Nuvan".

Fig. 2H: Variation in blood serum alkaline phosphatase (ALP) of Liza parsia exposed to "96 hr LC50" of "Nuvan".

Fig. 2 E

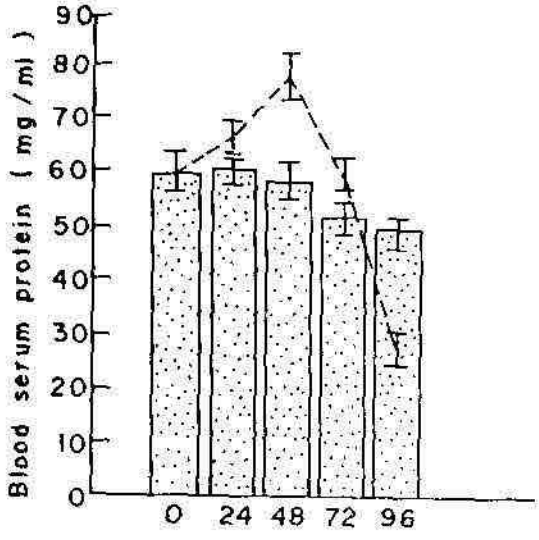


Fig. 2 G

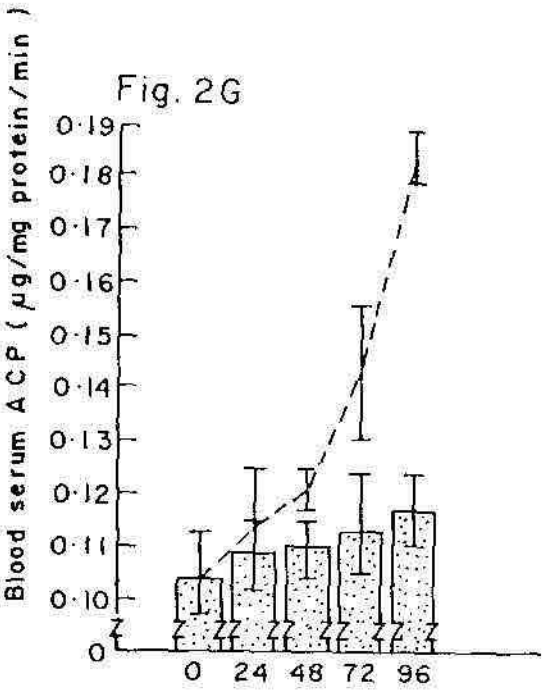


Fig. 2 F

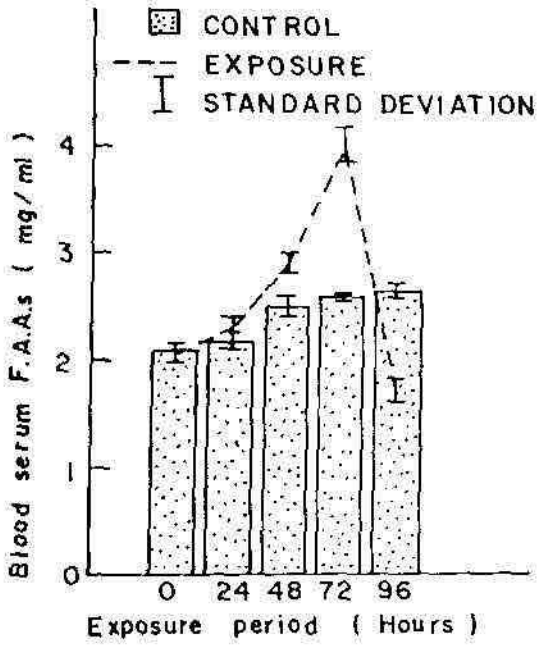


Fig. 2 H

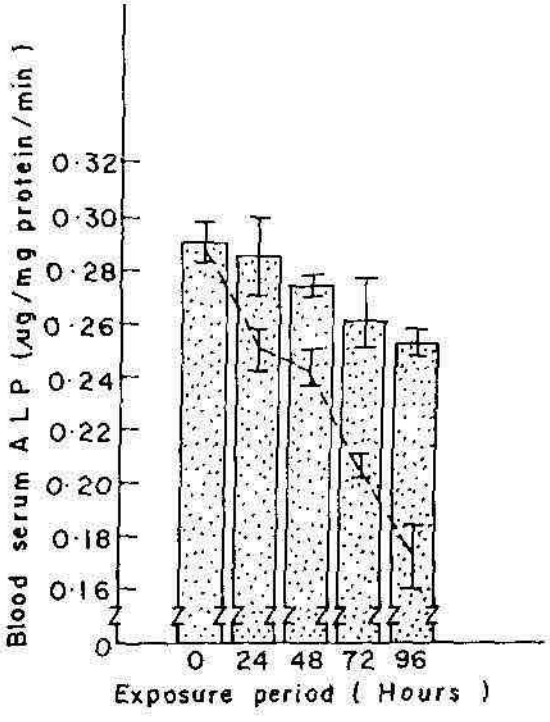


TABLE - 3E

Variation in blood serum protein of Liza parsia after acute exposures to "Nuvan"

(Salinity 10.0 ± 1‰ , Temperature 27.5 ± 1.5°C, pH 5.5)

Exposure period (Hours)	CONTROL		EXPOSURE			
	Protein (mg/ml)	% coefficient of variation	"Nuvan" Conc. (ppm)	Protein (mg/ml)	% coefficient of variation	% variation from control
0	59.804 ± 7.499	12.538	0	59.804 ± 7.499	12.538	0
24	60.294 ± 5.403	8.961	1.01	43.333 ± 2.079	6.237	-44.715
48	58.333 ± 7.277	12.475	0.75	28.431 ± 2.751	9.846	-51.261
72	52.941 ± 6.239	11.785	0.55	27.941 ± 4.766	16.762	-47.222
96	49.510 ± 5.789	11.692	0.48	27.451 ± 5.546	20.203	-44.554

± Standard deviation.

2.2.1.2. On sub-lethal exposures

Table 4E and Fig. 4E show the variation in the serum protein of fishes exposed to sub-lethal concentrations of "Nuvan". There was elevation of protein in all the three concentrations at 15th day and depletion at 45th day. On 30th day, increase was observed for 1/15th 96 hr LC50 and 1/10th 96 LC50 but decrease for 1/5th 96 hr LC50 over control. The differences between control and treatment for 15, 30 and 45 days were highly significant at 1% F-value.

2.2.2. Total free amino acids

2.2.2.1. On acute exposures

The total free amino acids content in fishes exposed to 0.48 ppm (96 hr LC50) for 24, 48 and 72 hours exposure period were elevated gradually followed by a drop at 96 hours exposure (Table 2F and Fig. 2F). From student 't' test it was seen that the means are not significantly different at 5% level between the control and exposure values. The calculated t-value was 0.922 against tabulated t-value 1.701.

There was depletion in serum total free amino acids (Table 3F and Fig. 3F) after acute exposures to 4 lethal concentrations (0.01 ppm, 0.75 ppm, 0.55 ppm and 0.48 ppm for 24, 48, 72 and 96 hours respectively). The depletion was maximum (-36.626%) at 0.48 ppm for 96 hours and minimum (-22.778%) at 1.01 ppm for 24 hours. The gradual elevation was observed for control value.

TABLE - 4E

Protein content (mg/ml) in blood serum of Liza parsia after sub-lethal exposures to "Nuvan"

(Salinity 10.0 ± 1‰ , Temperature 27.5 ± 1.5°C, pH 5.75 ± 0.25)

Exposure period (Days)	Protein in control	Sub-lethal concentrations					
		1/15th 96 hr LC50		1/10th 96 hr LC50		1/ 5th 96 hr LC50	
		Protein content	% variation from control	Protein content	% variation from control	Protein content	% variation from control
15	67.157 ± 6.631	82.353 ± 2.941	+22.63	81.373 ± 6.122	+21.17	77.451 ± 6.123	+15.33
30	62.255 ± 4.727	70.098 ± 3.061	+12.60	66.667 ± 1.698	+7.09	57.843 ± 4.493	-7.09
45	54.902 ± 3.701	53.922 ± 4.494	-2.92	49.019 ± 4.492	-10.72	43.628 ± 5.943	-20.53

± Standard deviation.

TABLE - 2F

Variation in blood serum total free amino acids of Liza parsia exposed to "96 hr LC50" of "Nuvan"

(Salinity 10.0 ± 1‰ , Temperature 27.5 ± 1.5°C, pH 5.5)

Exposure period (Hours)	CONTROL			EXPOSURE		
	Total free amino-acids (mg/ml)	% coefficient of variation	"Nuvan" Conc. (ppm)	Total free amino-acids (mg/ml)	% coefficient of variation	% variation from control
0	2.078 ± 0.147	7.074	0	2.078 ± 0.147	7.074	0
24	2.160 ± 0.143	6.597	0.48	2.316 ± 0.179	7.707	+ 7.222
48	2.480 ± 0.214	8.646	0.48	2.887 ± 0.179	6.183	+16.411
72	2.565 ± 0.072	2.807	0.48	4.028 ± 0.294	7.299	+57.037
96	2.632 ± 0.101	3.837	0.48	1.668 ± 0.159	9.532	-36.626

± Standard deviation.

TABLE - 3F

Variation in blood serum total free amino acids of Liza parsia after acute exposures to "Nuvan"

(Salinity 10.0 ± 1‰ , Temperature 27.5 ± 1.5°C, pH 5.5)

Exposure period (hours)	CONTROL		EXPOSURE			
	Total free amino-acids (mg/ml)	% coefficient of variation	"Nuvan" Conc. (ppm)	Total free amino-acids (mg/ml)	% coefficient of variation	% variation from control
0	2.078 ± 0.147	7.074	0	2.078 ± 0.147	7.074	0
24	2.160 ± 0.143	6.597	1.01	1.668 ± 0.167	9.982	-22.778
48	2.480 ± 0.214	8.646	0.75	1.787 ± 0.171	9.569	-27.943
72	2.565 ± 0.072	2.807	0.55	1.789 ± 0.209	11.655	-30.253
96	2.632 ± 0.101	3.837	0.48	1.668 ± 0.159	9.532	-36.626

± Standard deviation.

2.2.2.2. On sub-lethal exposures

From Table 4F and Fig. 4F depletion in total free amino acids content of serum at all sub-lethal concentrations for 15 days exposure and at 1/15th 96 hr LC50 for 30 days exposure were observed. The increase was observed at 30th day for 1/10th 96 hr LC50, 1/5th 96 hr LC50 and at 45th day for all sublethal concentrations. From anova analysis it was found that the total free amino acid contents between 15 days and 45 days are significant at 5% F-value.

2.2.3. Acid phosphatase content

2.2.3.1. On acute exposures

From Table 2G and Fig. 2G it is seen that there is gradual increase in the acid phosphatase content in fishes exposed to 96 hr LC50 at 24, 48, 72 and 96 hours. Using student 't' test, means were seen significantly differing at 5% level between acid phosphatase contents of control and exposed animals. The calculated 't' value was 2.716 against tabulated value 1.701.

Table 3G and Fig. 3G show the elevation in the acid phosphatase content in fishes exposed to all 4 lethal concentrations e.g., 24 hr LC50, 48 hr LC50, 72 hr LC50 and 96 hr LC50 for 24, 48, 72 and 96 hr respectively. The elevation was higher (58.182%) in fishes exposed to 0.75 ppm for 48 hours and lower (41.284%) in those exposed to 1.01 ppm for 24 hours. The gradual increase was noticed in control value at a lesser magnitude than treatment.

TABLE - 4F

Total free amino acids (mg/ml) in blood serum of Liza parsia after sub-lethal exposures to "Nuvan"

(Salinity 10.0 ± 1‰ , Temperature 27.5 ± 1.5°C, pH 5.75 ± 0.25)

Exposure period (Days)	Total free amino acids in control	Sub-lethal concentration					
		1/15th 96 hr LC50		1/10th 96 hr LC50		1/5th 96 hr LC50	
		Total free amino acids	% Variation from control	Total free amino acids	% variation from control	Total free amino acids	% variation from control
15	2.300 ± 0.091	2.181 ± 0.124	-5.174	2.121 ± 0.124	-7.783	1.883 ± 0.091	-18.130
30	2.488 ± 0.198	2.263 ± 0.080	-9.043	2.458 ± 0.327	+1.206	2.885 ± 0.106	+15.957
45	2.449 ± 0.061	2.872 ± 0.061	+17.272	3.330 ± 0.091	+35.974	3.630 ± 0.170	+48.224

± Standard deviation.

TABLE - 2G

Variation in blood serum ACP of Liza parsia exposed to "96 hr LC50" of "Nuvan".

(Salinity 10.0 ± 1‰ , Temperature 27.5 ± 1.5°C, pH 5.5)

Exposure period (Hours)	CONTROL			EXPOSURE		
	ACP (µg liberated/ mg protein/minute)	% coefficient of variation	"Nuvan" Conc. (ppm)	ACP (µg liberated/ mg protein/minute)	% coefficient of variation	% variation from control
0	0.104 ± 0.017	15.865	0	0.104 ± 0.017	15.865	0
24	0.109 ± 0.013	11.927	0.48	0.114 ± 0.023	19.737	+ 4.587
48	0.110 ± 0.011	9.545	0.48	0.121 ± 0.008	6.198	+10.000
72	0.113 ± 0.019	16.814	0.48	0.143 ± 0.026	17.832	+26.549
96	0.117 ± 0.014	11.538	0.48	0.183 ± 0.011	5.738	+56.410

± Standard deviation.

(Blood serum protein value from 'Table 2E' taken for calculation)

ACP = Acid phosphatase.

TABLE - 3G

Variation in blood serum ACP of Liza parsia after acute exposures to "Nuvan".

(Salinity 10.0 ± 1‰, Temperature 27.5 ± 1.5°C, pH 5.5)

Exposure period (Hours)	CONTROL			EXPOSURE		
	ACP (µg liberated/mg protein/minute)	% coefficient of variation	"Nuvan" Conc. (ppm)	ACP (µg liberated/mg protein/minute)	% coefficient of variation	% variation from control
0	0.104 ± 0.017	15.865	0	0.104 ± 0.017	15.865	0
24	0.109 ± 0.013	11.927	1.01	0.154 ± 0.017	10.714	+41.284
48	0.110 ± 0.011	9.545	0.75	0.174 ± 0.036	20.690	+58.182
72	0.113 = 0.019	16.814	0.55	0.171 ± 0.011	6.140	+51.327
96	0.117 ± 0.014	11.538	0.48	0.183 ± 0.011	5.738	+56.410

± Standard deviation.

(Blood serum protein value from "Table 3E" taken for calculation)

ACP = Acid phosphatase

Fig. 3G: Percentage variation in blood serum acid phosphatase (ACP) of Liza parsia after acute exposures to "Nuvan".

Fig. 3H: Percentage variation in blood serum ALP of Liza parsia after acute exposures to "Nuvan".

Fig. 3I: Percentage variation in oxygen consumption of Liza parsia after acute exposures to "Nuvan".

Fig. 3G

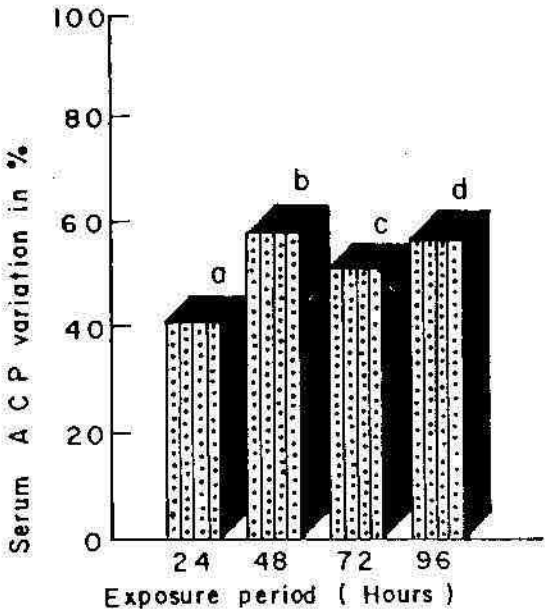


Fig. 3H

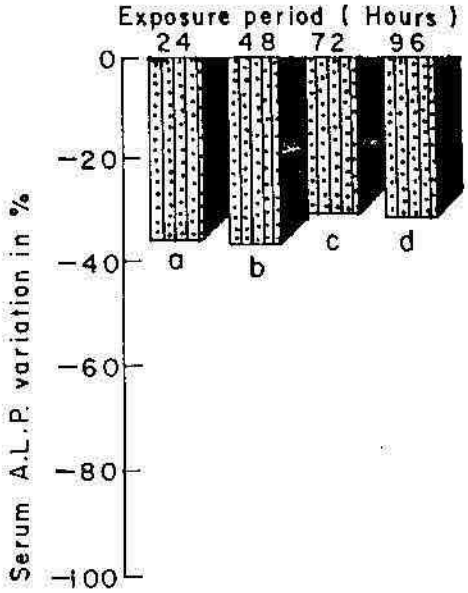
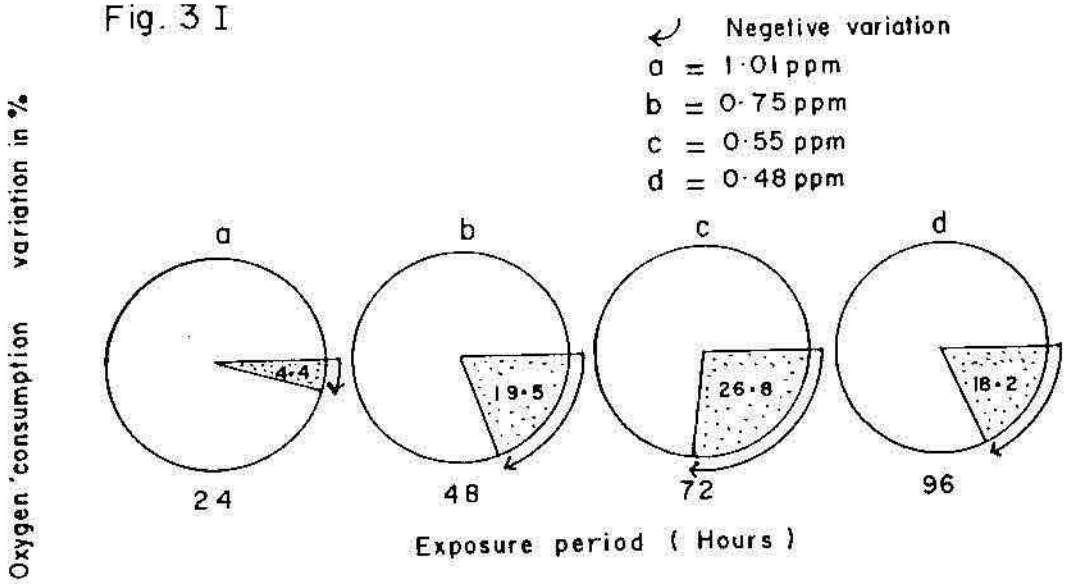


Fig. 3 I



2.2.3.2. On sub-lethal exposures

Increase in acid phosphatase content of serum were observed following sub-lethal exposures (Table 4G and Fig. 4G) for 15, 30 and 45 days at 1/15th, 1/10th and 1/5th 96 hr LC50. Anova analysis showed significant differences between the treatments and exposure periods at 1% F-value.

2.2.4. Alkaline phosphatase content

2.2.4.1. On acute exposures

The depletion was observed in serum alkaline phosphatase content (Table 2H and Fig. 2H) following acute exposure to 0.48 ppm (96 hr LC50) for 24, 48, 72 and 96 hours. Using student 't' test the means were seen differing significantly at 5% level between control and exposure values. The calculated value (4.367) was higher than that of tabulated 't' value (1.701).

Fishes exposed to lethal concentrations of 1.01 ppm, 0.75 ppm, 0.55 ppm and 0.48 ppm for 24, 48, 72 and 96 hours respectively had depleted alkaline phosphatase in blood serum (Table 3H and Fig. 3H). The decrease was greater (-36.496%) in serum of fishes exposed to 0.75 ppm for 48 hours and lower (-30.651%) in serum of fishes exposed to 0.55 ppm for 72 hours. The values in controls were also seen decreasing slowly upto 96 hours.

2.2.4.2. On sub-lethal exposures

Table 4H and Fig. 4H show the depletion in alkaline phosphatase content in blood serum exposed to sub-lethal concentrations of "Nuvan".

TABLE - 4G

ACP (µg liberated/mg Protein/minute) of blood serum of Liza parsia after sub-lethal exposures to "Nuvan"

(Salinity 10.0 ± 1‰ , Temperature 27.5 ± 1.5°C, pH 5.75 ± 0.25)

Exposure period (Days)	ACP in control	Sub-lethal concentrations					
		1/15th 96 hr LC50		1/10th 96 hr LC50		1/5th 96 hr LC50	
		ACP	% variation from control	ACP	% variation from control	ACP	% variation from control
15	0.109 ± 0.010	0.118 ± 0.005	+8.257	0.123 ± 0.003	+ 12.844	0.137 ± 0.014	+25.688
30	0.115 ± 0.003	0.133 ± 0.010	+15.652	0.120 ± 0.003	+4.348	0.147 ± 0.007	+27.826
45	0.121 ± 0.009	0.143 ± 0.013	+18.182	0.155 ± 0.010	+28.099	0.173 ± 0.011	+42.975

± Standard deviation.
(Blood serum protein value from 'Table 4E' taken for calculation)
ACP = Acid phosphatase.

FIG. 4 G: Percentage variation in acid phosphatase (ACP) content of blood serum on chronic exposures to "Nuvan".

Fig. 4H: Percentage variation in alkaline phosphatase (ALP) content of blood serum on chronic exposures to "Nuvan".

Fig. 4I: Percentage variation in oxygen consumption of Liza parsia on chronic exposures to "Nuvan"

Fig. 4 G

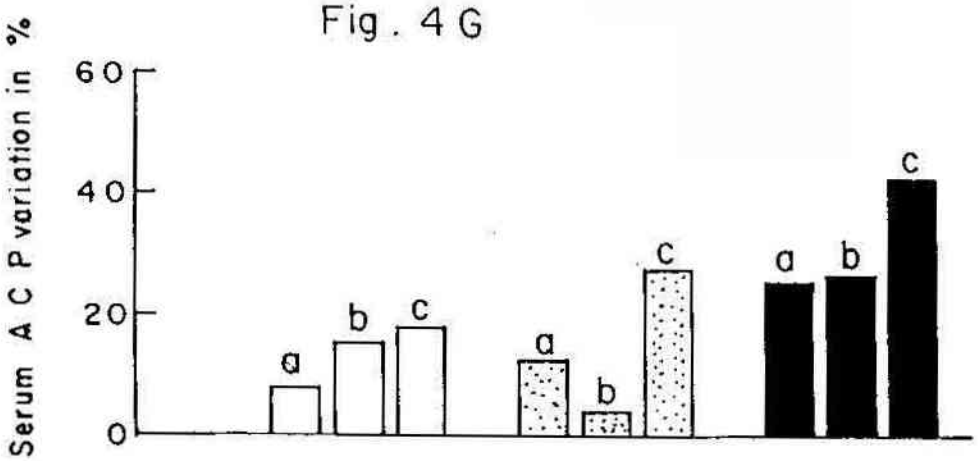


Fig. 4 H

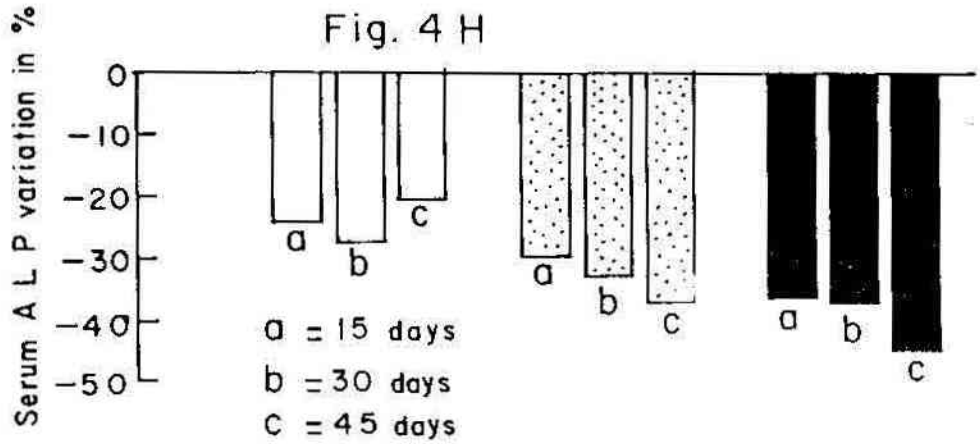


Fig. 4 I

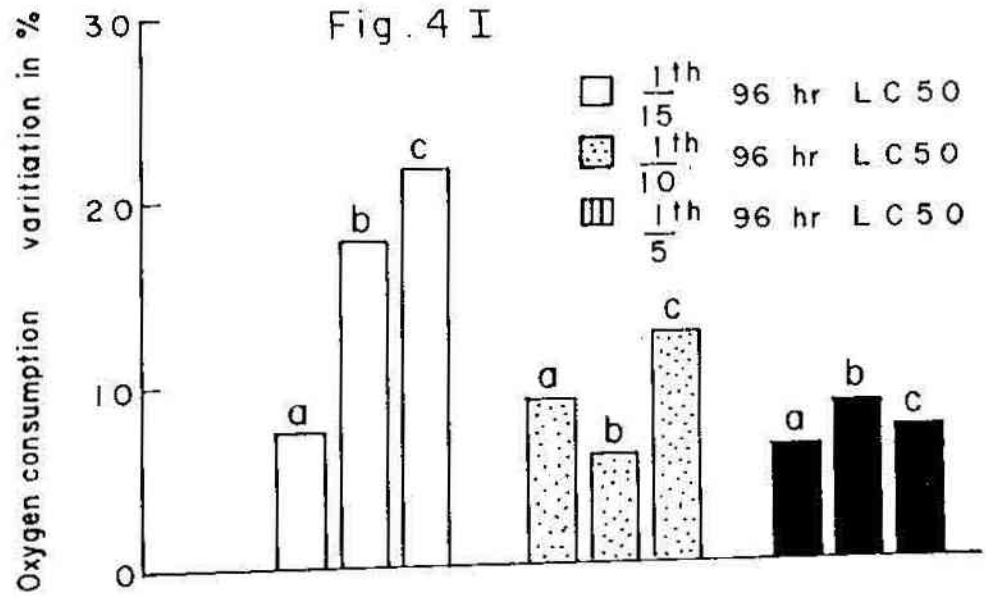


TABLE - 2H

Variation in blood serum ALP of Liza parsia exposed to "96 hr LC50" of "Nuvan"

(Salinity 10.0 ± 1‰ , Temperature 27.5 ± 1.5°C, pH 5.5)

Exposure period (Hours)	CONTROL		EXPOSURE			
	ALP (µg liberated/ mg protein/ minute)	% coefficient of variation	"Nuvan" concentration (ppm)	ALP (µg liberated/ mg protein/minute)	% coefficient of variation	% variation from control
0	0.290 ± 0.016	5.517	0	0.290 ± 0.016	5.517	0
24	0.285 ± 0.025	8.772	0.48	0.250 ± 0.010	4.157	-12.281
48	0.274 ± 0.008	2.920	0.48	0.242 ± 0.013	5.372	-11.679
72	0.261 ± 0.026	9.962	0.48	0.205 ± 0.008	3.902	-21.456
96	0.252 ± 0.010	3.898	0.48	0.173 ± 0.023	13.295	-31.349

± Standard deviation.

(Blood serum protein value from 'Table 2E' taken for calculation)

ALP = Alkaline phosphatase.

TABLE - 3H

Variation in blood serum ALP of Liza parsia after acute exposures to "Nuvan"

(Salinity 10.0 ± 1‰ , Temperature 27.5 ± 1.5°C , pH 5.5)

Exposure period (Hours)	CONTROL			EXPOSURE		
	ALP (µg liberated/mg protein/minute)	% coefficient of variation	"Nuvan" Conc. (ppm)	ALP (µg liberated/mg protein/minute)	% coefficient of variation	% variation from control
0	0.290 ± 0.016	5.517	0	0.290 ± 0.016	5.517	0
24	0.285 ± 0.025	8.772	1.01	0.183 ± 0.012	6.630	-35.789
48	0.274 ± 0.008	2.920	0.75	0.174 ± 0.028	16.092	-36.496
72	0.261 ± 0.026	9.962	0.55	0.181 ± 0.017	9.144	-30.651
96	0.252 ± 0.010	3.898	0.48	0.173 ± 0.023	13.295	-31.349

± Standard deviation.

(Blood serum protein value from 'Table 3E' taken for calculation)

ALP = Alkaline phosphatase

TABLE - 4H

ALP (µg liberated/mg protein/minute) in blood serum of Liza parsia after sub-lethal exposures to "Nuvan"

(Salinity 10.0 ± 1‰ , Temperature 27.5 ± 1.5°C, pH 5.75 ± 0.25)

Exposure period (Days)	ALP in control	Sub-lethal concentrations					
		1/15th 96 hr LC50		1/10th 96 hr LC50		1/5th 96 hr LC50	
		ALP	% variation from control	ALP	% variation from control	ALP	% variation from control
15	0.282 ± 0.009	0.214 ± 0.020	-24.113	0.197 ± 0.013	-30.142	0.177 ± 0.020	-37.234
30	0.276 ± 0.010	0.201 ± 0.014	-27.174	0.183 ± 0.012	-33.696	0.174 ± 0.008	-37.956
45	0.274 ± 0.019	0.217 ± 0.028	-20.803	0.169 ± 0.006	-38.321	0.147 ± 0.033	-46.350

± Standard deviation.
(Blood serum protein value from 'Table 4E' taken for calculation)
ALP = Alkaline phosphatase

Anova analysis showed that the variations in alkaline phosphatase contents between treatments were highly significant at 1% F-value.

3. Physiological studies:

3.1. Oxygen consumption

3.1.1. On acute exposures

The oxygen consumption of fishes exposed to 96 hr LC50 (i.e., 0.48 ppm) for 24, 48, 72 and 96 hours are shown in Table 2I and Fig. 2I. An initial increase was observed upto 48 hours of exposure followed by decline in subsequent exposure periods upto -18.24% at 96 hours. Student 't' test showed the significant difference between control and exposure animals at 5% level (where t-cal = 1.926 and t-tab = 1.701).

Table 3I and Fig.3I indicate the decrease in oxygen consumption in fishes exposed to all the 4 lethal concentrations (e.g., 24 hr LC50, 48 hr LC50, 72 hr LC50 and 96 hr LC50 for 24, 48, 72 and 96 hours respectively). The maximum decrease (-26.82%) was observed at 0.55 ppm for 72 hours against minimum value (-4.41%) at 1.01 ppm for 24 hours. But gradual increase in oxygen consumption was noticed in control fishes for 96 hours.

3.1.2. On sub-lethal exposures

The increase in oxygen consumption of fishes exposed to different sub-lethal concentrations for 15, 30 and 45 days are given in Table 4I and Fig. 4I. At lower concentration the increase was maximum than that

TABLE - 21

Variation in oxygen consumption of Liza parsia exposed to "96 hr LC50" of "Nuvan"

(Salinity 10.0 ± 1‰ , Temperature 27.5 ± 1.5°C, pH 5.5)

Exposure period (Hours)	CONTROL		EXPOSURE			
	Control value in "mg/kg body wt/hour"	% coefficient of variation	"Nuvan" concentration (ppm)	Exposure value in "mg/kg body wt./hour"	% coefficient of variation	% variation from control
0	383.3 ± 25.7	6.71	0	383.3 ± 25.7	6.71	0
24	397.0 ± 15.6	3.93	0.48	445.9 ± 15.0	3.37	+12.30
48	433.2 ± 15.6	3.60	0.48	446.4 ± 19.3	4.33	+3.06
72	462.6 ± 21.8	4.72	0.48	407.5 ± 35.4	8.70	-11.91
96	484.6 ± 41.2	8.50	0.48	396.2 ± 14.5	3.66	-18.24

± Standard deviation.

TABLE - 31

Variation in oxygen consumption of Liza parsia after acute exposures to "Nuvan"

(Salinity 10.0 ± 1‰ , Temperature 27.5 ± 1.5°C, pH 5.5)

Exposure period (Hours)	CONTROL		EXPOSURE			
	Oxygen consumption in control (mg/kg body wt/hour)	% coefficient of variation	"Nuvan" Conc. (ppm)	Oxygen consumption of exposed animals (mg/kg body wt/ hour)	% coefficient of variation	% variation from control
0	383.3 ± 25.7	6.71	0	383.3 ± 25.7	6.71	0
24	397.0 ± 15.6	3.93	1.01	379.5 ± 20.8	5.47	-4.41
48	433.2 ± 15.6	3.60	0.75	348.9 ± 37.2	10.66	-19.46
72	462.6 ± 21.8	4.72	0.55	338.5 ± 15.7	4.64	-26.82
96	484.6 ± 41.2	8.50	0.48	396.2 ± 14.5	3.66	-18.24

± Standard deviation.

TABLE - 41

Oxygen consumption (mg/kg body wt/hour) of Liza parsia after sub-lethal exposures to "Nuvan"

(Salinity 10.0 ± 1‰ , Temperature 27.5 ± 1.5°C , pH 5.75 ± 0.25)

Exposure period (Days)	Oxygen consumption in control	Sub-lethal concentrations					
		1/15th 96 hr LC50		1/10th 96 hr LC50		1/5th 96 hr LC50	
		Oxygen consumption	% variation from control	Oxygen consumption	% variation from control	Oxygen consumption	% variation from control
15	411.7 ± 34.3	442.2 ± 26.4	+7.41	449.3 ± 45.8	+9.13	437.8 ± 37.9	+6.34
30	446.0 ± 40.6	525.6 ± 47.9	+17.85	472.3 ± 34.7	+5.90	485.7 ± 17.2	+8.74
45	488.5 ± 36.8	594.7 ± 27.0	+21.74	550.7 ± 38.1	+12.73	525.3 ± 45.4	+7.47

± Standard deviation.

of higher concentrations. Anova indicated that the differences between control - 1/15th 96 hr LC50 and control - 1/10th 96 hr LC50 were significant at 5% F-value and the differences between exposure periods highly significant at 1% F-value.

3.2. Histological studies

Toxic effects in liver and kidney of Liza parsia exposed to lethal and sub-lethal concentrations of "Nuvan" for different exposure periods were studied.

3.2.1. Liver

In normal liver the hepatocytes are polygonal and have a distinctive central nucleus with densely staining chromatin margins and a prominent nucleolus. The portal triad (Central vein, Plate I.A.a; Bile duct Plate I.A.b; and Portal vein, Plate I.A.c) and hepatocytes photomicrographed from the transverse section of normal liver of Liza parsia is shown in (Plate I.A.)

Fishes sacrificed after acute exposures to "Nuvan" (e.g., 48 hr LC50, 72 hr LC50 and 96 hr LC50 for 48, 72 and 96 hr respectively) showed the extensive coagulative necrosis with pyknosis, karyorrhexis, karyolysis and vacuolar degeneration of cytoplasm of hepatocytes (Plate I.B, C and D). As seen in plate 1E congested central vein and centrilobular hypertrophy of hepatocytes with hyper chromatin nuclei is observed in fish that died on 12th day of exposure to 0.48 ppm in 96 hr LC50 experiment. In sub-lethal concentration (e.g., at 30th day in 1/15th 96 hr LC50) the distinctive

degenerative changes with karyolysis and karyorrhexis were more pronounced (Plate I.F), but 1/15th 96 hr LC50 for 45 days showed coagulative necrosis with proliferation of kuppfer cells (Plate I.H). In fishes exposed to higher sub-lethal concentrations i.e., 1/15th 96 hr LC50 for 30 and 45 days, and 1/10th 96 hr LC50 for 45 days, pushing of nuclei to one side, vacuolar degeneration, pyknosis and karyolysis of hepatocytes were observed (Plate I.G, J and I).

3.2.2. Kidney

The nephron of the typical euryhaline teleost comprises of a well vascularised glomerulus, neck segment and two or three proximal segments. Photomicrograph of the transverse section of kidney of an unexposed Liza parsia shows normal size and structure of reneal tubules and epithelial cells (Plate II.A). Fishes sacrificed after acute exposures to 48 hr LC50 and 96 hr LC50 showed enlargement of renal tubules (Plate II.B, C). In the section after sub-acute exposure to 1/15th 96 hr LC50 for 30 days vacuolation of epithelial cells of renal tubules are observed (Plate II.D). On exposure to 1/5th concentration of 96 hr LC50 for 45 days marked necrosis and extensive desquamation and flattening were observed in the tubular epithelial cells (Plate II.E).

Plate I.A: Cross section of normal liver of fish showing hepatocytes and portal triad (central vein, a; bile duct, b; and portal vein, c). H & E X 100.

Plate I.B: Cross section of liver after exposure to "48 hr LC50" of "Nuvan" for 48 hours showing extensive coagulative necrosis with pyknosis, karyorrhexis (Kh), karyolysis (Kl) and vacuolar degeneration of cytoplasm of hepatocytes (V). H & E X 400.

Plate I.C: Cross section of liver after exposure to "72 hr LC50" of "Nuvan" for 72 hours showing extensive coagulative necrosis with pyknosis (P), karyorrhexis, karyolysis and vacuolar degeneration of cytoplasm of hepatocytes H & E X 400.

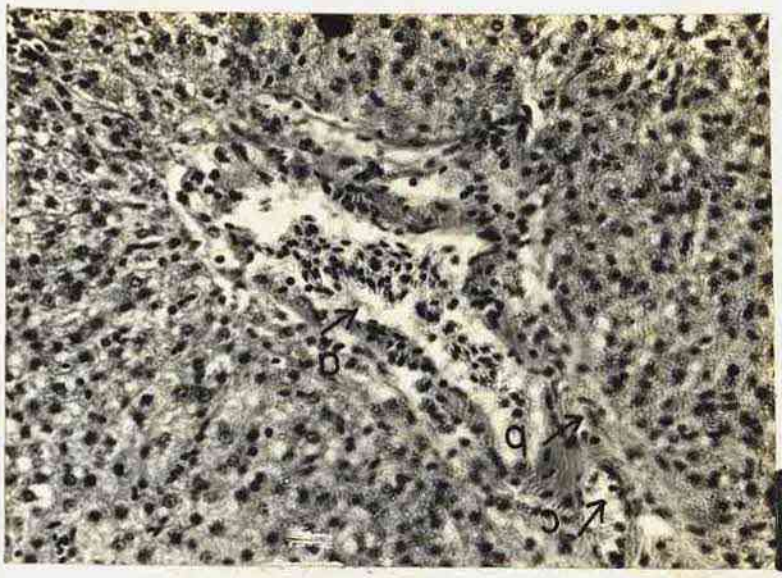


Plate I.A

Plate I.B

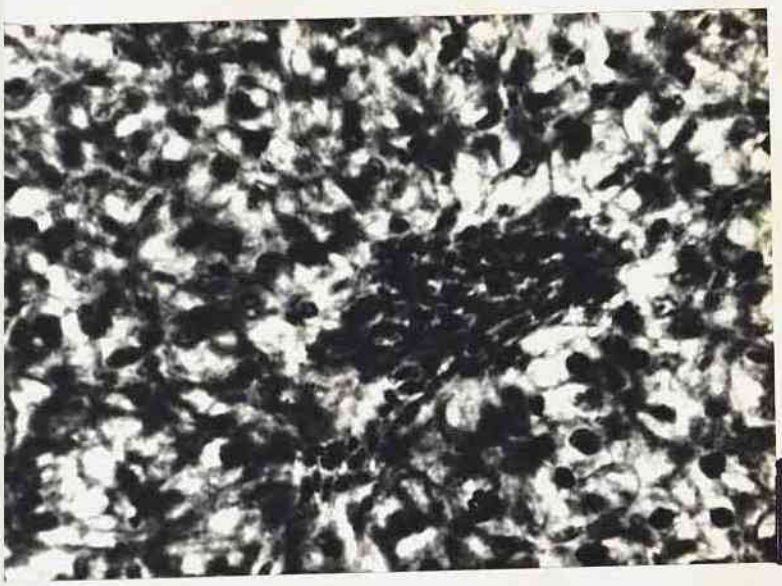
Plate I.C

Plate I.D: Cross section of liver after exposure to "96 hr LC50" of "Nuvan" for 96 hours showing extensive coagulative necrosis with pyknosis, karyorrhexis (Kh), karyolysis (Kl) and vacuolar degeneration of cytoplasm of hepatocytes (V). H & E X 400.

Plate I.E: Cross section of liver of fish that died on 12th day of exposure to "96 hr LC50" of "Nuvan" showing congested central vein and centrilobular hypertrophy of hepatocytes with hyper chromatin nuclei. H & E X 400.

Plate I.F: Cross section of liver after exposure to "1/15th 96 hr LC50" of "Nuvan" for 30 days showing distinctive degenerative changes with karyolysis and karyorrhexis (Kh). H & E X 200.

Plate I.D



Plae I.E

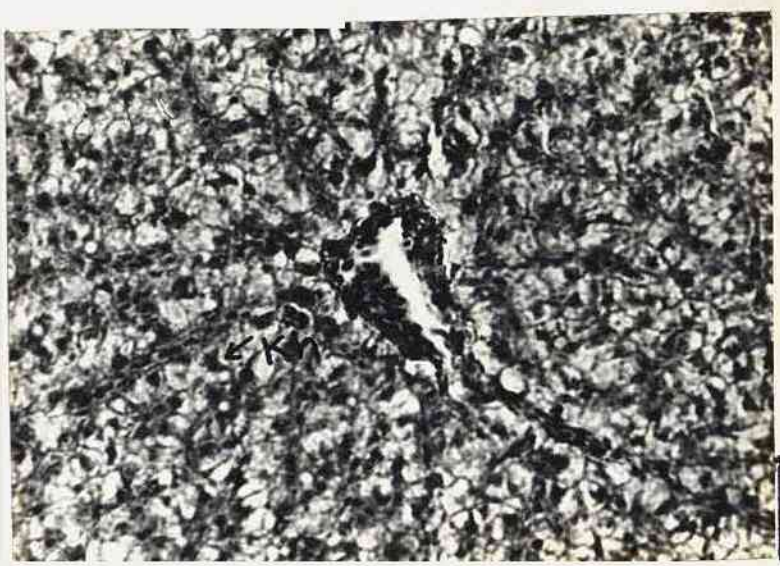


Plate I.F

Plate I.G: Cross section of liver after exposure to "1/5th 96 hr LC50" of "Nuvan" for 30 days showing vacuolar degeneration of cytoplasm of hepatocytes (V), pushing of nuclei to one side, pyknosis and karyolysis. H & E X 400.

Plate I.H: Cross section of liver after exposure to "1/15th 96 hr LC50" of "Nuvan" for 45 days showing coagulative necrosis with proliferation of kuppfer cells (K). H & E X 200.

Plate I.I: Cross section of liver after exposure to "1/10th 96 hr LC50" of "Nuvan" for 45 days showing vacuolar degeneration, pushing of nuclei to one side, pyknosis and karyolysis. H & E X 400.

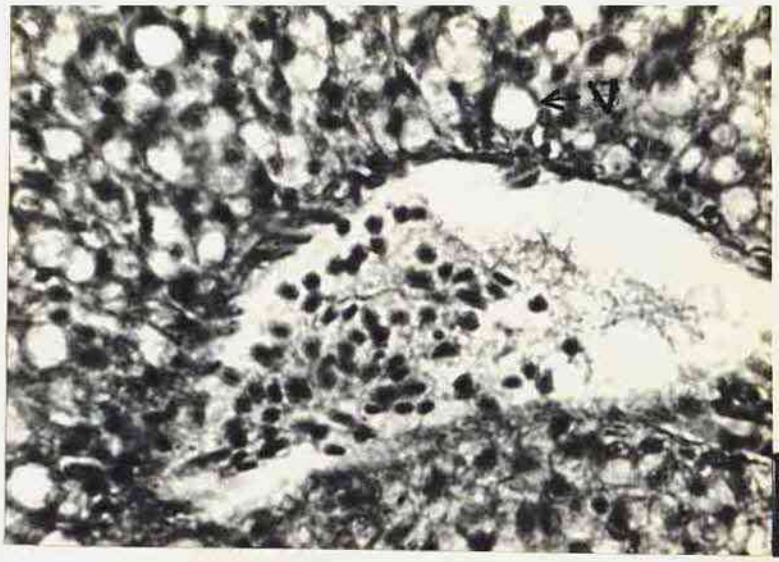


Plate I.G

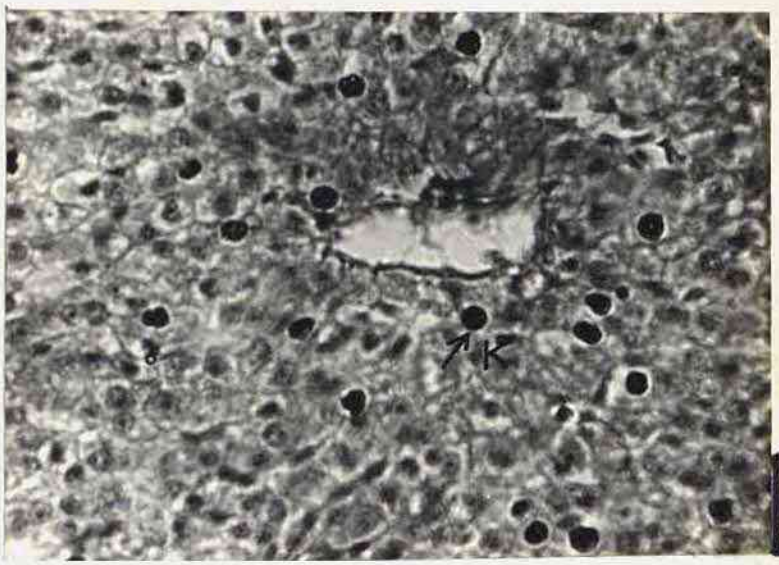


Plate I.H

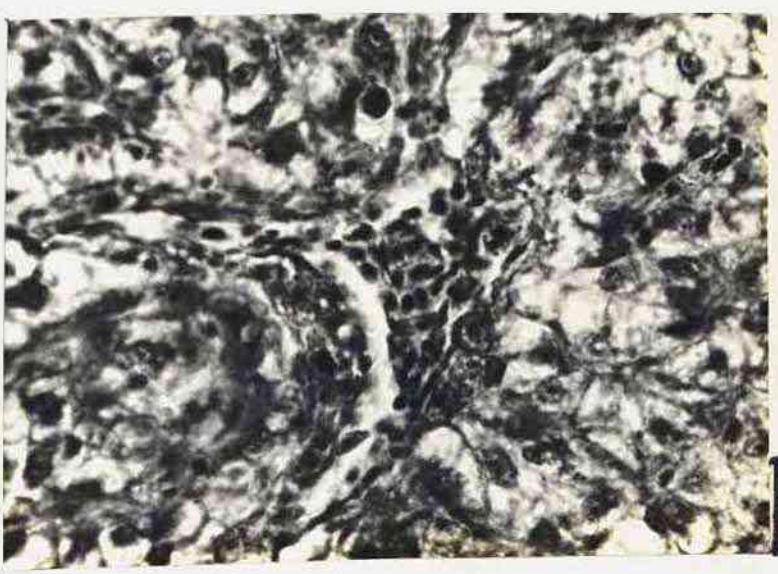


Plate I.I.

Plate I.J: Cross section of liver after exposure to "1/5th 96 hr LC50" of "Nuvan" for 45 days showing pyknosis, karyolysis, pushing of nuclei to one side and vacuolar degeneration of cytoplasm of hepatocytes (V). H & E X 400.

Plate II.A : Photomicrograph of kidney section of fish showing renal tubules (Rt) and epithelial cells (Ec). H & E X 200.

Plate II.B: Cross section of kidney after exposure to "48 hr LC50" of "Nuvan" for 48 hours showing the enlargement of renal tubules. H & E X 200.

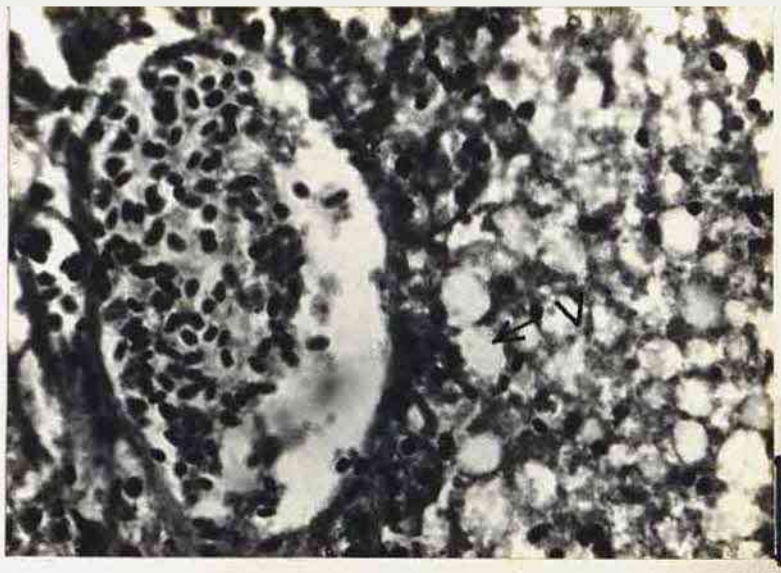


Plate I.J.

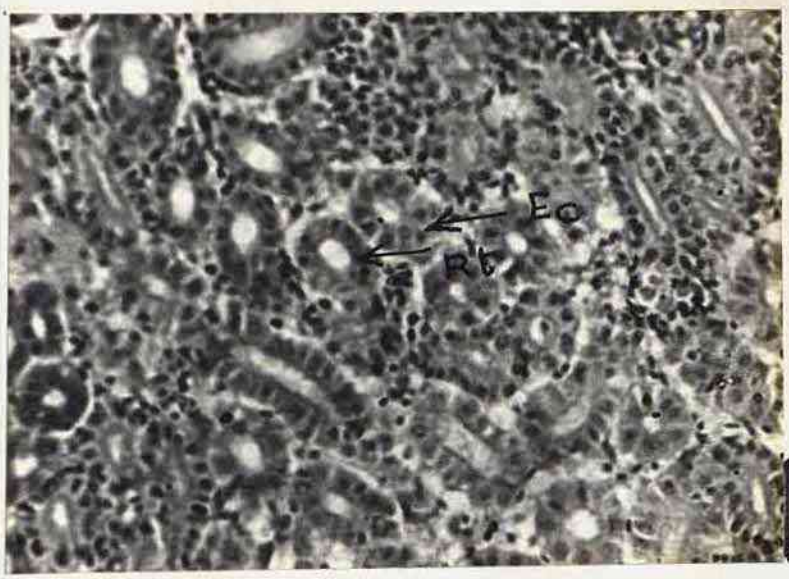


Plate II.A

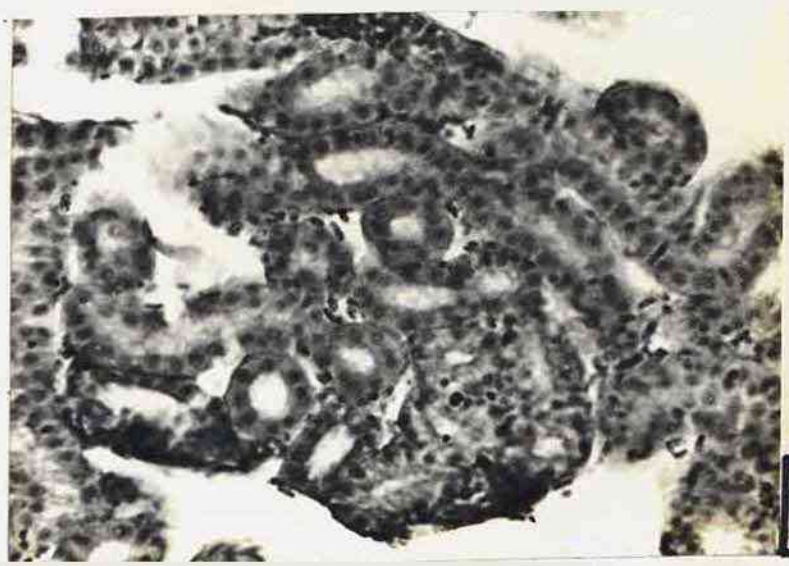


Plate II.B

Plate II.C: Cross section of kidney after exposure to "96 hr LC50" of "Nuvan" for 96 hours showing the enlargement of renal tubules. H & E X 200.

Plate II.D: Photomicrograph of kidney section after exposure to "1/15th 96 hr LC50" of "Nuvan" for 30 days showing vacuolation of epithelial cells of renal tubules. H & E X 40.

Plate II.E: Cross section of kidney after exposure to "1/ 5th 96 hr LC50" of "Nuvan" for 45 days showing marked necrosis of tubular epithelial cells and extensive desquamation and flattening (F). H & E X 200.

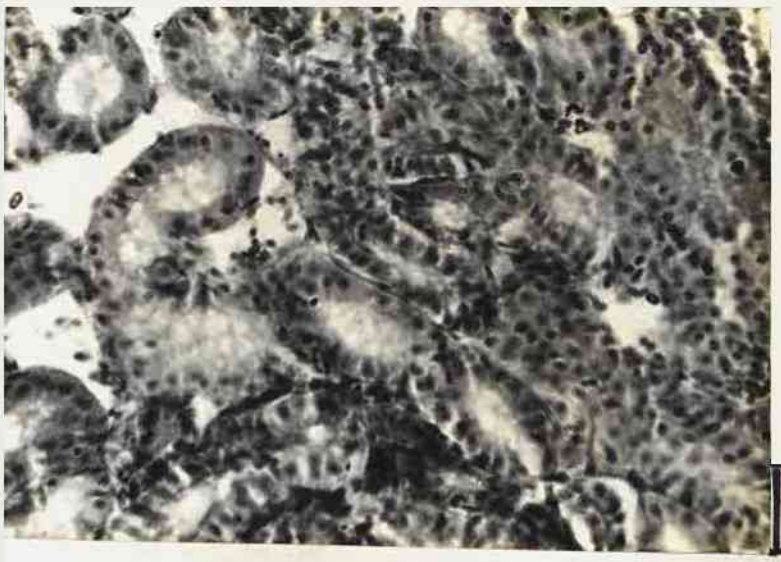


Plate II.C

Plate II.D

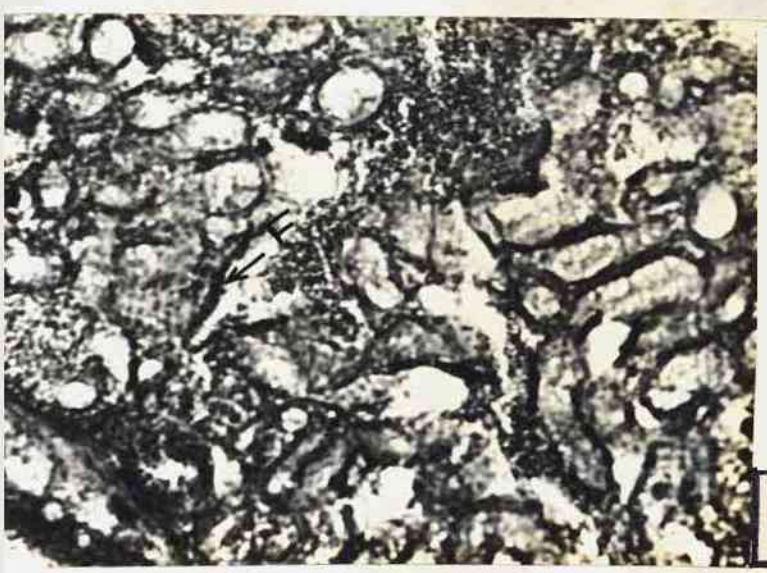


Plate II.E

D I S C U S S I O N

Acute toxicity studies:

The slope function, 95% confidence limit, and 95% fiducial limits as already given in results were found to gradually decrease in all the 4 exposure periods of 24, 48, 72 and 96 hours. The variations in percentage mortality (Table 1A-D), LC16, LC50, LC84 values (Table 1E) at different exposure periods indicate differential toxicity of "Nuvan" to Liza parsia Presently only a few reports are available pertaining to this type of work and on "Nuvan" as given in the next paragraph it is scanty. Though the LC-50 for 48 hours was showing a little higher value than the rest (Table 1E . it stays within the 95% fiducial limits (Fig.1E) and supports gradual decrease of all LC50 values by increase of time.

Verma et al. (1982) reported acute toxic ranges and LC50 values for 7 organochlorine, 14 organophosphorus, and 2 carbamate pesticides on Saccobranchus fossilis and found the acute toxicity range of 6-10 ppm and 15-20 ppm for DDVP (Nuvan) and malathion respectively. From their result it was proved that malathion is 2-3 times more toxic to fishes than DDVP. Pal (1983) reported 1.7 ppm and 12.9 ppm of DDVP as 48 hr LC5 and LC95 for Tilapia mossambica respectively. According to Sailatha et al. (1981) the commercial grade malathion was found to be 14.8 times more toxic than the technical grade malathion on Tilapia mossambica (e.g., 48 hr LC50 for tgM 5.542 ± 0.04 ppm and for cgM 0.337 ± 0.04 ppm).

In the present bioassay study 0.75 ppm of commercial grade "Nuvan" was found as 48 hr LC50 for Liza parsia. As the reports are not available on acute toxicity of "Nuvan" for brackishwater teleosts the present data cannot be compared with other results. Studies undertaken by Protic and Sabjlic (1989) to test the ability of molecular connectivity model for predicting the level of acute toxicity of different pollutants have shown that the molecular size of chemicals is directly proportional to acute toxicity. From their report it may be said that the acute toxicities for different chemicals can not be compared directly without going to the molecular level of it.

Biochemical studies:

Biochemical studies are gaining recently a lot of importance as indicators of toxicity or pollution stress. Bayne (1986) reported that biochemical responses can be useful in assessment of environmental impact on organism due to its increased specificity and sensitivity. The same report was supported by Connor and Huggett (1988).

The data presented in the present investigation revealed marked fluctuations in the organic constituents of muscle and blood serum on acute and chronic exposures to "Nuvan".

Depletion in the muscle protein content on acute (Table 2A and 3A) and sub-acute (Table 4A) exposures was observed in the present study. Similar observations were made by Ramalingam and Ramalingam (1982)

in Sarotherodon mossambicus exposed to malathion. They reported the conversion of tissue proteins into soluble fractions reaching the blood for utilisation at pesticidal stress. Ahamad et al. (1978) observed decrease in the mantle protein in Lamellidens marginalis exposed to sub-lethal concentrations of malathion. Decrease in liver protein of fishes exposed to malathion was also reported by Mukhopadhyay and Dehadrai (1980),Awasthi et al. (1984) and Kumar and Alam Ansari (1986) in Clarias batrachus, Channa punctatus and Brachydanio rerio respectively. Several other investigators (McLeay and Brown 1974, Panigrahi and Mishra 1980, Sashikala et al.1985, Parveen et al. 1987, Sastry et al. 1987) have also reported the depletion of protein in fish muscle tissue exposed to various toxicants. The possible causative factor for such a decrease may be due to the extensive proteolysis taking place in the muscle. The decline in muscle tissue protein in Liza parsia following acute and sub-acute exposures to "Nuvan" in the present study may be due to the utilization of muscle protein under stressed condition to meet the increased metabolic demands of the animals.

Increase in total free amino acids in liver due to sub-lethal exposure to malathion has been reported in Brachydanio rerio by Kumar and Alam Ansari (1986). Elevation of muscle tissue amino acids has been studied in Sarotherodon mossambicus exposed to ammonia (Sashikala et al. 1985). However, in the present study, elevation of total free amino acids in muscle tissue on acute exposures to "Nuvan" was accompanied by decrease in the protein level. It may be due to the break down of proteins into amino acids at pesticidal stress. But in case of sub-lethal exposures the

depletion of total free amino acids was observed initially followed by the gradual elevation (Table 4B). The depletion may be due to the conversion of amino acids into glucose in the process of gluconeogenesis followed by oxidation to CO_2 via the tricarboxylic acid cycle (Lehninger 1975). As the animals in the present study were fed daily with pellet feed, Lehninger's assumption may not hold good, and the possible reason may be the oxidative de-amination of amino acids. Latter increase may be related to the proteolysis of proteins and peptides by proteolytic enzymes to amino acids for energy production at biomolecular level.

Mustafa (1977) working on the maturation stress in Clarias batrachus, reported a decrease in the RNA content of skeletal muscle. He suggested that this decrease was due to the food deprivation leading to depletion of cellular components which accompanied maturation of fish. Decrease in RNA content of liver of fish subjected to sub-lethal concentration of Cadmium was reported in Channa punctatus (Dubale and Shah 1981a). A decrease in RNA of liver was reported in Brachydanio rerio sub-lethally exposed to malathion in 1986 by Kumar and Alam Ansari. The decrease in RNA of muscle tissue following acute (Table 2C and 3C) and sub-acute (Table 4C) exposure to "Nuvan" in the present study may be due to food deprivation in fishes during lethal exposures and reduction in apetite during sub-lethal exposures. The decrease of protein content of muscle as already dealt with early was observed in the present study. As RNA has direct relation with protein content, it may be concluded that the reduction of RNA was due to the proteolysis of protein by releasing the components

to the blood rather than the protein synthesis. The statement "Growth in term of accumulation of protein is always accomplished by high turn-over rate of RNA concentration which is a prime factor of protein synthesis machinery" by Buckley (1984) supports the possible cause given for the reduction of RNA content in the present study.

Increase in DNA concentration of body tissues has been reported by Mustafa (1977) in Clarias batrachus. According to him the depletion of cellular components leads to reduction in the weights of individual cells and decline in the cytoplasmic volume. As a result of this a large number of cells are required to make a given weight of muscle tissue sample and thus DNA per unit weight increases. That the concentration of DNA in a given weight of tissue is related to the number of cells contained in it has been established earlier (Bulow 1970). Besides this, the depletion of cellular constituents is also understood to cause shrinkage of the cells which in turn brings the cell nuclei into greater proximity. Therefore, it is quite logical to expect that the increase in the concentration of nuclei also increases the DNA concentration per unit weight of the tissue. Denizeau and Marion (1984), Stueber and Zahn (1985), and Kumar and Alam Ansari (1986) have reported the inhibition of DNA in liver of fishes exposed to different pesticides. In the present study, both in acute (Table 2D and 3D) and sub-lethal (Table 4D) exposures the elevation of DNA content in muscle tissue was observed. The possible reason for this may be the star-vation of animals in acute toxicity test and significant effect of "Nuvan" in both acute and sub-lethal experiments.

Growth is generally faster in early phases of the life cycle of animals (Dagg and Littlepage 1972). Therefore small animals with high RNA-DNA ratio would be expected to grow at faster rate than those of lower ratio. The decrease in RNA-DNA ratio following acute and sub-lethal exposures to "Nuvan" is observed in present investigation(Table 2J, 3J and 4J) and the decrease was maximum for the animals in acute exposure. Gradual decrease with increase in concentration and exposure period was observed in chronic experiment. In control the reduction in ratio may be related to the starved condition of fishes. In all the exposures the reduction in RNA-DNA ratio may be related to the reduction in growth rates. Maintenance of the high RNA-DNA ratio and continuity of growth at faster rate also depend upon the availability of sufficient food (Buckley 1979). The non-availability of food to experimental animals may be a reason for reduction of RNA-DNA ratio in the present acute exposure experiment also. Bulow (1970) had proved the RNA-DNA ratio as the sensitive indicator of growth rates of a fish. According to him the ratio and growth rates have the direct relationship. With no work other than the present one on exposure of fish to pesticide toxicity further comparison is not possible.

Shakoori et al. (1976) noticed a increase of serum protein in Channa punctatus (bloch) exposed to acute and sub-acute levels of malathion, dieldrin and endrin. Similar results were obtained by DiMichele and Taylor (1978) in Fundulus heteroclitus exposed to low levels of napthalene and they suggested that the increase might be due to the metabolic stress at exposures. In Carp, exposed to "Arochlor 1248", Ito and Murata (1980) have reported

increase in serum protein. They have mentioned that increased globulin content accounted for the increased total protein content. Elevation in plasma protein concentration has been reported by Oikari et al. (1983) in their work on Salmo gairdneri exposed to acute levels of dehydroabietic acid (DHAA). They felt that this was due to the increased content of globulin in the plasma. The increased serum protein in Liza parsia exposed to 96 hr LC50 for 24, 48 and 72 hours (Table 2E), chronically to 1/5th, 1/10th and 1/15th 96 hr LC50 for 15 days and in 1/15th 96 hr LC50, 1/10th 96 hr LC50 for 30 days (Table 4E) in the current investigation may also be related to the possible cause reported by DiMichele and Taylor (1978), and Ito and Murata (1980).

Total protein levels of serum decreased in Channa punctatus chronically exposed to endosulphan (Sastry and Siddiqui 1983) and it was reported to be due to low assimilation of food. Reduced serum protein was observed in Ictalurus punctatus exposed to acute level of "Arochlor 1254" and was explained Camp et al. (1974) to be due to an attendant reduction in the albumin-globulin ratio. Changes in quantity of serum proteins of Catla catla after 0.5 ppm mercury treatment was reported by Rai (1987). Several other investigators (NewComb 1974, Oikari and Soivio 1977, and Saxena and Mani 1985) have reported the depletion of plasma protein in fish exposed to various toxicants. The decrease in protein level of serum in acute (Table 2E and 3E) and sub-lethal (Table 4E) exposures for longer duration in present study may also be due to the low assimilation of food, inhibition in amino acids uptake for protein synthesis, or low levels of albumin and globulin content in blood.

Increased total free amino acids in blood serum was observed in Clarias batrachus after sub-lethal exposure to malathion (Mukhopadhyay and Dehadrai 1980). Similar result was obtained in blood by Dabrowska and Walasow (1986) in Cyprinus carpio exposed to sub-lethal concentration of ammonia. The increase in total free amino acids in plasma in the present study after exposure to 96 hr LC50 upto 72 hours (Table 2F) and longer period in sub-lethal concentrations (Table 4F) may be due to the breakdown of protein in muscle tissue releasing the amino acids to the blood stream. But in all 4 lethal exposures (Table 3F) and in short duration to sub-lethal concentration (Table 4F) the depletion of amino acids content in serum was observed in the present work. This may be due to the withdrawl of amino acids from blood stream to the surrounding active tissues for the energy production or for the protein synthesis needed in production of detoxifying enzymes at pesticidal stress as reported by Ramaswamy (1987) in Sarotherodon mossambicus after acute exposure to "sevin". The decrease in free amino acids in blood also reported by Shakoori et al. (1976) after acute exposure to malathion, dieldrin, and endrin in Channa punctatus (Bloch).

Elevations in the serum acid phosphatase on acute (Table 2G and 3G) and sub-acute (Table 4G) exposures were observed in the present study. Similar observation was made by Dalela et al. (1980b) in blood serum of Notopterus notopterus exposed to sub-lethal concentration of phenolic compounds and by Sharma et al. (1982) in Heteropneustes fossilis under sub-lethal stress of Congo Red (Diphenyl disazo binapthionic acid). Verma

et al. (1984) also reported an increase of serum acid phosphatase in Mystus vittatus during sub-acute (1/5th, 1/10th, 1/15th 96 hr LC50) exposures to thiotox, dichlorvos, carbofuran and their three combinations. In freshwater prawn Macrobrachium lamarrei the elevation of acid phosphatase activity has been recorded by Shukla and Shukla (1985) after sub-lethal dichlorvos exposure under static conditions. Sastry and Siddiqui (1983) reported the higher level of lactic acid in blood of Channa punctatus chronically exposed to endosulphan and thus reduces the pH of blood. Reports on stimulations of acid phosphatase in acute toxicities are scanty. In present investigation, the elevation in the acid phosphatase in serum may be due to the low pH of blood. The low pH of test media (e.g., 5.5) also may be a cause to reduce the pH of blood through diffusion.

In degenerating or damaged cells the activity of metabolites is less as alkaline phosphatases are involved in anabolic processes. As a group of hydrolysing enzymes, at stress it affects the digestion, intermediary metabolism, and membrane transport. Increase in alkaline phosphatases due to pollution stress has been studied in fishes by a number of workers (Ito and Murata 1980, Shaffi 1980, Sharma et al. 1982, and Verma et al. 1984). In contrast to these reports, decrease in serum alkaline phosphatase of fishes subjected to sub-lethal exposure of nitrogen supersaturation was reported in juvenile steelhead trout Salmo gairdneri (NewComb 1974). In fishes subjected to sub-lethal concentration of organochlorine, organophosphate, and phenylcarbamatic pesticide, the inhibition of alkaline phospha - tase has been reported in Barbus stigma by Khillare and Wagh (1988).Reduction

of alkaline phosphatase in the liver and kidney of Nemacheilus denisonii exposed to acute concentration of phosphamidon was reported by Rashatwar and Ilyas (1984). The inhibition of alkaline phosphatase activity was recorded in a freshwater prawn Macrobrachium lamarrei after sub-lethal dichlorvos exposure under static condition by Shukla and Shukla (1985). In all the above cases the inhibition was brought about by the fall of pH in blood following the rupture of tissue membrane. The same may be the cause for the reduction of alkaline phosphatase in the serum of Liza parsia in the present study in both lethal (Table 2H and 3H) and sub-lethal (Table 4H) exposures.

Physiological studies:

The inhibition in respiration and death of fish due to anoxia has been reported in fathead minnow (Pimephales promelas) exposed to chloramine by Grothe and Eaton (1975). These investigators suggested that the cause of chloramine induced death in fish was probably associated with "methemoglobinemia". The formation of methemoglobin resulted from chloramine-induced oxidation of red cells as they pass through the gills of the fish and can no longer deliver an adequate supply of oxygen to the tissues thus resulting the reduction of oxygen uptake and death. Tsai and Mckee (1980) working on the acute toxicity of chloramines, copper, and linear alkylate sulfonate on Carassius auratus noted the diminishing of oxygen uptake due to the oxidation of hæmoglobin to methemoglobin resulting in the death of fish from anoxia. Several other investigators (Lee et al. 1975, Lingaraj and Venugopalan 1978, Singh and Singh 1979, Rao et al. 1980, Skadsen

et al. 1980, Bakthavathsalam and Srinivasa Reddy 1985 and Pal and Konar (1985) have also reported the decrease in oxygen uptake over control in fishes exposed to various toxicants. The cause reported by Tsai and Mckee (1980) may be the possible explanation for the decrease in oxygen uptake in Liza parsia exposed to 4 lethal concentrations (Table 3I).

Jawale (1985) reported all pesticides to increase metabolic rate of fish over the control at LC50 concentrations initially followed by sudden decrease and death. His results were based on the use of DDT, endosulphan, rogor and dimecron on the metabolism of fish Rosbora daniconius. Brafield and Matthiessen (1976) observed the oxygen uptake trend to rise and then become extremely erratic and declining as death approaches in fish Gasterosteus aculeatus exposed to Zinc. Increasing respiration rate in fish Macropetrus salmoides exposed to pentachlorophenol (PCP) has been reported by Mathers et al. 1985. They suggested that PCP caused general metabolic stress by uncoupling oxidative phosphorylation in fishes. In the present study the results for oxygen uptake in 96 hr LC50 experiment obtained (Table 2I) is same as the result of Brafield and Matthiessen (1976) but the possible reasons are not clearly known. Initial increase however, may be due to increased activity to overcome the stress. But later decrease may be due to anoxia especially of the brain cells.

Increased O_2 uptake was observed in sockeye salmon exposed to sub-lethal concentration of bleached kraft pulp mill effluent - BKME(Davis 1973). He indicated that the arterial oxygen tension in sockeye salmon

declined rapidly and remains depressed following upto 24 hr exposure to BKME (33-47% of 4 day *LC50*). On an average according to him the decline represented a 20% decrease in oxygen saturation of the blood and decreased arterial oxygen tension increases the oxygen uptake through the gills to maintain the equilibrium. Sastry and Siddiqui (1983) reported the high level of lactic acid and haemoglobin in blood of Channa punctatus chronically exposed to endosulphan. The lowering of pH by lactic acid may be a cause for the increased content of haemoglobin. In the present study higher acid phosphatase content (Table 4G) was observed in blood serum of Liza parsia chronically exposed to "Nuvan". The lowering of blood pH (Table 4G) by acid phosphatase may be a cause for the increased haemoglobin content which increases the oxygen carrying capacity of blood. Bakthavathsalam and Srinivasa Reddy (1983) reported that the increase in oxygen consumption at higher concentration was lower than those in lower concentration in Anabas testudineus chronically exposed to lindane. Oxygen uptake was significantly decreased at higher concentration and highly elevated at lower concentrations of phenol on fishes was observed by Gupta (1987). Reports of Bakthavathsalam and Srinivasa Reddy (1983) and Gupta (1987) support the results obtained in the present study at chronic stress on fish.

Histological studies:

Disturbance in orientation of hepatocytes and hepatic ducts of liver, their loose arrangement and vacuolation were observed in the present study both in acute and sub-lethal exposures of Liza parsia to "Nuvan". Casillas et al. (1983) reported the disturbance in the orientation of hepatic ducts

in liver sections of Parophrys vetulus exposed to lethal concentrations of carbontetrachloride. They opined that the destruction of connective tissue may be the possible cause for the study. Konar (1977) in his study on the effect of acute exposure of phosphamidon, hepatochlor on Heteropneustes fossilis and Labeo rohita observed vacuolation, degeneration of cytoplasm and swelling of hepatocytes in liver sections of both the species. The vacuolation in liver also reported by Razani et al. (1986) in Brachydanio rerio chronically exposed to phenol compounds. Sastry and Malik (1979)have observed the vacuolation of cytoplasm of hepatocytes and enlargement of nuclei of Channa punctatus after sub-lethal exposure to dimecron. In the present observation the vacuolar degeneration of cytoplasm of hepatocytes may be related to the reports of Konar (1977). Observations of Slooff et al. (1983) suggested that the enlargement of liver of fish from polluted surface water in the Netherlands was mainly caused by hypertrophy of hepatocytes. Similarly hypertrophy of liver cells was observed in the present investigation as in the case of catfish exposed to heptachlor reported by Konar (1977). Such increase in size or swelling of cells is one of the earliest recognizable events following stress or injury which may lead to disruption of its normal physiological function.

Gupta and Dalela (1987) reported histological changes in kidney of Notopterus notopterus including degeneration and disolution of epithelial cells of renal tubules, hypertrophy and necrosis following sub-lethal exposure to phenolic compounds. Similar observations were made by Csepai (1978) in Cyprinus carpio chronically exposed to Anthio 40 EC, Satox 20 WSC,

and Basudin 10G. Vacuolation of renal cells and enlargement of renal tubules were reported by Konar (1977) in kidney sections of Heteropneustes fossilis and Labeo rohita chronically exposed to DDVP, phospamidon and heptachlor. In his study the possible causes for histological disorders are not reported. In the present study also, like Konar (1977) the vacuolation of renal cells of kidney in Liza parsia chronically exposed to "Nuvan" cannot be related to any particular reason. The deformation of renal tubules was observed by Bakthavathsalam et al. (1984) on Anabas testudineus chronically exposed to Furadon. Radhaiah et al.(1986) reported pronounced histopathological changes in Tilapia mossambica exposed for longer periods in sub-lethal concentration of heptochlor. According to Dubale and Shah (1981b) histological study revealed that, in general, the process of destruction was the function of dosages and period of exposure and also opined that the renal tubules of kidney are the first to be affected at pesticidal stress. Rashatwar and Ilyas (1984) reported the histopathological changes in kidney leading to the cloudy swelling of renal tubules in a freshwater teleost Nemacheilus denisonii acutely exposed to phosphamidon. In the present study also the swelling of renal tubules in acute exposures was noticed. The pronounced changes observed in the histological sections at higher sub-lethal concentration and prolonged exposure period is supported by the observations of Dubale and Shah (1981b).

The present study is of short duration. For long-term effects further long duration experiments are needed. However, the pesticide having found

to affect and alter the physiology, biochemistry and histology of the fish even during short-term exposure, it indicates need for careful use of it in the culture systems. For instance, changes in the amino acids and protein of blood serum appear to be not significant between control and exposure in the 96 hour lethal concentration tests. But in 45 days exposure to even sub-lethal concentrations they seem to be highly significant indicating harm to the fishes.

S U M M A R Y

1. The toxicity of "Nuvan" to Liza parsia was evaluated by using static bioassay test. The LC50 values were found to be 1.015 ppm, 0.750 ppm, 0.554 ppm and 0.482 ppm for 24, 48, 72 and 96 hours respectively. Results of computer analyses were tallying well with graphical presentation.

2. The fishes subjected to 96 hr LC50 (0.48 ppm) for 24, 48, 72 and 96 hours; lethal concentrations of 1.015 ppm, 0.750 ppm and 0.554 ppm for 24, 48 and 72 hours respectively and to sub-lethal concentrations of 1/15th 96 hr LC 50, 1/10th 96 hr LC50 and 1/5th 96 hr LC50 for 15, 30 and 45 days, were used in biochemical and physiological studies. The variations in protein, total free amino acids, RNA and DNA content of muscle; protein, total free amino acids, acid phosphatase and alkaline phosphatase of blood serum; and oxygen consumption of fish were studied. Liver and kidney tissue sections were taken for histological observations.

3. A drop in protein content of muscle tissue was noted in fishes subjected to acute and chronic toxicities of "Nuvan". The reverse relationship was seen for muscle tissue total free amino acids in acute toxicity tests but marked fluctuations were found out in sub-lethal exposures for varying periods.

4. There was a decrease in muscle tissue RNA, but increase in muscle tissue DNA content of fishes exposed to lethal and sub-lethal concentrations.

5. Initial increase in serum protein upto 72 hours in 96 hr LC50 experiment and drop in the content was observed for all lethal concentrations. The similar result was noted for serum total free amino acids in acute exposures. But marked fluctuations were observed for protein and total free amino acids of serum in chronic exposures.

6. Exposure to lethal and sub-lethal concentrations resulted in the elevation of acid phosphatase and depletion of alkaline phosphatase in blood serum of fish.

7. Initial increase followed by decrease in oxygen consumption of fishes was noted in 96 hr LC50 experiment. But fishes exposed to all four lethal concentrations showed decrease of oxygen consumption over control. In sub-lethal exposures the increase in oxygen consumption at higher concentration was lower than those in lower concentrations.

8. Lethal and sub-lethal exposures to "Nuvan" resulted in histological disorders such as vacuolation, disorganisation in the architecture of hepatic ducts of liver tissue and enlargement of renal tubules and displacement of epithelial cells in kidney sections.

9. The pesticide shows significant variation in physiological conditions in fishes subjected to 45 days of exposure to sub-lethal concentrations of 96 hr LC50 value. Further long duration study is needed to find out long-term effects of "Nuvan" on the fish.

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