EFFECT OF ORGANOCHLORINE ON AN AIR-BREATHING FISH

CLARIAS BATRACHUS (Linn)

THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN ZOOLOGY

NORTH EASTERN HILL UNIVERSITY
Date.....

Gagan Kumar Thakur
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I have great pleasure in forwarding the Ph. D. thesis entitled "THE EFFECT OF ORGANOCHLORINE ON AN AIR-BREATHING FISH, CLARIAS BATRACHUS (LINN.)" of Sri Gagan Kumar Thakur, for the award of 'Doctor of Philosophy' degree in Zoology of Bhagalpur University, Bhagalpur.

Sri Thakur has put more than four years of research work, in Aquatic Patho-biology Laboratory of Post-Graduate Department of Zoology, Bhagalpur University, Bhagalpur. The subject matter of the thesis is a record of bonafide research work done by Sri Thakur. By character and habit he is fit for the award of Ph. D. degree.

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Content

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Preface

Undesirable change in physical, chemical or biological characteristics of our air, land and water that may or will harmfully affect human life or that of desirable species, our industrial processes, living conditions and cultural assets; or that may or will waste or deteriorate our raw-material resources is pollution. Pollutants are residues of the things we make, use and throw away (Waste Management and Control", a report by the committee on pollution, National Academy of Sciences, U.S.A., 1966). Recognition of "pollution" thus surely depends upon the observation of environmental or biological damage and this leads us to identify the toxic agent or contributing circumstances. Increasing attention has been given to the protection of the aquatic environment against pollution, both nationally and internationally; in the freshwater field, the presence or absence of fish has been widely used as a biological indicator of the degree of pollution.

Pesticides have been used in increasing quantity in the past 70 years, and the compounds of different type have succeeded one another in popularity. Contamination of water by pesticides can have deleterious effects on organisms. The growing contamination of the environment with toxic agents is a matter of increasing awareness, in modern times. Potentially hazardous substances, regarded as foreign to the body and appropriately termed xenobiotics, confront mankind in nearly all spheres of human activity. Xenobiotics may be either selectively toxic or display a rather broad spectrum of adverse biological effects. The use of synthetic organic compounds for controlling insect pests of crops and forests, as well as the insect vectors of human and animal disease has created several problems in aquatic environment. These include exposure of non target organisms to lethal and sublethal amounts of pesticides and their byproducts. The non-target organism may be affected directly through nutritional contamination. The chemical and physical factors influence the pesticide impact on freshwater.

It has generally been accepted that the chlorinated hydrocarbons present a greater damage to non target animals' than to the organophosphate pesticides, because of their greater acute toxicity and the persistence of their residues

(Rudd, 1964; Johnson, 1968). Available literature indicate that effect of biocides on fishes have been studied variously in relation to toxicity, survival and behaviour (Cope, 1966; Saunders, 1969; Woodworth and Pascoe, 1982; Chun et al., 1981; Tonogai et al., 1983), haematology (Esler and Edmunds, 1966; Hunn, 1967; Howkins and Mawdesley-Thomas, 1972; Choudhary, 1979; Storozhuk and Guleva, 1983), oxygen consumption (Ranke-Rybicka, 1975; Lunn et al., 1976; Hose et al., 1983), blood biochemistry (Ishihara et al., 1967; Holmberg et al., 1972; Nemesok et al., 1982; Larsson et al., 1984), histopathology (Haga, 1970; Couch, 1975; Walsh and Ribelin, 1975; Choudhary, 1979; Rashatwar and Ilyas, 1984; Gupta and Dalela, 1986).

Literature dealing the toxicity of chlorinated hydrocarbons on Indian fishes are few, viz., toxicity and behaviour (Chakrawarti and Chaurasia, 1981; Singh and Srivastava, 1982; Manoharan, 1982), on blood biochemistry (Gupta, 1980; Ramalingam and Ramalingam, 1982; Gupta and Dhillon, 1983) and haematology (Sastry and Sharma, 1979; Shafi and Choudhary, 1980; Gupta and Singh, 1982). A systematic investigation concerning the effect of organochlorine insecticides on the physiology of freshwater Indian fishes have not been undertaken in detail. Hence, the present work entitled "Effect of an organochlorine (BHC) on a freshwater air-breathing fish,

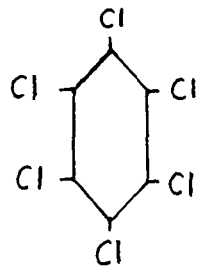
Clarias batrachus (Linn.) has been undertaken to investigate the pathobiology of this fish.

Clarias batrachus possess accessory respiratory organs in the form of supra-branchial chambers (Munshi, 1961) thus, provided with bimodal gas exchange mechanism. This fish dwells in marshy ponds, ditches and derelict water areas unsuitable for quality fish culture operations. The introduction of cage culture and the possibilities of culture in paddy fields and city sewage have raised the prospects of air-breathing fishes for the supply of additional fish flesh to the population (Jhingran, 1975). As it is evident, the air-breathing fishes under culture may be exposed to a number of pesticides, inorganic chemicals and other biocides introduced directly or indirectly as the result of runoff from treated land areas in order to protect and safeguard important crops and human life. The fishery biologists will have to compromise with the situation and to help in formulating safe concentration of these pesticides for preserving the aquatic ecosystem.

BHC or benzene hexachloride is rather less important than DDT. Though it is an extremely powerful insecticide its uses are much more limited owing to tendency to impart unpleasant odour and flavour to plants. It is produced by the

chlorination of benzene in presence of light. There are various possible positions for the hydrogens and chlorine atoms in the molecule, and so the compound exists in a number of different isomers which are remarkably different in their degree of toxicity. The 'Y' isomer is one of the most toxic of all insecticides where β and ε isomers are practically non-toxic to insects. The δ isomers is moderately active but variable in its effects in different insects, and is more toxic isomer to snails.

Chemical name of BHC is lindane (gamma BHC) or 1,2,3, 4,5,6-hexa chlorocyclo-hexane. The structural formula of BHC is given below:



Chlorinated hydrocarbon insecticides such as DDT, dieldrin, aldrin, endrin, lindane have very low solubility in water with a high solubility in oils and fats. Organic compounds which are chemically stable, and hence persistent, are recognized as presenting an element of risk to one or

more forms of life. Organochlorine pesticides and their metabolites, e.g., DDT, DDE, DDD, dieldrin, isomers of BHC, hexa chlorobenzene, chlordane, toxaphene are persistent organic compounds. Accumulation of organic insecticides by fish can proceed by two routes: absorption through the gills (Holden, 1962; Murphy, 1971), and ingestion of contaminated food (Macek, Rodgers, Stalling and Korn, 1970). In general, the efficiency of OCs accumulation by fish follows the sequence PCB > DDT > dieldrin > lindane. Elimination of OCs by fish proceeds more slowly than does their accumulation and there are some consistent differences between compounds (Macek et al., 1970; Grzenda et al., 1970). The tendency to eliminate OCs follows the sequence Lindane > dieldrin > DDT ≫ PCB.

The present dissertation has been divided into four chapters. Chapter 1 deals with the toxicity, behaviour and body composition. In this part LC_0, LC_{100} and TLm 96 hours estimated safe concentration values were calculated. The subjective visual observations on the behavioural responses of fish exposed to pollutant water were made in detail. Further, the effect of 15 days exposure on the body composition, growth rate, liver somatic-index (LSI) and gonadosomatic index (GSI) were investigated at various test concentrations.

The Chapter 2 of the dissertation includes the effect of various concentrations of BHC on the haematology namely on erythrocyte and leucocyte counts, blood haemoglobin, haematocrit, absolute values (MCH and MCHC) and rate of oxygen consumption in this fish.

The Chapter 3 deals with the effect of various concentrations of BHC after 96 hours on blood biochemistry, viz., plasma protein, glucose, total, free and esterified cholesterol and plasma electrolytes, viz., Na^+, K^+, Ca^{++}, Cl^- and PO_4^{---}.

The histopathological effect of BHC for 96 hours exposure on the skin, gills, liver, kidney and gonad of Clarias batrachus have been studied and critically evaluated for identifying characteristic lesions of diagnostic value. The results of this study has been incorporated in Chapter-4.

It is hoped that the present work will provide sufficient information concerning the effect of BHC on toxicity, behaviour, body composition and haematology of Clarias batrachus. This study further warrants the necessary scientific information to establish safe concentration for the use of BHC to various water bodies.

CHAPTER-I

EFFECT OF BHC ON MORTALITY, BEHAVIOUR AND BODY COMPOSITION

In recent years pesticide use and application has attracted the long overdue attention to its merits and has led to a great deal of careful scientific research (West, 1966; Westlake and Gunther, 1966; Middelem, 1966; Dykstra, 1968).

Pesticides and insecticides have been used in increasing quantities in past 70 years, and compounds of different type have succeeded one another in popularity. The possibility of water pollution through insecticides have recently been the main focal theme and the volume of published research works on the effect of pollution on aquatic organisms have increased enormously (Mc Kim et al., 1975).

Pesticides may reach the freshwater of rivers, lakes and streams in a variety of ways. They may be applied deliberately to water bodies for the control of undesirable

aquatic fauna or the aquatic stages of insects of public health importance. They may also make an incidental impact as a result of aerial application of insecticide for control of terrestrial pests in agriculture and forestry in surrounding area. They may also reach lakes and rivers by aerial drift or by run off from land. Finally gross impact of pesticide may occur from time to time as a result of accident or carelessness involving chemical concentrates.

Most of the pesticides applied for the pest control in agriculture and forestry enter the aquatic environment through various routes, polluting the aquatic ecosystem. The persistence of these toxic chemicals in aquatic environment may be dangerous for the survival of fish (Johnson, 1968; Saunders, 1969; Mawdesley-Thomas, 1971). Therefore, the study of toxicity to organism is an essential first step concerning evaluation of pesticide impact on the freshwater environment for achieving the objective, viz., to determine safe concentrations and to formulate the safe application rate of insecticide concerned (Sprague, 1969, 1970, 1973).

The chlorinated hydrocarbons such as DDT, DDD, DDE, dieldrin, endrin, and BHC have already been proved to be more toxic to fish (Muirhead-Thomson, 1971; Dimond et al., 1968). The chlorinated hydrocarbon insecticides combine a very low

solubility in water with a high solubility in oils and fats. They also show relative unreactivity and appreciable volatibility at normal environmental temperatures. Thus, since they are unreactive and hence fairly stable in various environmental reservoirs, they are persistent; they are volatile and are transported in vapour phase, which accounts for their ubiquity and their lipid solubility accounts for their accumulation in depot fat reserves of organisms (Lockwood, 1976).

The persistent organo-chlorine insecticides frequently show a delayed toxicity in that the lethal effect is not seen until sometime after contact with the chemical.

Toxicity effect of organo-chlorine insecticides has been investigated by a number of investigators (Datta, 1980; Vardia and Durve, 1980; Thakur et al., 1981; Singh and Srivastava, 1982; Hall et al., 1982; Mahboob Basha, 1983; Kaur and Virk, 1983).

In the present investigation effort has been made to determine the toxicity tolerance limit (TLm), behavioural response and overall effect on the body composition of Clarias batrachus (Linn.) to Benzene hexachloride (BHC), which is a widely used pesticide in India.

Materials and Methods

Live specimens of Clarias batrachus were procured from Katihar (Bihar) and were acclimatized in laboratory before experimentation. The fishes were kept in big aquaria (50 gallon capacity). The animals were fed with chopped goat liver and earthworms. Care has been taken to keep the animals healthy and free from parasites.

The 96 hr bioassays were conducted employing the technique of static test (Doudoroff et al., 1951) in glass aquaria (60x30x30 cm) in the laboratory at ambient water temperature (29.5-33.3°C) in the month of April-May, 1979. The physico-chemical characteristics of water were analysed according to standard methods published by APHA et al. (1975). These were: water temperature 29.5-33.3°C; pH 7.9; dissolved oxygen 6.0 mg/l; free CO_2 4.6 mg/l and HCO_3^- 284.0 mg/l. The

benzene hexachloride (BHC) used in the present work was of technical grade(50% purity) containing 6.5%'Y' isomer. Stock solution was prepared by dissolving BHC in AR-grade acetone, the strength of which was calculated based on w/v. The controls were treated with pure acetone only, similar to experimental group with BHC dissolved in acetone. The fishes used for bioassay were of average body weight of 62.54±0.802 g. Preliminary tests were carried out to estimate the LC_0 and LC_{100} values, followed by 96 hr exposure of 10 animals each group in 40l of solution in test concentrations between 2.5 to 30.0 mg/l of BHC. The mortality of fish in each test concentration was recorded. The bioassays were repeated thrice, so that in all 30 animals (10x3) were exposed to each test concentration. The toxicity response data were calculated at confidence limits of 95 per cent level and graphically represented on semi-logarithmic paper with test concentrations on the logarithmic scale and mortality percentage on arithmatic scale (Annes, 1975). The point at which the straight line thus obtained crosses the 50% mortality level has been taken as median lethal concentration (LC_{50}).

The subjective visual observations on the behavioural responses of the fish exposed to BHC were made. The number of aerial excursions and opercular movements were recorded in 2.5, 5.0, 10.0, 20.0 and 100.0 mg/l exposed animals from 1 to 24 hr.

In second set of experimental condition 10 male fishes of about 55-60 g body weight were kept each in two aquaria. Group 'I' was treated as control, while Group 'II' was subjected to longer exposure of 12.0 mg/l BHC for 15 days. The effect of BHC exposure on body weight, liver weight, gonadal weight and hepato-somatic index (HSI), gonado-somatic index (GSI) were determined.

The water content of liver and gonad was obtained from the loss of weight that occurred during drying at 55°C.

The hepato-somatic index (HSI) and gonado-somatic index (GSI) were calculated by the following formula:

$$HSI = \frac{\text{Liver weight}}{\text{Body weight}} \times 100$$

$$GSI = \frac{\text{Gonad weight}}{\text{Body weight}} \times 100$$

The effect of extended exposure to BHC, i.e., 15 days on hepato-somatic index (HSI) and gonado-somatic index (GSI) was investigated on five fishes from each test concentrations of 2, 5, 5.0 and 10.0 mg/l. The 15 days exposure experiments were conducted in the laboratory in circular plastic pools (2 x 0.8 m) and the BHC treated water of these pools were renewed at every 24 hrs as per recommendations of APHA et al. (1975).

Students 't' test was applied to evaluate the significance of difference at 5% level.

Observations

The results of acute toxicity test has been shown in Table-1 and the toxicity response curves have been represented in Plates I and II. On the basis of interpolation of the data, the LC_{100} and LC_0 were calculated to be 24.44 mg/l and 9.67 mg/l. Although it was observed in practice that LC_0 lies between 5.0 to 9.0 mg/l and LC_{100} between 25.0 to 26.0 mg/l. These discrepencies between observed data and those derived from graphical estimate are obvious, but the LC_{50} value of 15.944 mg/l is much close to the observed values (Plates I and II).

The behavioural response of fish towards toxicant was grossly dependent on concentration and length of exposure. When fishes were suddenly exposed to higher concentrations of BHC, hyper excitibility, increased aerial excursions and

opercular movements were observed as immediate response of fish towards the toxicant. In this case the restlessness of fish increased with the increasing exposure time. Fishes were often observed swimming with jerky movements on the surface of the water and tried to jump out of aquaria.

In higher concentrations of BHC fishes showed white wound patches on the skin surface at the sides of the body. Marked discolouration and depigmentation of the skin after BHC exposure has been observed. Fishes exposed to higher concentrations of BHC have tried to remain almost in vertical position perpendicular to the base of aquaria with the mouth facing the water/air interphase were frequently observed. The terminal phase was characterized by agitated movements, tremors, convulsions and loss of balance. The close examination of dead fish showed several wound patches on either sides of the body, near the spine, sometimes bent body and whittish gills with copious mucus.

The visual respiratory response and rate of surfacing of fish after exposure to various concentrations of BHC has been shown in Table-3 and graphically represented in Plate-III. The 24 hr mean values of opercular movement and rate of surfacing increases significantly with increasing concentrations of BHC. But there is no correlation between the two,

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i.e., rate of surfacing or opercular movement and increasing concentrations of BHC. At higher concentration, i.e., 100 mg/l the fish dropped the opercular movements considerably followed by increased rate of surfacing to the extent that the animal remains almost on the surface of water.

The feeding rates decreased with the increasing concentration of BHC. This visual observation was further supported from the analysis of the gut content. The gross food conversion rate could not be worked out since the discarded feed left in the aquarium was not accounted. The growth rate of tagged animals in 15 days decreased to 7.72 per cent against control.

The effect of 15 days exposure of BHC at a concentration of 12.0 mg/l has been shown in Table-4 and represented by bar histograms in Plates IV and V. The body weight has reduced from 60.4 ± 0.906 to 55.74 ± 0.815. There is significant decrease (7.72%) in the body weight of treated fishes in comparison to controls. The liver weight and gonad weight have reduced to 0.416 ± 0.012 ($P < 0.001$), 3.358 ± 0.524 ($P < 0.01$) in comparison to controls 0.898 ± 0.012 and 6.828 ± 0.468 g respectively. The liver water and gonadal (testis) water has significantly increased ($P < 0.01$ and $P < 0.001$ respectively). There is 50.25% and 47.67% decrease in hepato-somatic index and gonado-somatic index in 15 days exposed fishes to BHC in comparison to controls.

There is gradual decrease in hepato-somatic index and gonado-somatic index with increasing concentrations of BHC after 15 days exposure (Table-5).

TABLE -1

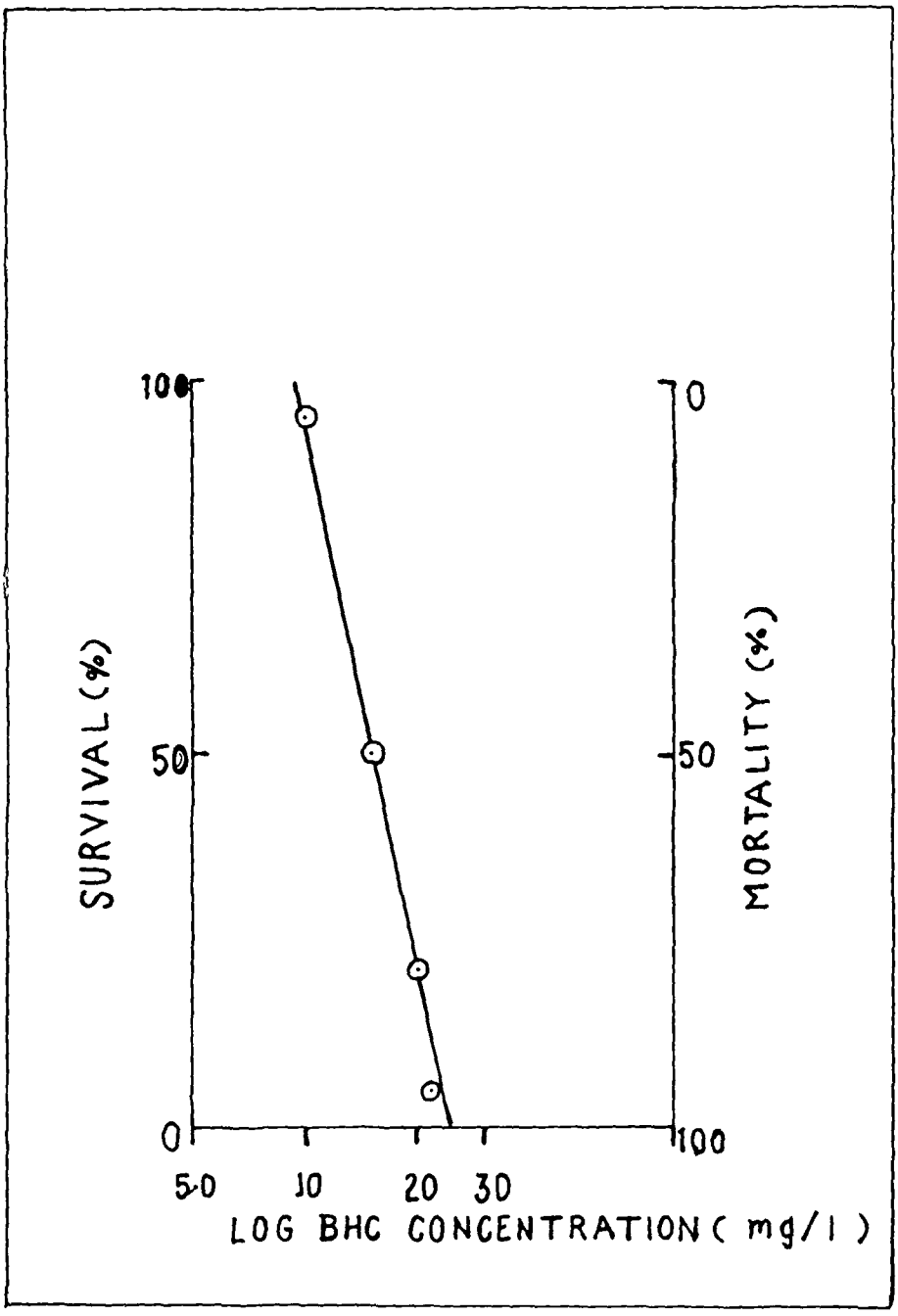
Physico-chemical composition of water and 96 hours LC_{50} value of BHC for Clarias batrachus (Linn.)

Parameters	Values
LC_{50} for BHC	15.94 mg/l
Free CO_2 content	4.6 mg/l
O_2 content	6.0 mg/l
HCO_3^- content	284.0 mg/l
Temperature of the water	29.5 - 33.3°C
pH	7.9

EXPLANATION OF PLATE -I

Fig. 1: Per cent mortality and survival rate of C. batrachus exposed to various concentrations of BHC for 96 hours.

PLATE I


100
50
0
SURVIVAL (%)
0
50
100
MORTALITY (%)
5·0
10
20
30
LOG BHC CONCENTRATION (mg/l)

EXPLANATION OF PLATE - II

Toxicity response curve on observed values of C. batrachus exposed to BHC at confidence limit of 95% level (96 hours).

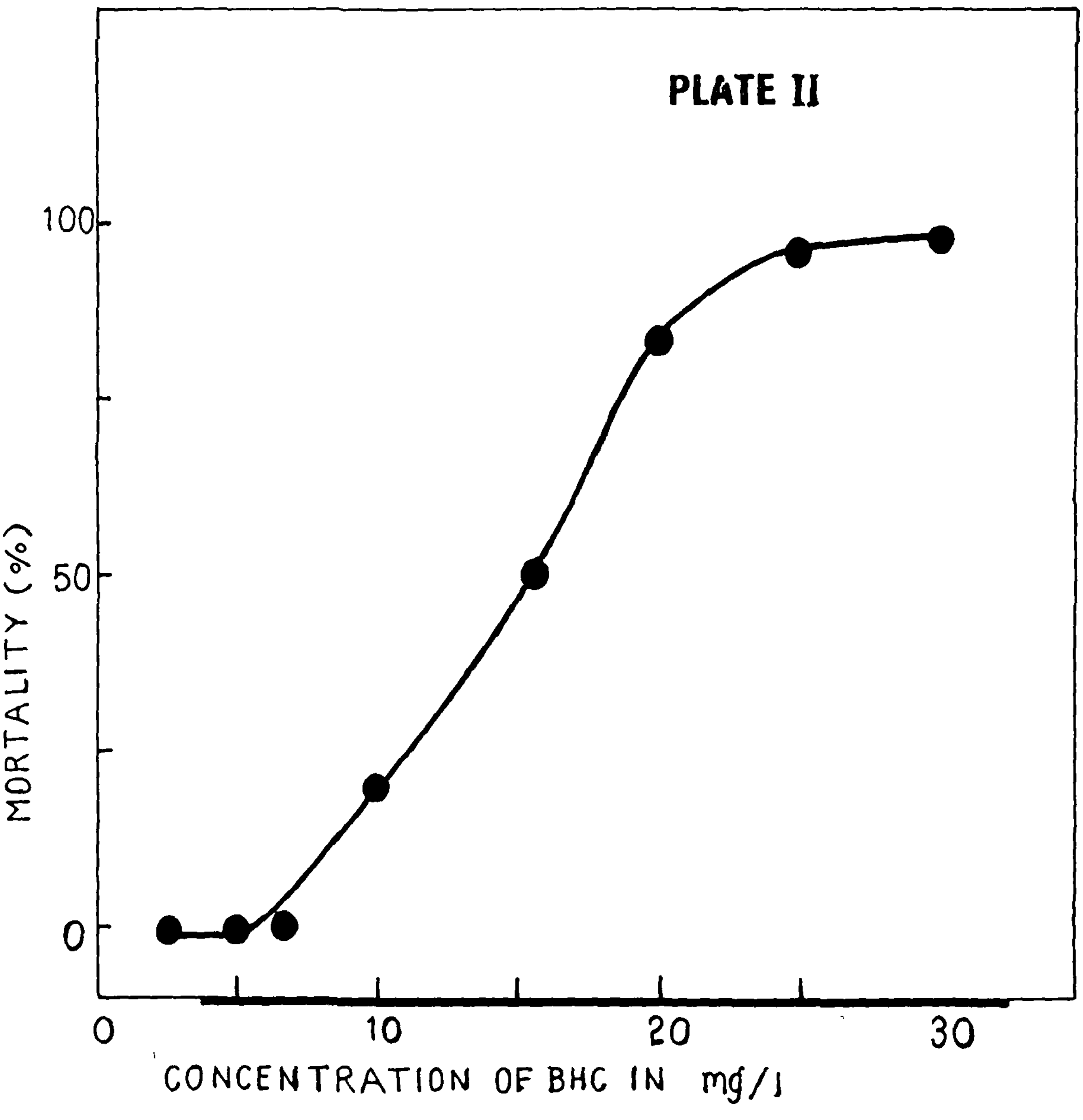


PLATE II
100
50
0
MORTALITY (%)
0
10
20
30
CONCENTRATION OF BHC IN mg/l

TABLE -2

Acute toxicity (96 hrs) and estimated safe concentrations of BHC to Clarias batrachus

Parameters	Values
Number of animals	30
Water temperature	29.5-33.3°C
Lethal concentrations (mg/l)	
LC_0	9.67
LC_{50}	15.94
LC_{100}	24.44

Estimated Safe Concentrations

LC_{50} of BHC 7.58 mg/l in Heteropneustes fossilis for 168 hours at 20-35°C (Basak and Konar, 1977)

TABLE -3

Respiratory behaviour of Clarias batrachus after exposure to various concentrations of BHC
(Mean value of 24 hrs; observations made at 4 hrs interval)

Concentration of BHC in mg/l	Number of Fishes	Rate of Surfacing per minute ± S.E.	Rate of opercular movement per minute ±S.E.
Control 0	6	0.15±0.008	4.53±0.125
2.5	6	0.16±0.122 (NS)	4.90±0.093 (NS)
5	6	0.19±0.010 (P< 0.05)	5.50±0.147 (P< 0.05)
10.0	6	0.72±0.039 (P< 0.001)	12.40±0.082 (P< 0.05)
20.0	6	2.18±0.005 (P< 0.001)	14.43±0.239 (P< 0.001)
30.0	6	5.97±0.459 (P<0.001)	19.63±0.376 (P < 0.001)
100.0	3	11.21±0.543 (P < 0.001). (initial reading just after exposure)	-

EXPLANATION OF PLATE - III

Graph to show the relationship between BHC concentration Vs opercular movement and rate of surfacing in *C. batrachus*.

PLATE III

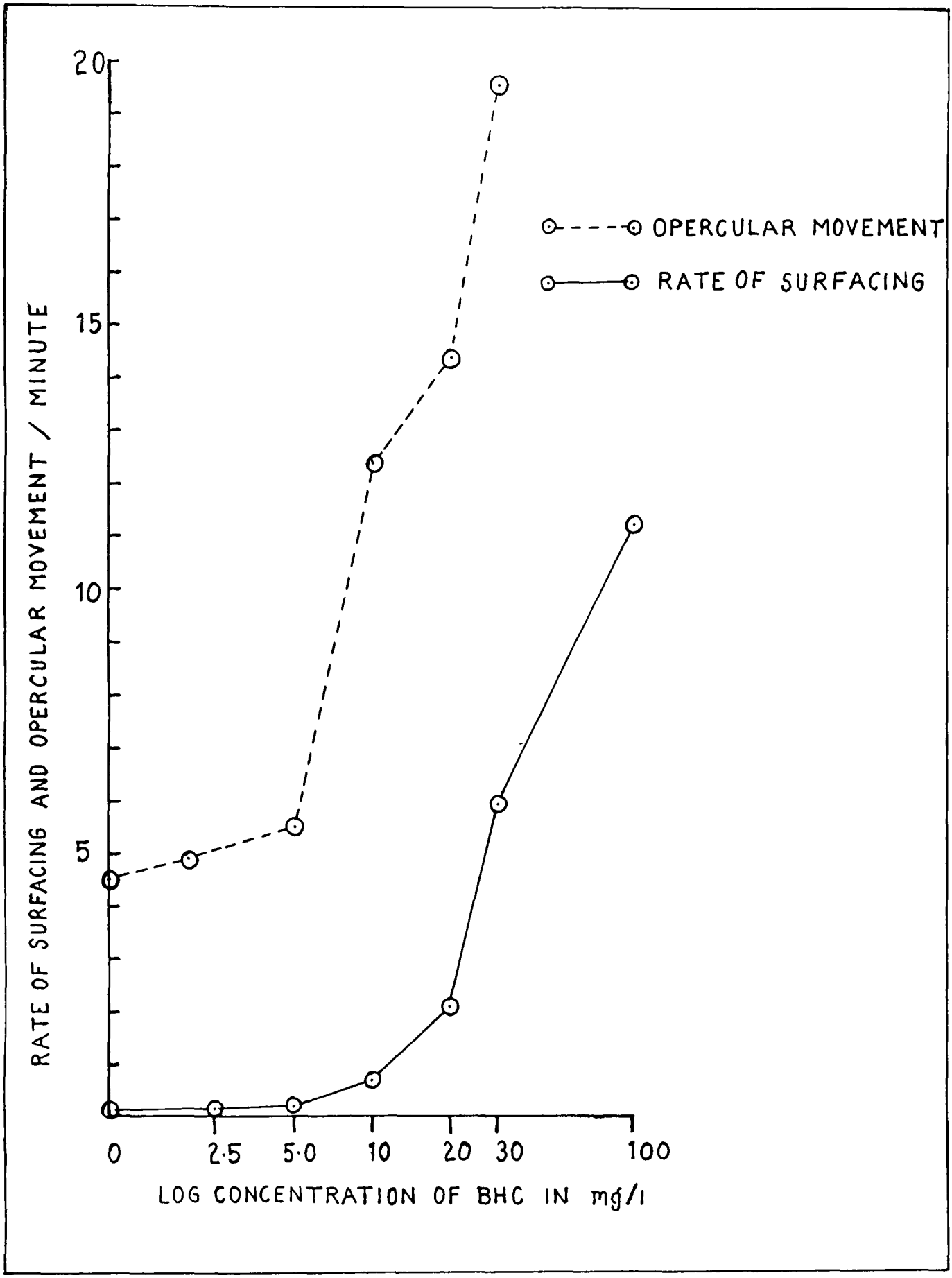

20
15
10
5
RATE OF SURFACING AND OPERCULAR MOVEMENT / MINUTE
OPERCULAR MOVEMENT
RATE OF SURFACING
0
2·5
5·0
10
20
30
100
LOG CONCENTRATION OF BHC IN mg/l

TABLE -4

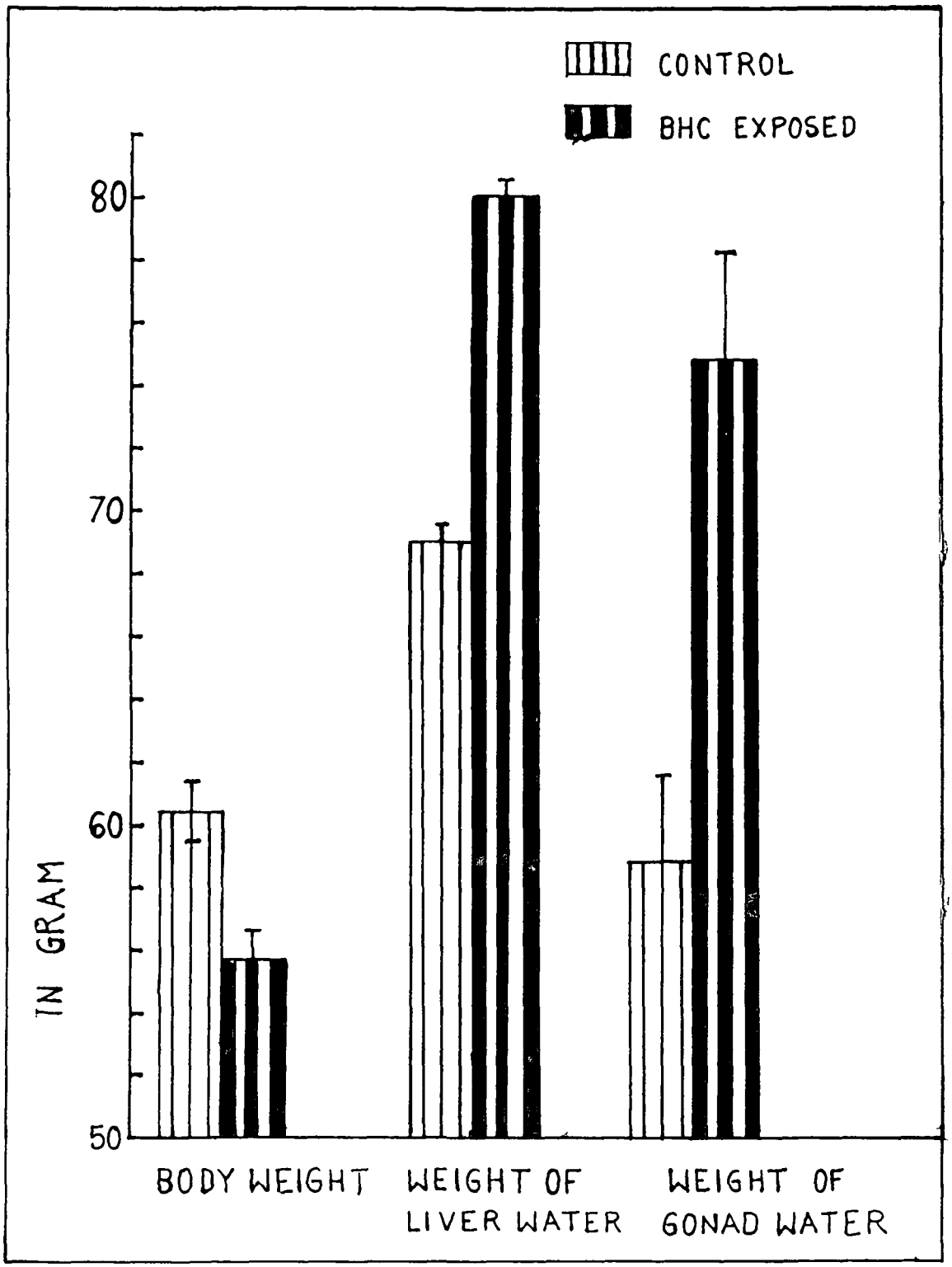
Liver, gonado-somatic index, liver and gonadal water in 12.0 mg/l BHC exposed *Clarias batrachus* for 15 days

Parameters	Control (n = 10±S.E.)	12.0 mg/l BHC exposure for 15 days (n = 6±S.E.)
Body weight (g)	60.4±0.906	55.74±0.815 (7.72% decrease)
Liver weight (g)	0.898±0.012	0.416±0.012 (P< 0.001)
Hepato-somatic Index	1.495±0.038	0.744±0.046 (P< 0.001) 50.24% decrease
Weight of liver water in 100g liver weight(g)	69.086±0.484	80.112±0.468 (P< 0.01) 13.76% increase
Gonad weight (g)	6.828±0.468	3.358±0.524 (P< 0.01)
Gonado-somatic Index	11.224±0.641	5.873±0.856 (P< 0.01) 47.67% decrease
Weight of gonad water in 100g of gonad weight (g)	58.898±2.653	74.834±3.422 (P< 0.001) 21.29% increase

EXPLANATION OF PLATE - IV

Histogram showing the effect of 15 days exposure of BHC on body weight and water percentage of liver and gonad of *C. batrachus*

PLATE IV


CONTROL
BHC EXPOSED
80
70
60
50
IN GRAM
BODY WEIGHT
WEIGHT OF LIVER WATER
WEIGHT OF GONAD WATER

EXPLANATION OF PLATE - V

Histogram showing the effect of 15 days exposure of BHC on gonad weight, gonadosomatic index, liver weight and liver somatic index of C. batrachus

PLATE V

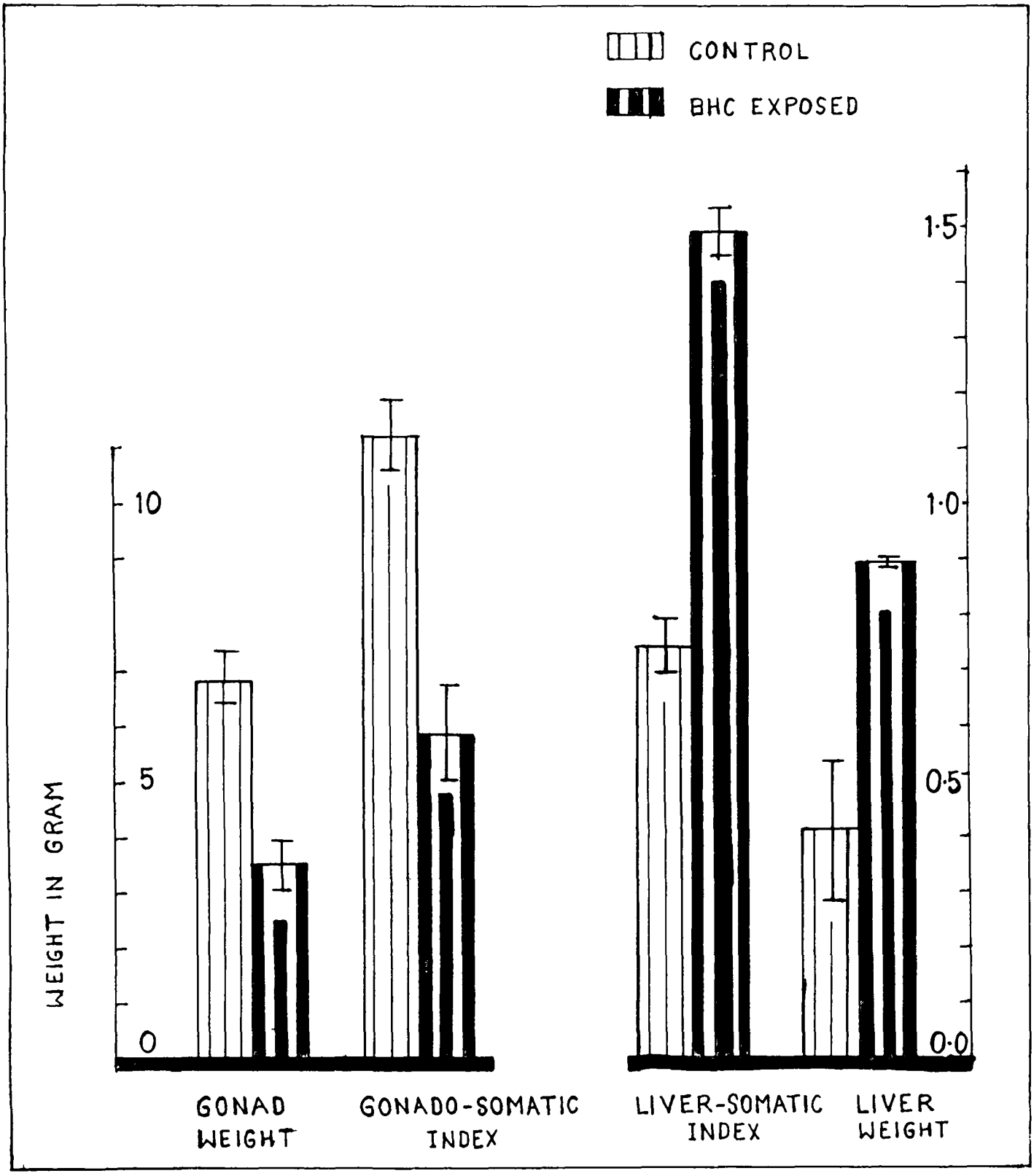

CONTROL
BHC EXPOSED
WEIGHT IN GRAM
10
5
0
1·5
1·0
0·5
0·0
GONAD WEIGHT
GONADO-SOMATIC INDEX
LIVER-SOMATIC INDEX
LIVER WEIGHT

TABLE -5

Effect of various concentrations of BHC on HSI, GSI of Clarias batrachus after 15 days exposure

Concentrations of BHC	Hepato Somatic Index	Gonado Somatic Index
Control 0	1.495±0.038	11.224±0.641
2.5	1.24 ±0.068 (P < 0.02)	11.02 ±0.058 (P < 0.001)
5.0	0.96 ±0.046 (P < 0.001)	10.72 ±0.116 (P < 0.001)
10.0	0.656±0.022 (P < 0.001)	6.44 ±0.172 (P < 0.001)

5 animals in each group; ± standard error; water temperature

The role of chemicals in controlling pest of crops and forests, as well as the insect vectors of human and animal disease has been recognised and accepted from the beginning of the century. Synthetic pesticides usually have high toxicity and some of them showing persistent properties. Many of the major rivers record almost chronic contamination with such organochlorine compounds as DDT, dieldrin, endrin, gamma BHC (Weaver et al., 1965; Novack and Rao, 1965; Holden, 1965; Holden and Marsden, 1967; Water Pollution Research Laboratory, 1967).

Sublethal doses of toxic chemicals may affect both the survival and reproduction (Moriarty, 1975) of animals. The straight forward static test to determine the median tolerance limit (TLm) has been adopted by public health authorities

(Doudoroff et al., 1951; Henderson and Pickering, 1958; Henderson and Pickering, 1958; Henderson et al., 1959; American Public Health Association, 1960).

The studies on pesticide impact deals in general with the reaction of a living organisms in an aquatic environment and according to Muirhead-Thomson (1971) the objective of such investigation is either to evaluate the impact of known pesticide against a particular undesirable freshwater animal or aquatic plant, or is concerned with the undesirable effects of a pesticide contaminating the habitat of freshwater food fish or other protected aquatic forms. Investigations on the impact of known pesticide (concentration and exposure time) to a particular species involves the estimation of mortality or survival rate and evaluation of sublethal exposure on growth, behaviour, reproductive capacity and other physiological parameters.

In the present investigation it was observed that the system of Clarias batrachus can function within broad range of pollution by BHC and the toxicity response of this fish towards BHC is a function of concentration and duration of exposure. The 96 hr LC_{50} value has been calculated to be 15.94. Basak and Konar (1977) reported 168 hours LC_{50} of BHC for Heteropneustes fossilis to be 7.58 ppm at 20-35°C water

temperature having 7.0 pH. Temperature conditions in toxicity tests are important parameter to be considered (Cope, 1966; Walker et al., 1964; Henderson et al., 1959). In countries where there is heavy application of pesticide and where fish kills are frequently reported, emphasis has naturally been on the acute toxic effects on the fish. The time/concentration factor was also shown to be an important consideration in evaluating fish toxicity (Muirhead-Thomson, 1971).

Water temperature is one of the most important factors in the environments of aquatic organisms and plays a vital role in determining their distribution, growth, reproduction, metabolism and behaviour. In toxicity investigations the temperature may range from 7°C to 29°C (Cope, 1966). The hardness of water, alkalinity and pH have no major effect on the toxicity to fish of chlorinated hydrocarbons (Henderson et al., 1960). Gamaxene is reported to be more toxic to Clarias batrachus and Heteropneustes fossilis in comparison to Anthio-25, Dimecron and Sevin (Chakrawarti and Chaurasia, 1981); and on common carp, Cyprinus carpio (Kaur and Virk, 1983).

The acute toxicity of a number of organochlorine compounds on Indian fishes have been reported by several investigators (Datta, 1980; Chakrawarti and Chaurasia, 1981;

Raizada, Sen and Raizada, 1981; Singh and Srivastava, 1982; Manoharan and Subbiah, 1982; Jacob, Nair and Balasubramanian, 1982; Mahboob Basha et al., 1983; Kaur and Virk, 1983).

Since toxicity bioassay is influenced by a number of factors including the technique of bioassay employed, therefore, a comparison of LC_{50} values obtained for several fish species by various authors would not be meaningful (Chaudhary, Pandey, Dubey, 1981).

Hasler (1970) has reviewed certain aspects of chemical ecology of fish and documented the importance of chemical communication in maintaining the behaviour patterns of fish as well as other marine organisms. Animals are sensitive to chemical signals at low concentration and they may rely on this sensory input to control their attitude and behaviour (Todd et al., 1967). According to Muirhead-Thomson (1971), "there is increasing realization that the effect of pesticides on the reactions of fish other than the easily observable mortality effects, must be taken into account in evaluating the complete ecological impact of a contaminating substance".

The subjective observations on behavioural responses of C. batrachus to lethal and sublethal concentrations of BHC are in agreement with those of Cyprinus carpio (Datta,

1980), Cyprinus carpio, Poecilia reticulata (Jivasek, 1981), Barbus stigmata (Manoharan and Subbiah, 1982) and Atherinops affinis, Rhaecochilus vacca, Oxyjulis californica, Chromis punctipinnis, Girella nigrica nus, Lythrypnus dalli, Hypsoblenius jenkinsi (Hope, Stoffel and Zerba, 1983) exposed to different organo-chlorine pesticides. The report of lindane poisoning on brown trout, Salmo trutta L. by Drewett and Abel (1983) is in agreement with the findings on Clarias batrachus exposed to BHC. The reaction of fish to various organo-chlorine insecticide poisoning can be in general characterized by erratic movements, muscle tetanus and loss of equilibrium, marked wound patches, discolouration and depigmentation of skin, atoxia and intermittent paralysis. Similar behavioural response to zinc poisoning has also been reported in Channa punctatus by Khangarot (1982), malathion poisoning on Heteropneustes fossilis by Chaudhary, Pandey and Dubey (1981), on Notopterus notopterus by Varma, Tonk and Kumar (1983).

Although there have been many reports of pathological lesions caused by pollutant in fish, there have been few systematic descriptions of the toxic syndromes associated with exposure to pollutants. Such descriptions are clearly desirable as an aid to understanding the mechanism of action of pollutants, and could be diagnostic aids in the investi-

gation of fish mortalities where the accidental or negligent dischargeof pollutants is suspected. The symtematology,leading to death produced by the exposure to BHC in the present study was indicative of the general toxic effects produced by most of the organo-chlorine insecticides. Drewett and Abel (1983) reported effect of lindane poisoning on Salmo trutta, at death the gill filaments were a bright red colour, indistinguishable from control fish. This colour gradually faded until at approximately 2 h after death the gills were white. This occurred in all lindance-exposed fish irrespective of the poison concentration. Gills of control fish faded slightly within the first 2 h after death to a pink colour, and did not turn white after rigor mortis had passed and decomposition begun after 20 h.

Death of fish exposed to lethal levels of zinc involves tissue hypoxia (Skidmore, 1970; Burton et al., 1972) and it may be therefore that fish killed by pollutants may show pathological signs of hypoxia in addition to signs specifically associated with the poison. Similarity of the signs of lindane poisoning and of hypoxia in Salmo trutta suggests that death from lindane poisoning may be associated with tissue hypoxia (Drewett and Abel,, 1983). Physiological and biochemical studies on fish exposed to lethal levels of zinc (Skidmore, 1970; Burton et al., 1972) indicate that tissue

hypoxia is a result of zinc poisoning and probable immediate cause of death, since zinc is not a potent internal poison (Skidmore, 1970). The convulsions and ataxia shown by BHC-poisoned fish suggests that BHC has a neurotoxic action in fish. If tissue hypoxia occurs as a result of BHC poisoning, it is likely to be due to loss of co-ordination in the cardiovascular and/or respiratory systems. Similar observations have been made by O'Brien (1967) in insects and mammals with lindane poisoning.

Hughes (1976) observed that one of the likely effects of pollution affecting the respiratory system is that it limits the metabolic scope of activity, i.e., the differences between the resting and active level of metabolism. Such an effect, according to Hughes (1976), would arise because of both an increase in the resting metabolism and a decrease in the upper level of active metabolism. It is generally recognized that fish respond to toxic chemicals by increased opercular movements (Belding, 1929). The stickleback breathes normally about 120 times per minute at 17°C. When heavy metal salts (Copper sulphate, 64 ppm and lead nitrate 500 ppm) are put into the apparatus the rate of opercular movement and rate of oxygen consumption rise. This is due to increased activity, as the fish senses the unfavourable changes in its

environment and struggles. As the toxic process advances the respiratory movements become more and more rapid, more regular and of increased amplitude. Despite the animal efforts to maintain its oxygen supply, the oxygen consumption falls, returns to normal and becomes subnormal. After periods of struggling and rest, and minor ups and downs in the opercular movement rate, the fish sooner or later becomes exhausted. When the oxygen consumption rate sinks to about 20 per cent of normal the opercular movement rate begins a precipitous descent and fish dies (Jones, 1948; 1964). The effect of an organo-chlorine insecticide BHC on Clarias batrachus also shows increased surfacing and increased opercular movement (Table-3; Plate-III). A number of published works are in agreement with the present finding (Jones, 1948, 1964; Annes, 1975; Kumar, Pant and Khanna, 1979; Sriwastava and Srivastava, 1979; Datta, 1980; Khangarot, 1982; Arunachlam and Palanichamy, 1982). Pandey et al. (1976) reported decrease in the opercular frequency in Channa punctatus exposed to malathion. Chaudhary, Pandey and Dubey (1981) observed irregular opercular frequency in Heteropneustes fossilis exposed to malathion. In Heteropneustes fossilis, the opercular movement increased enormously for Ist few minutes, but the average opercular frequency has decreased in 24 hr time. They showed high correlation of coefficient against concentration.

Nagendran and Shakuntala (1979) observed that under the exposure of sublethal concentrations of sodium pentachlorophenate, Puntius ticto exhibited significant increase in the opercular/surfacing activity.

Basak and Konar (1977) observed deformed taste buds and reduced feeding rate in Cyprinus carpio exposed to DDT and increased growth rate in Tilapia mossambica by 28% in DDT treated ponds. Holmberg et al. (1972) recorded considerable loss of body weight in Anguilla anguilla exposed to pentachlorophenol (PCP). Chaudhary, Pandey and Dubey (1981) found reduced feeding, and growth rate in Heteropneustes fossilis exposed to malathion. In the present study on Clarias batrachus exposed to BHC, a reduction in body weight as well as in feeding rate has been observed. Similar results have been obtained in fingerlings of Cyprinus carpio exposed to endrin (Datta, 1980); Poecilia sphenops (black molly) exposed to chloroform, tetra-chloroethylene and trichloroethylene (Lockie, Schecter and Christian, 1983). Manoharan and Subbiah (1982) showed that sublethal concentrations of endosulfan causes marked reduction in feeding rate from 5.94% to 9.02% and assimilation by 6.44 to 9.60%. Growth (weight) retarded from 11.6 mg/day in control to 7.3, 6.0 and 5.1 mg/day in the endosulfan treated Barbus stigma.

Verma, Tonk and Kumar (1983) reported that Thiotox (T), and organo-chlorine and malathion (M), an organophosphate insecticides exposed Notopterus notopterus consume lesser amount of food compared to unexposed fishes. The rainbow trout, Salmo gairdneri after prolonged exposure of hexavalent chromium showed no growth retardation (Pulte, Galieen and Strik, 1982). Food intake of Macropodus cupanus reared in different concentrations of carbaryl did not vary significantly while growth decreased with increased concentration of carbaryl possibly due to increased expenditure of energy on metabolism (Arunachalam and Palanichamy, 1982). Thus it appears quite clearly that reduction of body weight and feeding rate after toxicant exposure is species specific or toxicant specific reaction.

The body composition of fish has been reported in relation to size, age (Lovern, 1938), sex, locality (Zinevici 1970), nature of food (Hornell and Nayudu, 1924) and season (Philips, 1969) but informations related to the effect of pollutants on body composition are scanty. The moisture content of freshwater fishes has been reported mostly between 70-80% although values as low as 53.7% in Hilsa ilisha and as high as 88.8% in Cyprinus carpio are not uncommon (Anonym, 1962).

In experiment, where Clarias batrachus are subjected to 15 days exposure of 12.0 mg/l of BHC, there is a loss in body weight, liver weight, HSI, gonad weight and GSI, while water content of liver and gonad in exposed animals have increased significantly (Table-4; Plates-IV & V). Chaudhary, Pandey and Dubey (1981), demonstrated that the water and lipid contents of the whole body and ovary decreased compared to control. The liver water increased from 68.989±2.607% to 75.629±4.693% with the increasing concentration of malathion in Heteropneustes fossilis. Verma, Tonk and Kumar (1983) reported increased HSI and GSI and decreased water content of ovary, liver and kidney of Notopterus notopterus exposed to thiotox (T), an organochlorine, Malathion (M), an organophosphate and M/T and T/M combinations. A significant decrease between 27% and 43% in the liver size and liver somatic index of flounders were observed as a result of cadmium exposure (Larsson et al., 1976). The effect was not due to a drop in the water content of the liver, as this parameter was unchanged. It has also been shown in mammalian experiments that cadmium induces various changes in liver, from altered activity to certain liver enzymes to severe liver cirrhosis (Nilson, 1970). However, cadmium induced effects on the liver have probably been over looked in the past (Nordberg, 1974).

The decreased liver weight and liver somatic index in BHC exposed fishes as observed in present investigation is similar to those reported by various authors (Larsson et al., 1976; Nilson, 1970; Nordberg, 1974). The decrease in liver and gonad weight is not due to decrease in water content but possibly because of decrease in protein, fat and other solid materials. Increase in liver and gonadal water suggest very strongly that there is degradation of protein and fat of the liver and gonad. It has been reported that an animal may loss practically all of its fat and half of its protein and live but loss of only 19% its water causes death (Maynard and Loosli, 1962).

The LC_{50} value for this fish has been calculated to be very high. Generally BHC is applied at a rate of 10-15 kg per acre in the field. This would rarely produce a concentration of LC_{50} or would possibly reach a concentration considered not a safe value for this fish. The persistent organo-chlorine insecticides frequently show a delayed toxicity in that the lethal effect is not seen until sometime after contact with the chemical. These chemicals are fat soluble and so can accumulate in the body fat of an animal. Lethal doses may then be released into the blood stream when fat reserves are used. This delayed toxicity is strictly

speaking, a lethal not a sublethal effect although the toxic chemical may accumulate as a result of repeated sublethal exposures of the chemical (Moriarty, 1975). All chlorinated hydrocarbons except BHC-proved more toxic to fish than the organophosphorus-compounds under the same experimental conditions. Accumulation of organochlorines by fish can proceed by two routes: absorption through the gills (Holden, 1962; Murphy, 1971) and ingestion of contaminated food (Macek, Rodgers, Stalling and Korn, 1970; Grzenda, Paris and Taylor, 1970). Residue uptake through gills is related to metabolic rate and body size (Murphy, 1971). In practice food seems to be the main source of OCs to fish (Macek and Korn, 1970; Norstrom, McKinnon, de Freitas and Miller, 1975). Further investigations are to be encouraged to finally establish the effect of BHC contamination in the environment before this compound is declared safe on various water bodies as the insecticide of choice.

Summary

Investigations were undertaken to record the effect of BHC, an organochlorine insecticide on toxicity, behaviour and body composition of an air-breathing fish, Clarias batrachus (Linn.).

It was observed that the system of Clarias batrachus can function within broad range of pollution by BHC and toxicity response of this fish towards BHC exposure is a function of concentration and duration of exposure. The 96 hours toxicity tolerance limit (TLm) value for this fish has been calculated to be 15.94 mg/l, which is higher compared to several freshwater fishes.

The behavioural response of the fish towards toxicant was grossly dependent on concentration and length of exposure. Increased aerial excursions and opercular movement was observed as immediate response of fish towards the toxicant.

Liver weight, liver somatic index (LSI), gonad weight, gonado-somatic index (GSI) decreased with increasing concentration of BHC. There was marked increase in liver water (13.75%) and gonadal water in animals exposed to BHC for 15 days. Decreased feeding rate, growth rate and hydration of liver and gonad indicate the toxic effect of BHC.

In higher concentrations of BHC fishes showed white wound patches on the skin surface at the sides of the body. Marked discolouration and depigmentation of the skin after BHC exposure has been observed. Fishes exposed to higher concentrations have tried to remain almost in vertical position perpendicular to the base of aquaria. The terminal phase was characterized by agitated movements, tremors, convulsions and loss of balance. The dead fish showed several would patches on either sides of the body and whitish gills with copious mucus.

CHAPTER-II

ACUTE TOXIC EFFECTS OF BHC ON CORPUSCULAR HAEMATOLOGY AND RATE OF OXYGEN CONSUMPTION

The contamination of water by pesticides can have deleterious effects on organisms. The nature of the effects varies but can include structural and functional modification at both the cellular and sub-cellular levels in a variety of organisms (Verma et al., 1978, 1981). The general haematological tests are used to establish the normal health status and to diagnose diseases caused by various factors, viz., nutrition, environmental stress, toxicant, parasitic infections etc. in human and veterinary medicine but such faculties are not well established in poikilotherms especially fishes (Wedemeyer and Yasutake, 1977). The increasing use of fish as test species in toxicological studies in relation to pollution necessitated the establishment of normal haematological and physiological values. Fishes are intimately associated with their aquatic environment and readily reflects the measurable physiological change (Klontz, 1972).

Recently haematological studies has widely been made on fishes by a number of investigators to establish normal values (Hall and Gray, 1929; Albritton, 1952; Ostromova, 1960 Preston, 1960; Pradhan, 1961; Haws and Goodnight, 1962; Srivastava, 1968a, b, c, 1969a, b, 1970; Srivastava and Griffith, 1974; Mulachi, 1970; Joshi, 1978; Murachi, 1978; Gupta and Gupta, 1981; Goel et al., 1981; Banerjee, 1981, 1982; Lowe-Jinde and Nimi, 1983; Thakur, 1986).

The cyclic changes in the environmental time cues, viz., temperature, daylength (photoperiod), humidity, rainfall, food and physico-chemical composition of medium have pronounced effect on the life of poikilothermic animals and are intimately related to rhythmic physiology of animals (Wolfson, 1964; Bunning, 1967). With the increasing emphasis on fish haematology, under various physiological conditions of the animal a good deal of literature is available (review by Hawkins and Mawdesley-Thomas, 1972), which indicate the seasonal variability of haematological values with changing environmental conditions round the year. Similar observations have also been made by recent workers (Khan, 1977; Pandey, 1977; Prasad et al., 1978; Agrawal and Srivastava, 1976a, 1978; Chanchal et al., 1979; Mahajan and Dheer, 1979; Thakur, 1986).

Attempts to correlate the changes in the number of circulating blood corpuscles with reproductive activities and other endocrine factors, too seems to be scant and the results are conflicting. Cernikova (1967) failed to find any seasonal difference in the blood of Rutilus rutilus, Abramis brama and Lota lota. Khan (1977) in C. batrachus, Joshi and Tandon (1977) in H. fossilis and Tugarina and Ryzhova (1970) in Thymallus arcticus baicalensis have shown an increase in erythrocyte number and haemoglobin concentration during spawning period in these species on the other hand. Robertson et al. (1961) in Oncorhynchus tscharyntscha and Orecka (1970) in Tinca tinca have reported a decrease in the erythrocyte counts during spawning season. Schlicher (1927) is of the opinion that erythrocyte number decreases after spawning whereas, Prasad et al. (1978) and Chanchal et al. (1979) found biannual increase (once during breeding season and again during pre-winter) in the haematological values of M. aculeatum and A. testudineus respectively. Vernidub and Kolovaba (1971) have reported that during the first year of life, young Salmon, Salmo trutta, living in the transpolar region have two peaks of thyroidal activities and both are related to high erythropoiesis intensity. Slicher (1961) has described the influence of gonadal steroids and cortisol on the WBC responses in Fundulus heteroclitus. Influence of

endocrine factors on haematology has been investigated by Srivastava and Meier (1972), Meier and Srivastava (1975), Srivastava and Pickford (1972), Pickford et al. (1971).

A perusal of the above reports reveals a very controversial situation and one is left with the impression that no definite pattern is followed in the variability of haematological indices in relation to cyclic environmental variations and breeding cycle in fishes, as it differs from one species to another. The need therefore for the base-line data on haematological valuesof each species for the diagnosis of health and diseases is evident. The literature on the role of various endocrine factors, which are associated with the adaptability of animals under changing environmental conditions (Hoar, 1959) in relation to haematology of fishes is not well documented.

The contamination of fish habitat by different pollutants is of great concern, sometimes causing heavy mortality of a localized fish population and their food organisms. Most information about the effects of environmental pollutants on aquatic animals has been obtained from mortality studies. Very little is known about the damage to different internal organs or processes within an organism following exposure to environmental poisons. Consequently

knowledge about the mode of action of toxicants, of cause of death in poisoned aquatic animals is often lacking. A better understanding of these mechanisms is necessary if we want to predict the potential harmfulness of various chemicals to the environment (Larsson et al., 1976).

Blood is an important physiological system participating in the internal and external metabolism reacts sharply to any change in the organism. A toxicological study of blood fascilitates timely detection of the pathological shift in the organism and helps to identify the extent and nature of deviations from the normal conditions. Changes in blood under the influence of different environmental factors including toxic substances are mainly studied from quantitative parameters (Strorozhuk and Guleva, 1983).

In recent years attempts have been made to elucidate the toxic effects of various chemicals on haematological parameters, as it is believed to be the sensitive indicator to test the extent of harmfulness of pollution on aquatic organisms especially fishes (Bhimachar, 1946; Bhaskaran, 1946; Palmer, 1951; Weinreb, 1958; Frolova, 1960; Ostromova, 1960; Preston, 1960; Krylov, 1964; Esler and Edmunds, 1966; O'Brien, 1967; Vasil'ev, 1969; McKim, 1970; Pickford et al., 1971; Boiler 1973; Saad, 1973; Sangalang and Hollaran, 1973; Mcleay, 1973;

Newman and Maclean, 1974; Kawatski and McDonald, 1974; Saad et al., 1974; Kumar and Bhattacharya, 1975; Svobodova, 1975; Maziarka, 1975; Goldin, 1975; Mcleay, 1975; Qayyum et al., 1976; Agrawal and Srivastava, 1976; Lunn et al., 1976; Lone and Javaid, 1976; Sakoori et al., 1976; Pandey et al., 1976b; Larson et al., 1976; Panigrahi, 1977; Tishinova Nanova, 1977; Nayak and Madhyastha, 1977; Shami et al., 1978; Bilgrami and Qayyum, 1978; Hodson et al., 1978; Pandey et al., 1979; Choudhary, 1979; Panigrahi and Mishra, 1980; Shafi, 1980; Goel and Gerg, 1980; Mahajan and Juneja, 1980; Pandey et al., 1981; Ollenschlaeger, 1981; Rai and Qayyum, 1981; Singh and Srivastava, 1981; Sharma and Gupta, 1982; Goel et al., 1982; Kodama et al., 1982; Sarasquette et al., 1982; Mishra and Srivastava, 1983; 1984; Pandey et al., 1984; Thurston et al., 1984; Strorozhuk and Guleva, 1983; Rai and Qayyum, 1984; Natarajan, 1984). A perusal of the literature indicates that the effects of various toxicants on haematological parameters of Indian fishes is comparatively of recent origin.

The metabolic rate in fishes, as expressed in terms of oxygen consumption is influenced by a number of external and internal factors (review by Fry, 1971). The measurement of oxygen consumption is a sensitive method of establishing the relative importance of various environmental changes which is reflected in metabolic rate. Amongst the various

factors affecting the metabolic rate in fishes are age, and body size (Job, 1955; Winberg, 1956; Kramer, 1972; Munshi and Dube, 1973; Ojha and Munshi, 1975; Munshi et al., 1976; Ojha et al., 1977), temperature (Beamish, 1964; Kramer, 1972; Rajgopal and Kramer, 1974), season (Wells, 1935; Privolnev, 1948; Pandey 1978), photoperiod (Robert, 1960; Withy and Saunder, 1973), thyroid (Hoar, 1958; Pritchard and Gorbman, 1960; Gabos et al., 1973) and gonadal activities (Hoar, 1958; Beamish, 1964; Pandey 1976). In recent years attempts have also been made to study the effect of various pollutant on the respiratory physiology of fishes (Ranke Rybicka, 1975; Hughes, 1975; Waiwood and Johnson, 1974; Kawatski and McDonald, 1974; Lunn et al., 1976; Pandey et al., 1976b; Hughes, 1976; Singh and Singh, 1977; Reddy et al., 1977; Pandey et al., 1979; Choudhary, 1979; Tort, Crespo and Balasch, 1982; Sinhaseni et al., 1983; Hose, Hunt and Stoffel, 1983; Patil and Kaliwal, 1983; Pandey et al., 1984).

Literature related to the effect of pollutants on the oxygen consumption in Indian fishes is fragmentary. The present work is an attempt to ellucidate the acute toxic effects of an organochlorine insecticide, Benzene hexachloride (BHC) on haematological parameters and rate of oxygen consumption of an air-breathing fish, Clarias batrachus (Linn.).

Nomenclature of Blood Cells

With the increasing emphasis on fish haematology for diagnostic purposes under various physiological conditions of the animal a good deal of literature is available (review by Hawkins and Mawdesley-Thomas, 1972). Although several reviews of selected literatures have been published by various authors quite recently (Haider, 1967a, b, 1968; Hunn, 1967; Hawkins and Mawdesley-Thomas, 1972; Blaxhall, 1972), but the controversy has not yet been finally resolved. The trend of recognizing various cells in peripheral blood smears of fishes is based mainly on mammalian observations.

Erythrocytes

Mature erythrocytes in peripheral blood of fishes have been identified and described quite clearly in several fish species by many investigators. There is now general agreement that the immature erythrocytes (also called polychromatocytes) may be differentiated from matured one by the appearance of the nucleus and by the staining reactions of the cytoplasm. Polychromatocytes represent approximately 1% of the total number and are rounder and bluish-grey in giemsa stained smears (Ellis *et al.*, 1978). According to varying degrees of maturity they have been distinguished into early, mid and later polychromatocytes (Klontz *et al.*, 1964).

Leucocytes

There is no sufficient evidence to prove the usefulness of leucocyte differential counts on fish at present and it was assumed that differential counts on fish as in human medicine could prove its diagnostic utility when sufficient amount of evidence is gathered (Blaxhall and Daisley, 1973).

Lymphocytes

Lymphocytes are most numerous cells in fish blood. Bombrowski (1953) differentiated the lymphocytes on the basis of presence or absence of cytoplasm around the nucleus. Jordan and Speidel (1923) and Topf (1955) on the basis of their size differentiated them to small and big ones, while Haider (1968) recorded big, middle and small lymphocytes. But, there is now a general agreement that lymphocytes can only be differentiated as small and big (Blaxhall and Daisley, 1973; Pandey, 1974; Chaudhary, 1979; Thakur, 1986). They represent different functional states within the cell population rather than varying in functional capacity.

Monocytes

Monocytes have remained a subject of controversy among fish haematologists (Ellis, 1977). Schäperclaus (1954) a

pioneer fish haematologist and pathologist described the morphological details of monocytes in carps. According to him the monocyte is rich in cytoplasm, of about 14 µm in diameter and the nucleus is reniform in structure, made up of loose chromatin threads. Catton (1951), Dombrowski (1953) and Topf (1955) suspected the presence of monocyte in fish blood. Ellis et al. (1978) observed that only 1% of the circulating leucocytes are monocytes. The ultra structural details of monocyte has been investigated by Ferguson (1975a, b). Monocytes are partially differentiated cells and can develop into mature cells of mononuclear phagocyte system. They have also been termed as macrophage (Klontz, 1972; Connoy, 1972; Chaudhary, 1979). According to Klontz (1972) macrophage is derived from differentiating large lymphocytes. The nucleus of macrophage is large, irregular and is small in proportion to the total cell volume. The cytoplasm is basophilic and possess numerous small vacuoles.

Granulocytes

Various authors have claimed the presence of various kinds of granulocytes such as neutrophils, acidophils and basophils but some failed to recognize acidophils and still others to basophils in fish leucocytes. The neutrophil is often termed polymorphonuclear leucocyte or heterophil.

Neutrophil strongly reacts to benzidin-peroxidase reaction and therefore, could easily be recognized by this stain. Histochemical characters of plaice neutrophil has been extensively investigated by Ellis (1975) and he concluded that the neutrophil of fish in most respect resemble the mammalian neutrophil. They react positive with periodic acid Schiff (PAS), Sudan black-B, acid and alkaline phosphatase tests. The nucleus may show definite segmentation, but in many definite lobation may not be present. Pseudopodia like structure may be occasionally present (Haider, 1968) and there are enough evidences to show the phagocytic activities (Ellis et al., 1978).

It is evident from the review of literatures (Ellis, 1977) that presence or absence of eosinophil and basophil granulocytes in fish blood claimed by several authors have remained a matter of controversy. Ellis (1978) argues beyond doubt about the presence of these two granulocytes, but one type of leucocyte could easily be mistaken for the other. Conroy (1972) in Salmo salar bifurcated the cells of granulocytic series under fine and coarse granulocytes. He further placed neutrophil and metamyelocyte under fine granulocyte and the rest, probably eosinophil under coarse granulocyte. Blaxhall and Daisley (1973) grouped the cells of

granulocytic series of Salmo trutta into neutrophil, metamyelocyte and myelocyte. Eosinophil and basophil leucocytes are peroxidase negative (Haider, 1968). Dombrowski (1953) and Topf (1955) differentiated the cells of granulocytic series by peroxidase reaction into peroxidase positive and negative cells. They further noticed that the basophilic granulocytes to be the forerunner of thrombocytes and hence called them thrombocytoblast, but the term is confusing and misleading. Pandey (1974) while working on the haematology of Cyprinus carpio suffering from air bladder inflammation disease also used the method of Dombrowski (1953) and Topf (1955) for differentiating the cells of granulocytic series.

Thrombocytes

The presence of thrombocytes in the form of typically elongated cells had been a matter of discussion in the past. These are often termed spindle cells. Haider (1967b) recognized the different developing stages of spindle cells by Unna-Ziehl staining and proved that spindle cells are independent cell type and can only be grouped under thrombocytes. The general structure of spindle cells have been described by Ellis et al. (1978) and have correctly stated that a spent thrombocyte may frequently be confused with lymphocyte.

Developing cell series

Primitive cells of developing cell series are often found in the circulating blood (Catton, 1951; Weinreb and Weinreb, 1969; Pandey, 1974). Since there is great overlap in morphological criteria for identification of more primitive cells found in fish blood, therefore Blaxhall and Daisley (1973) grouped them as "blast cells".

On the basis of above discussion, in the present investigation following nomenclatures have been used:-

1. Erythrocytic series
2. Leucocytes
   - A. Non-granulocytic series:
     - a. Lymphocytes
       - i. Small
       - ii. Large
     - b. Macrophage
   - B. Granulocytic series:
     - a. Peroxidase positive - Neutrophil
     - b. Peroxidase negative - Myelocyte
       (with fine and coarse granules, i.e., basophil and eosinophil).
3. Thrombocytes
4. Blast cells.

The methods for procurements of animals and their maintenance have already been described in the previous chapter.

Experimental condition I(Haematological investigations)

After the acclimation of the fish in the laboratory conditions for a week, the animals of same sex and body weights (male: 59-65g) were exposed to test concentrations of 2.0, 4.0, 8.0 and 16.0 mg/l BHC for 96 hours, during which the pollutant water of each concentrations were renewed afresh every 24 hours. The water temperature remained during this period at 30-32.5°C (April-May). A control of similar size and weight was maintained separately along with the experimental animals. All fishes were fed daily with chopped

goat liver. Haematological investigations were undertaken after 96 hours exposure to various concentrations of BHC together with a separate control for each utilizing ten animals.

At the time of experiment, the fishes were taken out of the aquarium with least stress, using a hand net. Each fish was lightly anaesthesized (Klontz and Smith, 1968) by MS222 (Sandoz) solution in water. Blood samples were collected by cardiac puncture using hypodermic needle no.24 fixed onto a 2 ml sterilized syringe, each fish being sampled once. The blood is collected slowly and steadily, thereby, reducing the mechanical breakdown of red cells as far as possible. After taking out the blood each fish was blotted with turkish towel and their weights were recorded. In the present investigation,heparin (a solution of 5000 I.U.) has been used as an anticoagulant since it is the best anticoagulant as it does not alters the size of the red cells and minimizes the chance of haemolysis, as well as it does not interfere during the estimation of electrolytes in comparison to other anticoagulants. However, for differential counts of leucocytes trisodium citrate (TSC) was used as prescribed by various authors.

Total erythrocytes and leucocyte counts

Several diluting fluids suggested by different workers were tried: Shaw's (1930), Ress Ecker Fluid (Lucas and Jamroz, 1961) and modified Dacie's fluid (Blaxhall and Daisley, 1973). The latter fluid stored well, stained the cell nuclei effectively and was found most suitable for use with fish blood, when slight modifications were made. The method adopted was to keep stock solution of this fluid and this was diluted four times with tri-sodium citrate solution before use. In place of 1 in 50 dilution of blood, as suggested by Blaxhall and Daisley (1973), in the present method the blood was diluted to 1:200 in the conventional glass erythrocyte pipette to avoid the clumping and agglutination of cells as suggested by Thakur (1986). The total erythrocyte and leucocyte counts per mm^3 were determined by Neubauer improved double haemocytometer.

Haemoglobin estimation

The haemoglobin concentration in g/100 ml of blood was determined colorimetrically by Cyanomethaemoglobin method using Drabkin's reagent as diluting fluid and commercially available standards. This method was found superior because of the consistency of the results, stability of the reagents and the commercial availability of standards.

Haematocrit (Packed cell volume)

Haematocrit value was measured by heparinized micro haematocrit tube (1.1-1.2 mm) after centrifugation for 5 minutes at 10,000 rev/min. The per cent haematocrit value was determined with the aid of a microhaematocrit reader.

From these data absolute values, i.e., mean corpuscular haemaglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated by the following formula:

$$(\text{Pico}^{\text{MCH}}\text{grams}) = \frac{\text{Haemoglobin in g/100 blood}}{\text{Erythrocyte counts(million/cu mm)}}$$

$$\text{MCHC (\%)} = \frac{\text{Haemoglobin in g/100 ml of blood}}{\text{Haematocrit value/100 ml}} \times 100$$

Differential blood cell counts and their measurements

The thin blood smears were prepared on the alcohol-cleaned slides and after air drying were fixed in methanol for 10 minutes and then stained with May-Grünwald-Giemsa stains (Hayhoe et al., 1964). This stain is considered by many medical haematologists to have given superior results to other stains. The blood cell morphology was studied through light microscopy and on the basis of morphological differences and cytochemical reactions, the relative number of each type of leucocytes in the blood were counted and expressed in percentage.

The dimensions of red blood cells were determined by ocular (eye piece) micrometer on May-Grunwald Giemsa stained blood smears. The surface area and the thickness of erythrocytes were calculated from the observed data.

Student's 't' test were applied to evaluate the level of significance of all the parameters analyzed at 5% level. All the values of blood parameters estimated under different experimental conditions, were calculated and analyzed in the "Research Service Centre" of the Bhagalpur University with the help of Computer Programming.

Experimental condition II ($\dot{V}o_2$ measurements)

Live specimens of Clarias batrachus were procured from local fish dealer at Bhagalpur. The fishes were acclimatized in the laboratory conditions for a week before beginning the experimentation. Care has been taken to keep the animals healthy and free from parasites. The animals were fed daily with the pieces of goat liver. The water temperature remained during this period at 24-26°C (October). The animals were exposed to the test concentrations of 2.0, 4.0, 8.0 and 16.0 mg/l BHC for 96 hrs during which the pollutant water of each concentrations were renewed afresh every 24 hours.

Determination of $\dot{V}o_2$

The details of the method employed in the determination of rate of oxygen uptake through gills and skin under experimental conditions in which surfacing either prevented (i.e., free access to air not allowed) or allowed (i.e.,free access to air allowed) were those of Munshi and Dube (1973). In the first series of experiment,i.e., surfacing prevented, Munshi and Dube (1973) used a cylindrical glass respirometer with provision of continuous flow of water through it and removal of enclosed air (Plate-XI). Under this condition the fish was not in a position to utilize its air-breathing or accessory respiratory organ, therefore, the fish extracted the oxygen from the water for its requirements only through the gills and skin.

In the next series of experiment a rectangular acroplex box of approximately 4 liter capacity, having a small air chamber at its top to minimize gaseous exchange at the air water interface, was used (Plate-XI). Under this condition the fish can obtain air from the small air chamber in addition to extracting oxygen from water through gills and skin.

The concentration of dissolved oxygen in water samples were determined by Wrinkler's volumetric method (Welch, 1948).

Measurement of $\dot{V}o_2$ in various
concentrations of BHC exposure

The effect of BHC on the rate of oxygen uptake was measured at four different concentrations from 2.0, 4.0, 8.0 and 16.0 mg/l and a separate control was maintained in fresh aerated tap water. Fishes having almost same body weight (60 g) were used in the present experiment in order to avoid any change in the rate of oxygen consumption due to body size. Moreover, feeding was stopped at least 24 hours before measuring the rate of oxygen-consumption. The Vo_2 has been expressed in ml/kg/h.

Ten fishes in each experimental and control group were used and the mean values were compared. The difference of significance, if any was calculated by student's t-test at the level of 5%. All the values of Vo_2 estimated under different experimental conditions were calculated and analyzed in "Research Service Centre" of the Bhagalpur University with the help of Computer Programming.

Experimental condition I (Corpuscular Haematology)

Erythrocytes

The number of circulating erythrocytes decreased with increasing concentrationsof BHC. Maximum decrease was observed at the highest concentration level, i.e., 16.0 mg/l BHC exposure for 96 hrs (1.490±0.009; $P < 0.001$) in comparison to the control (2.191±0.089; Table-6).

The erythrocytes of this fish were elliptical or circular in shape. The erythrocyte diameter of the control fish were 31.312±0.109 μm which decreased to 26.179±1.050 after 16.0 mg/l BHC exposure for 96 hrs (Table-6). Erythrocyte diameter showed inverse relationship with the increasing concentrations of BHC. The percentage of spherocytosis was

proportional to the concentration of BHC exposure. The number of smudge cells (Plate-VII) also increased in BHC exposed animals. Vacuolization of erythrocytes were also evident. Sometimes disappearnace of red blood cell membrane was also observed. The nuclei of the erythrocytes of exposed animals were irregular and eccentrically placed. Howell-Jolly bodies were observed in the cytoplasm, a symptom of distructive anaemia.

Haemoglobin

The blood haemoglobin of exposed animals showed a decreasing trend in comparison to control group(12.074±1.521), although the decrease in thaemoglobin was statistically not significant (Table-6; Plate-IX).

Haematocrit values

Haematocrit or the packed cell volume (PCV) significantly decreased with increasing concentrations of BHC for 96 hours (Table-6; Plate-IX).

MCH and MCHC

MCH and MCHC showed inverse relation to the number of circulating erythrocytes. MCH showed an increasing trend and was significantly affected only at the highest concentration

(i.e., 16.0 mg/l for 96 hours; $P < 0.05$) while MCHC had significantly increased with increasing concentrations of BHC for 96 hours (Table 6;Plate-IX).

Leucocytes

Leucocyte total counts produced a clear trend of increase in leucocyte number after BHC exposure for 96 hours from 4.0 mg/l concentration onwards to 16.0 mg/l. In 2.0 mg/l the total leucocyte count showed significant decrease (14.525 ± 0.854) from the control (29.39 ± 0.037) while in 8.0 mg/l and 16.0 mg/l a significant increase of leucocyte count was noticed ($P < 0.01$; $P < 0.001$ respectively; Table-6; Plate-IX).

Classification of leucocytes of Clarias batrachus and effect of BHC exposure on differential leucocyte counts

The leucocytes of Clarias batrachus as evident from the present observation showed clearly that only 4 different kinds of cells were present. They were classified into agranulocytes and granulocytes respectively on the basis of absence or presence of granules in their cytoplasm. Further agranulocytes were distinguished as lymphocytes and monocytes. Similarly granulocytes were further classified as neutrophils and eosinophils only.

TABLE 6

Acute toxic effects of BHC (96 hours) at various concentrations on corpuscular haematology of *Clarias batrachus* (Linn.) (30-32.5°C; ± Standard error; n = 10 in each group)

Parentheses	Exposure Time	Control	BHC TREATED			
			2.0 mg/l	4.0 mg/l	8.0 mg/l	16.0 mg/l
Erythrocytes million/ mm^3	96 hrs	2.191±0.089	1.905±0.035 P <0.01	1.817±0.149 P<0.05	1.598±0.063 P<0.001	1.490±0.009 P<0.001
Mean erythrocyte diameter um	-do-	31.312±0.109	32.929±0.574	28.02 ±0.391	27.369±0.141	26.179±1.050
Haemoglobin g/100 ml	-do-	12.074±1.521	11.175±0.269 NS	10.357±0.366 NS	9.957±0.258 NS	9.815±0.141 NS
Haematocrit (%)	-do-	41.65 ±0.298	35.60 ±1.228 P<0.001	32.29 ±1.642 P<0.001	30.65 ±1.845 P<0.001	30.25 ±1.719 P<0.001
Mean corpuscular haemoglobin	-do-	54.179±3.103	56.495±1.118 NS	55.706±2.399 NS	61.201±3.014 NS	62.816±1.607 P<0.05
Mean corpuscular haemoglobin concentration	-do-	29.39 ±0.037	31.934±0.102 P<0.001	32.959±0.316 P<0.001	33.955±2.252 P<0.05	33.784±2.169 P<0.05
Leucocyte counts thousand/mm^3	-do-	17.475±0.869	14.525±0.854 P 0.05	20.96 ±3.336 NS	39.392±6.227 P<0.01	47.97 ±2.086 P<0.001

TABLE 7

Correlation coefficients and equations to show the relationships between concentrations of BHC (X) Vs erythrocyte counts (Y), mean corpuscular haemoglobin concentration (Y_1) and total leucocytes (Y_2) in

Clarias batrachus

Parameters Analyzed	Equations Y = a + b.X	Correlation coefficients (r)	Test stat for 'r'	Test stat for fo 'b'
Total erythrocyte counts in million/mm^3 (Y)	Y = 2.0368-0.39400. X	-0.90824	3.7594 P < 0.05	0.1630867 NS
Mean corpuscular haemoglobin concentration (Y_1)	Y = 54.77817+0.55063.X	+0.934467	4.5458 P < 0.02	2.67866 NS
Total leucocytes counts (Y_2)	Y = 14.80037+2.271337.X	0.947631	5.13937 P < 0.02	11.9929 P < 0.01

Lymphocytes

Lymphocytes were characterized by a prominent nucleus and with or without basophilic cytoplasm. Lymphocytes were further distinguished as small and big on the basis of their size. Total percentage of lymphocytes showed marked increase in all experimental animals (Table - 8; Plate - X). The maximum increase of lymphocytes were observed in 2.0 mg/l and 16.0 mg/l BHC exposed animals for 96 hours (76% and 80%; $P < 0.001$ and $P < 0.001$ respectively).

Monocytes

Monocytes were not located in the animals exposed to the lowest BHC concentration, i.e., 2.0 mg/l but in 4.0 and 8.0 mg/l they showed maximum increase although this increase was statistically non-significant (1.3 to 1.6% respectively). In the highest concentration the percentage of monocyte again declined (Table-8; Plate-X).

Neutrophils

Significant decrease in the number of neutrophils was observed with increasing concentrations of BHC. Maximum decrease in neutrophil percentage was observed at 16.0 mg/l BHC exposure for 96 hours (13.6 ± 618; $P < 0.001$; Table-8; Plate-X).

TABLE 8

Acute toxic effects of various concentrations of BHC (96 hours) on differential leucocyte counts of *Clarias batrachus* (Linn.) (30-32.5°C; ± Standard error; n = 10 in each group)

Parentheses	Exposure Time	Control	BHC TREATED			
			2.0 mg/l	4.0 mg/l	8.0 mg/l	16.0 mg/l
Lymphocytes	96 hrs	35.9 ±2.364	76.8 ±0.827 P< 0.001	65.2 ±3.749 P< 0.001	56.7 ±0.624 P< 0.001	80.2 ±0.733 P< 0.001
Monocytes	-do-	0.5 ±0.506	-	1.3 ±0.597 NS	1.6 ±0.258 NS	0.2 ±0.2 NS
Eosinophils	-do-	13.4 ±0.897	4.6 ±0.267 P< 0.001	9.7 ±2.817 NS	6.6 ±0.777 P< 0.001	6.0 ±1.193 P< 0.001
Neutrophils	-do-	51.34±4.804	18.5 ±0.637 P< 0.001	25.6 ±3.144 P< 0.001	34.4 ±3.144 P< 0.001	13.6 ±0.618 P< 0.001

EXPLANATION OF PLATE - VI

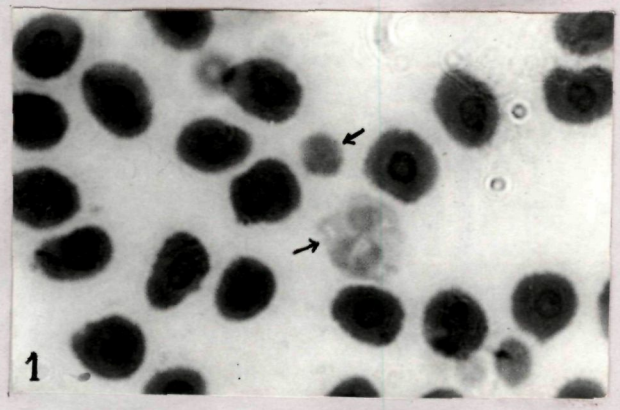
Fig. 1: Showing elliptical erythrocytes, basophils and neutrophils (arrow) from blood smears of normal animals (100x4x).

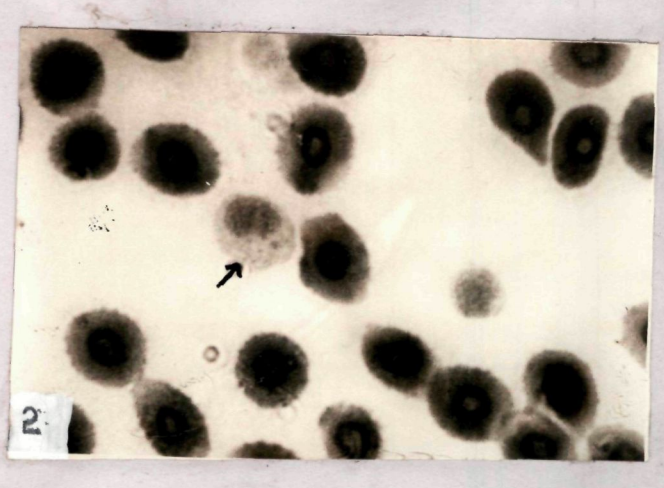
Fig. 2: Showing large lymphocyte (arrow) and basophils in blood smears of normal animals (100x4x).

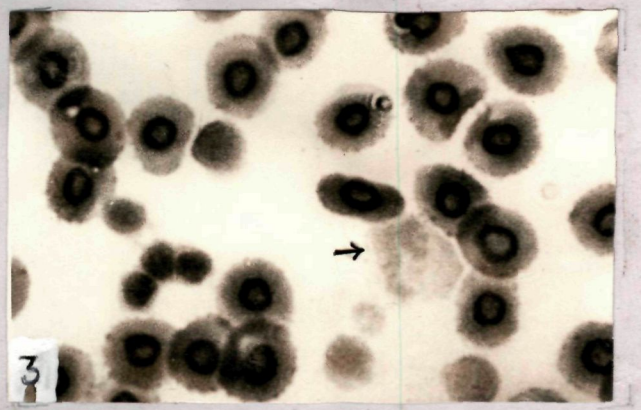
Fig. 3: Showing multinucleated neutrophil from normal animal (100x4x).

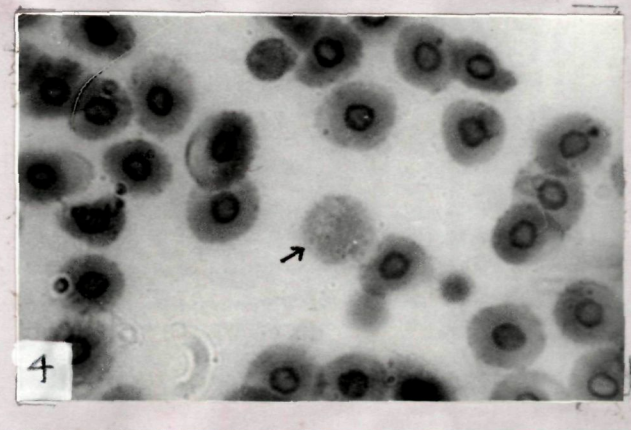
Fig. 4: Showing eosinophil from normal animal (100x4x).

PLATE VI







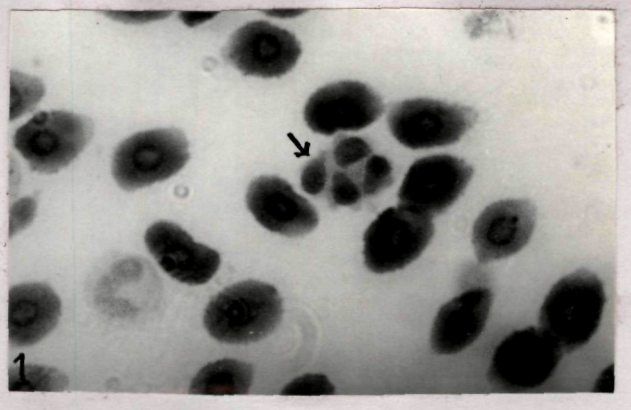


EXPLANATION OF PLATE - VII

Fig. 1: Showing blast cells in the blood smears of normal animals (100x4x).

Fig. 2: Showing large number of smudge cells in the blood smears of experimental animals (100x4x).

Fig. 3: Showing thrombocyte (spindle shaped) in the blood smears of normal animals (100x4x).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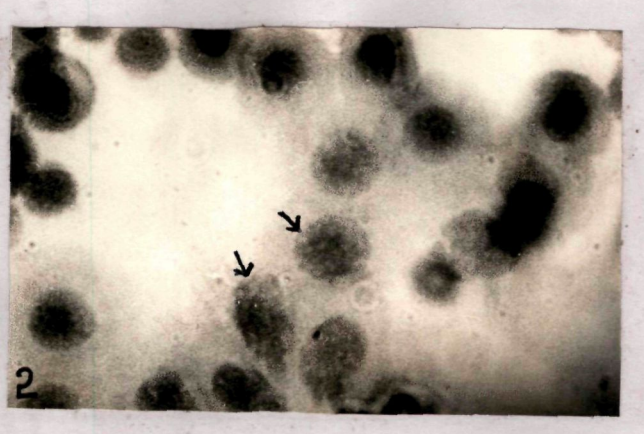
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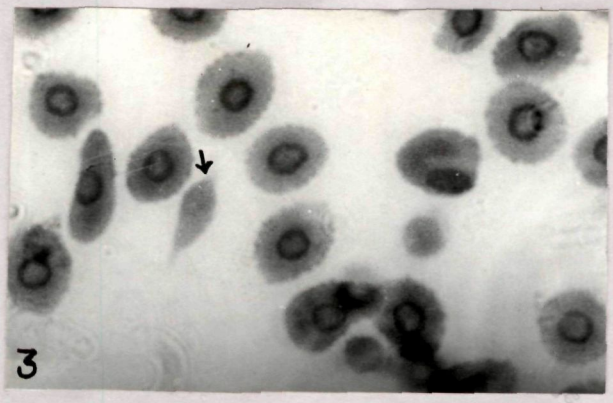
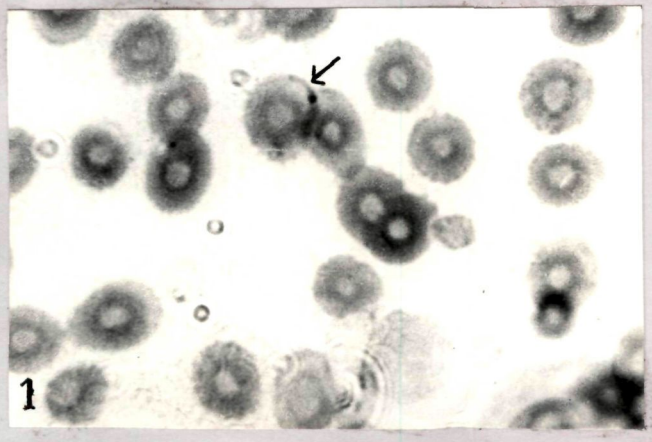

3

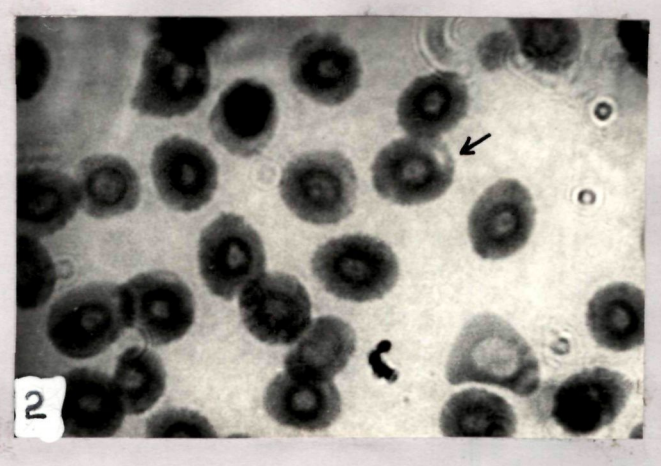
EXPLANATION OF PLATE - VIII

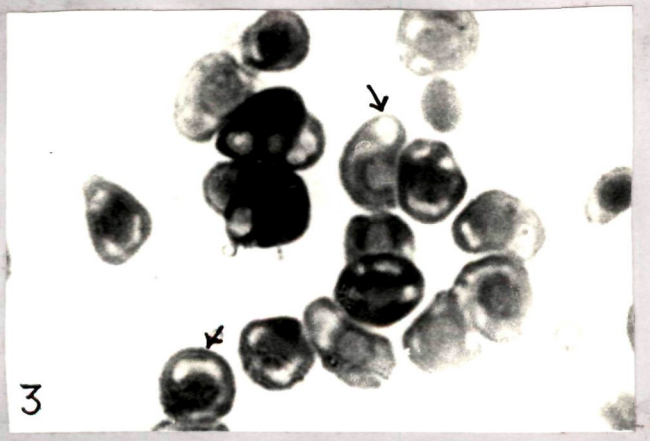
Fig. 1: Showing vacuolation of erythrocytes in the blood smears of BHC (8.6 mg/l) exposed animals (100x4x).

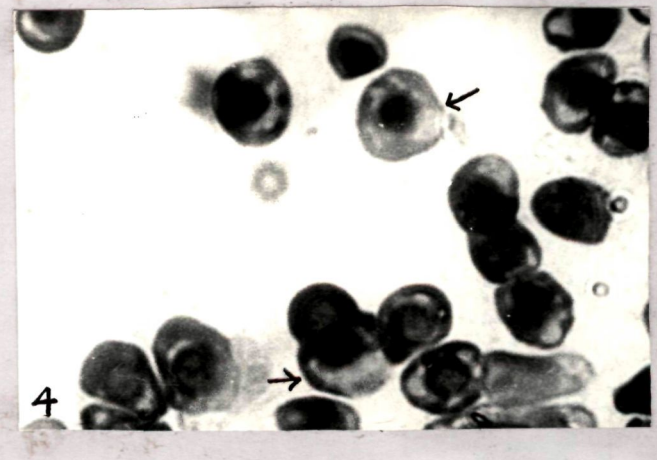
Fig. 2: Showing large number of vacuoles in the erythrocytes (arrow) and shifting of nuclei towards the periphery in BHC (16.0 mg/l) exposed animals (100x4x).

PLATE VIII









EXPLANATION OF PLATE - IX

Histograms showing the effect of various concentrations of BHC exposure for 96 hours on erythrocytes, leucocytes, erythrocyte diameter, PCV, MCH and MCHC of *C. batrachus*.

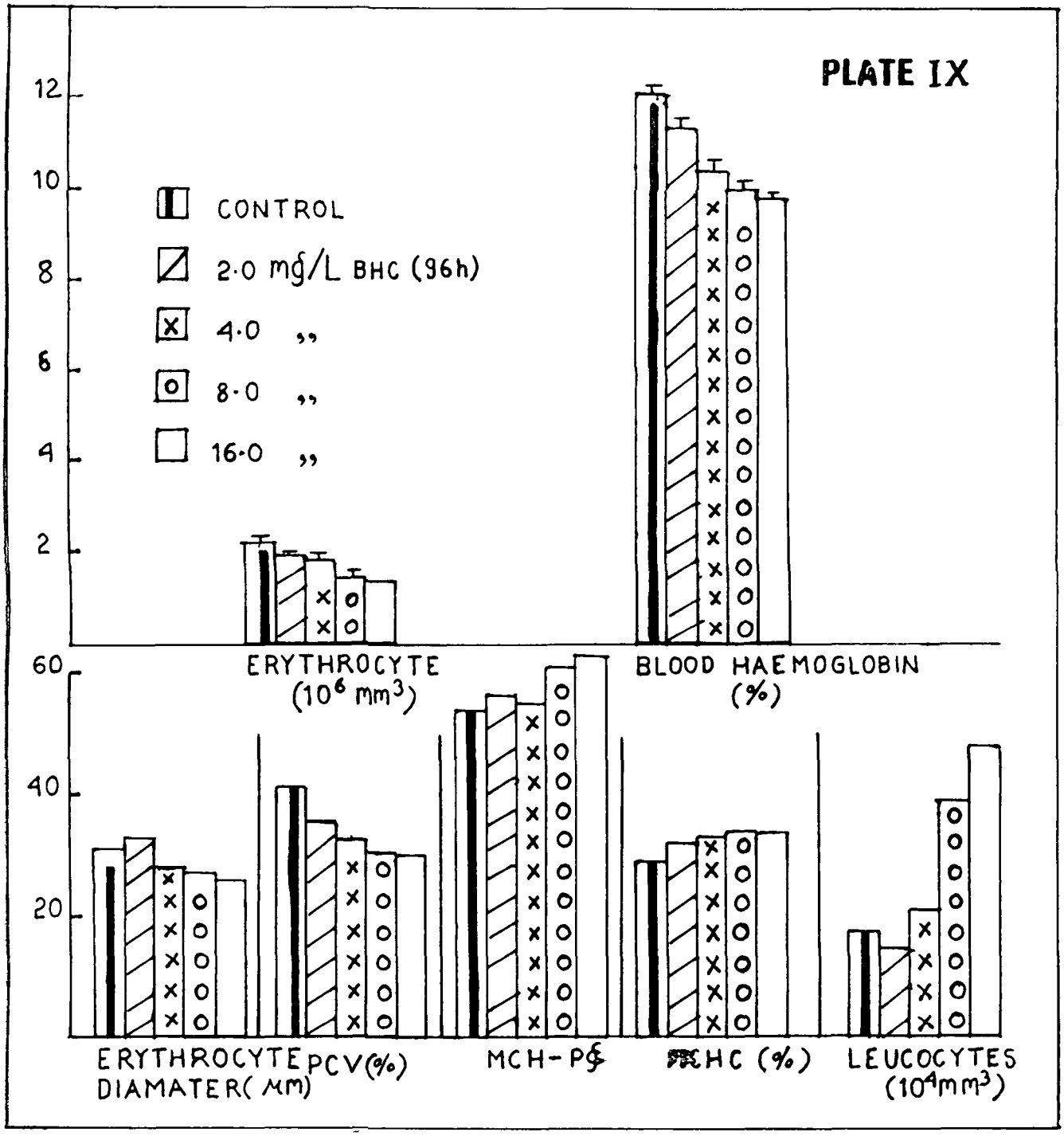
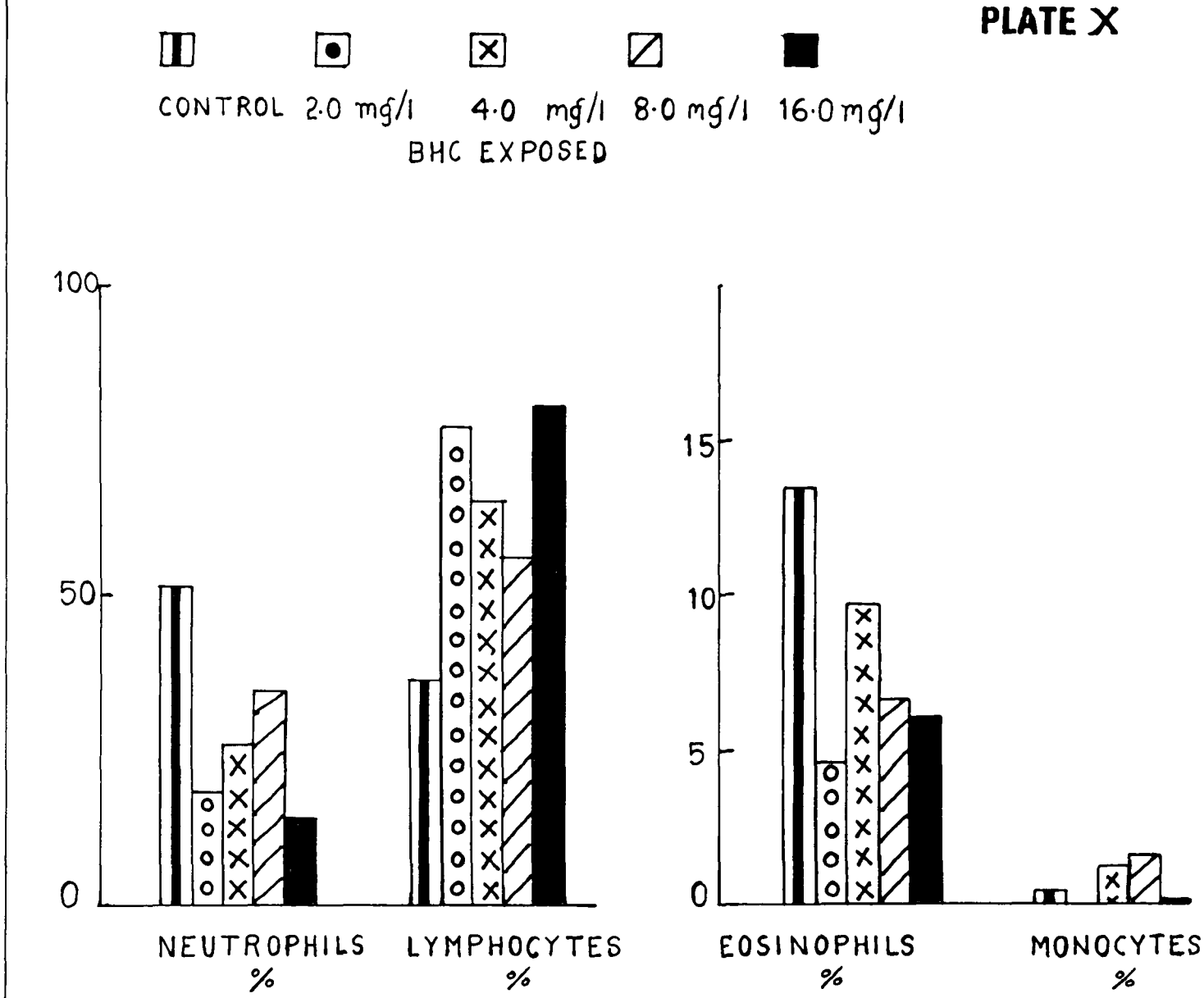


PLATE IX
CONTROL
2·0 mg/L BHC (96h)
4·0 ,,
8·0 ,,
16·0 ,,
12
10
8
6
4
2
60
40
20
ERYTHROCYTE (10⁶ mm³)
BLOOD HAEMOGLOBIN (%)
ERYTHROCYTE DIAMATER (MM)
PCV (%)
MCH-Pg
MCHC (%)
LEUCOCYTES (10⁴mm³)

EXPLANATION OF PLATE - X

Histograms showing the effect of various concentrations of BHC exposure for 96 hours on differential leucocyte counts in C. batrachus.

PLATE X


CONTROL
2·0 mg/l
4·0 mg/l
8·0 mg/l
16·0 mg/l
BHC EXPOSED
100
50
0
15
10
5
0
NEUTROPHILS %
LYMPHOCYTES %
EOSINOPHILS %
MONOCYTES %

Eosinophils

Eosinophils showed a decreasing trend in all the experimental animals. Maximum decrease of eosinophils was observed at 2.0 mg/l BHC exposure for 96 hours (P 0.001; Table-8; Plate-X).

Regression and correlation analysis

A linear relationship has been established between concentrations of BHC (X) and erythrocyte counts (Y), Mean Corpuscular Haemoglobin (Y_1) and total leucocytes (Y_2). This relationship has been established by the general equation:

$$Y = a + b. X$$

It has been observed that with the unit increase in the concentration of BHC the erythrocyte counts decreases by a factor of 0.394 (b value). The correlation coefficient (r) have been computed to be high. The value of 'r' is -0.9082 (Table-7). The mean corpuscular haemoglobin concentration (MCHC;Y_1) and total leucocytes (Y_2) increases by a factor of 0.551 and 2.211 respectively with the unit increase in the concentration of BHC. The values of 'r' of MCHC and total leucocytes are +0.9344 and +0.9476 respectively (Table-7).

Experiment II

$\dot{V}o_2$ in relation to concentration of BHC

The rate of oxygen consumption in Clarias batrachus at different concentrations of BHC exposure under both the experimental conditions (surfacing prevented and allowed) are summarized in Table-9. The mean value of $\dot{V}o_2$ of control animals were 74.183±6.144 and 71.923±2.947 under surfacing prevented and surfacing allowed conditions respectively (Table - 9). A perusal of the Table-9 indicates that exposure of BHC causes an increase in the $\dot{V}o_2$ but the values are not consistent. There was maximum increase in $\dot{V}o_2$ during surfacing prevented condition at 8.0 mg/l exposure of BHC for 96 hours (90.837 ± 2.629; $P < 0.01$) but in 2.0, 4.0 and 16.0 mg/l BHC concentration the data showed only non-significant increase. There is no correlation between increasing concentrations of BHC to $\dot{V}o_2$ in this animal, while during surfacing allowed condition the $\dot{V}o_2$ showed a decreasing trend at 2.0 and 4.0 mg/l BHC exposures but at concentrations 8.0 and 16.0 mg/l there was significant increase in $\dot{V}o_2$. Maximum rate of oxygen consumption was observed at 8.0 mg/l BHC exposure (138.182±5.528; $P < 0.001$; Table-9; Plate-XII).

TABLE 9

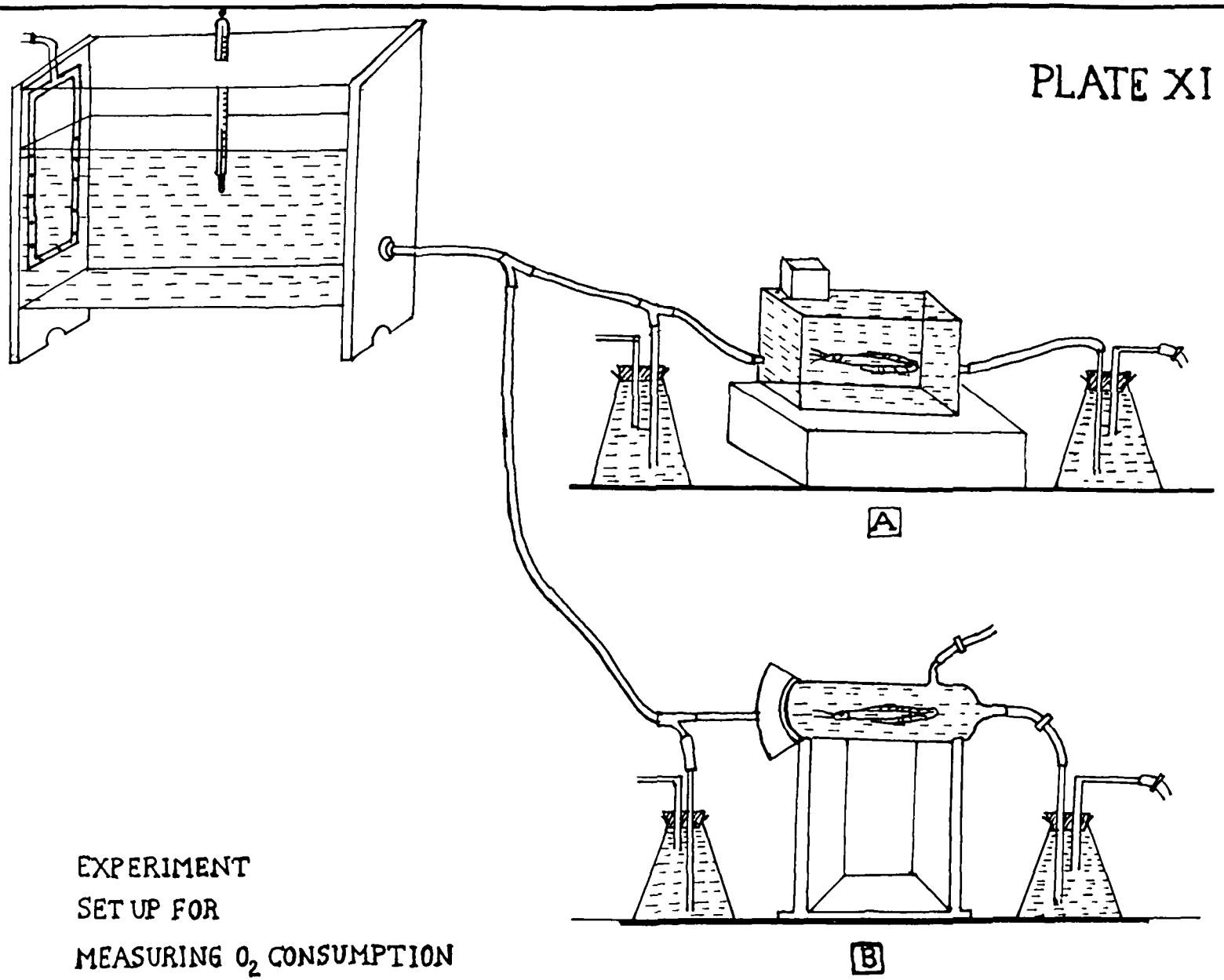
Effect of acute toxicity (96 hours exposure) of various concentrations of BHC on rate of oxygen consumption of Clarias batrachus (± Standard error, n = 10 in each group)

Parameters analyzed	Surfacing prevented (ml/kg/hr)	Surfacing allowed (ml/kg/hr)
Control	74.183±6.144	71.923±2.947
2.0 mg/l BHC	75.25 ±0.316 NS	71.724±0.352 NS
4.0 mg/l BHC	92.471±8.428 NS	69.732±4.606 NS
8.0 mg/l BHC	90.837±2.629 P < 0.01	138.182±5.528 P < 0.001
16.0 mg/l BHC	82.492±1.869 NS	113.005±3.324 P < 0.001

EXPLANATION OF PLATE - XI

Diagram of apparatus used to determine the oxygen consumption by the fish under surfacing allowed and prevented conditions in a continuous flow of water.

PLATE XI

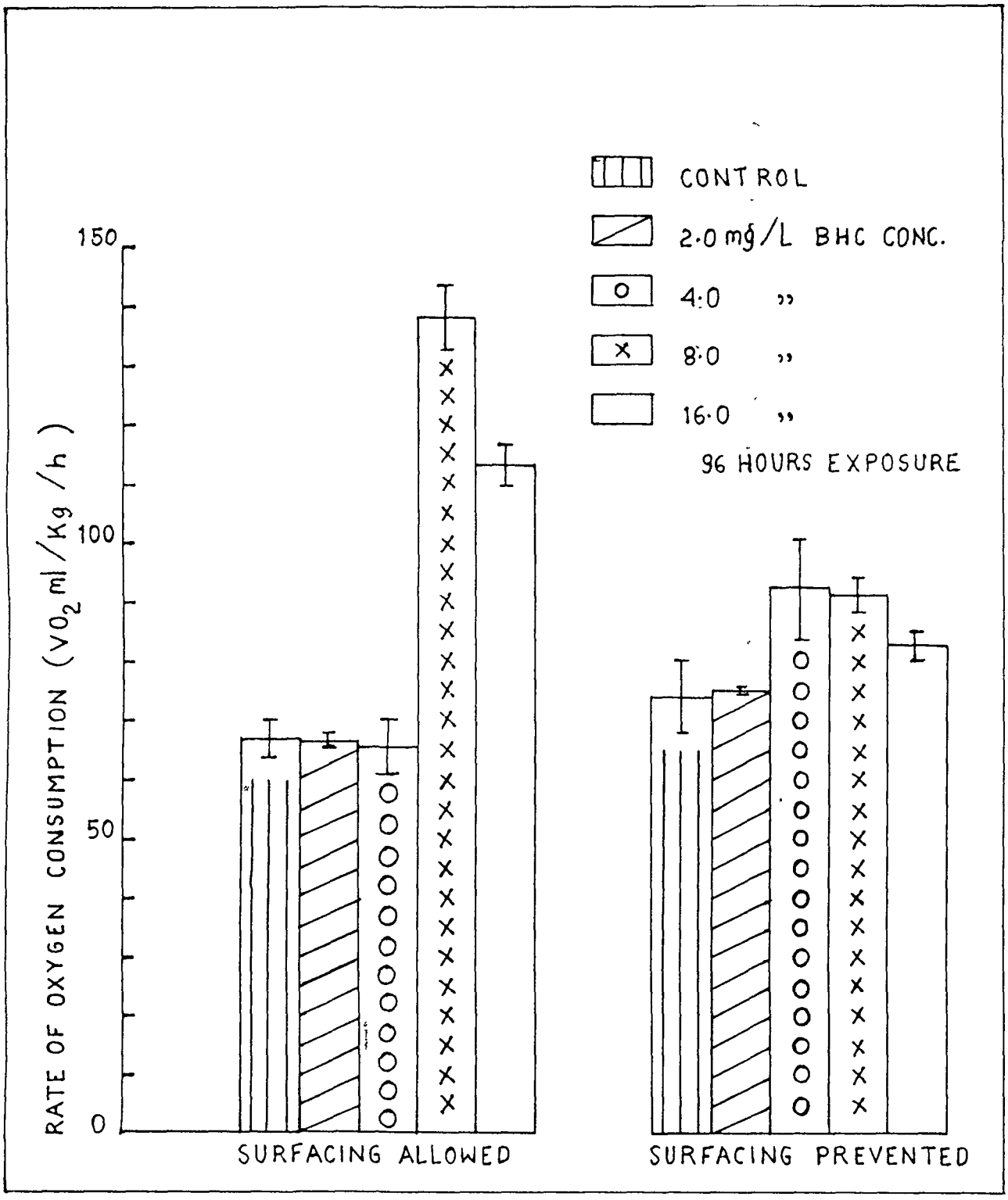


EXPERIMENT
SET UP FOR
MEASURING O_2 CONSUMPTION
A – AIR ALLOWED
B – PREVENTED

EXPLANATION OF PLATE - XII

Histograms showing the effect of various concentrations of BHC exposure for 96 hours on the rate of oxygen consumption during surfacing allowed and prevented conditions.

PLATE XII


CONTROL
2·0 mg/L BHC CONC.
4·0 ,,
8·0 ,,
16·0 ,,
96 HOURS EXPOSURE
RATE OF OXYGEN CONSUMPTION (VO_2 ml/Kg/h)
150
100
50
0
SURFACING ALLOWED
SURFACING PREVENTED

Discussion

The haematological parameters are used as the sensitive indicators of toxicity of different pollutants. It is interesting to note that the different groups of pesticides or even the pesticides of the same group do not have the same effect on fishes. The mode and site of action of different pesticides also differ greatly and therefore no generalization is possible regarding the effect of different pesticides in fishes.

Significant decrease in the erythrocyte counts and erythrocyte diameter was observed. There was also non-significant reduction in haemoglobin concentration, while haematocrit value declined significantly with increasing BHC concentrations. Mean corpuscular haemoglobin (MCH) showed marked increase but the significant increase in MCH was only obser-

ved at the highest concentration (16.0 mg/l), while the mean corpuscular haemoglobin concentration (MCHC) increased significantly in all the concentrations of BHC exposure for 96 hours.

Toxicant induced anaemia has been observed by many investigators using various kinds of chemical toxicants on different fish species (Pandey et al., 1976b; Larson et al., 1976; Pandey et al., 1979; Shafi, 1980; Panigrahi and Mishra, 1980; Pandey et al., 1981; Rai and Qayyum, 1981; Raizada and Gupta, 1982; Ollenschaeger, 1983; Mishra and Srivastava, 1983; Rai and Qayyum, 1984; Pandey et al., 1984; Thurston, 1984).

Significant decrease in the number of erythrocytes and haemoglobin content was also observed in CCl_4 intoxicated Clarias batrachus (Sharma and Gupta, 1982). DDT, Metacid and Unizeb caused anaemia in Channa punctatus (Pandey et al., 1979). In Clarias batrachus poisoned by Kelthane (an organochlorine) caused anaemia (Pandey et al., 1984). Buckley(1982) investigated the effect of monochloramine (NH_2Cl), a widely used drinking and waste water disinfectant, on human erythrocytes in vivo indicated oxidation of haemoglobin (Hb), and secondary to oxidant damage, haemolysis of erythrocytes in rainbow trout, Salmo gairdneri.

Malathion (an organophosphate) caused significant decrease in RBC counts, haemoglobin concentration, PCV and MCHC of Channa punctatus (Pandey et al., 1976). Panigrahi (1977) studied the toxicological effect of mercury poisoning on Anabas and reported a sharp fall in RBC counts, Hb concentration, PCV and MCHC. In flounder (Pleuronectes flesus) living in brackish water of Baltic, a dose dependent depression of the haematocrit and haemoglobin values after 15 days cadmium exposure was observed (Larson et al., 1976). They further suggested that the depressions in the haematocrit and haemoglobin values is due to anaemia in cadmium exposed fish. The probable cause of anaemia in flounders intoxicated by cadmium was suggested to be due to an inhibited production of iron and/or to an increased loss or destruction of red blood cells.

Cadmium induced anaemia is well documented in higher vertebrates (Nilson, 1970). A cadmium induced blood anaemia has also been observed in experiments with birds (Feldman, and Cousins, 1973), and mammals (Friberg, Piscator and Nordberg, 1971). The exact mechanism for cadmium induced anaemia is not fully understood but seems to be associated at least partly, with disturbed iron metabolism caused by decreased intestinal absorption (Feldman and Cousins, 1973; Richardson, Fox and Fry, 1974).

DDT,Metacid and Unizeb caused anaemia while treatment of Endrin, Urea and phenol produced polycythaemia (i.e., an increase in RBC counts and Hb content) in Channa punctatus (Pandey et al., 1979). Raizada and Gupta (1982) presented the data on the effect of RH-2161 (a fungicide) on total red blood cell (RBC) and haemoglobin (Hb) content of Trichogaster fasciatus, the values observed decreased with the increasing concentration of the fungicide. The lowest values were obtained in 25 and 30 ppm of concentration after 72 and 96 hrs. The ozone (O_3) appeared to attack the tissue primarily, and not the blood which caused at first an increase and then a decrease in the blood values of rainbow trout, Salmo gairdneri (Ollenschaeger, 1981). Goel et al. (1982) studied the effect of malathion (an organophosphate) on haematological values in Heteropneustes fossilis. The data on the anomalies induced by malathion exposure for 15 days in the blood parameters of H. fossilis revealed that erythrocyte count and Hb% decreased significantly by more than 21 and 34 per cent respectively causing anaemia. The findings further supported the observations of Pandey et al. (1976b) and Mishra and Srivastava (1983).

Heavy metals such as zinc, copper, lead, cobalt and nickel haemolyzed erythrocytes of several kinds of animals. The haemolytic activity of each heavy metal was species

specific to test animals and zinc affected the erythrocytes most profoundly of rainbow trout, S. gairdneri. There was a close relationship between the degree of haemolysis and loss in elasticity of cellular membrane as far as efficiency of heavy metals were concerned. Thus it is concluded that zinc had a special kind of acute cytotoxicity in rainbow trout (Kodama et al., 1982).

Similar change of reduction in erythrocyte count and Hb content was also observed in Macrognathus aculeatum after Dimecron exposure (Shafi, 1980). In Anabas scandens, depletion in Hb and red cells was reported and it was suggested that the decrease in erythrocytes may be mostly due to increase in erythrocyte life span, haemolysis, osmotic resistence and mechanical fragility of red blood cells, finally to haemolytic anaemia. Further, increase in haematocrit upto maximum period of 21 days and then a depletion on further exposure was noticed. Swelling and vacuolization of red blood cells was also noticed. On further exposure disappearance of red blood cell membrane and finally haemolysis of red blood cells was reported. In vitro studies showed that on exposure, shrinkage of red blood cells was evident (Panigrahi and Mishra, 1980). Thurston et al. (1984) in rainbow trout after chronic ammonia exposure also reported the case

of microcytic anaemia. Rai and Qayyum (1984) studied the effect of lead poisoning on haematological parameters of Catla catla. They observed gradual decrease in erythrocyte number. Haemoglobin concentration was significantly elevated in the lower concentration but in higher concentrations there was also gradual decrease in Hb content. Mean cell haemoglobin value also changed similarly but the value noted after higher exposure was significantly higher than the normal. They further suggested that the decrease in total erythrocyte due to lead intoxication can be attributed to haemolysis or erythropoietic disorders which seems to be brought about after the lead treatment. Similarly the case of haemolytic anaemia is also evident from the toxicological studies of lead on Colisa fasciatus (Srivastava and Mishra, 1979).

In the present study on Clarias batrachus the BHC and MCHC value bears an inverse relationship with erythrocyte count, i.e., the lower the count the higher the MCH and MCHC. The increase in MCH and MCHC values in BHC exposed animals are quite similar to the observations of Srivastava (1967). Such correlation was also noted in Catla catla in controls as well as in lead treated fish (Rai and Qayyum, 1984).

Thus from the above reports it is evident that present finding of decrease in RBC counts, Hb per cent and haematocrit values may be due to haemolytic anaemia after BHC exposure. Although the mechanism of action of BHC on red blood cells is not quite clear which requires further attention and research efforts for complete understanding. It appears that BHC is causing destruction of red cell membrane leading to dissolution of red cells as evident from the study of blood smears of BHC exposed animals. But the question still remains, how BHC is affecting this destruction ?

Chaudhary (1979) observed an increase in the number of erythrocytes, blood and plasma haemoglobin, haematocrit, MCV and MCH and a decrease in MCHC at various concentrations of malathion after 24 hours exposure in Heteropneustes fossilis. Similar observation was made on Cyprinus carpio exposed to several organophosphate pesticides, viz., Neren; Trifenox, TM 1:1, Metation E-50, Brevinyl E-50 and Decemtion P-6 (Svobodova, 1971, 1975). An increase in haematocrit values in rainbow trout after phenol and iron exposure was reported by Halsband (1963). Lone and Javaid (1976) observed that 24 hour exposure of malathion reduced the erythrocytes, haemoglobin content, blood clotting time but increased in MCH and colour index values but after 96 hour exposure there was increase in erythrocytes, leucocytes and haemoglobin content

compared to 24 hour exposure. Quantitative composition and morphology of blood cells in coho Salmon, Oncorhynchus kisutch exposed to mercury was studied by Storozhuk and Guleva (1983) and they observed an increased number of erythrocytes. While studying the toxicity of lead to rainbow trout, Salmo gairdneri, Hodson et al. (1978) noted significant increase in RBC number and decrease in RBC, volume, RBC cellular iron content and RBC-6-aminolevulinic acid dehydrogenase activity. They suggested that these changes might be due to increased erythropoiesis to compensate the inhibition of Hb production. A case of polycythemia was reported after Dithane exposure in Clarias batrachus (Pandey et al., 1984).

Informations on the effect of pollutants on total and differential leucocyte counts in fishes are inadequate (Palmer 1951; Weinreb, 1958; McLeay, 1973; Newman and Maclean, 1974; Qayyum, et al., 1976; Pandey et al., 1976; Nayak and Madhyastha, 1977; Sham, 1978; Choudhary, 1979; Rai and Qayyum, 1981; Snaski, 1982; Sharma and Gupta, 1982; Gerg, 1982; Storozhuk and Guleva, 1983; Rai and Qayyum, 1984).

The increased value of total leucocytes in the present investigation on Clarias batrachus corroborates the findings of Pandey et al. (1976) in Channa punctatus, Choudhary (1979) in Heteropneustes fossilis, Gerg (1982) on Channa punctatus,

Snaski (1982) on Salmo gairdneri and Natarajan (1984) on Channa striatus.

Clarias batrachus in present investigation showed decrease in the number of neutrophils and eosinophils while lymphocyte number increased causing lymphocytosis.

Similar observations were made by Pandey et al. (1976) on Channa punctatus exposed to malathion. They have also noticed a clear trend of increase in percentage of lymphocytes and a decrease in percentage of basophil, neutrophil and eosinophils. Carbon tetrachloride induced haematological alterations was studied in Clarias batrachus (Sharma and Gupta, 1982). They noticed increased number of lymphocytes and steady fall in neutrophils and eosinophils. The number of basophils and monocytes remained the same in both treated and normal fishes. Further they suggested that the increase in the number of lymphocytes might be due to increased phagocytic action of lymphocytes during intoxication of CCl_4.

2 -4 -diamine, 3 -aminoazobenzene (DAAB) induced alterations in the total leucocyte and differential counts of Channa punctatus was investigated by Gerg (1982). The total leucocyte counts showed a gradual increase in their number during both lethal and sublethal exposure of the dye. There

was gradual decrease in small lymphocytes accompanied by an increase of large lymphocytes. Phagocytic neutrophils increased in response to dye exposure and thrombocyte count, as well, showed gradual and significant decrease. The increase of neutrophils which are phagocytic in nature is considered non-specific adaptive response to remove the tissue debris. Similar increase of neutrophils was also reported by McCleay (1973) in coho Salmon exposed to industrial effluents. Commercial detergents produced the similar increase in neutrophil level (Nayak and Madhyastha, 1977). Newman and Mclean (1974) observed thrombocytopenia and lymphocytopenia and increase in neutrophils in Tantogolabrus adspersus after cadmium exposure. Coho Salmon, Oncorhynchus kisutch after mercury exposure showed higher number of neutrophils, lower lymphocyte and monocyte counts. The vacuolized neutrophils started appearing in mercury exposed animals (Storozhuk and Guleva, 1983).

Rai and Qayyum (1981) studied the mercury intoxicated teleost fish Catla catla (Ham.) and reported monocytopenia, thrombocytopenia and lymphocytosis. Similar increase in the number of neutrophils was also observed by Pandey (1976b) in Channa punctatus and Choudhary (1979) in H. fossilis after malathion exposure. The increase in neutrophils is due to non-specific response to a variety of stress stimuli (Ellis et al., 1978).

Fishes are primarily water breathers where gills and skin take part in metabolic gas exchange. Many species of teleostean fishes inhabiting water of low O_2 and high CO_2 content, have however, accessory respiratory organs which enable them to breathe atmospheric air. This cat fish, Clarias batrachus has special air-breathing organs which allow direct gas exchange with atmospheric air (Munshi, 1961).

The earliest classic research on the effects of aquatic pollution on respiratory physiology of fishes were carried out by Belding (1927), Steinmann (1928) and Jones (1938, 1964). In recent years the use of a number of physiological techniques directed at monitoring changes in respiration of fish may provide sufficient information to ellucidate the mechanism of environmental action on the metabolism (Choudhary 1979).

It is a well known fact that industrial wastes, pollutants, ammonia and heavy metals like copper and zinc, etc. have a direct effect on fishes or these effects in turn may be further influenced by the oxygen and the carbon dioxide content of water (Alabaster et al., 1957). Kawatski and McDonald (1974) using 3-trifluromethyl 4 nitrophenol (TFM) studied the oxygen consumption of brain and liver of four species of fish while sucker, rainbow trout, large mouth bass

and blue gill sunfish and observed that a concentration of 10.0 mg/l caused a significant decrease in oxygen consumption of these tissues. Waiwood and Johanson (1974) reported that methoxychlor (an organochlorine) at low concentration had no effect on oxygen consumption of white sucker but a high concentration of it increased oxygen consumption. Lunn et al. (1976) found that in Salmo gairdneri DDT (350 ppb) caused a significant increase in oxygen uptake capacity during the first two hours of exposures. A lower concentration of DDT (52.5 ppb) showed no significant change in respiration rate in this animal. They further reported that 4, EOD-dieldrin (250 ppb) interrupted the ventilation which resulted in the decline in mean respiratory rate. Lull et al.(1976) were of the opinion that carbaryl at 2000 ppb caused a significant decrease in Vo_2 in Salmo gairdneri. Ranke-Rybica (1975) reported a significant decrease in Vo_2 in Lebistes reticulatus after the treatment of three organophosphates namely malathion, phoschlor and dichlorofos. Pandey et al. (1976b) also found a significant decrease in Vo_2 in Channa punctatus after malathion treatment. Reddy et al. (1977) while working on the toxicity of the commercial organophosphorus compound Disyston to anabantid fish Colisa lalita observed that both sub-lethal and lethal concentrations of pesticides caused an elevation of oxygen consump-

tion during the first hour of exposure followed by a depression after two hours. Singh and Singh (1977) reported considerable decrease in oxygen uptake capacity of Mystus vittatus in all the concentrations of copper and zinc and concluded on the basis of regression lines that copper is more toxic than zinc. Exposure to chlorinated sea water had been shown to interfere with normal respiratory and osmo-regulatory function (Block et al., 1978). Depressed oxygen consumption rates were documented in killifish, Fundulus heteroclitus after the exposure to chlorinated sea water (Capuzzo et al., 1977). However, some fishes showed a tendency of avoiding chlorinated sea water (Schumacher and Ney, 1980; Giattina et al., 1981; Hose et al., 1983).

Jauch (1980) made investigations on the toxic effects of the organophosphates. Lebaycid R on respiration and circulation of Cichlid fishes, Herotilapia multispinosa and Tilapia leucostica. He further observed that 25 ppm concentration leads to a strong increase of ventilation rate. Ventilation rate decreases abruptly only at the end of survival time and apnoea occurred. Death occurs after a long period of intoxication probably also by a peripheral paralysis of respiration. Sublethal exposure of metasytox (LC_{50}, 1-7 mg/l) decreased the tissue respiration in Channa striatus (Natarajan, 1984).

In the present investigation on Clarias batrachus after 96 hours exposure of various concentrations of BHC there is gradual decrease in the rate of oxygen uptake (surfacing allowed) upto 4 mg/l concentration and then there is a maximum increase at 8.0 mg/l concentration. But it again decreased in the highest concentration (16.0 mg/l) in comparison to 8.0 mg/l exposure (Table 9;Plate-XII). While under surfacing prevented condition a reverse trend was observed. There was a non-significant increase in oxygen consumption following 2 mg/l and 4.0 mg/l exposure, but at higher concentrations like 8.0 mg/l and 16.0 mg/l there was reduction in the rate of oxygen consumption compared to that of 2.0 mg/l and 4.0 mg/l concentrations. The significant increase in the rate of oxygen consumption (surfacing prevented) was observed only at 8.0 mg/l. Thus it is clear that 8.0 mg/l concentration is most effective under both the conditions, viz., surfacing allowed and prevented. The mean values of rate of oxygen consumption of control Clarias batrachus observed in the present investigation are close to that reported by Munshi et al. (1975).

Choudhary (1979) in H. fossilis reported decrease in Vo_2 in both the short and long term exposures of malathion. Ranke-Rybicka (1975) in Lebistes reticulatus also reported

decrease in rate of oxygen consumption after malathion exposure. Pandey et al. (1976b) observed a significant decrease in the oxygen consumption of Channa punctatus after malathion exposure. O'Brien (1967) was of the opinion that DDT (organochlorine) causes a sharp and substantial increase in oxygen consumption in insects. Pandey et al. (1979) reported that DDT causes a significant decrease in oxygen consumption in Channa punctatus which stands in contrast to endrin which elevates the oxygen consumption by nearly 40%, though both these pesticides belong to the same group.

From the above discussions it is apparent that different biocides may have elevating or depressing effect on oxygen uptake capacity depending on the nature of pollutant and fish species, but the changes in the rate of oxygen consumption may not be detected at very low concentrations of pollutants. It is also interesting to note that all the three organic groups of pesticides, i.e., organochlorine, organophosphate and carbamate, do not produce group specific changes in fishes. This is possibly be attributed to wide variety of adaptations exhibited by fishes dwelling in different ecological niches. Thus it is impossible to formulate any specific rule concerning the effects of insecticides on the physiology of various fish species.

The increase in the rate of oxygen consumption after the exposure of BHC may be explained by assuming that excessive muscular activity takes place caused over stimulated nervous activity. For this enhanced nervous and muscular activities, energy is required in the form of ATP which in turn is a product of oxidative phosphorylation.

Summary

Investigations were undertaken to record the effect of BHC an organochlorine insecticide, on corpuscular haematology and rate of oxygen consumption of an air-breathing fish Clarias batrachus (Linn.).

The literature dealing the nomenclature of blood cells has been discussed in details.

The number of circulating erythrocytes decreased with increasing concentrations of BHC after acute exposure for 96 hours. Maximum decrease was observed at the highest concentration, i.e., 16.0 mg/l. RBC diameter showed inverse relationship with the increasing concentrations of BHC. The percentage of spherocytosis was proportional to the concentration of BHC exposure. The number of sumdge cells also

increased in BHC exposed animals. Vacuolization of red blood cells and sometime disappearance of red blood cell membrane was also observed. The blood haemoglobin and haematocrit showed a decreasing trend with the increasing concentrations of BHC, but statistically significant decrease was obtained in haematocrit values only. The MCH and MCHC displayed an inverse relation to the number of circulating erythrocytes. MCH showed an increasing trend and was significantly affected only at the highest concentration, i.e., 16.0 mg/l, while MCHC had significantly increased with increasing concentrations of BHC for 96 hours. A clear trend of increase was observed in leucocyte numbers from 4.0 mg/l concentration onwards. The total percentage of lymphocytes showed marked increase in all experimental animals. Significant decrease in the number of neutrophils and with few exception, eosinophils were observed with increasing BHC concentrations. A linear relationship has been established between concentrations of BHC and erythrocyte counts, MCH and total leucocytes.

The rate of oxygen consumption in Clarias batrachus at different concentrations of BHC exposure under both the experimental conditions, viz., surfacing prevented and allowed, were investigated, BHC exposure caused an increase

in the rate of oxygen consumption but the values were not consistent. Maximum increase in the rate of oxygen consumption under surfacing prevented condition was observed at 8.0 mg/l exposure of BHC for 96 hours. Under surfacing allowed condition the rate of oxygen consumption showed a decreasing trend at 2.0 and 4.0 mg/l BHC concentrations, but at 8.0 and 16.0 mg/l concentrations there were significant increase in the rate of oxygen consumption.

CHAPTER-III

ACUTE TOXIC EFFECTS OF BHC ON BLOOD BIOCHEMISTRY AND PLASMA ELEC TROLYTES

Blood chemistry has been recognized as an important indicator to access the physiopathological state in human and veterinary medicine, but the subject has remained neglected for long particularly in poikilothermic vertebrates especially in fishes. Fishes are water dwellers and are sensitive to any physical or chemical alterations of their environment. Increasing use of pesticides and other chemicals in agriculture have created a serious problem of water pollution. Despite many publications, fish haematology and blood chemistry in health and disease is in developing state. There are wide variations even in the normal values of these parameters (Klontz and Smith, 1968; Wedemeyer and Chatterton, 1971). Blood chemistry values of fishes have been found to be influenced by various factors such as age, sex, physiological states, nutrition and environment (review by Mulachy, 1975).

Carbohydrates, proteins and lipids constitute the major source of energy for animals, the alteration in these chemical constituents depends on the energy requirement with the body metabolic activities comprising breathing, swimming, fighting and courting (Kinne, 1963). The production rates and the utilization rates of these substances determine the amount of their availability at any moment and their concentration pattern in blood provide an index of these chemical substances in the body (Best and Taylor, 1969).

Regulation of water-electrolyte balance in fishes remained neglected for long. Osmoregulatory function in fishes have been found to be influenced by several external factors. Environmental temperature influences the body fluid system of teleost (review by Houston, 1973). A significant proportion of water electrolyte exchange takes place at several points which seems to be directly or indirectly dependent upon the metabolic activities of the animal (Epstein et al., 1967; Conte, 1969). Therefore, it is apparent that any change in physico-chemical composition of aquatic environment which affect the physiology of fish may also alter the water electrolyte-transport.

In recent years the fish toxicologists investigated the effects of aquatic pollution on blood biochemistry and/

or electrolyte metabolism of several fish species (Ishihara et al., 1967; McKim et al., 1970; Christensen et al., 1972; Holmberg et al., 1972; Annes, 1974; Larsson et al., 1976; Shakoori et al., 1976; Choudhary, 1979; Gupta, 1980; Dalela, 1981; Sastry, Siddiqui and Singh, 1982; Nemesok et al., 1982; Wagner and McKeown, 1982; Sastry, Rao and Singh, 1982; Natarajan, 1982; Gill and Pant, 1983; Eddy and Maloiy, 1983; Larsson, 1984).

A review of the above mentioned literature indicates that very little is known about the effects of various pollutants on the blood biochemical parameters and electrolyte balance in fishes.

Literature relating to the effects of organo-chlorine insecticides on the blood biochemistry and electrolyte metabolism of fishes are few (Esler and Edmunds, 1966; Grant and Mehrle, 1970; Janicki and Kniter, 1971; Gupta, 1980; Ramalingam and Ramalingam, 1982; Gupta and Dhillon, 1983).

The present investigation is an attempt to record the change in various chemical constituents together with plasma electrolyte levels after Benzene hexachloride (BHC) exposure in an air-breathing fish, Clarias batrachus.

The method for procurement of animals and their maintenance have already been described in previous chapters.

EXPERIMENTAL CONDITION I

Blood chemistry values after BHC exposure

After acclimation of the fish in the laboratory condition for a week, the animals of the same sex (males) and body weights (60-65g) at the average water temperature 33-34°C (in the months of May-June) were exposed to test concentrations of 2.0, 4.0, 8.0, 16.0 mg/l of BHC (Benzene hexachloride) for 96 hours during which the pollutant water of each concentrations were renewed afresh every 24 hours. A control of similar size and weight was maintained separately along with the experimental animals. All fishes were fed daily with chopped goat liver.

Separation of plasma: For all biochemical studies, except the blood glucose, plasma were used in the present investigation. After taking out the blood in heparinized syringe the needle was removed from the syringe and the blood was transferred to clean and dry centrifuge tubes, with least pressure on the plunger to avoid haemolysis. Haemolysis was also prevented by using sterlized, dry syringe and needle. Plasma was separated immediately by centrifuging the blood at low to moderate speeds and the clear supernatant (plasma) was taken out with the help of a well sterilized lumber puncture needle no. 19 and was transferred to clean and dry stoppered glass tubes.

It is easier to obtain blood serum almost free from haemolysis than plasma. Since, in the present investigation plasma has been used, therefore determinations of biochemical constituents were made immediately. Moreover, on keeping the blood overnight for obtaining the serum a numberof biochemical changes may take place in blood (Varley, 1978) such as:

1. Passage of substances may take place through the red cell envelope, especially potassium, which is present in much greater concentration in the cell than in plasma, Diffusion of potassium occurs more rapidly in blood at 4°C than at room temperature.

2. Plasma inorganic phosphate may increase due to formation of ester-phosphates present in the cells.

3. Any loss of carbon-dioxide from plasma to atmosphere disturbs the balance and to maintain the ratio of bicarbonate to carbon dioxide constant, the passage of CO_2 from the cells into the plasma and bicarbonates from plasma to cells occur. Even so, the bicarbonate content of the cells is reduced so that chloride passes from cells to plasma to keep the ratio of bicarbonate to chloride the same in both cells and plasma.

Estimation of plasma protein

After the separation of plasma from the blood, on the same day total plasma protein was determined colorimetrically by Biuret method as suggested by Gornall et al. 1949 (King and Wooton, 1959).

Estimation of Blood glucose

Immediately after taking out blood, it was deproteinized with freshly prepared 4% trichloroacetic acid, centrifuged and the supernatant was separated. Blood glucose was determined colorimetrically by Anthrone method (Seifter et al., 1950).

Estimation of total, free and esterified cholesterol

Total, esterified and free cholesterol in plasma was determined according to Webster (1962).

Experimental Condition I

Blood Biochemical Parameters

Plasma total protein

The plasma protein although increased after 96 hours of exposure of BHC but the significant increase in plasma total protein was observed only at 8.0 mg/l BHC concentration (Table-10; Plate-XIII).

Blood glucose

The blood glucose level increased significantly at all the test concentrations of BHC after 96 hours of exposure from the control value of 57.288±0.916 mg/100 ml blood. Maximum elevation of blood glucose (117.336±0.076 mg/100 ml blood;

$P < 0.001$) was observed at 16.0 mg/l BHC concentration (Table 10; Plate-XIII).

Plasma total cholesterol

The total cholesterol significantly decreased after 96 hours of BHC exposure from the control value (239.002± 14.415 mg/100 ml plasma; Table-10; Plate-XIV). Maximum decrease of total cholesterol (57.869±1.205 mg/100 ml plasma; $P < 0.001$) was observed at the highest concentration of BHC exposure, i.e., 16.0 mg/l (Table-10; Plate-XIV).

Free cholesterol

Free cholesterol also decreased significantly in various BHC concentrations. Maximum decrease (20.140±2.052 mg/100 ml plasma; $P < 0.001$) was observed at 8.0 mg/l concentration (Table-10; Plate-XIV).

Esterified cholesterol

The value of esterified cholesterol also declined but the significant decrease was only noticed at the highest concentration of BHC exposure, i.e., at 16.0 mg/l (12.149±3.184 mg/100 plasma; $P < 0.01$) from the control value (76.664±22.281).

TABLE 10

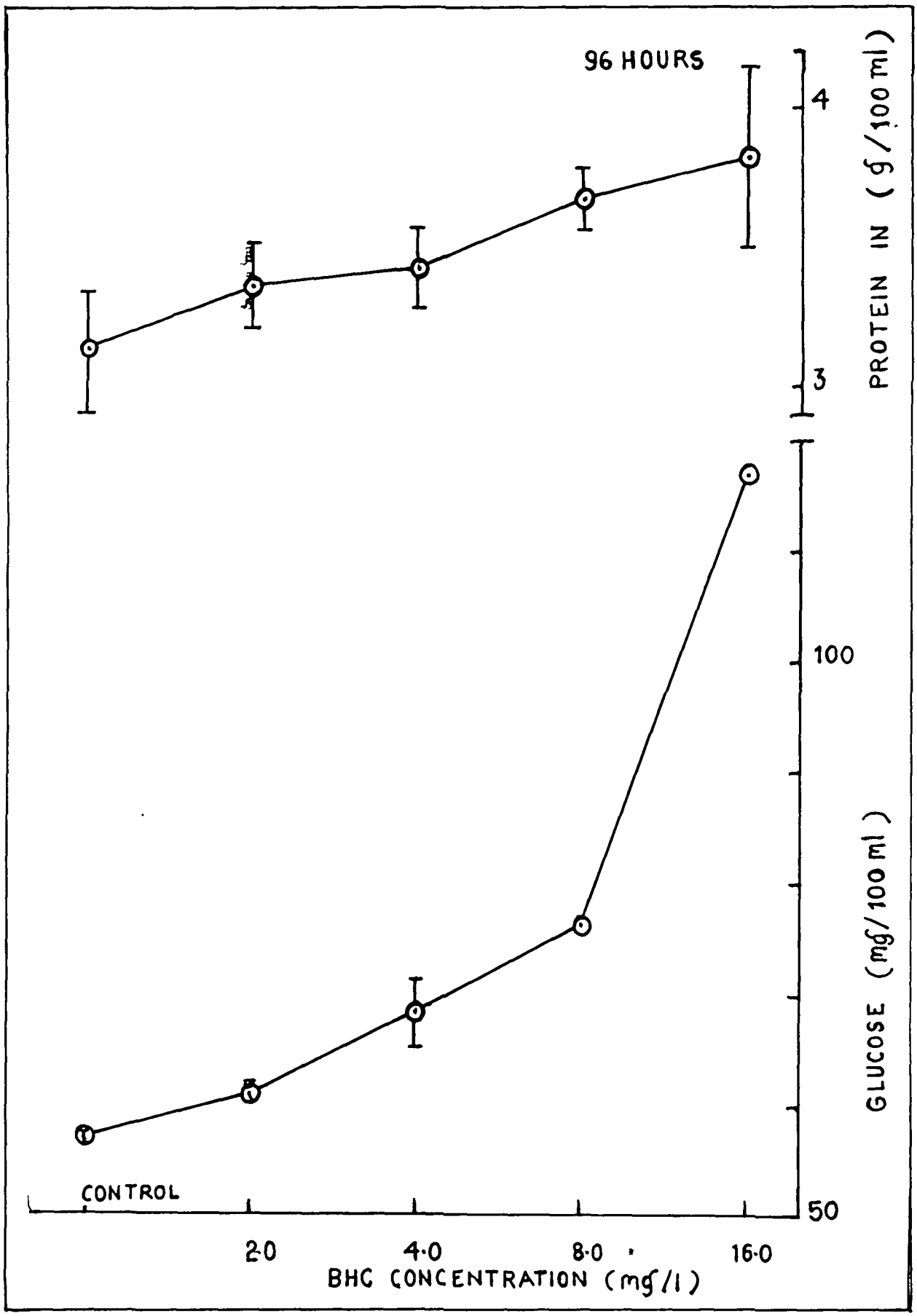
Acute toxic effects of various concentrations of BHC on plasma protein, glucose, and cholesterol level of *Clarias batrachus* (n = 10 each group; ± Standard error)

Parenthesis	Exposure Time	Control	BHC EXPOSURE			
			2.0 mg/l	4.0 mg/l	8.0 mg/l	16.0 mg/l
Total protein (g/100 ml)	96 Hours	3.144±0.225	3.358±0.329 NS	3.412±0.158 NS	3.683±0.11 P<0.05	3.812±0.318 NS
Blood glucose (mg/100 ml blood)	-do-	57.288±0.916	61.065±1.465 P<0.05	68.865±3.233 P<0.01	76.826±0.945 P<0.001	117.336±0.076 P<0.001
Total cholesterol (mg/100 ml plasma)	-do-	239.002±19.521	178.289±7.532 P<0.01	194.286±7.116 P<0.05	64.478±3.526 P<0.001	57.869±1.205 P<0.001
Free cholesterol (mg/100 ml plasma)	-do-	162.337±14.415	131.589±4.252 P<0.05	97.305±2.729 P<0.001	20.140±2.052 P<0.001	45.721±2.812 P<0.001
Esterified cholesterol (mg/100 ml plasma)	-do-	76.664±22.281	46.701±5.189 NS	96.981±8.049 NS	44.338±3.501 NS	12.149±3.184 P<0.01

EXPLANATION OF PLATE - XIII

Effect of various concentrations of BHC exposure for 96 hours on plasma protein and blood glucose levels in *C. batrachus*.

PLATE XIII


96 HOURS
4
3
PROTEIN IN (g/100 ml)
100
50
GLUCOSE (mg/100 ml)
CONTROL
2·0
4·0
8·0
16·0
BHC CONCENTRATION (mg/l)

EXPLANATION OF PLATE - XIV

Effect of various concentrations of BHC exposure for 96 hours on total, free and esterified cholesterol levels of plasma of *C. batrachus*.

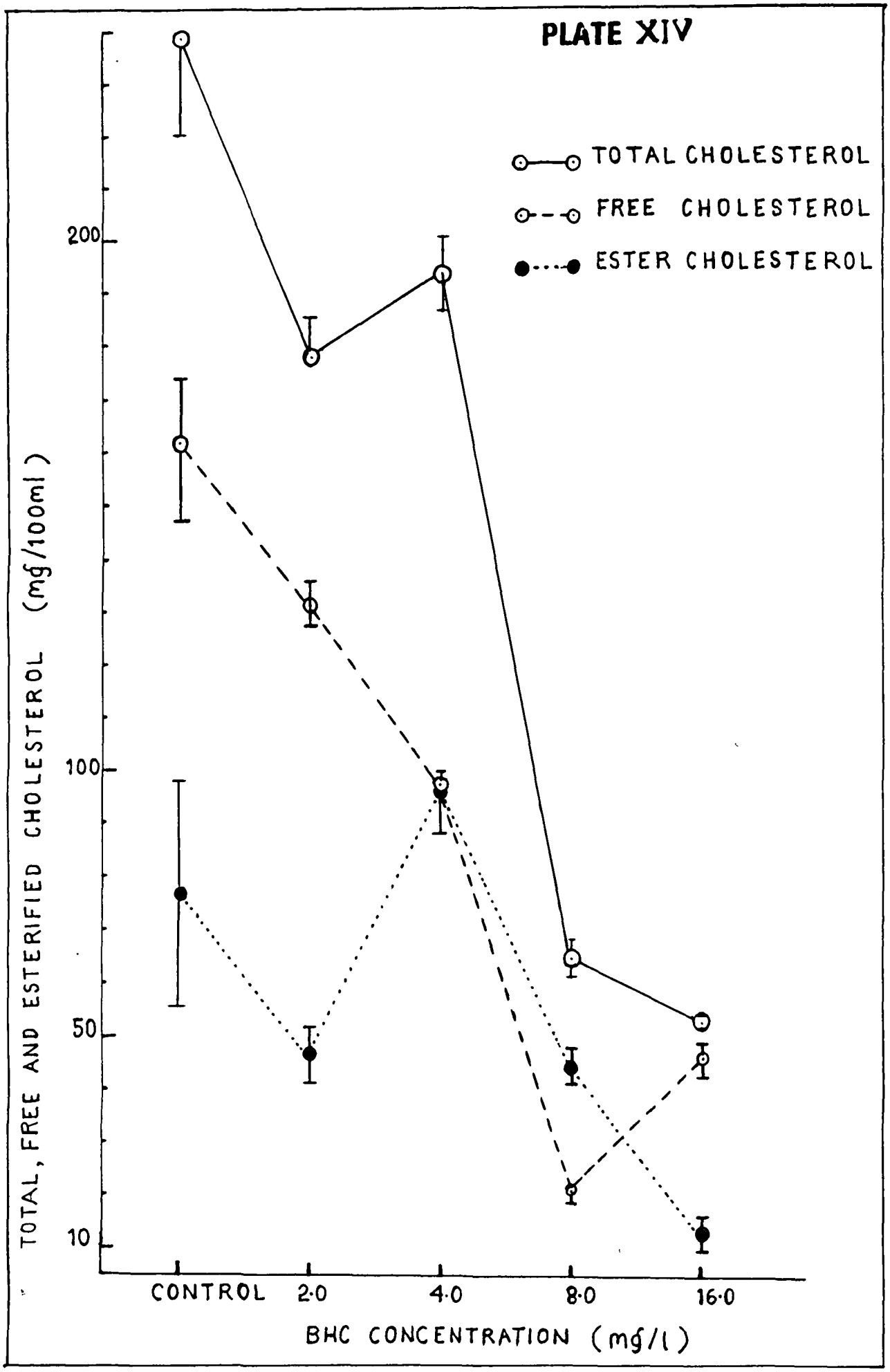


PLATE XIV
TOTAL CHOLESTEROL
FREE CHOLESTEROL
ESTER CHOLESTEROL
TOTAL, FREE AND ESTERIFIED CHOLESTEROL (mg/100ml)
200
100
50
10
CONTROL
2·0
4·0
8·0
16·0
BHC CONCENTRATION (mg/l)

Regression and correlation analysis

A linear relationship has been established between the concentration of BHC and total plasma protein, blood glucose and total plasma cholesterol values. The results are given in Table-11.

It has been found that with unit increase in the concentration of BHC the total plasma protein and blood glucose increased by a factor of +0.039 and +3.758 (b value) respectively. Correlation coefficient (r) have been calculated to be 0.945 and 0.984 respectively, whereas plasma total cholesterol decreases by a factor of -11.426 with unit increase in the concentration. Correlation coefficient (r) has been computed to be 0.889.

Experimental Condition II

Plasma electrolytes

Sodium: Plasma sodium level increased at lower concentrations, i.e., 2.0 and 4.0 mg/l of BHC exposure for 96 hours, but declined significantly at 8.0 and 16.0 mg/l BHC concentration (111.309±2.899 mEq./l and 112.172±1.503 mEq./l respectively) from the control (Table-12; Plate-XV).

TABLE 11

Correlation coefficient and equations to show the relationship between concentrations of BHC exposure and plasma protein, blood glucose and total cholesterol in *Clarias batrachus* (Linn.)

Parameters Analyzed	Equation Y=a+b. X	Correlation coefficient (r)
Concentration of BHC (X) Vs Plasma Protein	Y = 3.243+0.039. X	0.945 P < 0.02
Concentration of BHC (X) Vs Blood glucose	Y = 53.727+3.758.X	0.984 P < 0.01
Concentration of BHC (X) Vs Plasma total cholesterol	Y = 215.338-11.426.X	0.889 P < 0.05

Potassium

The plasma potassium level declined significantly in lower concentrations, i.e., 2.0 and 4.0 mg/l BHC exposure for 96 hours whereas in higher concentrations (8.0 and 16.0 mg/l BHC exposure) there was significant increase in plasma potassium level (Table-12; Plate-XV).

Calcium

Plasma calcium level decreased significantly in various concentrations of BHC exposure for 96 hours (Table-12; Plate-XVI).

Inorganic phosphate

Plasma level of inorganic phosphate increased consistently in various concentrations of BHC but the significant decrease was noticed in 16.0 mg/l BHC exposure.

TABLE 12

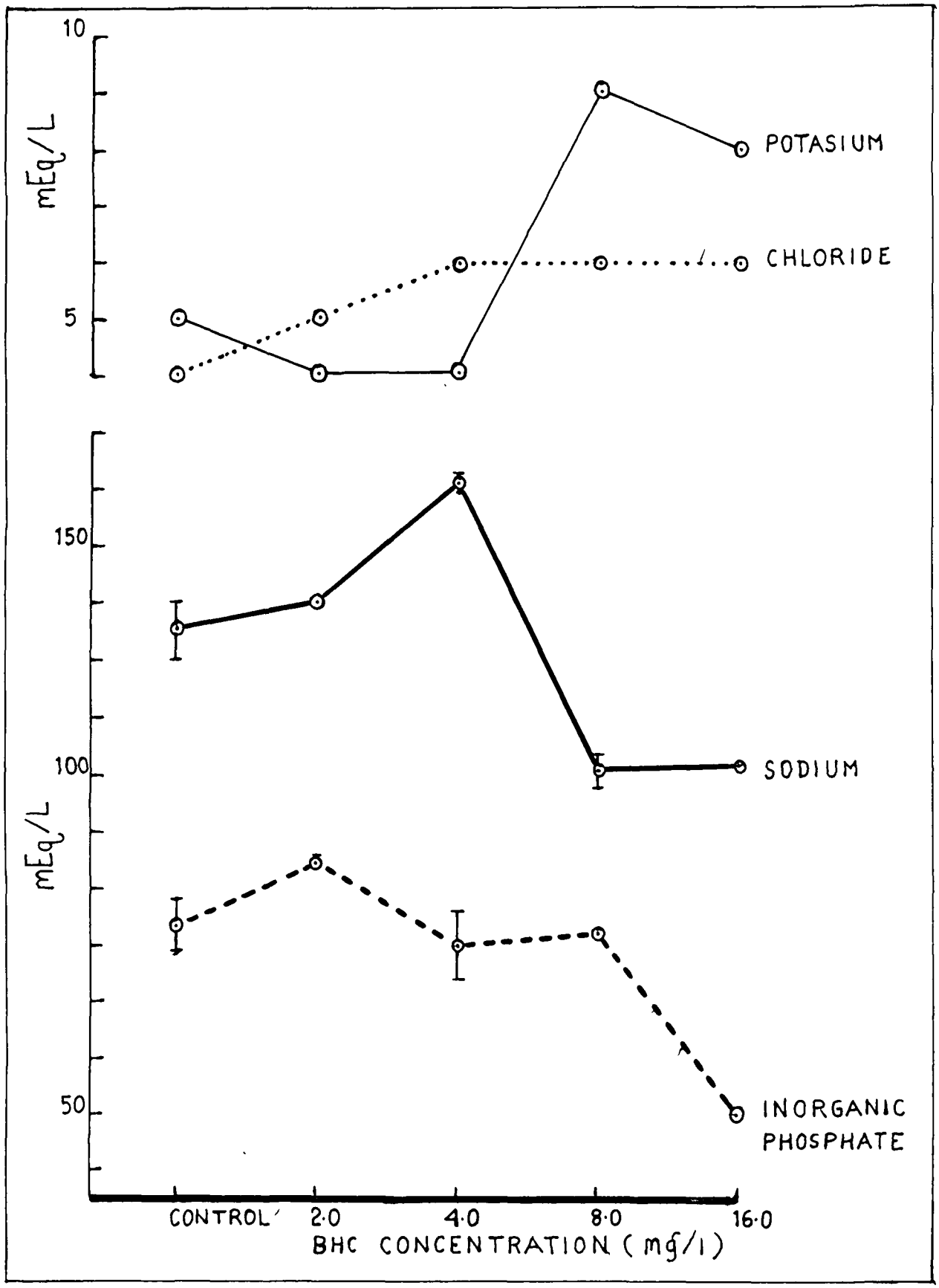
Acute toxic effects of BHC exposure on plasma electrolytes of *Clarias batrachus* (Linn.)

Parentheses	No. of Fish (n)	Control	BHC EXPOSURE			
			2.0 mg/l	4.0 mg/l	8.0 mg/l	16.0 mg/l
Sodium (Na^+)(mEq/L)	10	135.141±5.696	141.071±0.161 NS	161.025±1.445 P < 0.01	111.309±2.899 P < 0.01	112.172±1.503 P < 0.01
Potassium (K^+) (mEq/L	10	5.520±0.081	4.924±0.045 P < 0.001	4.681±0.140 P < 0.001	9.984±0.585 P < 0.001	8.557±0.260 P < 0.001
Calcium(Ca^{++}) (mEq/L)	10	57.996±0.129	52.645±0.044 P < 0.001	40.094±1.154 P < 0.01	52.144±0.577 P < 0.001	52.599±0.603 P < 0.001
Inorganic phosphate (PO_4^{--}) (mEq/L)	10	83.557±5.043	99.859±0.451 P < 0.01	80.589±5.684 NS	82.607±3.739 NS	50.105±0.265 P < 0.001
Chloride (Cl^-) (mEq/L)	10	4.880±0.073	5.136±0.351 NS	6.401±0.205 NS	6.837±0.122 P < 0.001	6.959±0.253 P < 0.001

EXPLANATION OF PLATE - XV

Graph showing the effect of various concentrations of BHC on plasma sodium, chloride, potassium and inorganic phosphate levels in C. batrachus.

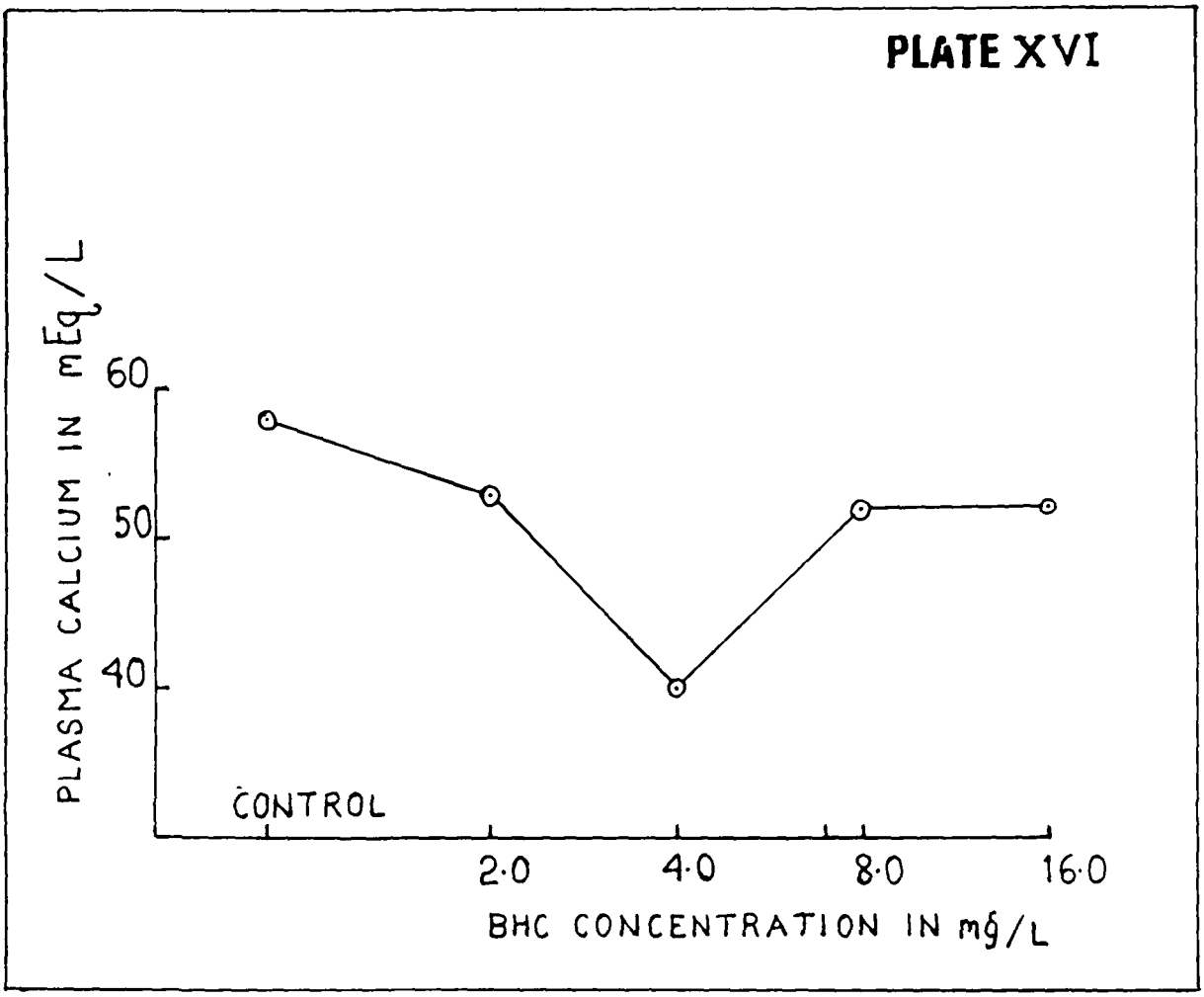
PLATE XV


10
5
mEq/L
POTASIUM
CHLORIDE
150
100
50
mEq/L
SODIUM
INORGANIC
PHOSPHATE
CONTROL
2·0
4·0
8·0
16·0
BHC CONCENTRATION (mg/l)

EXPLANATION OF PLATE - XVI

Graph showing the effect of various concentrations of BHC on plasma calcium levels in *C. batrachus*.

PLATE XVI


PLASMA CALCIUM IN mEq/L
60
50
40
CONTROL
2·0
4·0
8·0
16·0
BHC CONCENTRATION IN mg/L

Discussion

The present investigation on the effect of organochlorine insecticide, BHC on an air-breathing fish, Clarias batrachus showed increasing trend of plasma protein with increasing concentrations of BHC. Similar increase in plasma protein has been noticed in some fishes after pollutant treatment (Ishihara, 1967; McKim, 1970; Shakoori et al., 1976; Choudhary, 1979).

Shakoori et al. (1976) reported an increase in plasma protein and a decrease in free aminoacid levels of Channa punctatus exposed to malathion and observed no change in electrophoretic patterns of plasma protein. Heteropneustes fossilis exposed to malathion showed an increase in plasma protein level (Choudhary, 1979). Similarly Ishihara et al., (1967) observed increase in globulins of carp serum. Apparent

increased protein level after 6 days and decreased level after 21 days was reported in brook trout, Salvelinus fontinalis exposed to copper (McKim et al., 1970).

Toxicant induced decreased plasma protein level has been observed by many investigators using various kinds of chemicals on different fish species (Ester, 1970; Dalela et al., 1981; Dubale and Awasthi, 1982; Ramalingam and Ramalingam, 1982).

Dalela et al. (1981) studied the effect of three pesticides, thiotox, dichlorovos and carbofuran on Mystus vittatus and observed decreased plasma protein level and further suggested that decreased plasma protein was due to excretion of proteins by kidney resulting from kidney disorder (albuminuria) or impaired protein synthesis due to liver disorder. Ester (1970) reported decreased serum protein contents of Sphaeroides maculatus exposed to methyl parathion. Blood protein level of brown bull-head, Ictalurus nebulosus was significantly decreased after 30 days and remained unchanged after 60 days of exposure to copper. Dubale and Awasthi (1982) reported decreased protein content in catfish, Heteropneustes fossilis exposed to a sublethal concentration of dimethoate for 48 days. Sastry, Rao and Singh (1982) noticed that intestinal absorption of glycine

and tryptophan decreased significantly after mercury exposure in murrel, Channa punctatus after 15 days and 30 days. Liver and muscle total proteins decreased in S.mossambicus subjected to sublethal concentration of DDT, malathion, and mercury (Ramalingam and Ramalingam, 1982). Banerjee et al., (1977) reported marked alterations in the levels of serum indices after malathion poisoning in Clarias batrachus and Tilapia mossambica.

The probable cause of increased protein level might be due to alpha, 2 globulin increased value. In human beings during cirrhosis, nephrotic syndrome or malnutrition state there is increase in α-2 globulin protein-level. But the real cause of increased protein level is still not clear.

Hyperglycaemia has been proved to be the main bio chemical change induced by several pollutants (Havu, 1969; Holmberg et al., 1972; Ghafghazi and Mennear, 1973; Larsson, 1975, 1976; Choudhary, 1979; Verma et al., 1979; Bansal et al., 1979; Singh, 1979; Gupta, 1980; Dalela et al., 1981; Nemesok et al., 1982; Wagner and McKneown, 1982; Sastry and Siddiqui, 1982; Gill and Pant, 1983).

Choudhary (1979) observed an increase in blood glucose level after 24 hours and decrease in 8 week's exposure of malathion in an air-breathing fish, Heteropneustes fossilis.

Malathion induced alteration of glucose was observed in Clarias batrachus and Tilapia mossambica (Banerjee, 1977). Gupta (1980) studied the impact of sublethal concentrations of Rogor (organophosphate) in Indian catfish, Heteropneustes fossilis at 3rd, 6th, 12th, 48th and 96th hours after exposure. Hyperglycaemia was observed at all the specified time intervals except 6th hours, significant increase in lactic acid were noticed except at 48th hour, marked increase in pyruvic acid however was observed at 3rd hour. He further suggested that glycogenolysis in the liver mobilizes glucose which results in increase in concentrations of intermediary metabolites. In Channa punctatus exposure of methyl parathion caused hyperglycaemia.

Holmberg et al. (1972) reported marked increase in blood glucose in Anguilla anguilla after pentachlorophenol treatment. Ammonia induced hyperglycaemia was reported in carp, Cyprinus carpio, silver carp, Hypophthalmichthys molitrix and sheet fish, Silurus glanis. Ammonia as a toxic agent caused stress in fishes by enhancing their metabolic processes as it was reflected by the enhanced blood sugar and catecholamines level of the blood sera (Nemesok et al., 1982). Dalela et al. (1981) studied the biochemical effects of three pesticides thiotox, dichlorovos and carbofuran on certain blood chemistry values of a fish, Mystus vittatus and

observed increased blood glucose level after 30 days of exposure. Hyperglycaemia was caused due to enhanced breakdown of liver glycogen and perhaps mediated by adrenocorticotrophic hormone (ACTH) and glucagon hormone and reduced insulin activity.

In present investigation, Clarias batrachus showed hyperglycaemic response. There was also high correlation between increasing concentrations of BHC with increasing level of blood glucose. Organochlorine insecticides act as adrenal-pituitary glucocorticoid mediated stressors which affect blood glucose levels by the pathway of stress response (Hart and Straw, 1971; and Hart et al., 1971). Effect of chlordane on Saccobranchus (Heteropneustes) fossilis and Labeo rohita as observed by Verma et al. (1979) and Bansal et al., (1979) showed severe nephritis and pancreatic/or hepatic disorders might cause increased glucose level.

Larsson et al. (1976) observed increased blood glucose in flounder, Pleuronectes flesus and concluded that increased glucose level was due to enhanced breakdown of liver glycogen. They further suggested that the effect of cadmium on carbohydrate metabolism was mediated by adrenocortical hormones or reduced insulin secretory activity. Such an explanation was further supported by the findings of Havu (1969)

that cadmium appeared to accumulate in the pancreatic islets of fish and caused damage of insulin producing beta cells. Similar cadmium accumulation was observed in mammalian pancreas (Berlin and Ullberg, 1963) and reduced circulating serum insulin (Ghafghazi and Mennear, 1973). An increase of glucose and decrease of lactate in blood with increase of glycogen in liver and decrease in muscle was observed by Larsson (1975) and Larsson et al. (1976) in flounders after exposing to various concentrations of cadmium. Punctius conchonius exposed to acute (24 h) and chronic (90 days) cadmium poisoning showed a condition of hyperglycaemia (Gill and Pant, 1983).

Zinc stressed hyperglycaemia accompanied by plasma insulin and glycogen depression was reported in rainbow trout, Salmo gairdneri (Wagner and McKeown, 1982). These changes were due to possible influences of epinephrine which was elevated in stressed fish and/or a direct effect of zinc metals on pancreatic beta cells. Natarajan (1982) reported decreased glycogen levels in liver, muscle, brain, kidney and gill tissue in zinc sulphate exposed climbing perch, Anabas scandens.

Mercury exposed (3 ug/l) Channa punctatus showed decreased intestinal absorption of glucose and fructose (Sastry Rao and Singh, 1982). Sastry and Siddiqui (1982) studied the

effects of a sublethal concentration of the carbamate pesticide, sevin (1.05 mg/l) on Channa punctatus and reported hyperglycaemia. They further suggested that anaerobic metabolism was favoured and aerobic oxidation of pyruvate was impaired in fish exposed to sevin. Choudhary (1979) observed decreased glucose level (hypoglycaemia) in H. fossilis after long term exposure (8 weeks) of malathion. Similarly Sastry, Siddiqui and Singh (1982) observed decreased blood sugar and lactic acid and increased glycogen content of liver and muscles in the snake-head Channa punctatus.

Holmberg et al. (1972) in eel after pentachlorophenol, Choudhary (1979) in H. fossilis after malathion exposure, Esler and Edmunds (1966) in puffes after endrin exposure observed increased plasma cholesterol level.

In the present study the BHC exposure significantly reduced the plasma total cholesterol level in Clarias batrachus. Similar decrease in blood cholesterol was observed in Mystus vittatus after 30 days of exposure of three pesticides, thiotox, dichlorovos and carbofuran (Dalela et al., 1981). A decreased level of cholesterol in Mystus vittatus might be due to increased breakdown of cholesterol into free fatty acids as observed by Verma et al. (1979). Gill and Pant (1983) reported increased liver, kidney and ovary cholesterol

in Punctius conchonius exposed to acute (24 hr) and chronic (90 days) cadmium poisoning. BHC exposed Clarias batrachus showed decreased level of esterified cholesterol. The decrease in the level of esterified cholesterol in plasma might be due to liver damage and impairment of hepatic functions, which is further confirmed by the histopathological examinations of the liver (Chapter 4th of the present thesis). The report further supports the finding's of Choudhary (1979).

Very little is known as regards to toxicant induced changes in the plasma/serum electrolytes of Indian freshwater fishes. However, a r e v i e w of the literature suggests that most of the works have been performed on the marine and estuarine fishes and the studies are associated mainly with the problem of temperature acclimation. The literature dealing the effects of pollutants on the electrolyte balance in fishes is few (Esler and Edmunds, 1966; Grant and Mehrle, 1970; McKim et al., 1970; Janicki and Kniter, 1971; Larsson et al., 1976; Banerjee et al., 1977; Choudhary and Pandey, 1980; Gupta, 1980; Dalela, 1981; Larsson et al., 1984).

Esler and Edmunds (1966) recorded increased serum sodium followed by a fall in liver sodium in marine teleost exposed to endrin. McKim et al. (1970) observed no change in plasma chloride of Salvelinus fontinalis after long term exposure of copper.

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Larsson *et al.* (1976) obtained high plasma sodium and chloride values and reduced potassium, calcium and magnesium levels in flounder (*Pleuronected flesus*) exposed to cadmium. Choudhary and Pandey (1980) observed that the malathion exposure depressed the blood plasma sodium and chloride but elevated the potassium and calcium levels. Dalela *et al.* (1981) studied the biochemical effects of three pesticides thiotox, dichlorovos and carbofuran on *Mystus vittatus* after 30 days of exposure and observed increase in total blood phosphorus, sodium, potassium, magnesium, calcium, iron and chloride. Total phosphorus increased with increasing concentration of the toxicant. Gupta (1980) reported increased potassium, magnesium, calcium, phosphorus and non-protein nitrogen in *Clarias batrachus* and *Cirrhina mrigala* after intoxication by aldrin and Swascofic CD-38.

In the present investigation, BHC exposure induced an increase in plasma sodium level at two lower concentrations (2.0 and 4.0 mg/l), which declined in higher concentrations (8.0 and 16.0 mg/l), while potassium level declined in lower concentrations (2.0 and 4.0 mg/l) and increased in higher concentrations (8.0 and 16.0 mg/l). The calcium level declined significantly whereas the inorganic phosphate increased at all the concentrations except at the highest concentration, i.e., 16.0 mg/l BHC exposure. Plasma chloride level

only increased at the lowest concentration (2.0 mg/l) where as in higher concentration the chloride level decreased. It is apparent that plasma sodium and potassium were inversely related to each other, i.e., an increase in Na^{++} level was marked with a decrease in K^{+} level of plasma in BHC exposed animals. Plasma calcium and phosphate was also found to be inversely related.

Janicki and Kniter (1971) observed marked inhibition of Na^{+}, K^{+}, Mg^{2+} ATPase in the gills and intestinal mucosae of DDT exposed Pseudopleuronectes americanus.

Eddy and Maloiy (1983) examined the effect of 24 hour acid water exposure (pH 4) on ion regulatory response of a catfish, Clarias mossambicus, net loss of body Na^{+} accompanied by smaller losses of Cl^{-} and K^{+} ions was observed. Larsson et al. (1984) reported disturbed chloride balance in perches (Perca fluviatilis) exposed to simulated heavy metal containing effluent from a sulfide ore smelting. Additional stress treatment (asphyxia) further strengthened the toxic effects of heavy metal containing effluent. Marked alteration in the level of inorganic phosphate was observed in Clarias batrachus after malathion poisoning (Banerjee et al., 1977).

It is difficult to formulate a generalized pattern of electrolyte behaviour of diagnostic value for sub-lethal and lethal toxic exposures. De Bruin (1978) has rightly emphasized the need for consideration of major electrolytes such as cations (Na^+, K^+, Mg^{++}) and certain anions (Cl^-, HCO_3^{---}), in toxic conditions. The mammalian electrolyte disorders and particularly in human being have been investigated quite extensively. Taylor (1970) observed that the metabolism of sodium and potassium is closely linked with the maintenance of fluid balance and with regulation of acid-base status. A decrease in plasma chloride has been reported in conditions where there is low plasma sodium, when there is retention of organic acids together with excessive urinary loss of chloride in the acidosis of chronic renal failure. The primary hyponatraemia occurs in salt-losing nephristis and in Addison's disease. The secondary hyponatraemia results due to fluid retention as is found in congestive cardiac or renal failure and other pathological status. The sodium and chloride deficits are confined for the most part to gastro-intestinal, renal, or endocrinal disorders, but the abnormalities of potassium metabolism have broader range of causes and may arise from extra renal pathological states. A primary increase in plasma potassium may occur after the infusion of several blood or of potassium salts and may be a

feature of Addison's disease. Secondary hyperkalaemia due to loss of potassium from the cells occurs in acidosis of renal failure, diabetic coma and shock. High calcium values are found in hyperthyroidism, and sometimes in patients with carcinoma, myeloma and sarcoid (Wootton, 1974). Choudhary (1979) suggested that the increased potassium after malathion exposure in H. fossilis was due to intra vascular haemolysis. De Bruin(1976) reported primary disruptive effect of pollutant on the red cell membrane which is reflected by the altered pattern of distribution and exchange of cations across the erythrocyte membrane resulting in defective "active transport" pump mechanism. Clarias batrachus poisoned by BHC showed an increased plasma K^+ level and reduction of Na^+ in higher concentrations whereas in lower concentrations there was reduced plasma K^+ and increased Na^+ level. These observations are similar to the findings on H. fossilis after malathion exposure (Choudhary and Pandey, 1980). Grant and Mehrle (1970) observed that a high dose of endrin (430 mg/kg body weight) to gold fish caused the loss of Na^+ and Cl^- resulting in complete failure of osmoregulatory processes. Dalela (1981) suggested a similar process of failure of osmoregulation in Mystus vitattus due to severe renal damages. BHC exposed animals in the present investigation

showed increased phosphate level at various concentrations which seems to be due to toxicant induced nephritis. Similar explanation has been suggested by Dalela et al. (1981) and Gupta (1980). Calcium is a general regulator of permeability of cell membranes of water and ions, low calcium usually increases permeability and high calcium decreasesit (Prosser, 1973). Regulation of calcium metabolism by a small epithelial cell mass, the ultimobranchial organ has been found in several species of fishes (review by Barr, 1965).

Summary

Investigations were undertaken to record the effect of BHC on blood biochemistry and electrolytes of an air-breathing fish, Clarias batrachus.

The plasma protein increased after 96 hours of exposure of BHC in all the concentrations. The blood glucose increased significantly at all the concentrations. The total cholesterol decreased significantly after BHC exposure. Free and esterified cholesterol level also declined after BHC exposure. A linear relationship was established between the concentrations of BHC and total protein, blood glucose and plasma-cholesterol values. Plasma total protein and blood glucose increased with increasing concentrations of BHC while total plasma cholesterol level declined with increasing BHC concentrations.

Plasma sodium level increased at lower concentrations (2.0 and 4.0 mg/l) but declined at higher concentrations (8.0 and 16.0 mg/l). Plasma potassium level declined significantly at the two lower concentrations (2.0 and 4.0 mg/l) whereas in higher concentrations (8.0 and 16.0 mg/l BHC exposure) there was increase in K^+ level. Plasma calcium level was found to be decreased in animals exposed to the various BHC concentrations, while inorganic phosphate level showed consistently increasing trend, which however decreased at the highest concentration. The plasma chloride level first increased in the lowest concentration (2.0 mg/l) but at 4.0 to 16.0 mg/l concentrations of BHC exposure there was marked decrease in the level of plasma chloride.

CHAPTER-IV

ACUTE TOXIC EFFECTS OF BHC ON HISTO PATHOLOGY

Introduction

Rapid industrialization, urbanization and recent drive to enhance farm productivity, all are polluting inland waters by various kinds of pesticides and other chemicals which are greatly detrimental to the aquatic fauna including fishes. Treatment of fish with contaminated water causes severe pathogenic changes at tissue level and histology gives useful data concerning tissue lesions. Evidences for the organochlorine accumulation in aquatic ecosystem is abundant and therefore organochlorine insecticides are more detrimental in comparison to other chemicals and produces long term effects. The liver, gills, kidney, gonad and brain have usually been the organs of choice for histological studies of pesticide induced changes in fishes.

The histo-pathological effect of various pesticides and related chemicals on liver (Duke and Wilson, 1971; Eller, 1971; Mukherjee and Bhattacharya, 1975; review by Couch, 1975; review by Walsh and Ribelin, 1975; Choudhary, 1979; Solangi and Overstreet, 1982; Rashatwar and Ilyas, 1984; Gupta and Dalela, 1986), kidney (Trump and Ginn, 1968; Rucker and Amend, 1969; Haga et al., 1970; review by Walsh and Rebelin, 1975; Choudhary, 1979; Kumar and Srivastava, 1980; Srivastava and Tripathi, 1981; Rashatwar and Ilyas, 1984; Haniffa and Sudaravadhanam, 1984) and gills (Khagarot, 1982; Shimada et al., 1982; Goel et al., 1982; Kumaraguru et al., 1982; Solangi and Overstreet, 1982; Tuurala and Soivio, 1983; Mitchell and Cech, 1983; Fukuda, 1983) have been well documented. However, literature on pathology and poisoning of fishes especially organochlorine are few (Sastry and Sharma, 1979; Shafi and Choudhary, 1980; Gupta and Singh, 1982; Matthiessen and Roberts, 1982; Drewett and Abel, 1983).

In India still the use of organo-chlorine compounds in pest control in in practice and very little is known about their toxic effects on Indian fishes. Therefore the present investigation has been undertaken to evaluate the effect of BHC, an organochlorine insecticide on non-specific and specific tissue response of an air-breathing fish, Clarias batrachus in order to assess the diagnostic value of the pesticide toxicity.

Methods of procurement of animals and their maintenance have already been described in the previous chapters. After the acclimation of fish in the laboratory conditions for a week, the animals of the same body weight (60-65g) were exposed to 16.0 mg/l (LC_{50} concentration) of BHC for 96 hours and this level of pollutant was maintained throughout the period of experimentation by renewing pollutant water afresh at every 24 hours. The average water temperature remained at 32.5°C. At the end of the 96 hours exposure, 5 fishes each from experimental and similarly kept control were taken randomly and sacrificed. Skin fragments of about 6/10 mm between the dorsal spine and lateral line canal were cut and fixed in 10% neutral formalin, Bouin's fluid and Zenker's

fluid. Small pieces of gills were cut off and carefully washed in physiological saline to remove their adhering mucous and then fixed in Zenker's fluid. Liver, kidney and gonad preserved in Bouin's fluid and all the fixed tissue were further processed using standard histological technique. Paraffin sections were cut at 5-7 microns and stained with haemotoxylin and eosin.

Skin

The integumentary system in Clarias batrachus is distinguished into epidermis, dermis and subcutis. The epidermis consists of an outermost epithelial layer, the middle layer and the basal layer called stratum germinativum. The outermost epithelial layer is composed of stratified epithelial cells in which numerous flask shaped mucous glands are located. The mucous glands are unicellular and open on the surface of the skin. The middle layer is the thickest layer of epidermis. Club cells which are large and usually round in shape are located in the middle layer. The cytoplasm of club cells are homogenous. In the middle layer large number of spherical mucous glands are also present. The basal layer is composed of single layer of cells. These cells are cuboidal in shape with spherical nucleus. The dermis is characterized by the presence of

bundles of muscles and collagen fibres, supplied with blood capillaries and nerve fibres. A uniform and continuous layer of pigment cells are present in the upper layer of dermis in close contact with the basal layer of epidermis.

The acute toxic exposure of BHC for 96 hours produced sloughing of epidermal layer with hyperplasia of cells. The mucous cells also increased and mucous coats were also observed on the surface of the skin. In the middle layer mucous cells also increased in number. The uniform distribution of pigment cells were also affected and they became localized and shrunken. Necrosis of muscle bundles in the dermis was also observed (Plate-XVII; Figs. 2,3).

Gills

The structure of the gills of Clarias batrachus followed the typical plan of teleostean gills as described by Munshi (1960). The hemibranchs consist of several long thin filaments called primary lamellae. The secondary gill lamellae arise at regular intervals as semifolds from the dorsal and ventral side of primary gill lamellae, which further increase the respiratory surface area for gaseous exchange. The cartilaginous gill rays are located in the main shaft of the primary gill lamellae, and the adductor muscles are attached with the gill

EXPLANATION OF PLATE - XVII

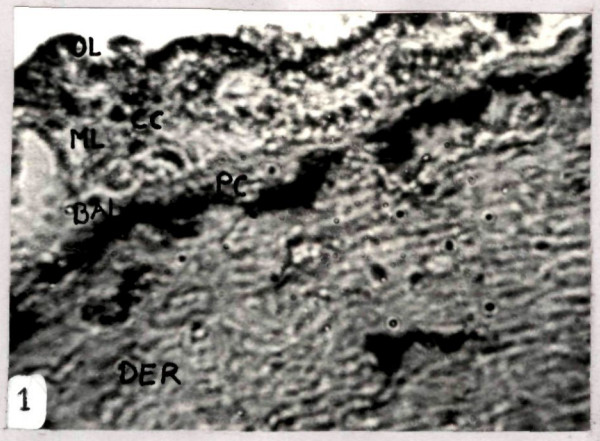
Fig. 1: Cross section of the skin of control *C. batrachus* (45x 10x).

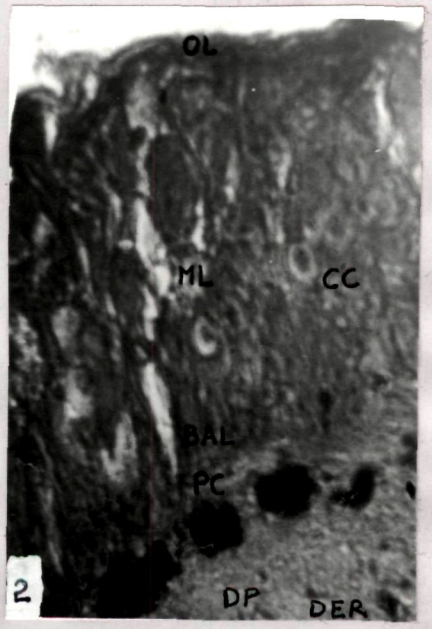
Figs. 2, 3: Different stages of histopathological changes in the skin of *C. batrachus* after the exposure to BHC (45x 10x).

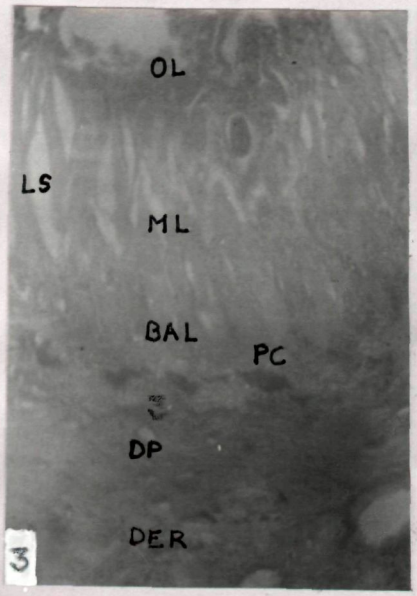
Abbreviations

BAL	..	Basal layer
CC	..	Club cell
DER	..	Dermis
DP	..	Dermal papilla
FMC	..	Flask shaped mucous cells
LS	..	Lymphatic space
OL	..	Outer-most layer
PC	..	Pigment cells
SMC	..	Spherical mucous cells

PLATE XVII







rays to adjust the amount of ventilation of lamellae. The afferent and efferent branchial arteries are also located within primary gill lamellae. The blood used to pass from afferent artery of the primary gill lamellae to the efferent artery through the secondary gill lamellae. The pillar cells are present between upper and lower basement membrane layer of secondary lamella and separates the adjacent blood channels. The middle vascular layer of secondary gill lamellae are surrounded on either side by a thin basement membrane and covered externally by single layered cuboidal epithelial cells. The mucous glands and the acidophil granular cells are located in the gill epithelium.

The gills of BHC exposed animals showed several histopathological changes. Acute exposure caused copious mucous secretion. At death the gill filaments were a bright red colour, indistinguishable from those of control fish. This colour gradually faded until at approximately 2h after death the gills were white. Gills of control fishes faded slightly within the first 2h after death, to a pink colour and did not turn white until after rigor mortis had passed and decomposition begun after 20h. The gills exhibited greatest damage. Swelling of secondary lamellae leading to detachment of the epithelium from the pillar cells and partial degeneration with loss of rigidity of the pillar cell system and hypertrophy in the

EXPLANATION OF PLATE - XVIII

Fig. 1: Horizontal section through the gill of control *C. batrachus* showing secondary lamella and associated structure (H & E x 450).

Figs. 2, 3: Horizontal section through the gill of control *C. batrachus* showing secondary lamella and associated structure (45x 10x).

Fig. 4: An early stage of lamellar odema and hyper trophy and curling of secondary lamellae after the exposure of BHC (45x 10x).

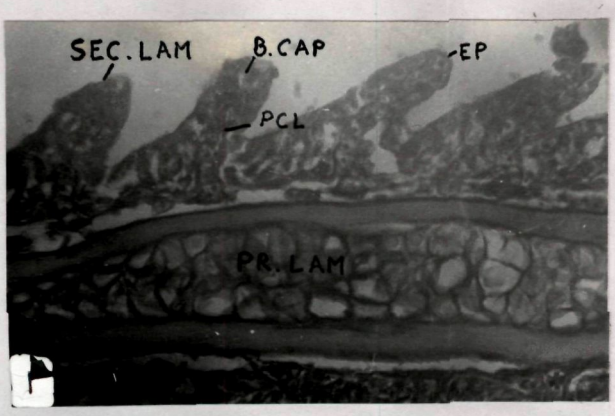
Fig. 5: Proliferative interlamellar epithelium with fused secondary lamella shown by arrow (45x 10x).

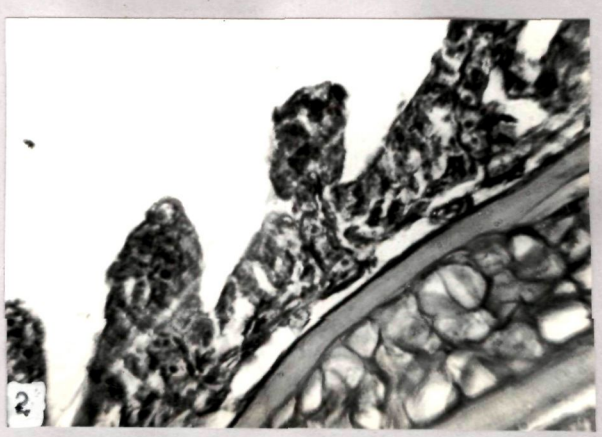
Fig. 6: Gill showing lamellar hypertrophy and hyperplasia in lamellar epithelium with thrombosed lamellar telangiectasis shown by arrow (45x 10x).

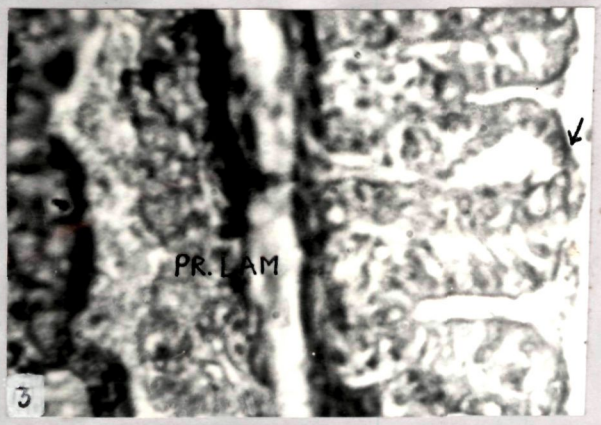
Abbreviations

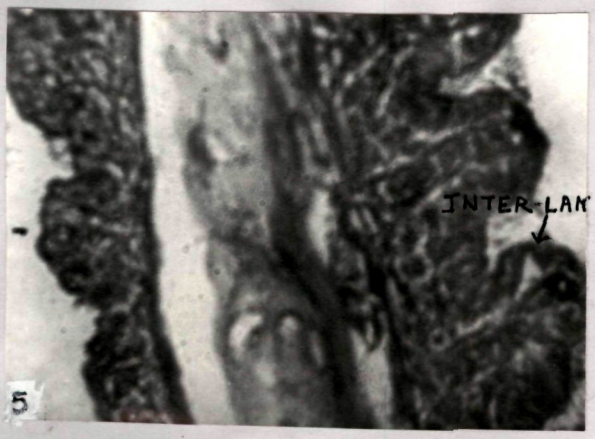
B.Cap.	..	Blood capillary
EP	..	Epithelium
Int. ep.	..	Inter lamellar epithelium
PCL	..	Pillar cell
Pr. Lam	..	Primary gill lamella
Sec. lam.	..	Secondary gill lamella

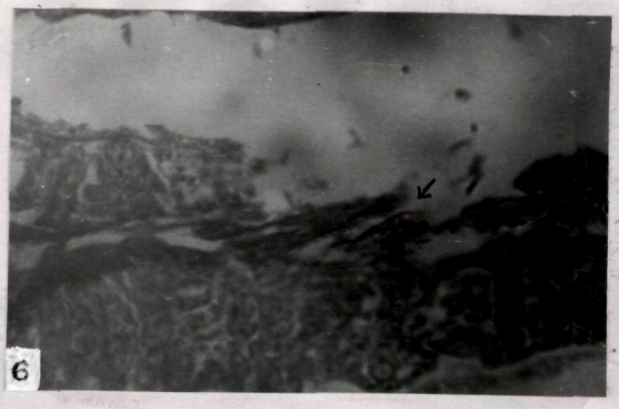
PLATE XVIII











lamellar channels were the major changes induced (Plate -XVII; Fig.4). In BHC exposed fish the tips of adjacent lamellae fused (Plate-XVIII; Fig.5). The pillar cells showed extensive damage.

Liver

The liver of Clarias batrachus is a bilobed reddish brown organ. Each liver lobe is a homogeneous mass of polygonal cells and the hepatocyte have centrally placed spherical nuclei with prominent nucleoli. The hepatocytes are arranged in the form of anastomosing parenchymal sheet of poorly defined lobular organization. Sinusoids are fewer in number and irregularly distributed in the liver parenchyma.

The acute toxic exposure of BHC produced pronounced histopathological change in the liver of Clarias batrachus. The colour of the liver from reddish brown changed to yeelowish red. The cellular details of the hepatocytes was obliterated. Other pathological changes were hypertrophy of hepatic cells, disarray of the liver cord and vacuolation of cytoplasm and necrosis (Plate-XIX;Fig.2). The liver as a whole showed distorted appearance.

Kidney

The kidney of Clarias batrachus is a pair of dark brown elongated structure located in the retroperitoneal position,

ventral to the vertebral column, and may be distinctly divided into head and trunk region. The trunk kidney is elongated extending the entire length of the body cavity and supplied by renal arteries arising directly from the aorta. The head kidney is roughly triangular in shape. Histologically, the trunk kidney is composed of renal capsules, nephrons and collecting tubules, and the inter tubular space filled with lymphoid tissue, The head kidney is composed of lymphoid, haemopoietic and adrenocortical tissues.

BHC exposure caused glomerular shrinkage in some glomeruli. Renal tubules collapsed and the distal segment showed strong fibrosis and tubular necrosis. Cellular debris in the renal tubule was also observed. The haemopoietic tissues of the head kidney were in degenerative phase (Plate-XX;Figs. 2,3).

Gonad

A pair of testis is present which are elongated and flattened structures, situated on either side, ventral to the kidneys in the posterior region of the abdominal cavity. Testis remain attached to the body wall by means of mesorchia. The testis is composed of large number of seminiferous tubules or lobules which are closely bound together by a thin layer of connective tissue. The lobules are surrounded by lobule

boundary cells which resemble with connective tissue cells. The spaces between the lobules are filled with connective tissue, blood capillaries and interstitial cells (Leydig cells). The testis of Clarias batrachus shows seasonal variation in spermatogenic activity.

In BHC exposed animals the spermatogonia were in degenerative condition. Degeneration of seminiferous tubules was also observed accompanied by cellular disintegration (Plate-XIX; Figs. 4, 5).

EXPLANATION OF PLATE - XIX

Fig. 1: T.S. of liver of control *C. batrachus* showing hepatocytes with central nuclei (45x 10x).

Fig. 2: T.S. of liver of BHC exposed animal showing, partial vacuolation of hepatocytes, cellular degeneration and stage of liver cirrhosis (45x 10x).

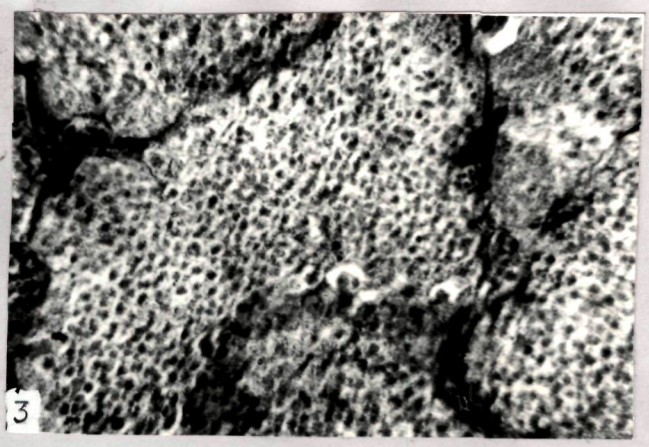
Fig. 3: T.S. of testis of control *Clarias batrachus* showing seminiferous tubules packed with spermatocytes (45x 10x).

Figs. 4, 5: Different stages of degeneration of spermatocytes (45x 10x).

PLATE XIX


1


2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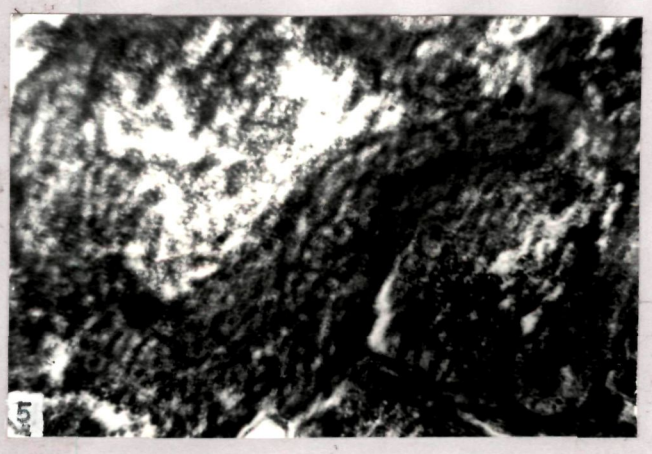
4

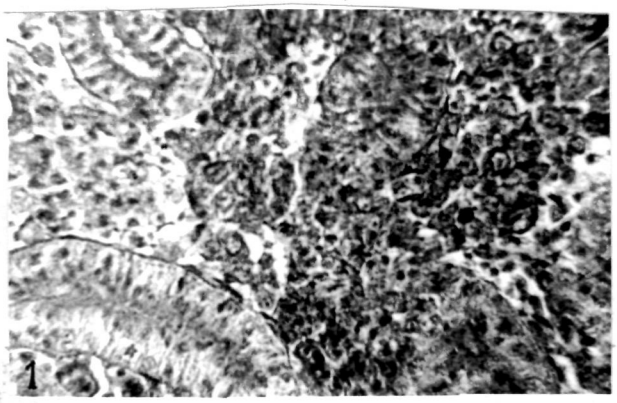

5

EXPLANATION OF PLATE - XX

Fig. 1: T.S. of trunk kidney of control *C. batrachus* showing renal tubules (45x 10x).

Fig. 2: T.S. of trunk kidney of BHC exposed animal showing shrinkage of glomerulus, tubular fibrosis, tubular epithelial necrosis and cellular debris in the lumen of the kidney tubules. (45x 10x).

Fig. 3: T.S. of trunk kidney of BHC exposed animal showing glomerular shrinkage (45x 10x).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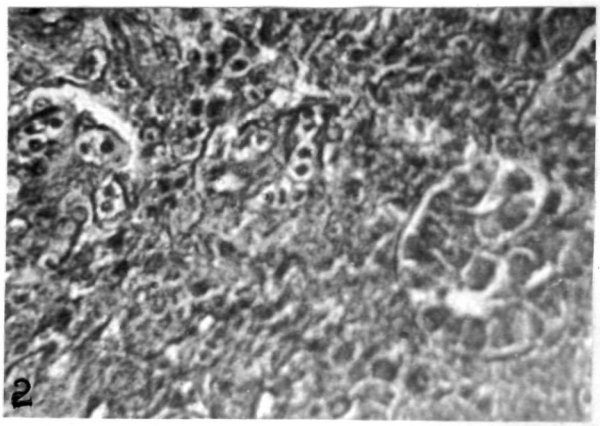
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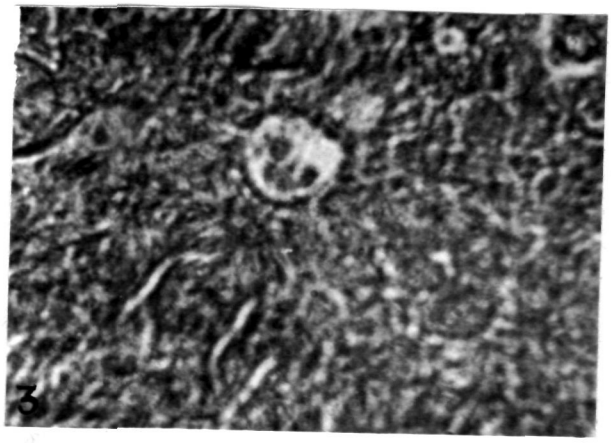
3

Discussion

The integument of the fish is its outermost defence against the surrounding environment. The skin is highly vulnerable to damage by various pathogens and polluted water with consequence of osmoregulatory distress. Several pathogens caused skin lesions, viz., virus (Wolf, 1966; Yasutake, 1970), bacteria (Snieszko and Ross, 1969; Bullock and Snieszko, 1970), fungi (Fijan, 1969), etc. Skin papillomas among fishes have been used as suitable, economical large scale screening of chemical carcinogen (Campbell *et al.*, 1974; Stich *et al.*, 1976). Sub-lethal effects of pollutants causing fish tumour has been reported by Stich *et al.* (1976).

Choudhary (1979) observed hyperplasia of epidermis of *H. fossilis* after long-term exposure of malathion. Such responses have been reported by low temperature (Roberts and

Bullock, 1976), chemical pollutants hormonal stimuli and putative viral agents (Roberts, 1975).

Thus the hyperplasia of skin as observed in the present investigation on Clarias batrachus after BHC exposure can not be considered as a specific reaction to pesticide. Production of copious mucous by flask shaped mucous cell is of interest from physiological point of view. Choudhary (1979) in H. fossilis after malathion exposure also observed copious mucous and suggested its protective function of integument. Mittal and Munshi (1971) reported weakly acidic sulphated mucopolysaccharide nature of slime produced by flask shaped unicellular gland.

The club cell or granular cell is found mainly in the skin of fishes belong to suborder siluroidei. Sato (1967, 1978) studied the staining reaction and fine structure of cottids. Mittal and Munshi (1971) reported albuminous nature of its secretion. Moreover, the nature and function of this cell has not been fully ascertained. The presence of relatively large number of lymphocytes in the epidermis of pollutant exposed animal shows the inflammatory response of the epidermal tissues. The function of increased lymphatic space is possibly to supply more nutrition to the stratum germinativum. The necrosis mainly due to specific action of BHC on the skin. The melanophores of fish are asteroid cells in the dermis. The melanophores are

under nervous or humoral control, any chemical agent or disease process which affect these centres will also affect the pigment cells. The present observation of shrinkage in pigment cells in skin coincide with the previous report of this dissertation (Chapter 1) in which change in colour has also been observed. Similar observation has also been made on H. fossilis after malathion exposure (Choudhary, 1979).

Teleostean gills are delicate leaf like structure which remain in contact with the surrounding water. Any physico-chemical agent in the water is responsible for marked gill lesions in fishes. A review on this problem has also been published by Eller (1975). He concluded that the gill damage is easily detected in pesticide studies designed to measure acute toxicity within one or two weeks. But, in long term, sub-acute studies, gill damage is subtle and is not always apparent and observed. A wide variety of pathological changes in gills of trout, blue gill and gold fish have been attributed to acute or chronic level of pesticides by various authors (Christie and Battle, 1963; Cope, 1965; Vanvalin et al., 1968; Matton and Lahan, 1969; Esler, 1971).

Kumaraguru (1982) observed epithelial separation or necrosis, mucous cell hyperplasia, clubbing of epithelial cells or hyperplasia and fusion of adjacent secondary lamellae

in Permethrin poisoned gills of rainbow trout, Salmo gairdneri. Khangarot (1982) studied zinc induced pathological changes in the gills of Puntius sophore and observed delamination of gill epithelium from the pillar cell system, oedema, fusion of secondary gill lamellae and degeneration of gill epithelial cells. Tuurala and Soivio (1983) studied the effect of dehydroabietic acid (DHAA) and zinc on the structural and circulatory changes in the gills of Salmo gairdneri. Dehydroabietic acid (DHAA) induced hypertrophy of the epithelium. Both substances induced a marked vasoconstriction in the secondary lamellar capillaries. Similar report of hyperplasia of the secondary gill lamellae have been given on gold fish, Carassius auratus after alkyl-benzene sulfonate (Fukuda, 1983) and on channel cat fish, Ictalurus punctatus after ammonia exposure (Mitchell and Cech, 1983). Choudhary (1979) studied the effect of organophosphate (malathion) and reported lamellar hypertrophy, hyperplasia, fusion of tips of adjacent lamellae, dilation of lamellar capillary and damage of pillar cells in the gills of H. fossilis. Swelling of secondary lamellae leading to detachment of the epithelium from the pillar cell system and partial degeneration with loss of ridigiry of the pillar cell system, hypertrophy in lamellar channels, fusion of tips of adjacent lamellae and overall damage of gill are features observed after BHC exposure in Clarias batrachus in present investiga-

tion. Present report is similar to the observations on Trichogaster fasciatus exposed to BHC (Gupta and Singh, 1982). Similarly in Salmo trutta exposed to lindane (BHC) gill damage and disarray of secondary gill lamella is observed by Drewett and Abel (1983).

Munshi and Singh (1971) investigated the histopathological effects of hepatochor and nicotine on the gills of H. fossilis. Separation of the respiratory epithelium from lamellae through proteinaceous fluid in gills of cutthroat trout after endrin exposure is noticed by Eller (1971). Andrews et at. (1966) also reported oedema in the gills of blue gill (Lepomis microchirus) by the exposure of hepatochor. The lamellar aneurysms progressing to fusion of lamellae by the exposure of dichlorobenil (Cope, 1966) and diuron (MaCraren et al., 1969) in blue gills have also been reported.

From the above account it is clear that the majority of histo-pathological changes are of limited diagnostic value and the tissue response are of non-specific nature. However, gill lamellar telangiectasis, aneurysms and oedema with epithelial separation produced after the exposure of toxins require careful evaluation in comparative fish pathology. Hughes and Perry (1976) emphasized the importance of morphometric study of gills through electron and light microscopy for evaluating pollutant action.

The shortening of gill lamellae along with thickening of the epithelial coverage of the secondary lamella should be considered as an adaptive tissue response to toxic environment which possibly provide more rigidity to gill filaments against collapse. Question may arise how the gills remain functional even after such wide ranging damage by the toxic effect of various pollutant ? Hughes (1976) gathered sufficient evidence to show that besides the normal course of blood flow through afferent and efferent artery via lacunar blood channels within the secondary lamellae, a different pattern of blood flow dose occur through the marginal channel and during pollution this becomes only one exposed to water current. Hughes (1976) observed that the trout exposed to pollutant continues to survive and depends entirely upon gas exchange across thin reduced surface.

Liver have usually been the organ of choice for histological studiesof pesticides induced changes in fishes because it is involved in vital metabolic processes of the body. Moreover, vertebrate liver is the chief detoxifying organ. In toxicological studies, liver had often been found to be the organ with highest concentrations of biocides or pesticides with relatively greater damage and impairment of the tissues (Ellias and Bengelsdorf, 1952; Hinton and Pool, 1976). Couch (1975) reviewed the pesticide induced changes in the liver

of fishes and demonstrated that hepatic damage occurs in both acutely lethal and chronic sublethal exposure of certain pesticides and related chemicals. Sriwastawa and Tripathi (1981) studied the histopathological changes in Esomus dandricus after sodium and ammonium sulphate poisoning. Endrin induced hepatic injury in Channa punctatus was reported by Sastry and Sharma (1979). They observed hypertrophy of hepatic cells and their nuclei, liver cord disarray, vocuolation of cytoplasm and necrosis. In some cells, the cell membrane was ruptured, centrolobular area was highly necrosed and liver on the whole showed distorted appearance. Colour change of liver from reddish brown to yellowish red after BHC exposure in Clarias batrachus is similar to the previous report on H. fossilis exposed to malathion (Choudhary, 1979). In present investigation on Clarias batrachus the cellular details of the hepatocyte was obliterated, hypertrophy of hepatic cells, disarray of liver cord and vacuolation of cytoplasm and necrosis and prominent splitting due to the widening of the sinusoids and loss of cell shape and rupture of the cellular membrane was observed. Similar observations were made on Trichogaster fasciatus exposed to BHC (Gupta and Singh, 1982). Endosulfan insecticide also produced liver lesions in Clarias garipinus and Tilapia rendall (Matthiessen and Roberts, 1982). Shafi and Choudhary (1980) observed endrin induced hepatic injury in Anabas testudineus.

They further observed rupture of hepatic cell membrane particularly in the centrolobular areas of the liver, where the nuclei also appeared partly disintegrated. The cytoplasm became clumped, vacuolated and in some cases appeared slightly pushed towards the cell border. Drewett and Abel (1983) noticed vascular congestion and cellular damage of the liver after lindane poisoning in brown trout, Salmo trutta.

Gupta and Dalela (1986) reported histopathological changes after sublethal concentrations of phenol (P), 2, 4 dinitrophenol (DNP), pentachlorophenol (PCP) individually, and in three combinations: (PCP + DNP)/P (highly antagonistic), (DNP + P)/PCP (additive) and (P + DNP)/PCP(highly synergistic) on Notopterus notopterus. In fish exposed to P and (PCP + DNP)/P histopathological changes included the loss of typical polygonal shape, displacement of nucleus from the center, splitting and degeneration of hepatic tissue, hypertrophy (enlargement) of blood vessels and vacuolation in hepatocytes were also observed. The fish exposed to DNP, hepatocytes lost their polygonal shape. Nuclei and cytoplasm of the cells migrated to the periphery. Splitting, hypertrophy, vacuolation, necrosis of hepatic tissue and degeneration of blood vessels were also observed. In fish exposed to PCP, and (DNP/P) PCP and (P + DNP) /PCP combinations showed degeneration of hepatic tissue and

complete loss of polygonal shape leading to necrosis and vacuolation. Hypertrophy of hepatocytes and their nuclei and blockage of sinusoid capillaries were also observed.

Thus from the above mentioned account the histological effects of organochlorine insecticides on the liver of freshwater fishes can be summarized as (i) hypertrophy of the hepatic cells; (ii) loss of cell shape and necrosis; (iii) widening of sinusoids; (iv) disarray of the liver cord; (v) rupture of cellular membrane; (vi) vacuolation of cytoplasm of hepatocytes.

Kidney principally serves as a means of water excretion and conservation of electrolytes by reabsorption through powerfull ion pumps in freshwater fishes. This osmoregulatory function of the kidney requires suitable cellular machinery. Since pollutants have toxic effects on a variety of membrane functions, there could be more possible ways in which pollutant-cell interaction may interfere with kidney function in fish.

DDT induced tubular degeneration have been reported by several authors (King, 1962; Mathur, 1962; Buhler et al., 1969). Mount and Putnicki (1966) observed vacuolated cells in glomeruli after the exposure of endrin. Choudhary (1979) observed shrinkage of glomeruli, epithelial necrosis and cellular debris

in the tubules of kidney of H. fossilis exposed to malathion. Kumar and Srivastava (1980) observed shrinkage in glomerulus and degeneration of arterioles of kidney in Channa punctatus exposed to sodium chloride. Similar observations were made on Esomus dandricus by Sriwastawa and Tripathi (1981). Wood (1975) reported protein like material in the bowman's space and collecting tubules by parathion in rainbow trout. Reddy et al., (1977) observed pathological changes in kidney of Colisa lalia exposed to organophosphorus compound, Disyston. In the present study on Clarias batrachus, glomerular shrinkage, collapsed renal tubules, strong fibrosis of distal segment and tubular necrosis and cellular debris in the renal tubules were observed. The shrinkage of glomeruli indicates partial dysfunction in filtration mechanism. The epithelial necrosis and cellular debris in the tubules can be considered as non-specific tissue response, since degeneration of kidney tubules have also been reported by several organochlorine insecticides and sometimes this may be suspected as a complication of autolysis procss.

Organochlorines are not only toxic to normal life processes, but also gametogenesis is affected by this insecticide. Endrin (10 ppb for 1 year) caused atresia of oocytes in the cutthroat trout, Salmo clarki, without apparently affecting the testis (Eller, 1976). Endrin (0.6 ppb "Hexadrin" for 96 hr or 4 weeks) also supressed gonadotropin secretion and gonadal

32P uptake in both male and female Indian catfish, H. fossilis (Singh and Singh, 1980 a, b, d) and gonadal steroid biosynthesis may also be reduced (Singh and Singh, 1980c, d). Similar findings have been obtained for Aldrin (17 ppb "Aldrin" for 4 weeks) in female Indian catfish (Singh and Singh, 1981). Also, DDT retarded ovarian development when fed to brook trout (S. fontinalis) at high sublethal level (Macek, 1968). Polychlorinated biphenyls (PCB's) administered orally or intraperitonially caused gonadal regression in Atlantic cod, Gadus morhua (Freeman and Sangalang, 1977; Freeman et al., 1978, 1980), brook trout, S. fontinalis (Freeman and Idler, 1975), rainbow trout, S. gairdneri, and carp, C. carpio (Sivarajah et al., 1978a, b). Reproduction is also adversely affected in other species (Nebeker et al., 1974; Bengtsson, 1980). Increasingly, both DDT and PCB's appear to have stimulatory effect on gametogenesis at low dosages (Macek, 1968; Freeman and Sangaland, 1977; Freeman et al., 1978).

Another organochlorine, 'r'-benzenehexachloride (BHC) (5 ppb for 4 weeks), causes atresia of oocytes and inhibits tuteinizing (LH)-induced in vitro ovulation in Japanese medaka O. latipes (Hirose, 1975).

In the present investigation on Clarias batrachus exposed to BHC showed marked inhibition of spermatogenesis and degeneration of seminiferous tubules.

Organophosphates although generally less hazardous to life, these compounds are not without any harmful effect on fish gametogenesis. Malathion (9 ppm "Cythion" for 96 hours or 4 weeks) and parathion (630 ppb "paramar M 50" for weeks) produced an effect on gonadotropin secretion, gonadal 32P uptake, and steroidogenesis similar to that of endrin and aldrin mentioned previously (Singh and Singh, 1980a, d, 1981). Parathion also inhibited spermatogenesis in the guppy (Billard and Kinkelin, 1970). Other non.chlorinated pesticides such as fenitrothion, carbaryl, lebaycid and diazinon all inhibit oogenesis to a greater or lesser extent in various species (Saxena and Garg, 1978; Carlson, 1971; Kling, 1981; Allison, 1977; Goodman et al., 1979). steroidogenesis may also be affected (Kapur et al., 1978). Decreased spermatogenic activity and haemorrhage in testis is observed in Colisa fasciatus exposed to manganese sulphate (Srivastava and Agarwal, 1983).

The present attempt to analyze the observed data on the histopathological effect of BHC, an organochlorine on Clarias batrachus have been encouraging, but requires cautious evaluation for identifying characteristic lesion of diagnostic value for this class of pesticide, as the extent of tissue response to various biocides and stress in most fishes are limited and unspecific. Moreover, the possible interaction of variables such as nutrition and pesticides and their effect on various organ systems require to be evaluated separately.

Summary

The present investigation has been undertaken to evaluate the effect of BHC, an organochlorine on specific tissue response of an air-breathing fish, Clarias batrachus.

Skin: The integument of Clarias batrachus consists of epidermis, dermis and subcutis. The epidermis consists of an outermost epithelial layer, the middle layer and basal layer. The epithelial is composed of stratified epithelial cells in which numerous flask shaped mucous glands are located. Middle layer is thickest layer of epidermis in which club cells are found. The basal layer, stratum germinativum is composed of single layer of cells. The dermis is characterized by the presence of bundles of muscles and collagen, supplied with blood-capillaries and nerve fibres, pigment cells.

Acute toxic exposure of BHC produced sloughing of epidermal layer with hyperplasia of cells. Increase in mucous glands and shrunken pigment cells as well as necrosis of dermal muscular bundles was observed.

Gills: The structure of gills of C. batrachus followed the typical plan of teleostean gills. The effect of acute exposure of BHC caused copious mucous secretion. After death the colour of gills changed to white within 2 hours swelling of secondary gill lamellae leading to detachment of the epithelium from the pillar cells and partial degeneration with loss of rigidity of the pillar cell system and hypertrophy in the lamellar channels were the major changes induced. In some cases the tips of the adjacent lamellae fused. The pillar cells showed extensive damage.

Liver: The liver of Clarias batrachus is a bilobed reddish brown organ. Liver lobe is a homogeneous mass of polygonal cells, hepatocytes have centrally placed nuclei. Hepatocytes arranged in the form of anastomosing parenchymal sheet of poorly defined lobular organization. The sinusoids are fewer in number and irregularly distributed in the liver parenchyma.

Acute exposure of BHC changed the reddish brown colour into the yellowish red. The cellular details of the hepatocyte was obliterated. Hypertrophy of hepatic cells, disarray of

liver cord, vacuolation of cytoplasm and necrosis was observed. The liver as a whole showed distorted appearance.

Kidney: The kidney is a paired elongated structure and distinctly divided into head and trunk region. Histologically the trunk kidney is composed of renal capsules, nephrons and collecting tubules and the inter tubular space filled with lymphoid tissues. The head kidney is composed of lymphoid, haemopoietic and adrenocortical tissues.

BHC exposure caused glomerular shrinkage. Renal tubules collapsed and distal segment showed fibrosis and tubular necrosis. Cellular debris in the renal tubule was also observed. The haemopoietic tissues of the head kidney were in degenerative phase.

Gonad: A pair of testis is present which are elongated and flattened structures. The testis is composed of large number of seminiferous tubules which are closedly bound together by a thin layer of connective tissue. The spaces between the lobules are filled with connective tissue, blood capillaries and interstitial cells (Leydig cells).

In BHC exposed animals spermatogenesis was inhibited and degeneration of seminiferous tubule and spermatogonial cells were observed.

Summary and Conclusion

Chlorinated hydrocarbons or organochlorines are notoriously toxic synthetic organic compounds presently being used extensively in many places for pest control in agriculture. It has now been accepted that the chlorinated hydrocarbons present a greater damage to non-target animals than other pesticides. These compounds are chemically stable thus they are persistent and owing to their fat solubility they are accumulated in adipose tissues within animal's body. The compounds frequently reach the aquatic environment, i.e., ponds, ditches, swamps and other derelict water areas where the fish, Clarias batrachus, thrive all the year round. Clarias batrachus is an air-breathing fish, is commercially important on account of its nutritive/therapeutic value and extra vigour. This fish has recently attracted the attention

of the fishery biologists and toxicologists exploring the possibility of its systematic and scientific culture in oxygen deficient water. Keeping in view the above account and considering the paucity of literature connected with the effect of organochlorine insecticides on Indian fishes, it was thought desirable to initiate work on this important aspect of chemical pollution. Hence, the present work entitled "Effect of Organochlorine, BHC on an air-breathing fish, Clarias batrachus (Linn.)." has been undertaken to investigate the patho-biology of this fish.

It was observed that the systems of Clarias batrachus can function within broad range of pollution by BHC and toxicity response of this fish towards BHC exposure is a function of concentration and duration of exposure. The 96 hours TLm value for this fish has been calculated to be 15.94 mg/l which is higher compared to several freshwater fishes. The behavioural response of the fish towards toxicant was grossly dependent on concentration and length of exposure. Increased aerial excursions and opercular movements were observed as immediate response of fish towards the toxicant. Liver weight, liver somatic index (LSI), gonad weight, gonadosomatic index (GSI) decreased with increasing concentration of BHC. There was marked increase in liver

water (13.75%) and gonadal water in 15 days BHC exposed animals. Decreased feeding rate, growth rate and hydration of liver and gonad indicate the toxic effect of BHC.

Effect of various concentrations of BHC for 96 hours on haematology indicate the linear relationship between concentration of BHC and erythrocyte counts, mean corpuscular haemoglobin (MCH) and total leucocyte counts. It has been observed that with unit increase in the concentration of BHC the erythrocyte counts decreases by a factor of 0.394. Mean corpuscular haemoglobin concentration (MCHC) and total leucocytes increase by a factor of 0.551 and 2.211 respectively with unit increase in the concentration of BHC. Haemoglobin and haematocrit values decreased in various concentrations of BHC while MCH increased in 96 hours BHC exposed animals. The lymphocyte per cent increased, eosinophil and neutrophil per cent decreased while monocyte per cent showed fluctuating result in BHC exposed animals. Erythrocyte diameter decreased with the increasing concentration of BHC exposure. The percentage of spherocytosis was also related to the concentration of exposure. The number of smudge cells also increased and Howell-Jolly bodies were present in the cytoplasm of BHC exposed animals indicating the symptom of destructive anaemia in BHC exposed animals. The haemolytic anaemia with spherocytosis were produced by BHC exposure.

BHC exposure caused increase in the rate of oxygen consumption, but the values at increasing concentrations were not consistent under both surfacing allowed and prevented conditions.

Plasma protein and blood glucose increased while plasma total cholesterol decreased significantly with increasing concentrations of BHC. Free and esterified cholesterol also declined after BHC exposure in this fish. A linear relationship was established between the concentration of BHC and total plasma protein and blood glucose, Plasma protein and blood glucose increased by a factor of 0.039 and 3.758 respectively, while the total plasma cholesterol decreased by a factor of 11.426. The decrease in ester cholesterol after BHC exposure indicate the impairment of hepatic function and liver damage.

Plasma sodium level increased at lower concentrations (2.0 and 4.0 mg/l) but declined at higher concentrations (8.0 and 16.0 mg/l) of BHC. Plasma potassium level declined significantly at lower concentrations but in higher concentrations there was increase in potassium level. Plasma calcium level declined in various BHC concentrations while inorganic phosphate increased at low concentration, which decreased at the highest concentration. The plasma chloride

level first increased at lower concentration (2.0 mg/l) but in higher concentration of BHC there was marked decrease in plasma chloride. The increased plasma potassium and decreased sodium level might have caused the primary desruptive effect of pollutants on the red cell membrane resulting in defective "active transport" pump mechanism.

A number of histopathological effects were observed in the tissues of 96 hours BHC exposed animals (16.0 mg/l BHC). Some of the important effects were sloughing of epidermal layer, hyperplasia of cells, increased mucous glands, shrunken pigment cells and necrosis of dermal muscular bundles of the skin; swelling of secondary lamellae, partial degeneration and loss of rigidity of pillar cell system, hypertrophy of lamellar channels, fusion of the tips of adjacent lamella in gills; loss of cell shape, hypertrophy of hepatic cells, disarray of liver cord, vacuolation of cytoplasm, necrosis and infiltration of lymphocytes in the liver; degeneration of haemopoietic tissue in the head kidney, glomerular shrinkage, renal tubules collapsed, fibrosis of the distal segment of the renal tubule, tubular necrosis in kidney; degeneration of seminiferous tubules with spermatocytes in the gonad etc.

The analysis of observed data on histopathological effect of BHC on this fish is encouraging, although charac-

teristic lesions of diagnostic value for this class of pesticides have not been identified. The BHC exposed animals may become susceptible to various germs of dreadful diseases due to reduced resistance of the animal.

The present investigation on the effect of BHC on an air-breathing fish, Clarias batrachus indicates that the sublethal toxicity of the insecticide may reduce many of the reflexes resulting in the altered behaviour and its inability to search food, maintain balance in water and increased respiration etc.

Further, the effects of BHC on the blood parameters clearly indicate the anaemic condition of the fish, due to destruction of erythrocytes and atrophic condition of erythropoietic tissues.

Physiological changes indicating increased respiration, disbalance in the ratio of free to esterified cholesterol, decrease in the total cholesterol, prolonged reduction in haemoglobin and increase in oxygen consumption are the result of sub-lethal toxicity of BHC, which indicate that although the animals can survive under these conditions but are definitely under stress in which growth rate is markedly reduced.

These results point out the need for further extensive investigations on BHC and other organochlorine compound, to determine the actual amount, their persistence in aquatic environment and their long term effect.

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