Effect of Lindane on reproduction in Heteropneustes fossilis

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INTRODUCTION

Lindane is an organochlorine insecticide and fumigant, which has been used on a wide range of soil-dwelling and plant-eating insects. Lindane is presently used primarily for seed treatment and in lotions, creams, and shampoos for the control of lice, and mites (scabies) in humans. Benzene hexachloride (BHC) is the 100% pure form of the product while lindane is slightly less pure (~99% pure). There are eight separate three dimensional forms (isomers) of BHC, the gamma configuration being one of those forms.

Lindane is highly toxic and a stimulant of central nervous system with symptoms usually developing within one hour. Symptoms of acute exposure in humans can include mental and motor retardation, central nervous system excitation, intermittent and continuous convulsions, respiratory failure, pulmonary edema and dermatitis. Other symptoms in humans are more behavioural in nature such as loss of balance and somersaulting (Smith, 1991), grinding of the teeth, and hyperirritability. Lindane can be absorbed through the skin, through inhalation or through direct ingestion. Most acute effects have been due to accidental or intentional ingestion, although inhalation occurred (especially among children) when it was used in vaporizers. Workers may be exposed to the product by absorption through the skin and through inhalation, if handled incorrectly.

The oral LD_{50} for rats is 88-270 mg/kg, for mice 59-246 mg/kg, and for rabbits 60 mg/kg. The lowest oral dose, which may be lethal for a child, is estimated to be 180 mg/kg. Lotions applied for scabies have resulted in severe intoxication in some children and infants. Small amounts of lindane fed to rabbits (1.5-12.0 mg/kg) for five to six weeks, and to rats (6.25-25 mg/kg) for 35 weeks suppressed their immune systems. This adversely affected the organisms' ability to fight off disease. In a two-year rat study, significant liver changes were attributed to the intake of moderately small amounts of lindane in the diets of the test animals (approximately 5 mg/kg/day).

Female rats experienced a disturbance of their reproductive cycle and inhibited fertility with doses of 0.5 mg/kg of lindane for four months. However treatments with 0.05 mg/kg of lindane did not produce these effects. Lindane was found to be slightly estrogenic to female rats and also caused the

seminiferous tubules in male rats to become atrophied at doses of 8 mg/kg/day over a ten day period (Smith, 1991). These tests suggest that the compound may have reproductive effects in human populations also.

Beagles given 7.5 or 15 mg/kg from day five throughout gestation did not produce pups with any noticeable birth defects. Pregnant rats given small amounts of lindane in their food had offspring unaffected by the pesticide (National Library of Medicine, 1992). Lindane, however, can be passed from the mother to the developing fetus. It appears that lindane will not cause developmental effects at low levels of exposure and causes reproductive effects at levels approaching the acute toxicity doses. However these effects have not been observed in human populations.

A variety of tests on mice and on microbes have shown no mutagenicity in the cells tested with lindane. It has been shown to induce some changes in the chromosomes of cultured human lymphocytes during cell division at fairly low doses. It is unlikely that lindane would pose a mutagenic risk in humans at very low exposure levels.

The carcinogenicity of lindane in experimental animals is low or limited as judged by the International Agency for Research on Cancer (U.S. Department of Health and Human Services, 1985). Mice fed with 100-500 mg/kg lindane containing diets for 24 weeks showed no signs of tumors. Rats fed for a lifespan at 5-1,600 mg/kg diet with a mean age at death of 58 weeks, had no increase in tumor incidence. One of the confounding factors in establishing a link between the insecticide and its carcinogenicity is the presence of three different dimensional forms (isomers) of the compound BHC. Each form has a slightly different toxicity. The International Agency for Research on Cancer has concluded that there is sufficient evidence to show that one of the lindane isomers is carcinogenic and limited evidence to establish the carcinogenicity of the beta and gamma isomers (ACGIH, 1991).

Of a single lindane dose of 40 mg/kg to rats, 80% was excreted in urine and 20% in faeces. Half of the administered lindane is excreted in three or four days. When administered for 18 days at 8 mg/kg, metabolites were found in blood, liver, kidneys, spleen, heart, and the brain. In humans, the mono, di, tri, and tetra-chlorophenolic metabolites were detected in urine with the trichlorophenols predominating. Residues disappear within three weeks after dosing ceases. Cows fed low doses of lindane in their daily ration for 35 days produced milk with residues from 0.002 to 0.015 ppm.

Lindane can be stored in the fat of mammals and birds. Birds of prey in the Netherlands contained up to 89 ppm of lindane in this tissue. Residues can also find their way into egg yolks at measurable concentrations for 32 days after dosing (Ulman, 1972). Harbor seals from the German North Sea and racoons from North America were found to have lindane in their fat at concentrations ranging from 0.3 ppm to 1.0 ppm (Howard, 1991).

Lindane is highly toxic to fish. The 96 hr LC_{50} ranges from 1.7 to 32 ppb for trout and salmon to 44 to 131 ppb for catfish, perch and goldfish. Water hardness did not seem to alter the toxicity to fish but temperature did. An increase in temperature from 2 to 18°C caused a 2.3-fold decrease in rainbow trout toxicity, but a 7 to 29°C increase caused a 2.6-fold increase in lindane toxicity to bluegill toxicity. Chronic and sublethal exposures to lindane produced liver and kidney problems in fish. Most of the lindane in the fish was unmetabolized. In the snail (Physa) most of the lindane was found as the metabolite, pentachloro-cyclohexene.

Birds are more tolerant of high doses of lindane than are mammals. Mallards have an LD_{50} of more than 5000 mg/kg. Pheasants, Japanese quail, and bobwhite quail have LD_{50} values of 561 ppm, 425 ppm and 882 ppm, respectively. Thus lindane is only slightly toxic to these organisms. Egg shell thinning and reduced egg production has occurred in birds exposed to lindane. Lindane is also highly toxic to bees and to aquatic invertebrates. The compound is believed to cause birth defects in amphibians.

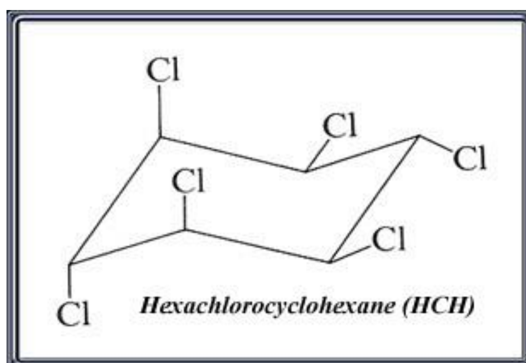
On eight types of soil, it was found that lindane residues decreased by 40 to 80% per year. When sprayed on the surface, the half- life was 4-6 weeks with 90% gone in 30-40 weeks. When worked into the soil, the half-life was 15-20 weeks with 90% gone in two to three years. At the end of 15 years, 0.2% remained. The typical half-life for lindane was 400 days. Lindane can be washed off into the soil, especially when humus content is low (Ulman, 1972).

The pesticide has been found in a significant number of groundwater samples in New Jersey, California, Mississippi, South Carolina, and in Italy at very low concentrations (maximum concentration of 0.9 ppb in New Jersey) (Howard, 1991). Lindane is a contaminant in water in the Great Lakes at very low concentrations as well.

Lindane is very stable in both fresh and salt water environments. It will disappear from the water by secondary mechanisms such as adsorption on sediment, biological breakdown by microflora and fauna, and adsorption by fish through gills, skin and food (Ulman, 1972). Storage in body fat is directly proportional to concentration in feed.

Plants pick up residues from not only direct application, but through water and vapor phases. While crops such as cauliflower and spinach had accumulated less than 0.1 ppm when grown in soil with residues of 0.1 to 0.5 ppm, carrots may accumulate high, persistent concentrations (Ulman, 1972). Persistence is seen when plants are rich in lipid content. The half- life of lindane in lettuce was found to be three to four days. The metabolism in plants is not well understood, but carrots were estimated to metabolize lindane at a rate of 43 to 47% after eight to ten weeks, based on the uptake by the plant.

This chlorinated hydrocarbon is commonly named as lindane. Other names/synonyms are gamma-HCH, gamma-BHC (refers to more than 99% gamma isomer), gamma-HKhTsH, ENT 7796 and OMS17. The chemical name is Gamma isomer of 1,2,3,4,5,6-hexachlorocyclohexane, 1-alpha, 2-alpha, 3-beta, 4-alpha, 5-alpha, 6-beta hexachlorocyclohexane (CAS No. 58-89-9). It is named asgamma 1α,2α,3β,4α,5α,6β-hexachlorocyclohexane by IUPAC and the chemical formula is $C_6H_6Cl_6$.



Hexachlorocyclohexane (HCH)

Technical lindane is a white crystalline solid with a melting point of 112.5-113.5°C and boiling point of 323.4°C. Its molecular weight is 290.85. It is stable to light, heat, air and strong acids but decomposes to trichlorobenzenes and HCl in alkali. It is soluble in most organic solvents such as acetone, benzene and ethanol and is soluble in water at 10 parts per million (ppm) at 200°C. Other chemical and physical properties include vapour pressure of 5.6 mPa at 20°C and specific gravity of 1.85. It has an adsorption coefficient of 1100.

It has several trade names such as Aalindan; Africide; Agrocide; Agrocide III; Agrocide WP; Ameisenmittel Merck; Ameisentod; Aparasin; Aphtiria; Aplidal; Arbitex; BBH; Ben-Hex; Bentox; Bexol; Celanex; Chloresene; Codechine; DBH; Detmol-Extrakt; Devoran; Dol; Drill Tox-Spezial Aglukon; ENT 7796; Entomoxan; Exagamma; Forlin; Gallogama; Gamaphex; Gammalin; Gammalin 20; Gammex; Gammexane; Gammaterr; Gexane; Grammapox; Hecltox; Hexa; Hexachloran; y-Hexachloran; Hexachlorane; Hexaverm; Hexicide; Hexyclan; HGI; Hortex; Inexit; Isotox; Jacutin; Kokotine; Kwell; Lacca Hi Lin, Lacca Lin-O-Mulsion; Lendine; Lentox; Linafor; Lindafor; Lindagam; Lindagrain; Lindagam; Lindagram; Lindatox; Lindasep; Lin-O-Sol; Lindagranox; Lindalo; Lindamul; Lindapoudre; Lindaterra, Lindex; Lindust; Lintox; Lorexane; Milbol 49; Msycol; Neo-Scabicidol; Nexen FB; Nexit; Nexit-Stark; Nexol-E; Nicochloran; Novigam; Omnitox; Ovadziak;Owadizak; Pedraczak; Pflanzol; Quellada; Sang-gamma; Silvanol; Spritz-Rapidin; Spruehpflanzol; Streunex; TAP 85; Tri-6; Vitron. It is available under following formulations like, emulsifiable concentrates (0.45% to 40% with 11% or 12% & 20% common); flowable concentrates (0.5%, 1.0%, 30%, & 40%) wettable powders (3%, 6%, 9%, 10%, 25%, 75%); pressurized liquids (0.25%, 0.75%, 3%); dusts (0.5% to

75% with 1% common); smoke generators (10.2, 11.2, 20.0%); liquid ready-to-use (0.5% to 25% with 0.1% & 0.5% common); tech. grade (99%, 99.5%, 100%), etc. The following are the some important global manufacturers of lindane: Agrolinz (Austria); All India Medical Corp. (India); Celamerck GmbH KG Ingelheim, (Fed. Rep. Germany); Drexel Chemical Co. (USA); Inquinosa (Spain); Mitsui, Inc. (Fukuoka, Japan); Rhône-Poulenc; Phytosanitaire (Lyons, France) and Tianjin International Trust & Investment Corp. (Tianjin, China).

With few exceptions (e.g., DES, dioxin, DDT and DDE), a causal relationship has not been established between exposure to a specific environmental agent and an adverse effect on human health operating via an endocrine disruption mechanism. An important consideration in evaluating endocrine-disrupting mechanisms is the concept of negative feedback control of hormone concentrations. Endogenous secretion and elimination of hormones are highly regulated, and mechanisms for controlling modest fluctuations of hormones are in place. Therefore, minor increases of exogenous hormones following dietary absorption and hepatic detoxification of these xenobiotics may be inconsequential in disrupting endocrine homeostasis in the adult. Whether the fetus and the young are capable of regulating minor changes to the endocrine milieu is uncertain.

An essential question in the analysis and discussion of the issue of environmental hormone disruption for risk assessment is whether the exposure and endocrine potency levels of the agents are sufficient to adversely affect human populations. If endocrine disruption is occurring through a hormone receptor mechanism, low ambient concentrations along with low-affinity binding of purported xenobiotics are probably insufficient to activate an adverse response. For example, exposure concentrations of weak estrogenic alkylphenols are on the order of ppm to ppb. White et al. (1994) reported effluent concentrations from sewage discharge plants in the United Kingdom at 0.1 ppm. Approximately 1/100 of the total (bound plus free) serum estradiol available is free to activate a physiologic response in female rats (Montano et al., 1995). According to White et al. (1994), of the alkylphenols tested, it requires some 1000 to 10,000 times more of the weak estrogen than estradiol to bind 50% of the estrogen receptor. If these data are correct, it means that 100,000 to 1,000,000 times more of the agent is needed to activate a physiological response. In other words, there would have to be 100 to 1000 times more of the agent in the water to activate an estrogenic response. Clearly, the normal human female is able to regulate parts per billion concentrations of estradiol without difficulty. In addition, Safe (1995) points out that dietary exposure to xenoestrogens derived from industrial chemicals is minimal compared to estrogen equivalents from naturally occurring bioflavonoids. Furthermore, in the case of environmental estrogens as endocrine disruptors, it is known that competition for binding sites by antiestrogens and downregulation of estrogen receptors via Ah receptor-mediated chemicals in the environment may mitigate estrogenic effects of some chemicals (Safe et al., 1991). Taken together, based on the available evidences, that exposure to a single xenoestrogenic chemical at current environmental concentrations is probably insufficient to evoke an adverse effect in human adults. More information is needed to determine whether this holds good for the human foetus and the neonate. Also, whether additional chemicals may overcome a body burden or operate at nonestrogenic receptor sites to stimulate or inhibit estrogenic or other responses needs to be determined.

Another unknown of relevancy is whether a mixture of chemicals with endocrine-disrupting potential via additivity (Soto et al., 1994; Harries et al., 1995) or synergy (Arnold et al., 1996) is sufficient to elicit a response and whether antagonists within the same mixture are sufficient to negate the response (Harris et al., 1990). These uncertainties will require considerable exploration.

There are a number of data gaps in our understanding of mechanisms of mammary gland carcinogenesis. Traditionally, safety and scaling factors and mathematical models have been employed to estimate the risk to humans based on study results in test animals. Such procedures are based on assumptions that may not be realistic for predicting human hazard/risk or mechanisms. Therefore, there is a need to develop and validate biologically based dose-response test animal-to-human extrapolation models for studying mechanisms of toxicity and chemical carcinogenesis, thus improving human risk assessment. Because environmental estrogenlike chemicals have been implicated as possible contributing factors in the

etiology of human breast cancer, these agents could be tested in various appropriate animal models.

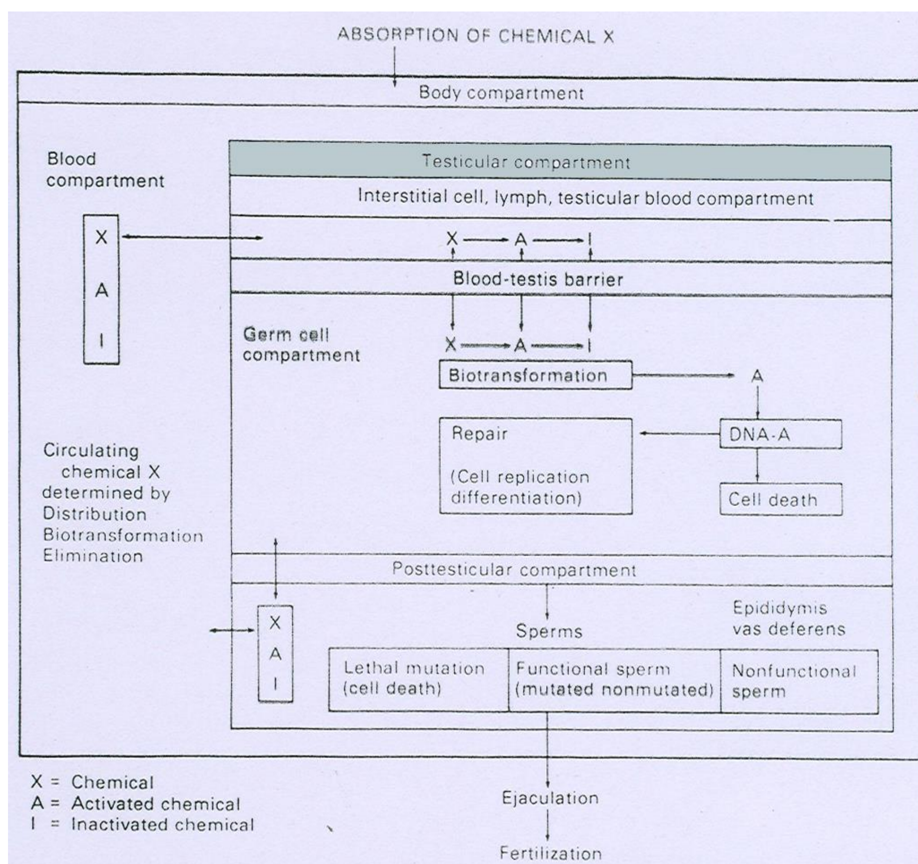


Figure 1: A pharmacokinetic model for the male gonad system (Lee and Dixon, 1978)

Testing for reproductive toxicity should include evaluation of both the quantity and quality of sperm produced. Such measures are emphasized in both the draft, *EPA Guidelines for Reproductive Toxicity Risk Assessment and the draft, Two-Generation Reproductive Toxicity Test Guidelines*. Recent revelations that agents such as estradiol and DES as well as the DDT metabolite DDE also have antiandrogenic activity place significantly increased importance on that mechanism of action. It is possible that the effects attributed to estrogenic activity are due to antiandrogenic activity instead of or in addition to estrogenic activity. Therefore, it is important that testing for endocrine-disrupting potential of environmental chemicals include the ability to detect antiandrogenic activity in addition to estrogenic activity. Testing also should be able to detect alteration in androgen receptor function as reflected in genome expression.

Further extensive research on populations exposed to DES might allow stratification of adverse effects by timing and level of exposure. Additionally, because retrospective examinations of existing data are likely to yield ambiguous results, it is important that prospective studies of human male sperm production be conducted. Such studies should include examination of trends in testicular cancer and sperm production over time and attempt to relate results to body and target tissue burdens of chemicals known to have antiandrogenic and/or estrogenic effects. The need for information relatively quickly dictates that existing populations of men be studied. For the long term, ideally a study would begin with the pregnancies from which the male study population was derived. Under those conditions, evaluation of the other known or developmentally induced reproductive system effects also could be done. Whether herbicide exposure contributes to the increasing incidence of human adenocarcinoma of the prostate and, if so, whether the mechanism is through an endocrine disruption have yet to be confirmed. If additional epidemiology studies support the above finding, then the next step is to identify specific herbicides that are causative agents and the

mechanisms by which these carcinogens act. Because an association between prostate cancer and herbicide spraying has been suggested, there is need to determine the most likely route (oral, inhalation, and/or dermal) of human exposure. If a dietary risk factor (increased fat intake) is confirmed, perhaps an oral route of exposure is most likely. Is a genotoxic effect operational, or is there an epigenetic mechanism working? Pertinent to this discussion, what is the evidence that a hormonal mechanism is contributing to the increased incidence of this disease? Are androgen-mimetic chemicals likely candidates? These and other questions require further research.

Future efforts should concentrate on developing improved tests to identify environmental agents that alter endocrine function through their action on the CNS and pituitary. Such tests are needed to identify any adverse neuroendocrine changes that occur in response to exposure during development and/or in adulthood. These tests might include direct measures of the gonadotropins and prolactin, as well as assessment of the functional reproductive end points regulated by the pituitary hormones. Further information is needed to better evaluate the extent to which normal sex differences in the neuroendocrine control of gonadal function may contribute to gender differences in response to reproductive toxicants. Because the CNS may develop tolerance to exposure to environmental agents, further studies are needed to evaluate the impact of tolerance on neuroendocrine/reproductive toxicity and to determine whether the current tests will identify this phenomenon.

Clearly, there is a need for protocols and multiple tests to identify chemicals that have the potential of disrupting thyroid hormone function. In rat studies, propylthiouracil treatment during development impairs CNS function (i.e., hearing) in adulthood (Davenport and Dorcey, 1972). Information on effects of chemicals in both sexes and the effects of exposure to the fetus, children, and adults are necessary. Once these apical tests are developed and validated, additional tests to ascertain mechanisms of action appear feasible. In an effort to extrapolate test animal to human equivalence, reasonable dose-response data are needed along with pharmacokinetics studies.

Many questions must be addressed before the overall magnitude, extent, and specific causes of this environmental concern can be resolved. Information is needed on what chemicals or class of chemicals are considered to be genuine endocrine disruptors. The quantity (dose) of a chemical necessary to cause an adverse effect is important. Next, there is a need to know whether chemicals suspected of being endocrine disruptors act in an additive, synergistic, or antagonistic manner. Although there are several available tests for evaluating chemicals for possible unique endocrine system disruption in some animal species, it is unclear which one or ones are the most useful. Apparently there are no avian reproductive tests to evaluate specific estrogenic effects in birds. Therefore, it is important to determine how well current screening assays predict adverse ecological effects due to endocrine disruption.

Methods need to be developed and validated to test for a cause-and-effect and a dose-response relationship to allow for sound risk assessment and regulatory decisions to be made. Additional research is needed to determine whether a chemical or its metabolites have hormonal activity, and if so, what mechanism of action is involved; rank chemicals in relative potency terms of toxicity; determine whether organisms are exposed to specific chemicals in the environment; ascertain whether there are sensitive species and individuals, and predict effects in the environment, including effects on organisms, populations, communities, and ecosystems. Specifically, test methods are needed to identify potential endocrine disruptors, quantify the potency of such action, and demonstrate any adverse outcome(s).

Sentinel species (organisms used to detect effects of hazardous exposures) have been used to identify environmental contaminants. Therefore, there is a need to determine whether current sentinel species are adequate surrogates for identifying endocrine disruptors in wild and aquatic life or if other sentinel species should be identified and validated for assessing the state of ecosystems. Perhaps the development, validation, and use of amphibian and/or reptilian models would be appropriate in view of the widespread distribution and lack of information on these classes of vertebrates. Evaluations of ecological effect generally do not consider factors such as disease resistance (immune system dysfunction), behavior (mating disruption), or reproductive viability of offspring (transgenerational effects). Consequently, there is a need to determine whether existing ecological effects/end points are adequate for assessing endocrine

system perturbation. If not, then additional effects/end points are needed. Finally, there is a need to know what effects that occur at the earliest response threshold are relevant for further risk characterization and what are the population, community, or ecosystem consequences of the effects observed in fish and wildlife.

MATERIALS AND METHODS

Heteropneustes fossilis is an air-breathing, fresh water teleost with a dendritic accessory respiratory organ. Adult *Heteropneustes fossilis* of both sexes weighing 225±25g were collected from local market. They were acclimatized to laboratory conditions for 2 weeks before being used in the experiments. During the acclimation period, fish were fed daily with minced goat liver and *ad libitum*.

1. Determination of lethal concentration (LC_{50}):

For LC_{50} determination, lindane (technical grade 98.5% pure) was used in this study, which was kindly provided by M/S. Fine Pest India Private Limited, Indore, India, and it was stored at 4°C until used. Initially it was dissolved in a known volume of acetone and further dilutions were made using tap water to make stock solutions of appropriate concentrations as per the guidelines of dilution techniques given in Standard Methods by APHA, AWWA, and WPCF (1975).Tap water (pH 7.3, hardness 23.2 $mg.L^{-1}$, dissolved oxygen 8 $mg.L^{-1,}$ and temperature 27 ± 2ºC was used . Appropriate amounts of stock solutions were added in the test aquaria (200 L) to make various concentrations. Feeding was stopped 24 hrs before starting the experiments. A wide range of concentrations were selected and used, including those that killed no fish and those that killed all fish. Twenty fish were used in each concentration. Mortality was examined for 24, 48, 72 and 96 hours. A control group consisting of 20 fish was also maintained to note any unexpected mortality of fish under these conditions. Water was renewed every 24hr along with the required amount of stock solutions of lindane. The dead fish were removed immediately from the aquaria to avoid oxygen depletion. Mortality and behavioral and morphological changes were recorded. The trimmed Spearman-Karber method was followed for the calculation of LC_{50} values and 95% confidence interval endpoints with trimming at 0 and 10%.

2. Determination of sublethal concentration of lindane:

Experiments were conducted using sublethal and toxicologically safe concentrations of the lindane for long term. The sublethal concentration (40$\mu g.L^{-1}$) used in this study corresponded approximately to one-tenth of the LC_{50} values of the compounds for 96 hr. The concentration was selected according to the guidelines of OECD (Organization for Economic Co-operation and Development, 1992) and following the procedure of Barnhoorn (2001). This concentration was chosen on the basis of several behavioural responses exhibited by the test organisms at various concentrations. In this experiment, groups of 10 fish each were exposed to safe concentrations of 40$\mu g.L^{-1}$ of lindane for 45, 90 and 180 days. One group was maintained in tap water as control. During the period of this experiment, water was renewed daily with the required amount of stock solution of lindane. The fish were fed on alternate days with mined goat liver. Five fish each from the experimental and controls were sacrificed 45, 90 and 180 days after the treatment. The required samples were separated and processed further according to the various parameters investigated in this study.

3. Gravimteric measurement:

The testes and seminal vesicles were dissected out and separated after exposing the fish to selected sublethal concentration of lindane for 45, 90 and 180 days. Weights of the testis and SV were expressed in 100 g body weight of fish to give respectively, gonado-somatic index (GSI) and seminal vesicle-somatic indesx (SVSI).

4. Estimation of total protein:

Principle

The protein reacts with $CuSo_4$ (in the protein reagent) to form a protein-copper complex. In the second step, this complex is allowed to reduce the phosphomolybdic-phosphotungstic acid complex. The reduced complex is blue in colour and read colorimetrically at 660 nm.

Preparation of Reagents

TCA 5 %

5 g of TCA was dissolved in 100 mL of DDW

NaOH 1 N

4 g of sodium hydroxide was dissolved in 100 mL DDW.

Protein reagent

This reagent was prepared fresh. 2 g of sodium carbonate was dissolved in 100 mL of 0.1 N NaOH (0.4 g NaOH in 100 mL of DDW). To this, 1 mL of 4% sodium potassium tartarate (4 g of sodium potassium tartarate in 100 mL of DDW) was added. Then, 1 mL of 2 %

copper sulphate (2 g of $CuSO_4$ in 100 mL of DDW) was added drop by drop and shaken vigorously.

Folin-Phenol Reagent

A mixture of 100 g of sodium tungstate, 25 g of sodium molybdate, 700 mL of DDW, 50 mL of 85% phosphoric acid and 100 mL of conc. HCl was refluxed in 1.5 L flask for 10 hours. Then 150 g of lithium sulphate, 50 mL of DDW and few drops of 10% bromine water were added and the mixture was boiled for 15 minutes without condenser to remove excess of bromine. The mixture was cooled and diluted to 1 L and filtered. Care was taken so that the reagent did not develop any greenish tint. The acid concentration was determined by titrating with 1 N NaOH to phenolphthalein endpoint. The reagent was diluted in such a way that the final acid concentration is 1 N.

Preparation of tissue sample

100 mg of weighed tissue was homogenized in 1 mL of 0.6% saline and 1 mL of 5% TCA was added to it. It was then left for an hour at room temperature and then centrifuged at 3000 rpm for 10 minutes and 1 mL of 1N NaOH was added to the precipitate. It was again vortexed briefly and centrifuged at 3000 rpm for 10 minutes and 20 μL supernatant was taken for protein estimation after adding 480 μL of DDW.

Preparation of standard solution

Bovine serum albumin (BSA) was used as the standard for protein assay. A stock solution was prepared by dissolving 100 mg of BSA in 100 mL of DDW. From the stock solution, 10, 15, 20, 30, and 40 μL were pipetted out separately and added to the test tubes containing 490, 485, 480, 470, and 460 μL of DDW respectively.

Estimation

500 μL of standard or sample was taken in a test tube. To this, 5 mL of protein reagent was added and the mixture was left for 10 minutes at room temperature. Then 0.5 mL of 1N Folin-phenol reagent was added, and left in the dark for 30 minutes. The O.D. was read at 660 nm.

Calculation

A standard graph was drawn using the O.D. values observed for different concentrations of standard protein and the concentration of protein in the sample was calculated by referring the O.D. obtained for sample using the standard graph. The protein concentration was calculated using the following formula:

$$\frac{\text{OD of unknown}}{\text{OD of std.}} \times \frac{\text{conc. of std.}}{\text{1000 (mg conversion)}} \times \frac{\text{vol. of homogenate}}{\text{vol. of homogenate}} \times \frac{1000}{\text{100 taken for analysis}}$$

= mg/g wet tissue wt.

5. Estimation of free amino acids:

Principle

Ninhydrin deaminates amino acids liberating ammonia and gets reduced to hydrindantin. The liberated ammonia condenses with hydrindantin to form a violet colored compound diketohydrindylidenediketohydrindamine (DHDA) at pH 5.0. Potassium cyanide prevents the oxidation of the reduced hydrindantin. The intensity of violet colour is directly proportional to the amount of amino acid.

Preparation of tissue sample

100 mg of tissue was homogenized in 1 mL of 80% ethanol. It was then centrifuged at 5000 rpm for 5 minutes. The supernatant was taken for analysis. 10 μL of supernatant was mixed with 990 μL of DDW and used for estimation.

Preparation of plasma sample

The blood (2.0 ml) was collected using a sterile syringe fitted with 26G needle by puncturing the blood vessel distributed at caudal region. The blood was allowed to clot at 4°C for 30 min. The serum was separated by centrifuging at 4°C for 10 min. 50 μL of serum was added to 2 mL of 80% ethanol and centrifuged at 5000 rpm for 5 minutes. 10 μL of the supernatant was added to 990 μL of DDW and taken for analysis.

Preparation of Reagents

Citrate buffer pH 5 (0.2 M)

21.008 g of citric acid was dissolved in 200 mL of DDW. Then it was added with 200 mL of 1 N NaOH and diluted to 500 mL. The reagent was stored in cold with thymol (200 mg/litre). The pH was adjusted to 5 using acetic acid.

Potassium cyanide (0.01M)

0.1628 g of potassium cyanide was dissolved in 25 mL with DDW.

Solution A

5 mL of 0.01 M potassium cyanide was made up to 250 mL with methyl cellosolve (It was stable for one month at room temperature).

Solution B

500 mg of ninhydrin was dissolved in 10 mL of methyl cellosolve (It was stable for 2 months at room temperature).

Solution C

10 mL of solution B was mixed with 50 mL of solution A. The resultant solution was initially red, but soon became yellow.

Preparation of amino acid standard solution:

Standard A: Glycine

0.0268 g of glycine was dissolved in 0.5 mL of DDW. To this 3.5 mL of 1N HCl and 0.1 g of sodium benzoate were added. The volume was made upto 50 mL with DDW.

Standard B: Glutamic acid

0.0525 g of dry glutamic acid was dissolved in 0.5 mL of DDW. To this 3.5 mL of 1N HCl and 0.1 g of sodium benzoate was added. The volume was made upto 50 mL with DDW.

Standard C

3 mL of Standard A and 3 mL of Standard B were mixed and the volume was made upto 100 mL with DDW. 1 mL of this solution contains 0.006 mg of aminoacid nitrogen.

Estimation

To 1 mL of sample and standard solution in the test tube, add 0.5 mL of citric acid buffer (pH 5). Then 1.2 mL of solution C (potassium cyanide and ninhydrin in methyl cellosolve) was added and the mixture was heated for 15 minutes at 90°C. Cooled under running tap water for 5 minutes, and then 2.3 mL of 60% ethanol was added. The O.D was read at 570 nm.

Calculation

Amount of aminoacid nitrogen present in the sample was calculated by using the following formula:

$$\frac{\text{OD of sample}}{\text{OD of standard}} \times 0.006 \times 100 \times 10 = \text{mg \% of aminoacid nitrogen}$$

Where 0.006 is the amount of aminoacid nitrogen present in the standard, and 100 X 10 is for converting to 1 g of tissue.

6. Estimation of protein-bound sugars:

Principle

The sulphuric acid hydrolyses polysaccharide bound to proteins into monosaccharides and dehydrates all monosaccharides into furfural or furfural derivatives. They combine with anthrone to form a colored complex that is proportional to the amount of monosaccharides complexed with the proteins

Preparation of reagents

Anthrone reagent

50 mg of anthrone and 1 g of thiourea were dissolved in 100 mL of 66 % con. H_2SO_4.

Preparation of sample

Protein-bound sugar was estimated by following the method of AOAC (1980). 100 mg of tissue was homogenized in 1 mL of 10% TCA, and centrifuged at 2500 rpm for 10 minutes. To the precipitate, 1 mL of 1N HCl was added, and the tube was closed with marble and placed in oven at 80°C for 18 hours for hydrolysis. The hydrolysate was used for analysis.

Estimation

To 0.05 mL of hydrolysate, 0.45 mL of DDW was added. To this, 5 mL of anthrone reagent was added, and the mixture was heated in a water bath for 15 minutes. Then it was cooled in dark at room temperature for 30 minutes. The O.D was read at 620 nm in a spectrophotometer.

Calculation

The standard graph prepared for total free sugars was used for protein- bound sugars. The protein-bound sugars could be calculated by referring the O.D obtained for sample using the standard graph. The concentration was calculated using the following formula:

$$\frac{\text{OD of unknown}}{\text{OD of std.}} \times \frac{\text{conc. of std.}}{\text{1000 (mg conversion)}} \times \frac{\text{vol. of homogenate}}{\text{vol. of homogenate taken for analysis}} \times \frac{1000}{100} = \text{mg/g wet tissue wt..}$$

7. Estimation of total free sugars:

Principle

Sulphuric acid in anthrone reagent hydrolyses di and oligosaccharides into monosaccharides and dehydrates all monosaccharides into furfural or furfural derivatives. These 2 compounds react with anthrone, which produces a complex coloured product, which could be read in a spectrophotometer.

Preparation of reagents

Anthrone reagent

50 mg of anthrone and 1 g of thiourea were dissolved in 100 mL of 66 % con. H_2SO_4.

Preparation of tissue sample

100 mg of tissue was homogenised in 1 mL of 10% TCA and centrifuged at 2500 rpm for 10 minutes. The supernatant was taken for analysis. 200 μL of supernatant was mixed with 300 μL of DDW, and this was taken for estimation.

Preparation of standard solution

Glucose was used as the standard for free sugars. A stock solution was prepared by dissolving 100 mg in 100 mL of 0.1% benzoic acid. From the stock solution 100, 200, 300, 400 and 500 μL were taken in separate test tubes and made up to 500 μL with DDW.

Estimation

To 500 μL of sample / standard, 5 mL of anthrone reagent was added and the mixture was boiled for 15 minutes in a water bath, and cooled in dark at room temperature for 30 minutes. The O.D was read at 620 nm.

Calculation

A standard graph was drawn using the O.D values observed for different concentrations of standard glucose. The concentration of total free sugars was calculated by referring the OD obtained for sample using standard graph. The free sugars can also be calculated using the following formula:

$$\frac{\text{OD of unknown}}{\text{OD of std.}} \times \frac{\text{conc. of std.}}{\text{1000 (mg conversion)}} \times \frac{\text{Vol. of homogenate}}{\text{Vol. of homogenate taken for analysis}} \times \frac{1000}{100} = \text{mg/g wet tissue wt.}$$

8. Estimation of glycogen:

Principle

Sulphuric acid in the anthrone reagent hydrolyses the glycogen into glucose and then dehydrates it into furfurals. This compound reacts with anthrone to produce a complex colored product, the intensity of which is proportional to the amount of glucose present in glycogen.

Preparation of reagents

Anthrone reagent

50 mg of anthrone and 1g of thiourea were dissolved in 100 mL of 66 % con. H_2SO_4.

Preparation of tissue sample

100 mg of tissue was homogenized in 1 mL of 80% ethanol followed by a centrifugation at 2500 rpm for 10 minutes. To the precipitate, 5 mL of 5% TCA was added which was followed by the incubation in boiling water bath for 15 minutes. Then 5 mL of 5% TCA was added and centrifuged again at 2500 rpm for 10 minutes. The supernatant wss taken for analysis.

Preparation of standard solution

Glucose was used as the standard for glycogen. A stock solution was prepared by dissolving 100 mg in 100 mL of 0.1% benzoic acid. From the stock solution 50, 100, 150, 200 and 250 μL were taken in separate test tubes and made up to 1000 μL with DDW.

Estimation

To 1 mL of sample and standard solutions, 10 mL of anthrone reagent was added and maintained in a boiling water bath for 15 minutes and later cooled at room temperature in dark. The O.D was read at 620 nm.

Calculation

A standard graph was drawn using the O.D values observed for different concentrations of standard glucose. The concentration of glycogen was calculated by referring the OD obtained for sample using the standard graph. The glycogen (in glucose equivalents) was calculated using the following formula:

$$\frac{\text{OD of unknown}}{\text{OD of std.}} \times \frac{\text{conc. of std.}}{\text{1000 (mg conversion)}} \times \frac{\text{Vol. of homogenate}}{\text{vol. of homogenate taken for analysis}} \times \frac{1000}{100} = \text{mg/g wet tissue wt.}$$

9. Estimation of glucose:

Preparation of tissue sample

100 mg of tissue was homogenized in 1 mL of 10% TCA. For haemolymph, 0.2 mL was added to 1.8 mL of 10% TCA. This was centrifuged at 2500 rpm for 10minutes. The supernatant was taken for analysis.

Preparation of reagents

Orthotoluidine boric acid reagent

0.25 g of thiourea and 0.24 g of boric acid were dissolved in 10 mL of DDW. This mixture was added to acetic acid and orthotoluidine in the ratio of 10:75:15 and kept overnight in the cold.

Preparation of standard graph

From a stock solution of D-glucose (100 mg in 100 mL of 0.1% benzoic acid), aliquots of 20, 40, 60, 80 and 100 μL were taken to obtain concentrations of 20, 40, 60, 80, and 100 μg, respectively. The standard solutions were analysed for glucose similar to the tissue sample, and a standard graph was drawn with concentration on the X-axis and OD on the Y-axis.

Estimation

To 0.2 mL of supernatant, 0.8 mL of DDW was added, and to this, 4 mL of O-toluidine boric acid reagent was added. This mixture was heated in a boiling water bath for 15 minutes, and then cooled under running tap water. The blue colour developed was read at 640nm.

Calculation

The concentration of glucose in sample was calculated by referring the OD obtained for sample using the standard graph. The glucose was calculated using the following formula:

$$\frac{\text{OD of unknown}}{\text{OD of std.}} \times \frac{\text{Conc. of std.}}{\text{1000 (mg conversion)}} \times \frac{\text{Vol. of homogenate}}{\text{Vol. of homogenate taken for analysis}} \times \frac{1000}{100} = \text{mg/g wet tissue wt.}$$

10. Estimation of total lipids:

Principle

The lipids in tissues are extracted with at least 20 volumes of 2:1 mixture of chloroform–methanol. From the tissue extract, the non-lipid matter is removed by Folch washing (volume of water to which different mineral salts are added).

Procedure

1 g of tissue was homogenized in 20 mL of chloroform–methanol mixture (in the ratio 2:1). It was then left undisturbed for 2 hours in the dark. It was then filtered through a Whatman filter paper (No.41), and the filtrate was preserved. The residue was re-extracted in the same solvent (half of the original volume) and filtered in Whatman's filter paper (No.41). Both the filtrates are pooled, and the total volume was noted. To the filtrate, 1/5th volume of 0.6% saline was added, and the mixture was transferred to a separating funnel and left undisturbed overnight in the dark. The lower layer was carefully taken out from the separating funnel in a pre-weighed beaker, and 4mL of benzene and 8mL of ethanol was added. This mixture was evaporated to dryness at room temperature. The beaker (after total evaporation) was weighed again, and the difference was concluded as the weight of lipid in the tissue.

11. Estimation of total cholesterol:

Preparation of reagents

Liebermann Burchard reagent (LB reagent)

1 mL of conc. $H_2SO_4$was added to 20 mL of ice-cold acetic anhydride. The mixture was kept for 10 minutes and brought to room temperature, and then 10 mL of glacial acetic acid was added and mixed thoroughly. The solution was prepared fresh, preferably in a room with dim lighting and stored in a dark bottle.

Preparation of standard graph

From a stock solution of cholesterol (100 mg in 100 mL of chloroform), aliquots of 50, 100, 150, 200, 250 and 300 μL were taken to obtain concentrations of 50, 100, 150, 200, 250 and 300 μg respectively. This was made up to 1000 μL with chloroform.

Estimation

The dry content of total lipid was dissolved in 5 mL of chloroform. 1 mL of this and 1 mL of standard solutions were taken in separate test tubes, and evaporated to dryness in a water bath at 80°C. Then 3 mL of Liebermann-Burchard reagent was added and the

mixture was kept for 30 minutes in the dark. The O.D was read at 620 nm.

Calculation

A standard graph was drawn using the O.D observed for different concentrations. The concentration of cholesterol was calculated by referring the O.D. obtained for the sample using the standard graph.

RESULTS & TABLES

RESULTS

Determination of LC_{50} values for lindane:

The mortality rate was examined after exposing the catfish, *Heteropneustes fossilis*, to various concentrations of lindane for 24, 48, 72 and 96 hours. LC_{50} values (Table 2) were determined by following the Trimmed Spearman-Karber method as described earlier. After 24 hours of exposure, trimming performed at 0% level showed LC_{50} value of 898.22 $\mu g.L^{-1}$ with lower and upper values of 799.33 and 963.34 $\mu g.L^{-1}$, respectively. 10% trimming performed for the same period indicated LC_{50} value of 810.05 $\mu g.L^{-1}$ with lower and upper values of 757.44 and 852.87 $\mu g.L^{-1}$, respectively. After 48 hours of exposure, trimming performed at 0% level showed LC_{50} value of 734.56 $\mu g.L^{-1}$ with lower and upper values of 644.89 and 780.65 $\mu g.L^{-1}$, respectively. 10% trimming performed for the same period indicated LC_{50} value of 694.33 $\mu g.L^{-1}$ with lower and upper values of 604.45 and 726.73 $\mu g.L^{-1}$, respectively. After 72 hours of exposure, trimming performed at 0% level showed LC_{50} value of 627.45 $\mu g.L^{-1}$ with lower and upper values of 580.12 and 678.24 $\mu g.L^{-1}$, respectively. 10% trimming performed for the same period indicated LC_{50} value of 570.09 $\mu g.L^{-1}$ with lower and upper values of 532.76 and 614.65 $\mu g.L^{-1}$, respectively. After 96 hours of exposure, trimming performed at 0% level showed LC_{50} value of 446.90 $\mu g.L^{-1}$ with lower and upper values of 394.78 and 485.90 $\mu g.L^{-1}$, respectively. 10% trimming performed for the same period indicated LC_{50} value of 403.78 $\mu g.L^{-1}$ with lower and upper values of 365.23 and 431.84 $\mu g.L^{-1}$, respectively. The concentrations of pesticides were linear over the period of observation in this study.

Effect of lindane on testicular somatic index:

Effect of lindane on the testicular somatic index in the catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days is shown in Table 1 and Figure 1. An overall significant variation was observed in the control catfish during the annual breeding cycle ($P< 0.01$; F= 35.37). Although the testicular somatic index did not exhibit any pronounced change after exposing to 45 days, it declined drastically after 90 ($P < 0.01$; F= 35.22) and 180 ($P< 0.001$; F= 142.64) days of exposure.

Effect of lindane on seminal vesicle-somatic index:

Effect of lindane on the seminal vesicle-somatic index in the catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days is shown in Table 2 and Figure 2. An overall significant variation was observed in the control catfish during the annual breeding cycle ($P< 0.01$; F= 31.69). Although the seminal vesicle-somatic index did not exhibit any pronounced change after exposing to 45 days, it declined drastically after 90 ($P< 0.01$; F = 48.45) and 180 ($P< 0.001$; F = 104.66) days of exposure.

Effect of lindane on the level of total protein:

Effect of lindane on the level of total protein in testis of the catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days is shown in Table 3 and Figure 3. An overall significant variation was observed in the control catfish during the annual breeding cycle ($P< 0.01$; F= 41.55). Although the level of total protein in testis did not exhibit any pronounced change after exposing to 45 days, it declined drastically after 90 ($P< 0.01$; F = 29.46) and 180 ($P < 0.001$; F = 196.43) days of exposure.

Effect of lindane on the level of total protein in seminal vesicle of the catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days is shown in Table 4 and Figure 4. An overall significant variation was observed in the control catfish during the annual breeding cycle ($P< 0.01$; F= 28.44). Although the level of total protein in seminal vesicle did not exhibit any pronounced change after exposing to 45 days, it declined drastically after 90 ($P< 0.01$; F = 29.46) and 180 ($P< 0.001$; F = 147.55) days of exposure.

Effect of lindane on the level of free amino acids:

Effect of lindane on the level of free amino acids in testis of the catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days is shown in Table 5 and Figure 5. An overall significant variation was observed in the control catfish during the annual breeding cycle ($P< 0.01$; F= 32.80). Although the level of free amino acids in testis did not exhibit any pronounced change after exposing to 45 days, it declined drastically after 90 ($P< 0.01$; F = 30.35) and 180 ($P< 0.001$; F = 162.54) days of exposure.

Effect of lindane on the level of free amino acids in seminal vesicle of the catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days is shown in Table 6 and Figure 6. An overall significant variation was observed in the control catfish during the annual breeding cycle ($P< 0.01$; F= 29.05). Although the level of free amino acids in seminal vesicle did not exhibit any pronounced change after exposing to 45 days, it declined drastically after 90 ($P< 0.01$; F = 41.59) and 180 ($P< 0.001$; F = 208.89) days of exposure.

Effect of lindane on the level of protein bound sugars:

Effect of lindane on the level of protein bound sugars in testis of the catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days is shown in Table 7 and Figure 7. An overall significant variation was observed in the control catfish during the annual breeding cycle ($P< 0.01$; F= 28.67). Although the level of protein bound sugars in testis did not exhibit any pronounced change after exposing to 45 days, it declined drastically after 90 ($P< 0.01$; F = 47.45) and 180 ($P< 0.001$; F = 178.30) days of exposure.

Effect of lindane on the level of protein bound sugars in seminal vesicle of the catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days is shown in Table 8 and Figure 8. An overall significant variation was observed in the control catfish during the annual breeding cycle ($P< 0.01$; F= 37.33). Although the level of protein bound sugars in seminal vesicle did not exhibit any pronounced change after exposing to 45 days, it declined drastically after 90 ($P< 0.01$; F = 29.98) and 180 ($P< 0.001$; F = 184.37) days of exposure.

Effect of lindane on the level of total free sugars:

Effect of lindane on the level of total free sugars in testis of the catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days is shown in Table 9 and Figure 9. An overall significant variation was observed in the control catfish during the annual breeding cycle ($P< 0.01$; F= 30.67). Although the level of total free sugars in testis did not exhibit any pronounced change after exposing to 45 days, it declined drastically after 90 ($P < 0.01$; F = 35.40) and 180 ($P< 0.001$; F = 241.08) days of exposure.

Effect of lindane on the level of total free sugars in seminal vesicle of the catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days is shown in Table 10 and Figure 10. An overall significant variation was observed in the control catfish during the annual breeding cycle ($P< 0.01$; F= 24.87). Although the level of total free sugars in seminal vesicle did not exhibit any pronounced change after exposing to 45 days, it declined drastically after 90 ($P< 0.01$; F = 44.43) and 180 ($P< 0.001$; F = 198.45) days of exposure.

Effect of lindane on the level of glucose:

Effect of lindane on the level of glucose in testis of the catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days is shown in Table 11 and Figure 11. An overall significant variation was observed in the control catfish during the annual breeding cycle ($P< 0.01$; F= 41.31). Although the level of glucose in testis did not exhibit any pronounced change after exposing to 45 days, it declined drastically after 90 ($P< 0.001$; F = 82.07) and 180 ($P< 0.001$; F = 159.45) days of exposure.

Effect of lindane on the level of glucose in seminal vesicle of the catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days is shown in Table 12 and Figure 12. An overall significant variation was observed in the control catfish during the annual breeding cycle ($P< 0.01$; F= 38.97). Although the level of glucose in seminal vesicle did not exhibit any pronounced change after exposing to 45 days, it declined drastically after 90 ($P< 0.01$; F = 107.85) and 180 ($P< 0.001$; F = 163.23) days of exposure.

Effect of lindane on the level of glycogen:

Effect of lindane on the level of glycogen in testis of the catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days is shown in Table 13 and Figure 13. An overall significant variation was observed in the control catfish during the annual breeding cycle ($P< 0.01$; F= 32.30). Although the level of glycogen in testis did not exhibit any pronounced change after exposing to 45 days, it declined drastically after 90 ($P< 0.01$; F = 39.95) and 180 ($P< 0.001$; F = 160.65) days of exposure.

Effect of lindane on the level of glycogen in seminal vesicle of the catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days is shown in Table 14 and Figure 14. An overall significant variation was observed in the control catfish during the annual breeding cycle ($P < 0.01$; F= 29.78). Although the level of glycogen in seminal vesicle did not exhibit any pronounced change after exposing to 45 days, it

declined drastically after 90 (P< 0.01; F = 48.90) and 180 (P< 0.001; F = 163.23) days of exposure.

Effect of lindane on the level of total lipids:

Effect of lindane on the level of total lipids in testis of the catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days is shown in Table 15 and Figure 15. An overall significant variation was observed in the control catfish during the annual breeding cycle (P < 0.01; F= 28.45). Although the level of total lipids in testis did not exhibit any pronounced change after exposing to 45 days, it declined drastically after 90 (P < 0.01; F = 30.35) and 180 (P< 0.001; F = 167.56) days of exposure.

Effect of lindane on the level of total lipids in seminal vesicle of the catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days is shown in Table 16 and Figure 16. An overall significant variation was observed in the control catfish during the annual breeding cycle (P< 0.01; F= 32.87). Although the level of total lipids in seminal vesicle did not exhibit any pronounced change after exposing to 45 days, it declined drastically after 90 (P< 0.01; F = 46.44) and 180 (P< 0.001; F = 139.40) days of exposure.

Effect of lindane on the level of total cholesterol:

Effect of lindane on the level of total cholesterol in testis of the catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days is shown in Table 17 and Figure 17. An overall significant variation was observed in the control catfish during the annual breeding cycle (P< 0.01; F= 31.20). Although the level of total cholesterol in testis did not exhibit any pronounced change after exposing to 45 days, it declined drastically after 90 (P< 0.01; F = 34.35) and 180 (P< 0.01; F = 74.69) days of exposure.

Effect of lindane on the level of total cholesterol in seminal vesicle of the catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days is shown in Table 18 and Figure 18. An overall significant variation was observed in the control catfish during the annual breeding cycle (P < 0.01; F= 38.41). Although the level of total cholesterol in seminal vesicle did not exhibit any pronounced change after exposing to 45 days, it declined drastically after 90 (P < 0.01; F = 38.47) and 180 (P< 0.001; F = 173.64) days of exposure.

Effect of lindane on the level of free cholesterol:

Effect of lindane on the level of free cholesterol in testis of the catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days is shown in Table 19 and Figure 19. An overall significant variation was observed in the control catfish during the annual breeding cycle (P< 0.01; F= 42.25). Although the level of free cholesterol in testis did not exhibit any pronounced change after exposing to 45 days, it declined drastically after 90 (P< 0.01; F = 48.20) and 180 (P< 0.01; F = 35.55) days of exposure.

Effect of lindane on the level of free cholesterol in seminal vesicle of the catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days is shown in Table 20 and Figure 20. An overall significant variation was observed in the control catfish during the annual breeding cycle (P< 0.01; F= 32.46). Although the level of free cholesterol in seminal vesicle did not exhibit any pronounced change after exposing to 45 days, it declined drastically after 90 (P< 0.01; F = 40.55) and 180 (P< 0.001; F = 148.26) days of exposure.

Effect of lindane on the level of esterified cholesterol:

Effect of lindane on the level of esterified cholesterol in testis of the catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days is shown in Table 21 and Figure 21. An overall significant variation was observed in the control catfish during the annual breeding cycle (P< 0.01; F= 45.27). Although the level of esterified cholesterol in testis did not exhibit any pronounced change after exposing to 45 days, it declined drastically after 90 (P< 0.01; F = 32.51) and 180 (P< 0.001; F = 125.89) days of exposure.

Effect of lindane on the level of esterified cholesterol in seminal vesicle of the catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days is shown in Table 22 and Figure 22. An overall significant variation was observed in the control catfish during the annual breeding cycle (P< 0.01; F= 28.62). Although the level of esterified cholesterol in seminal vesicle did not exhibit any pronounced change after exposing to 45 days, it declined drastically after 90 (P < 0.01; F = 30.93) and 180 (P< 0.01; F = 47.45) days of exposure.

Effect of lindane on the level of phospholipids:

Effect of lindane on the level of phospholipids in testis of the catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days is shown in Table 23 and Figure 23.

An overall significant variation was observed in the control catfish during the annual breeding cycle (*P*< 0.01; F= 34.28). Although the level of phospholipids in testis did not exhibit any pronounced change after exposing to 45 days, it declined drastically after 90 (*P*< 0.01; F = 42.78) and 180 (*P*< 0.001; F = 189.76) days of exposure.

Effect of lindane on the level of phospholipids in seminal vesicle of the catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days is shown in Table 24 and Figure 24. An overall significant variation was observed in the control catfish during the annual breeding cycle (*P*< 0.01; F= 46.90). Although the level of phospholipids in seminal vesicle did not exhibit any pronounced change after exposing to 45 days, it declined drastically after 90 (*P*< 0.01; F = 31.68) and 180 (*P*< 0.001; F = 115.80) days of exposure.

Effect of lindane on the level of free fatty acids:

Effect of lindane on the level of free fatty acids in testis of the catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days is shown in Table 25 and Figure 25. An overall significant variation was observed in the control catfish during the annual breeding cycle (*P*< 0.01; F= 32.67). Although the level of free fatty acids in testis did not exhibit any pronounced change after exposing to 45 days, it declined drastically after 90 (*P*< 0.01; F = 32.22) and 180 (*P*< 0.001; F = 129.40) days of exposure.

Effect of lindane on the level of free fatty acids in seminal vesicle of the catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days is shown in Table 26 and Figure 26. An overall significant variation was observed in the control catfish during the annual breeding cycle (*P*< 0.01; F=25.25). Although the level of free fatty acids in seminal vesicle did not exhibit any pronounced change after exposing to 45 days, it declined drastically after 90 (*P* < 0.01; F = 28.34) and 180 (*P*< 0.001; F = 163.57) days of exposure.

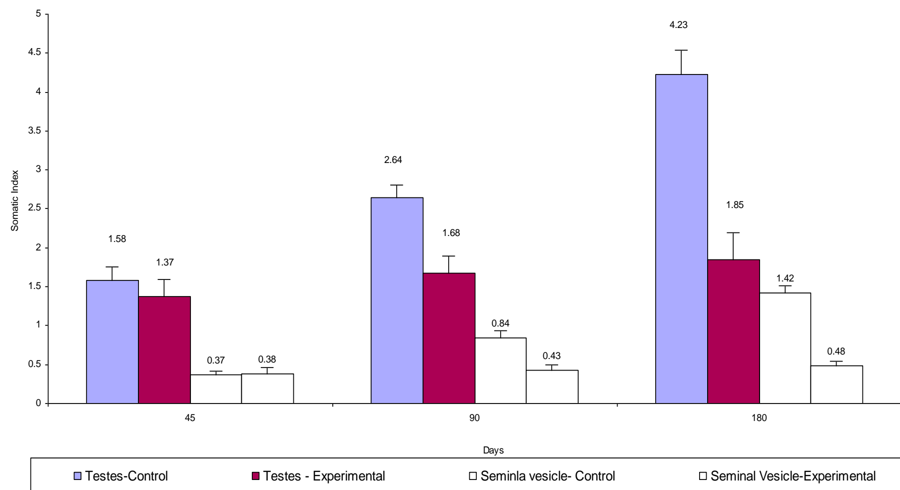
Table 1: Effect of lindane on the testicular somatic index in the catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days (Values are Means ± S.E.M.; n=5)

	45 days	90 days	180 days
Control	1.58±0.18	2.64±0.17	4.23±0.31
Experimental	1.37±0.22 NS	1.68±0.21*	1.85±0.34**

Table 2: Effect of lindane on the seminal vesicle-somatic index in the catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days (Values are Means ± S.E.M.; n=5)

	45 days	90 days	180 days
Control	0.37±0.04	0.84±0.09	1.42±0.09
Experimental	0.38±0.08 NS	0.43±0.07*	0.48±0.06**

Figure 2: Effect of lindane on the Testes and seminal vesicle-somatic index in the catfish *Heteropneustes fossilis*



Testes
*Significant at *P*< 0.01 (F = 35.22 for 90 days)
**Significant at *P*< 0.001 (F = 142.64 for 180 days)
NS: Not significant

Seminal Vesicle
*Significant at *P*< 0.01 (F = 48.45 for 90 days)
**Significant at *P*< 0.001 (F = 104.66 for 180 days)
NS: Not significant

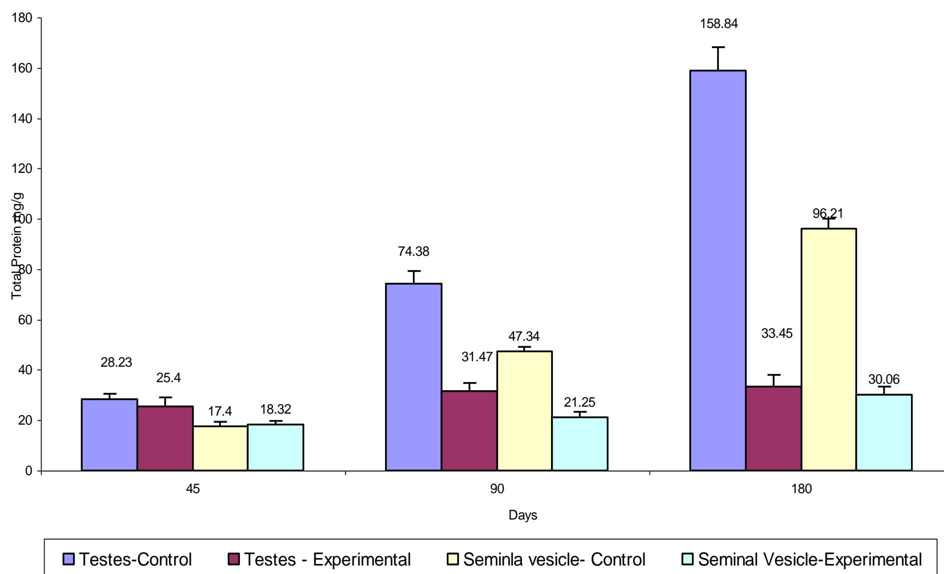
Table 3: Effect of lindane on the level of total protein in the testis of catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days (Values are Means ± S.E.M.; mg/g wet tissue wt.; n=5)

	45 days	90 days	180 days
Control	28.23±2.15	74.38±4.90	158.84±9.21
Experimental	25.40±3.56 NS	31.47±3.19	33.45±4.46

Table 4: Effect of lindane on the level of total protein in the seminal vesicle of catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days (Values are Means ± S.E.M.; mg/g wet tissue wt.; n=5)

	45 days	90 days	180 days
Control	17.40±1.97	47.34±1.82	96.21±3.77
Experimental	18.32±1.34 NS	21.25±2.18	30.06±3.36

Figure 4: Effect of lindane on the testes and seminal vesicle Total Protein in the catfish *Heteropneustes fossilis*



Testes	Seminal Vesicle
*Significant at *P*< 0.01 (F = 29.46 for 90 days) **Significant at *P*< 0.001 (F = 196.43 for 180 days) NS: Not significant	*Significant at *P*< 0.01 (F = 41.56 for 90 days) **Significant at *P*< 0.001 (F = 147.55 for 180 days) NS: Not significant

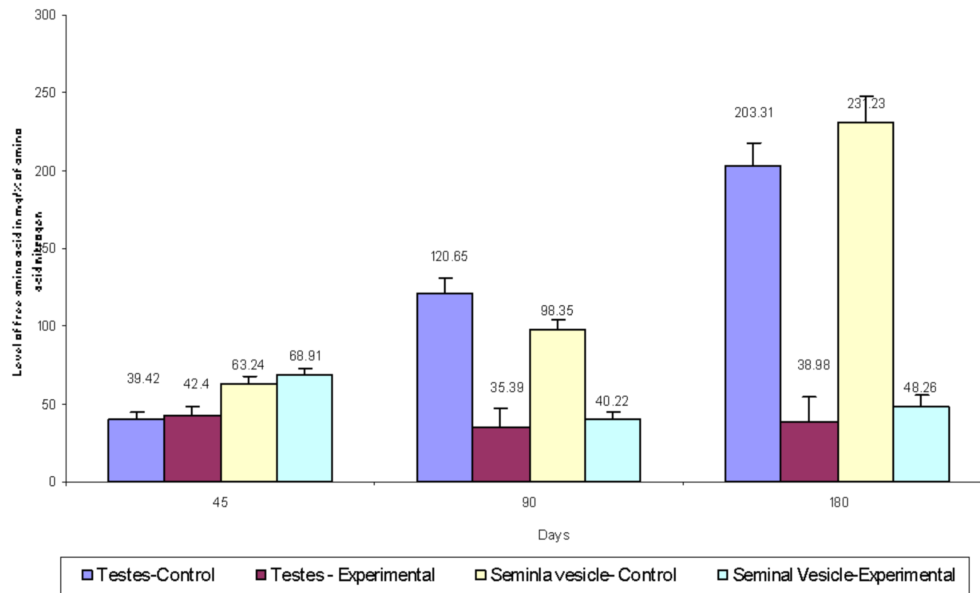
Table 5: Effect of lindane on the level of free amino acids in the testis of catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days (Values are Means ± S.E.M.; mg % of aminoacid nitrogen; n=5)

	45 days	90 days	180 days
Control	39.42±4.74	120.65±9.78	203.31±14.24
Experimental	42.40±5.67 NS	35.39±11.25**	38.98±15.80**

Table 6: Effect of lindane on the level of free amino acids in the seminal vesicle of catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days (Values are Means ± S.E.M.; mg % of aminoacid nitrogen; n=5)

	45 days	90 days	180 days
Control	63.24±4.87	98.35±5.60	231.23±17.20
Experimental	68.91±3.81 NS	40.22±4.50*	48.26±7.12**

Figure 6: Effect of lindane on the testes and seminal vesicle level of free aminoacids in the catfish *Heteropneustes fossilis*



Testes	Seminal Vesicle
**Significant at *P*< 0.001 (F = 30.35 and 162.54 for 90 and 180 days, respectively) NS: Not significant	*Significant at *P*< 0.01 (F = 41.59) **Significant at *P*< 0.001 (F = 208.89) NS: Not significant

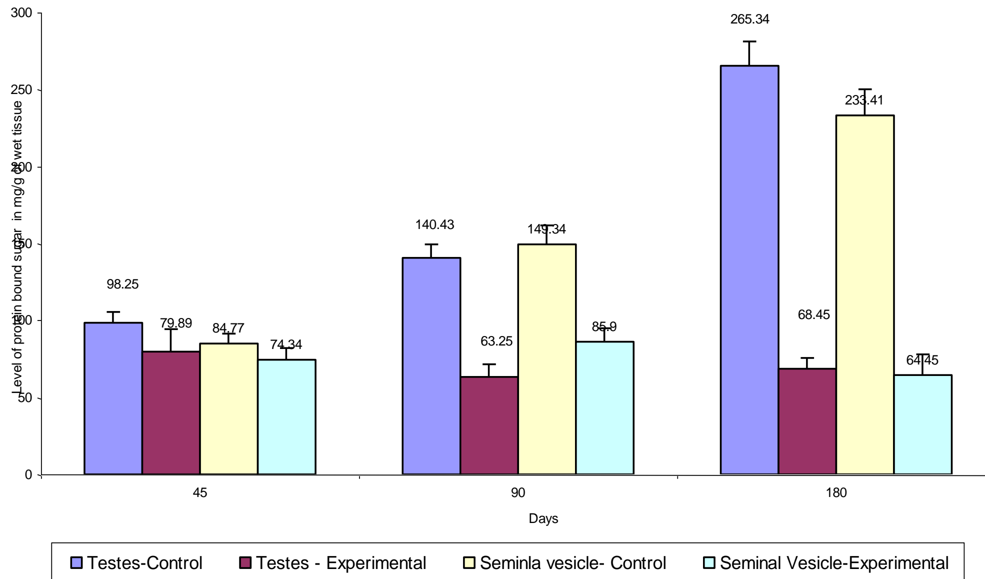
Table 7: Effect of lindane on the level of protein bound sugars in the testis of catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days (Values are Means ± S.E.M.; mg/g wet tissue wt.; n=5)

	45 days	90 days	180 days
Control	98.25±7.28	140.43±8.94	265.34±16.20
Experimental	79.89±14.27 NS	63.25±8.39**	68.45±7.32**

Table 8: Effect of lindane on the level of protein bound sugars in the seminal vesicle of catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days (Values are Means ± S.E.M.; mg/g wet tissue wt.; n=5)

	45 days	90 days	180 days
Control	84.77±6.89	149.34±12.10	233.41±16.95
Experimental	74.34±7.53 NS	85.90±9.17*	64.45±13.20**

Figure 8: Effect of lindane on the testes and seminal vesicle on the level of Protein bound sugar in the catfish *Heteropneustes fossilis*



Testes	Seminal Vesicle
**Significant at *P*< 0.001 (F = 47.45 and 178.30 for 90 and 180 days, respectively) NS: Not significant	*Significant at *P*< 0.01 (F = 29.98) **Significant at *P*< 0.001 (F = 184.37) NS: Not significant

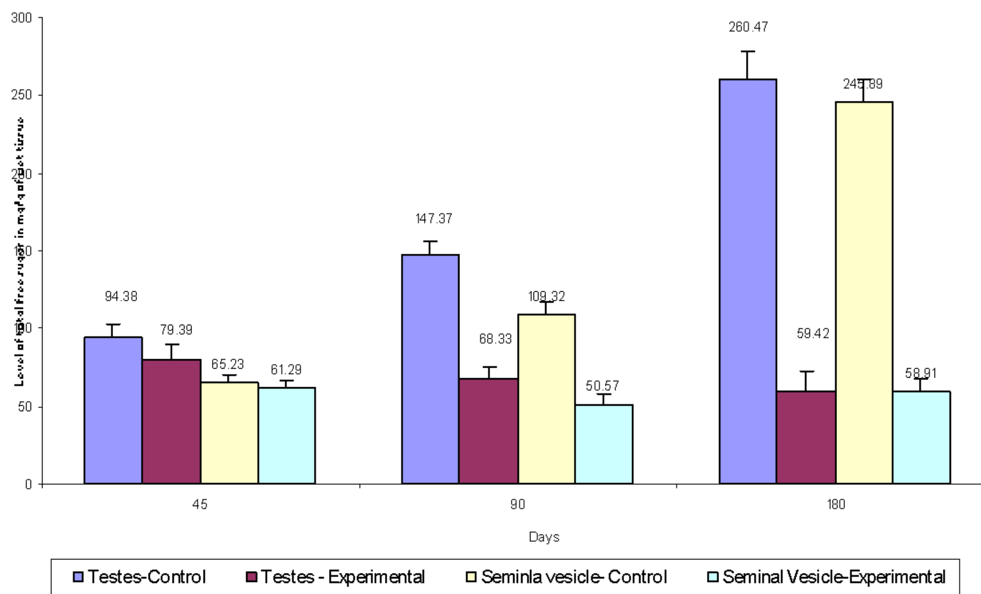
Table 9: Effect of lindane on the level of total free sugars in the testis of catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days (Values are Means ± S.E.M.; mg/g wet tissue wt.; n=5)

	45 days	90 days	180 days
Control	94.38±7.85	147.37±8.90	260.47±18.34
Experimental	79.39±10.42 NS	68.33±6.45*	59.42±13.40**

Table 10: Effect of lindane on the level of total free sugars protein in the seminal vesicle of catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days (Values are Means ± S.E.M.; mg/g wet tissue wt.; n=5)

	45 days	90 days	180 days
Control	65.23±4.96	109.32±8.05	245.89±14.43
Experimental	61.29±5.50 NS	50.57±7.23*	58.91±9.03**

Figure 10: Effect of lindane on the testes and seminal vesicle on the level of total free sugar in the catfish *Heteropneustes fossilis*



Testes	Seminal vesicle
*Significant at *P*< 0.01 (F = 35.40) **Significant at *P*< 0.001 (F = 241.08) NS: Not significant	*Significant at *P*< 0.01 (F = 44.43) **Significant at *P*< 0.001 (F = 198.45) NS: Not significant

Table 11: Effect of lindane on the level of glucose in the testis of catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days (Values are Means ± S.E.M.; mg/g wet tissue wt.; n=5)

	45 days	90 days	180 days
Control	60.45±6.51	174.35±12.59	260.44±18.28
Experimental	49.96±7.34NS	40.05±8.03**	55.83±7.43**

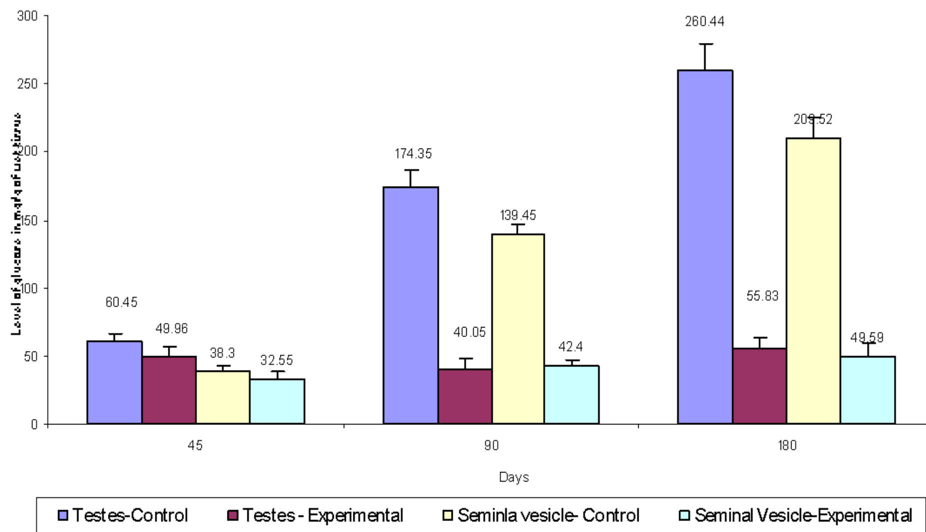
**Significant at *P*< 0.001 (F = 82.07 and 159.45 for 90 and 180 days, respectively)
NS: Not significant

Table 12: Effect of lindane on the level of glucose in the seminal vesicle of catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days (Values are Means ± S.E.M.; mg/g wet tissue wt.; n=5)

	45 days	90 days	180 days
Control	38.30±4.19	139.45±6.68	209.52±15.80
Experimental	32.55±5.77 NS	42.40±4.63**	49.59±10.28**

**Significant at *P*< 0.001 (F = 107.85 and 163.23 for 90 and 180 days, respectively)
NS: Not significant

Figure 12: Effect of lindane on the level of glucose in the testes and seminal vesicle of the catfish *Heteropneustes fossilis*



Testes	Seminal Vesicle
**Significant at *P*< 0.001 (F = 82.07 and 159.45 for 90 and 180 days, respectively) NS: Not significant	**Significant at *P*< 0.001 (F = 107.85 and 163.23 for 90 and 180 days, respectively) NS: Not significant

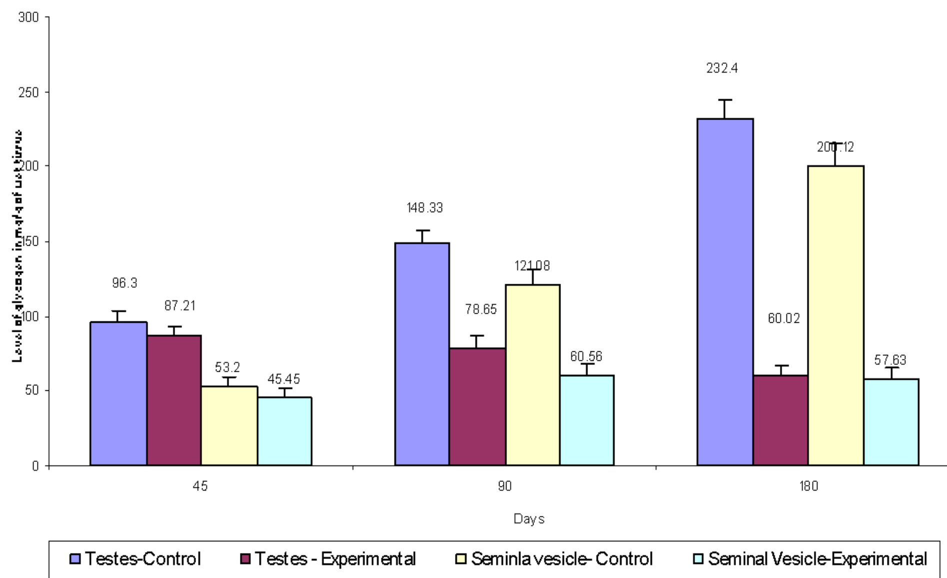
Table 13: Effect of lindane on the level of glycogen in the testis of catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days (Values are Means ± S.E.M.; mg/g wet tissue wt.; n=5)

	45 days	90 days	180 days
Control	96.30±7.31	148.33±9.34	232.40±12.56
Experimental	87.21±5.90 NS	78.65±8.80*	60.02±7.05**

Table 14: Effect of lindane on the level of glycogen in the seminal vesicle of catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days (Values are Means ± S.E.M.; mg/g wet tissue wt.; n=5)

	45 days	90 days	180 days
Control	53.20±6.18	121.08±10.34	200.12±14.87
Experimental	45.45±5.93 NS	60.56±7.84*	57.63±8.22**

Figure 14: Effect of lindane on the level of glycogen in the testes and seminal vesicle of the catfish *Heteropneustes fossilis*



Testes	Seminal Vesicle
*Significant at *P*< 0.01 (F = 39.95) **Significant at *P*< 0.001 (F = 160.65) NS: Not significant	*Significant at *P*< 0.01 (F = 48.90) **Significant at *P*< 0.001 (F = 173.23) NS: Not significant

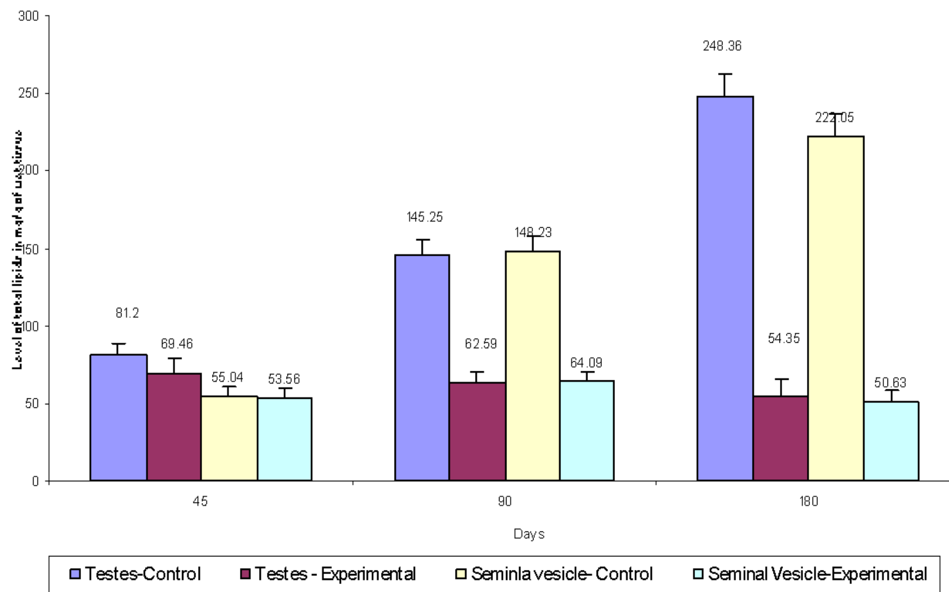
Table 15: Effect of lindane on the level of total lipids in the testis of catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days (Values are Means ± S.E.M.; mg/g wet tissue wt.; n=5)

	45 days	90 days	180 days
Control	81.20±7.31	145.25±9.87	248.36±14.30
Experimental	69.46±8.94 NS	62.59±7.74*	54.35±11.20**

Table 16: Effect of lindane on the level of total lipids in the seminal vesicle of catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days (Values are Means ± S.E.M.; mg/g wet tissue wt.; n=5)

	45 days	90 days	180 days
Control	55.04±6.12	148.23±9.45	222.05±14.32
Experimental	53.56±5.87 NS	64.09±6.45*	50.63±7.89**

Figure 16: Effect of lindane on the level of total lipids in the testes and seminal vesicle of the catfish *Heteropneustes fossilis*



Testes	Seminal Vesicle
*Significant at *P*< 0.01 (F = 30.35) **Significant at *P*< 0.001 (F = 167.56) NS: Not significant	*Significant at *P*< 0.01 (F = 46.44) **Significant at *P*< 0.001 (F = 139.40) NS: Not significant

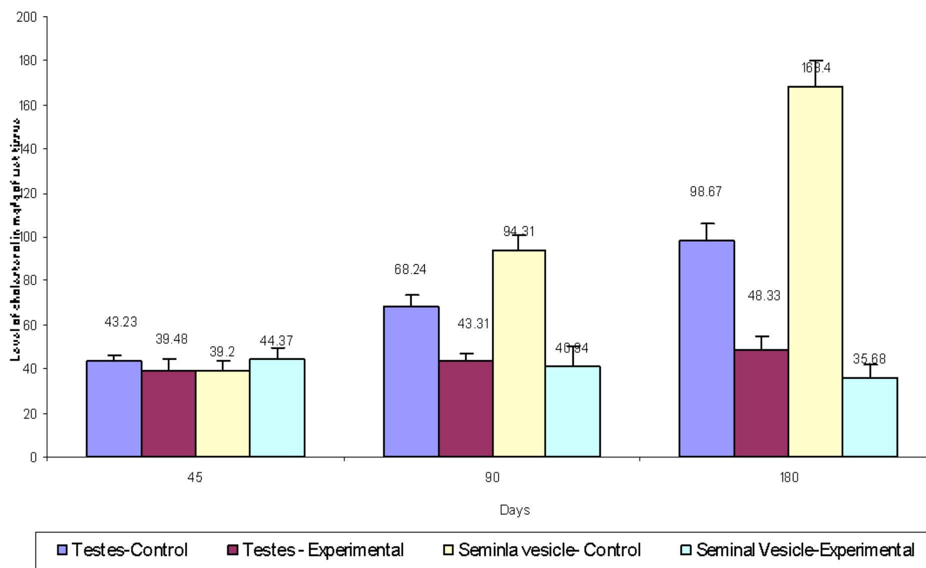
Table 17: Effect of lindane on the level of total cholesterol in the testis of catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days (Values are Means ± S.E.M.; mg/g wet tissue wt.; n=5)

	45 days	90 days	180 days
Control	43.23±3.32	68.24±5.50	98.67±7.34
Experimental	39.48±4.56NS	43.31±3.70*	48.33±6.25*

Table 18: Effect of lindane on the level of total cholesterol in the seminal vesicle of catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days (Values are Means ± S.E.M.; mg/g wet tissue wt.; n=5)

	45 days	90 days	180 days
Control	39.20±4.37	94.31±6.43	168.40±11.57
Experimental	44.37±5.23 NS	40.94±9.31*	35.68±6.19**

Figure 18: Effect of lindane on the level of Cholesterol in the testes and seminal vesicle of the catfish *Heteropneustes fossilis*



Testes	Seminal Vesicle
*Significant at *P*< 0.01 (F = 34.35 and 74.69 for 90 and 180 days, respectively) NS: Not significant	*Significant at *P*< 0.01 (F = 38.47) **Significant at *P*< 0.001 (F = 173.64) NS: Not significant

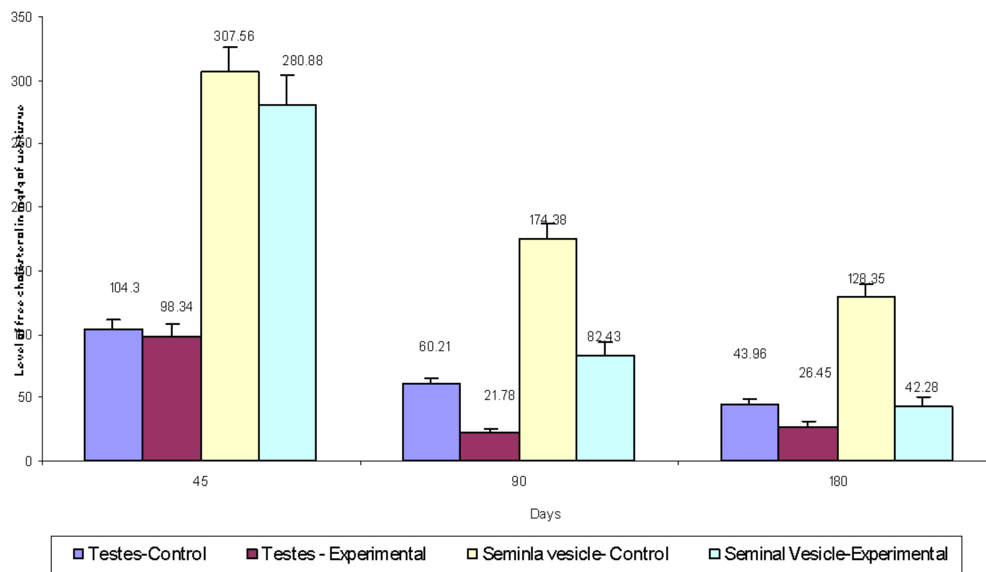
Table 19: Effect of lindane on the level of free cholesterol in the testis of catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days (Values are Means ± S.E.M.; µg/100 mg wet tissue wt.; n=5)

	45 days	90 days	180 days
Control	104.30±7.45	60.21±4.78	43.96±5.40
Experimental	98.34±9.67NS	21.78±3.57*	26.45±4.82*

Table 20: Effect of lindane on the level of free cholesterol in the seminal vesicle of catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days (Values are Means ± S.E.M.; µg/100 mg wet tissue wt.; n=5)

	45 days	90 days	180 days
Control	307.56±19.30	174.38±12.56	128.35±10.52
Experimental	280.88±23.36 NS	82.43±11.07*	42.28±8.03**

Figure 20: Effect of lindane on the level of free Cholesterol in the testes and seminal vesicle of the catfish *Heteropneustes fossilis*



Testes	Seminal Vesicle
*Significant at *P*< 0.01 (F = 48.20 and 35.55 for 90 and 180 days, respectively) NS: Not significant	*Significant at *P*< 0.01 (F = 40.55) **Significant at *P*< 0.001 (F = 148.26) NS: Not significant

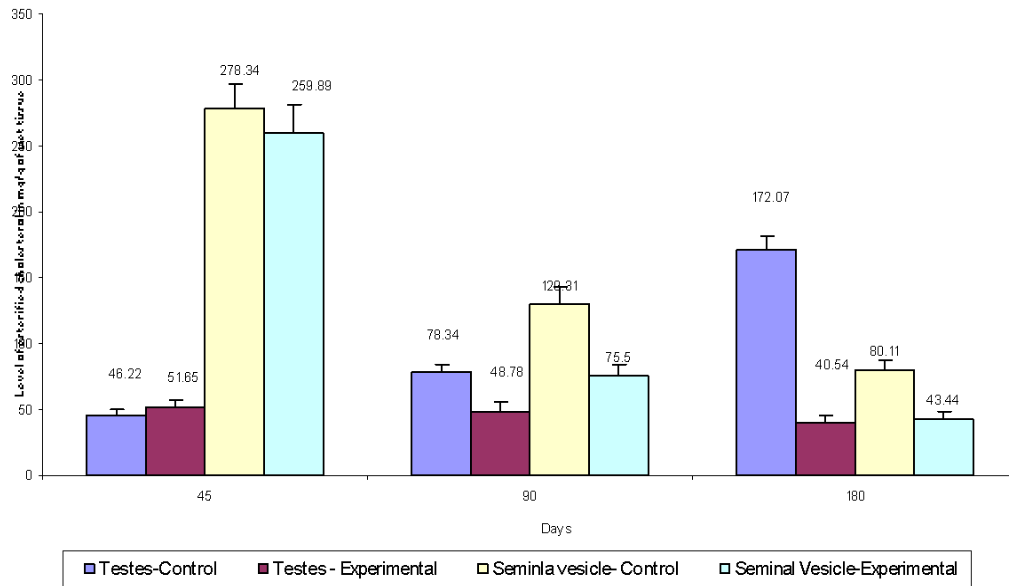
Table 21: Effect of lindane on the level of esterified cholesterol in the testis of catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days (Values are Means ± S.E.M.; µg/100 mg wet tissue wt.; n=5)

	45 days	90 days	180 days
Control	46.22±3.15	78.34±5.40	172.07±9.46
Experimental	51.65±5.89 NS	48.78±6.32*	40.54±5.03**

Table 22: Effect of lindane on the level of esterified cholesterol in the seminal vesicle of catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days (Values are Means ± S.E.M.; µg/100 mg wet tissue wt.; n=5)

	45 days	90 days	180 days
Control	278.34±18.39	129.31±13.90	80.11±7.38
Experimental	259.89±21.61 NS	75.50±8.46*	43.44±5.10*

Figure 22: Effect of lindane on the level of esterified Cholesterol in the testes and seminal vesicle of the catfish *Heteropneustes fossilis*



Testes	Seminal Vesicle
*Significant at *P*< 0.01 (F = 32.51) **Significant at *P*< 0.001 (F = 125.89) NS: Not significant	*Significant at *P*< 0.01 (F = 30.93 and 47.45, respectively) NS: Not significant

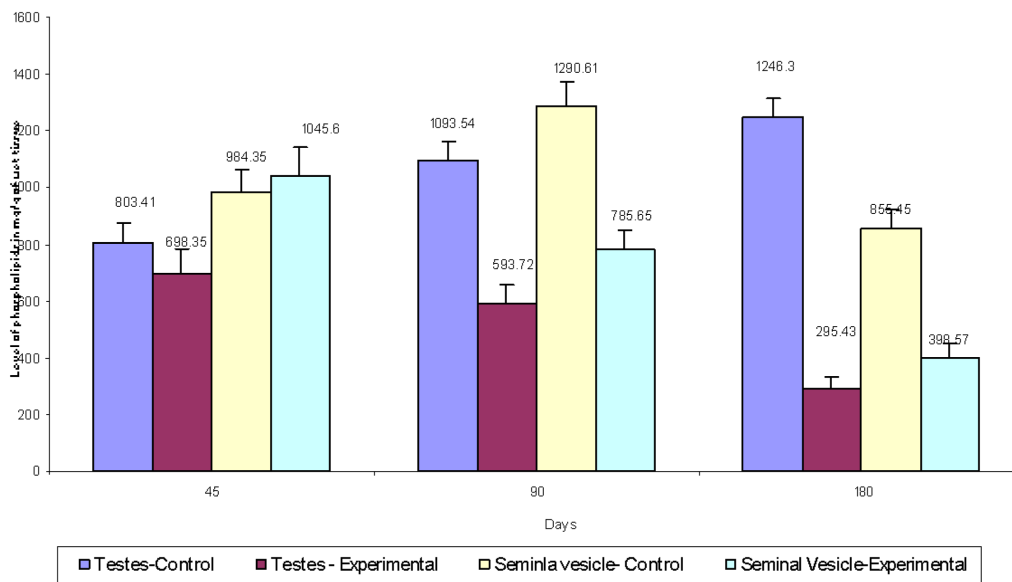
Table 23: Effect of lindane on the level of phospholipids in the testis of catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days (Values are Means ± S.E.M.; µg/100 mg wet tissue wt.; n=5)

	45 days	90 days	180 days
Control	803.41±72.72	1093.54±70.87	1246.30±68.31
Experimental	698.35±85.85 NS	593.72±61.80*	295.43±34.27**

Table 24: Effect of lindane on the level of phospholipids in the seminal vesicle of catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days (Values are Means ± S.E.M.; µg/100 mg wet tissue wt.; n=5)

	45 days	90 days	180 days
Control	984.35±78.30	1290.61±84.50	855.45±69.94
Experimental	1045.60±96.89 NS	785.65±65.90*	398.57±55.04**

Figure 24: Effect of lindane on the level of phospholipids in the testes and seminal vesicle of the catfish *Heteropneustes fossilis*



Testes	Seminal Vesicle
*Significant at *P*< 0.01 (F = 42.78) **Significant at *P*< 0.001 (F = 189.76) NS: Not significant	*Significant at *P*< 0.01 (F = 31.68) **Significant at *P*< 0.001 (F = 115.80) NS: Not significant

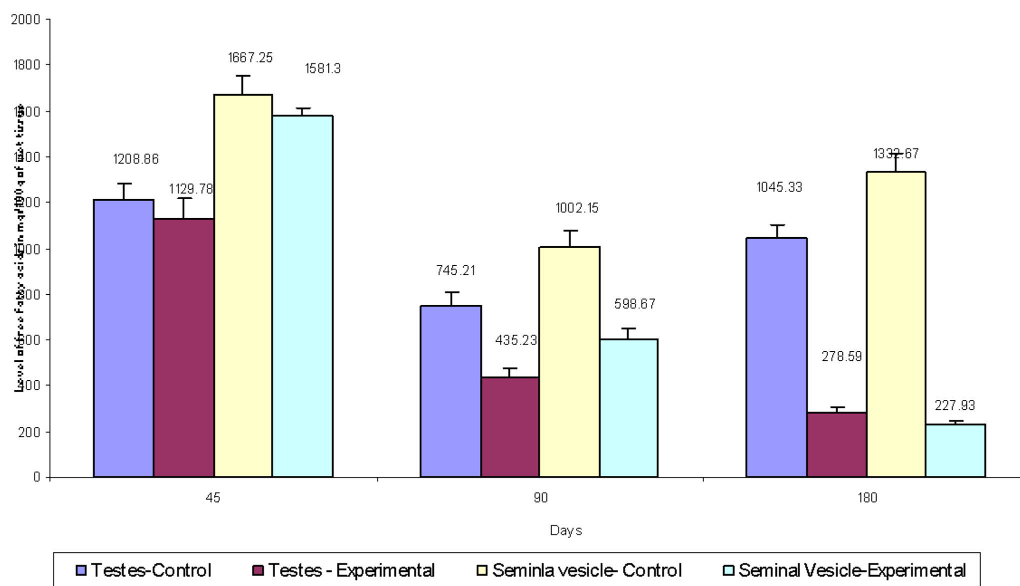
Table 25: Effect of lindane on the level of free fatty acids in the testis of catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days (Values are Means ± S.E.M.; µg/100 mg wet tissue wt.; n=5)

	45 days	90 days	180 days
Control	1208.86±78.34	745.21±64.60	1045.33±56.35
Experimental	1129.78±92.11 NS	435.23±45.38*	278.59±29.75**

Table 26: Effect of lindane on the level of free fatty acids in the seminal vesicle of catfish *Heteropneustes fossilis* after exposure for 45, 90 and 180 days (Values are Means ± S.E.M.; µg/100 mg wet tissue wt.; n=5)

	45 days	90 days	180 days
Control	1667.25±88.64	1002.15±74.40	1332.67±84.89
Experimental	1581.30± NS	598.67±50.03*	227.93±20.08**

Figure 26: Effect of lindane on the level of free fatty acids in the testes and seminal vesicle of the catfish *Heteropneustes fossilis*



Testes	Seminal Vesicle
*Significant at *P*< 0.01 (F = 32.22) **Significant at *P*< 0.001 (F = 129.40) NS: Not significant	*Significant at *P*< 0.01 (F = 28.34) **Significant at *P*< 0.001 (F = 163.57) NS: Not significant

DISCUSSION

Teleosts are known to accumulate high concentrations of pesticides in their tissue and are widely used as bioindicators for pollution in marine and freshwater environments (Casini et al., 1995). They usefully complement chemical analyses as they provide a time-integrated indication of environmental contamination. Biomarkers are measurements at the lower levels of biological complexity (molecular, cellular and tissue levels) that indicate whether the organisms are exposed to pollutants and/or the biological effects of such exposure. Since responses at molecular, cellular and tissue levels precede alterations at population, community or ecosystem levels biomarkers are considered "early warning" tools in environmental quality assessment (McCarthy and Shugart, 1990). A panel of different parameters is in current use in field and laboratory studies including behavioural analyses and/or measurements of biochemical biomarkers.

The present study clearly indicates a decrease in the testicular-somatic and seminal vesicle-somatic indices. Kulshrestha and Arora (1984) have reported reduction in the number of oocytes, reduction in size and deformity in different stages of oocytes, damage to yolk vesicles in maturing and mature oocytes, increase in the number of atretic oocytes, development of interfollicular spaces, dilation of blood vessels and reduction in the gonadosomatic index in *Channa striatus* after sublethal exposure of carbaryl and endosulfan. In an another study, fish exposed to sub-lethal doses of various contaminants, including organophoshphate, carbamate and organochlorine pesticides (endosulfan), ovarian alterations such as smaller diameter of oocytes compared to control fish are reported (Rastogi and Kulshrestha, 1990). Studies conducted in case of males indicated gross abnormality of reproductive system after exposure to chlorinated hydrocarbons (Harding et al. 1999). Although the suppression of vitellogenin synthesis and storage is the

causative factor in the case of females Palmer and Selcer, 1996) in decreased gonadosomatic index, such responsible factors in male fish are yet to be identified. However, an investigation conducted by Dorsey and Tchounwou (2004) clearly indicates the possibility of hepatic involvement in disrupting the routine endocrine events in male fish as well during organochlorine poisoning.

Additionally, in this study, the reduction in the level of total protein was also observed in testis and seminal vesicle after 90 and 180 days exposure to lindane. Similar changes were noticed in relation to the level of free amino acids and protein bound sugars as well in both the tissues examined. The decrease in protein content during the exposure is similar to the decrease reported by Singh et al. (1996) in liver and muscle protein content of the fish, *Heteropneustes fossilis* exposed to pesticide, and in *C. batrachus* exposed to carbofuran by Singh and Sharma (1998). A reduction in protein content of these organs in the exposed fish indicates a physiological adaptability of fish, possibly to compensate to overcome the stress of exposure (Vega et al., 2002). The changes in free amino acids during the pesticide exposure period appeared to be most essential in vital tissues, which are responsible for energy synthesis and other metabolic functions. Similar findings have been reported in *C. batrachus* exposed to trichlorfon (Shobha Rani and Janaiah, 1991). The elevated ammonia concentration observed by the above workers might be accountable towards increased ammoniogenesis that occurs in liver and muscle tissues. Ammonia is a toxic metabolite and excess ammonia is known to trigger the operation of detoxification or utilization systems, chiefly by way of formation of less toxic nitrogenous substances, namely urea and glutamine (Krebs, 1980). Although urea and glutamine were not measured in the present study, this mechanism might have been operative as reported in *C. batrachus*.

The impact of pollutants on growth, survival and fertility of fish is an important concern, yet few studies have investigated the effects of long exposures to certain environmental pollutants on intermediary metabolism and growth (Hontela, 1997). Fish subjected to metals have a reduced condition factor (Kearns and Atchinson, 1979; Munkittrick and Dixon, 1988; Laflamme et al., 2000), and a reduced growth efficiency (Sherwood et al., 2000), the capacity to convert consumed food into body mass. Reduced growth can be mediated through direct effects of pollutants on physiological functions of the fish (Sherwood et al., 2002). In the present investigation, a drastic decrease in the levels of protein bound sugars, total free sugars, glucose and glycogen in testis and seminal vesicle was observed during 90 and 180 days exposures to lindane. However, the plasma cortisol level was elevated from 45 days exposure onwards. Begum (2004) reported a decreased glycogen content in the liver and to some extent in muscle of *Clarias batrachus* by carbofuran.

Previous studies have shown that chronic exposure to metals blunted the normal cortisol stress response (Brodeur et al., 1997; Laflamme et al., 2000) and perturbed carbohydrate metabolism in perch (Sjöbeck et al., 1984). Exposure up to 30 weeks to Cd in the laboratory decreased growth as well as liver glycogen reserves in rainbow trout (Haux and Larsson, 1984; Ricard et al., 1998) and tilapia (Pratap and Wendelaar Bonga, 1990), through increase in the activity of aspartate transaminase (AST) and alanine transaminase (ALT), lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) in *Channa punctatus* (Sastry et al., 1997). Other studies have suggested that such a change is related to enhanced circulating levels of both catecholamines and glucocorticoids (Nakano and Tomlinson, 1967). Thus, the marked glycogenolysis observed after acute exposure to pesticide in the present study was most likely due to stress-induced increase in circulating catecholamines and glucocorticoids. Alanine and aspartate transaminase activities are used as sensitive markers in experimental insecticide intoxication in teleost fish (Beyer et al., 1996). Similar increases in ALT and AST activities reported in liver and muscle tissues of *C. batrachus* after short-term exposure to dimethoate (Begum and Vijayaraghavan, 1995, 1996) add support to the present observations. Enhanced activity of the transaminases provides the oxaloacetic acid, pyruvate, α-ketoglutarate and glutaric acid to meet the increased energy demand during pesticide imposed stress conditions. The oxaloacetic acid, pyruvate and α-ketoglutarate might have been channeled into the citric acid cycle. The glutamic acid formed from transamination may be

subsequently deaminated leading to the formation of ammonia (Bidigare and King, 1981).

An enzyme system is present to bring out the rapid mobilization of glycogen for energy release. The key step in this process is the production of glucose-1-phosphate from glycogen by the action of glycogen phosphorylase (Morgan and Parmeggiani, 1964). The increased phosphorylase-a activity in liver and muscle tissues of *C. batrachus* exposed to carbofuran confirms the active breakdown of tissue glycogen, apparently for metabolic processes to meet the increased energy demand imposed by pesticide intoxication (Begum and Vijayaraghavan, 1995, 1996). Thus, this study supports the hypothesis that sublethal exposures to pesticides impair testicular maturation of teleost fish through a perturbation of intermediary metabolism. The metabolic status, specifically liver reserves of glycogen and triglycerides, and enzyme activities might have been accounted for such a change in the intermediary metabolism. Fish use glycogen for immediate energy requirements during acute stress (Lowe-Jinde and Niimi, 1984; Vijayan and Moon, 1992), and they maintain their liver glycogen reserves by mobilizing other energy stores such as lipids and proteins (Sheridan and Mommsen, 1991). Fish with the highest tissue burdens of pesticides might not have the capacity to increase their plasma glucose concentrations following an acute stress, because of low glycogen reserves. However, the increased level of plasma cortisol level observed in this study reflects this case by its specific role in overcoming any stressful event since the exposure of the pesticide. This difference in the glycogen reserve-cycling, and probably in related metabolic parameters, may be linked through a disrupted condition to use energy, to the lowest condition factor and to the suppression of testicular and seminal vesicle recrudescence as observed through low somatic indices.

Several significant correlations were shown between hepatic or muscular glycogen and pesticides concentrations in the three fish species. They were generally positive with muscle glycogen and can be negative with liver glycogen, in particular in the crucian carps (bile fluoranthene) and the black bullheads (liver PCBs and bile fluoranthene). The variability of such energy parameters is largely demonstrated. Thus, the increase of the glycogen contents proves to be a significant marker of the organic contamination in the three species (Roche et al., 2002). Nevertheless, these observations are contradicting those classically described. Regarding glycogen concentration, a number of contradictory results have been described in ecotoxicological studies. Acute intoxication may lead to a glycogen depletion (Gimeno et al., 1995; Sancho et al., 1998; Strmac and Braunbeck, 1999; Walter et al., 2000) as a consequence of feed refusal and a decrease in gluconeogenesis, often dose-dependent, due to a diminished activity of key gluconeogenic enzymes (Feeley, 1995; Viluksela et al., 1999). Moreover, Braunbeck and Appelbaum (1999) described glycogen and lipid depletion and observed gut and liver ultrastructural changes at extremely low doses of endosulfan in carp. On the contrary, Thomas et al. (1999) have shown a lack of such physiological response after PAHs exposure. They suggest that chronically exposed organisms may develop a physiological tolerance to these pollutants. In the same way, Oruc and Uner (1998) concluded that in *Cyprinus carpio*, an elevation of glycogenolysis occurs following an acute chemical stress and compensatory mechanisms are developed during chronic exposure.The positive correlation between glycogen content and concentration of organic pollutants tends to show a coexistence of a pathological alteration and a general stimulation of hepatic metabolism in fishes.

These results suggest that fish challenged by environmental pollution may have a higher turnover of glucose and more glucose may be produced from non-carbohydrate substrates and used only to a limited extent. Further evidence for increased energetic costs and altered intermediary metabolism in fish during intoxication with pesticide was evaluated by estimating the levels of various classes of lipids in testis and seminal vesicle. This study also clearly demonstrates decreased level of total lipids, total cholesterol, free cholesterol, esterified cholesterol, phospholipids and free fatty acids after exposure of catfish to lindane for 45 and 90 days. Further, it also suggests that the energetic costs of detoxification processes might have an impact on fish gonadal maturation through alterations of intermediary metabolism.

Fish biomarkers for monitoring the anthropogenic chemicals are frequently used (Stein et al., 1993;

Strandberg et al., 1998). The effects of organic xenobiotics on fish have been extensively documented. A number of reports have brought evidence that contamination with lipophilic pollutants triggers many biochemical responses, like disturbance of endocrine functions, activation of metabolization systems or more generally, disorder of the normal physiology (Stein et al., 1993; Burgeot et al., 1996; van der Oost et al., 1996, 1997). However, the difficulty to select appropriate biological indicators is related to the chemical complexity of the potential contaminants and the nature of target organisms. Organic xenobiotic toxicity involves interactions among several metabolites operating by distinct mechanisms translating into a vast array of biological disorders.

Due to the nature of the contamination, previous investigations about validation of biomarkers were focused on numerous biological indicators including the detoxification mechanisms (biotransformation, antioxidant process), energy requirements, but also some non-specific metabolic processes (Roche et al., 2002). It has been shown that three hepatic activities involved in the protection against oxyradicals: catalase; glutathion peroxidase and superoxide dismutases; muscle and gill ATPases as well as muscle and brain acetylcholinesterase are more significant in term of biomarkers than the biotransformation enzymes ethoxyresorufine-*O*-deethylase and uridine diphosphate glucosyl transferase. Moreover, it is presumed that glycogen and protein tissue rates could also be sensitive biomarkers. Consequently, many studies have been conducted to assess the responses of energetic markers (glycogen, protein and lipids) after exposing to environmental contaminants (Roche et al., 2002).

The levels of energy metabolic reserves differed largely from one species to another, moreover, they vary according to season. No statistical correlation was detected between the neutral lipid contents and contaminant concentrations among the eels investigated, in spite of their muscle being particularly fatty. On the other hand, Roche et al. (2002) found a preferential accumulation in hepatic membrane structures through a correlation analysis for lindane and PCBs in the crucian carps and black bullheads. Nevertheless, in the latter non-fatty fishes, the rates of some lipophilic substances (liver γ-BHC and muscle PCBs) were also strongly correlated with the contents of neutral lipids. This observation suggests the role of lipid quality and composition (i.e. relative amount of fatty acids saturation) in the tendency of organochlorines to bioaccumulate and thereby involve in lipid metabolism (Ewald and Larsson, 1994).

Further evidence for increased energetic costs and altered intermediary metabolism in fish from polluted environments was provided by estimating lipid metabolism (Levesque et al., 2002). Whether fish rely on muscle protein instead and whether this underlies, at least in part, remains to be investigated after organochlorine exposure. In the earlier study, the activity of three enzymes involved in lipid metabolism, glucose 6-phosphate dehydrogenase (G6PDH), malic enzyme (ME) and triglyceride lipase (TGL), were measured after exposing to pollutants (Levesque et al., 2002). Both G6PDH and ME use NADP as coenzyme, generating NADPH, which is used for lipid synthesis. In fish sampled in the summer in the contaminated lakes, the activities of ME and G6PDH were lower than in fish from the reference lakes, indicating that less NADPH was produced. This could significantly compromise lipogenesis. There were no differences in the activity of these two enzymes among lakes in the fall. Importantly, the activity of G6PDH and ME in fish from the reference lakes exhibited a significant seasonal variation, being higher in the summer than in the fall, whereas activities remained low during both seasons in fish from the contaminated lakes. Similar variations were noticed in various classes of lipids in the control fish itself indicating their prominent role during testicular developmental processes. Concentration of liver TG lipase, an enzyme that hydrolyses triglyceride reserves, was enhanced and liver triglyceride reserves were significantly lower in the fall in fish from the most contaminated lake (Levesque et al., 2002). Plasma FFA levels were also lower in fish from the two most contaminated lakes, suggesting a significantly enhanced utilization of lipid reserves and FFA in fish challenged by chronic exposure to metals. Since the triglyceride reserves were higher in the summer compared with the fall in the reference lake, it appears that fish from the contaminated lakes may be unable to increase their lipid reserves in the summer whereas the fish from reference lakes can do so. This metabolic alteration

may, as has been postulated for glycogen reserves, contribute to the delayed gonadal recrudescence through altered lipogenesis after lindane exposure.

Seminal vesicles are accessory glands present in the male reproductive system of teleosts belonging to Gobiidae, Siluridae and Blennidae (van Tienhoven, 1983; vanden Hurk et al., 1987; Patzner, 1991; Singh and Joy, 1999. In catfish, the reproductive system consists of one to many pairs of SV lobes, which arise posterior to the testes laterally on the sperm duct. In parallel with the maturity of testis, the SV epithelial cellssecrete a mucopolysaccharide-protein–lipid-rich fluid called seminal vesicle fluid (SVF), whose content increases gradually and reaches the peak level in spawning phase (Nayyar and Sundararaj, 1970; van den Hurk et al., 1987; Singh and Joy, 1999). Different functions have been reported for the SVF such as production of sialomucins which help to attach eggs to the sea grass, concentrate spermatozoa and promote fertilization as in grass goby (Lahnsteiner et al., 1992a), secretion of nutrients, steroids, enzymes, and ions, enhancing sperm quality and fertilization of eggs, pheromonal functions, etc. as in gobies and catfish (van den Hurk et al., 1987; van den Hurk and Resink, 1992; Lahnsteiner et al., 1992a; Singh and Joy, 1998, 1999). Analysis of testicular fluid produced by Sertoli cells and epithelial cells of testicular main ducts and seminal fluid produced by the spermatic duct epithelium of teleosts demonstrates considerable intra- and inter-species variability in the physical and chemical composition (Stoss, 1983; Kruger et al., 1984; Linhart et al., 1991; Suquet et al., 1993; Lahnsteiner et al., 1995, 1996; Billard et al., 1996; Wang and Crim, 1997).

The high levels of proteins and hexosamines indicate the presence of mucosubstances as has been shown by earlier workers in both SV and testicular glands (Nayyar and Sundararaj,1970; Lahnsteiner and Patzner, 1990; Lahnsteiner et al., 1992a; Singh and Joy, 1998). Histochemical and qualitative biochemical studies have also demonstrated the presence of organic constituents such as mucoproteins, glycoproteins, acid mucopolysaccharides, phospholipids, proteases, creatinine, organic and inorganic phosphatases in *Heteropneustes fossilis* (Nayyar and Sundararaj, 1970) and *C. gariepinus* (van den Hurk et al., 1987). The organic components of the SVF not only enhance the osmotic concentration, but also may serve as nutrients fructose, glucose, hexosamines and lipids (Scott and Baynes, 1980; Stoss, 1983; Lahnsteiner et al., 1991, 1992b, 1994). The present study clearly shows a sharp decrease in the levels of fructose, hexosamine and sialic acid in testis and seminal vesicle after exposure to lindane for 90 and 180 days.

Although there is no similar studies available in fish, an inhibition of somatic growth and accessory sex organs weights, elevated pituitary and serum prolactin levels, and a suppression of testicular Leydig cell functions has been reported after exposure to methoxychlor at doses as low as 25 mg/kg body weight/day (Gray et al., 1989). In another study the effect of methoxychlor on the epididymis was studied by administering higher doses of methoxychlor (50, 100, and 200 mg/kg body weight/day) for 1, 4, and 7 days in order to identify an epididymal specific effect (Latchoumycandane et al., 2002). The body weight of animals treated with methoxychlor did not show any significant change, indicating that the general condition of the animals was normal. Testis weight did not change significantly at any dose; however, administration of methoxychlor at all doses caused a reduction in the weights of the epididymis, seminal vesicles, and ventral prostate. Neverrtheless, Gray et al. (1999) reported that administration of methoxychlor at doses ranging from 200 to 400 mg/kg/day for 11 months altered the body weight and weights of the liver and kidney. The shorter period of administration given by Latchoumycandane et al. (2002) in their study may probably account for the lack of observed effect on the organ weights.

There is now accumulating evidence indicating that organochlorine pesticides can compromise the integrity of the male reproductive system of fish, reptiles and mammals (Colborn et al., 1993; Guillette et al., 1994 and Kavlock, et al., 1998). Additionally, studies have shown that exposure to low levels of organochlorine residues can result in physiological disturbances and reproductive disruption in humans and other animals (Burow et al. 1999; Beard et al., 1997, 1999; Colosio et al., 1993; Daniel et al., 1995; Miller,1998; Peper et al., 1999; Rawlings et al., 1998). Organochlorine chemicals, including DDT, polychlorinated biphenyls (PCBs), dioxins, lindane, hexachlorobenzene, and pentachlorophenol (PCP) are generally cited as

potential endocrine-disrupting substances (US EPA, 1997; Danzo, 1997; Illinois EPA, 1997). Endocrine-disrupting mechanisms of these chemicals are ardently linked to mimicking hormonal estrogens (Burow et al. 1999; Danzo, 1997; Fry, 1995). Furthermore, chlorinated compounds, that mimic hormones, have the ability to induce proliferate responses (Burow et al. 1999). Exposure to organochlorine pesticides in the aquatic environment has resulted in towering occurrences of vitellogenin (Vtg) synthesis in species of male fish (Sumpter et al., 1995; Chritiansen et al., 1998; Hennies, 2003). Furthermore, the induction of Vtg in male fish exposed to environmental contaminants has become an attractive indicator of potential estrogenic potency (Sumpter et al., 1995; Wallace, 1985; Palmer and Palmer, 1985; Palmer and Palmer, 1995).

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