Toxicity of organochlorine pesticide, Lindane to fish: A review

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ABSTRACT

Lindane, an organochlorine pesticide, has been widely used in public health and agriculture in several countries including India. Lindane has long been associated with pollution due to its long persistence and quick accumulation in fatty tissues. It is considered a possible carcinogen, mutagen, teratogen, immunotoxin, and neurotoxin in mammals. However, data on toxicity of lindane to fish is inadequate to establish the mode of action of this pesticide in this group of vertebrate. The present study thus aims to review the works of toxic effects of lindane to fish.

Key words: Organochlorine, lindane, toxic, acute, chronic

INTRODUCTION

Lindane is an organochlorine pesticide that has been widely used in public health and agriculture production in developed and developing countries including India. In India, the first BHC technical plant was set up at Rishra, near Kolkata, in 1952 [1]. The use of the insecticide hexachlorocyclohexane (HCH), commercially available as lindane, increased greatly after DDT became legally restricted in many countries [2]. Lindane has been reported to be toxic, carcinogenic, endocrine disrupter and recalcitrant chlorinated insecticide widely used in developing countries in agriculture and public health. Lindane has been long used and has been associated with serious soil pollution. Lindane also has long-term effects on human health, including anemia as well as liver, testicular, bone marrow, and kidney damage [3]. In India, lindane formulations are registered for use in pharmaceutical products for control of head lice and scabies on people [4]. It is also registered for use to control fly, flea, cockroach, mosquito, bed bug, and beetle populations. In agriculture, it is registered for use to control pests in cotton, sugarcane, pumpkin, cabbage, onion, apple, walnut, maize, okhra, potato, tomato, cauliflower, radish, cucumber and beans. Lindane is commonly used on a wide variety of crops, in warehouses, and in public health to control some diseases brought about by insects. Other applications are in the manufacture of lotions and shampoos for the control of lice and mites in humans. Also, it may be found in formulations of fungicides. It is available as a suspension, emulsifiable concentrate, powder, and ultra low volume liquid [5]. The US Environmental Protection Agency has prohibited lindane products for direct application in aquatic environments. Lindane has been declared restricted for use in India [6]. Lindane is highly toxic to fish which absorbs it directly from water or by ingesting contaminated food and bioaccumulate in their fatty acids at ratios of 500:1200 due to its lipophilic nature [7]. Fish have been used extensively for monitoring purposes because they concentrate pollutants in their tissues, which are directly absorbed from water and also through their diet, reflecting the level of pollution in the aquatic environment [8]. Additionally, fish occupy different habitats in the ecosystem, having different feeding behaviours, thus offering the potential to study the influence of both environmental and biological factors on the bioaccumulation of pollutants [9]. It should also be considered that different organs of fish react in different ways or with different intensity to the presence of a toxic substance [10]. Being an important part of food chain, fish are at great risk of lindane exposure. The present study, thus, aims at reviewing the works on toxic effects of lindane in fish.
Structure of lindane
Lindane (Fig) is a colourless solid with a faint musty odour. Lindane belongs to the chemical family of chlorinated hydrocarbons, commonly known as gamma-HCH, gamma-BHC, gamma-benzene hexa chloride. Lindane is the gamma isomer of hexachlorocyclohexane. There are eight stereoisomers of hexachlorocyclohexane, of which only lindane has significant insecticidal activity. However, technical mixtures of all isomers have been widely used as commercial insecticides. The hexachlorocyclohexanes are hydrophobic and several isomers readily volatilize, thus they have become globally distributed and bioaccumulate in fatty tissues of animals [11]. Lindane has a low vapour pressure and solubility in water and dissolves in most organic solvents. Lindane is stable to light, air, heat and acids. Lindane is slightly soluble in water (10 ppm at 20°C) and in most organic solvents, including acetone and aromatic and chlorinated hydrocarbons. Lindane is stable to light, heat, air, and strong acids, but decomposes in alkali solutions to trichlorobenzenes and HCl. The minimum content of Lindane for commercial use is usually equal or above 99.5 % [12]. Lindane is very stable in both fresh- and salt-water environments, and it is resistant to photodegradation [13]. It disappears from the water by secondary mechanisms such as adsorption and absorption by sediment, flora and fauna, in the case of fishes through gills, skin, and food [14].

Acute toxicity of lindane
The acute toxicity of Lindane to African Catfish (Clarias gariepinus) juveniles was assessed in a static renewal bioassay for 96 h to examine the effects of this pesticide and ascertain their level of tolerance and their suitability as bio-indicator in freshwater ecosystems. The 24, 48, 72 and 96 h median lethal concentration (LC₅₀) of lindane to Clarias gariepinus are 1.45, 1.38, 1.32 and 1.29 ppm, respectively. The median lethal time (LT₅₀) of 0.9, 1.0 and 1.1 ppm was zero while the LT₅₀ of 1.2, 1.3 and 1.4 ppm are ~73 h, ~59 h and ~48 h, respectively. The minimum lethal concentration was 1.40 ppm while the minimum lethal time was ~73 h [15]. The 96-h LC₅₀ value for the same fish (C. gariepinus) was found to be much lower at 0.38 mg/L in another study [16]. This shows that the differences in LC₅₀ value of lindane to different fish species depends on many factors like age, body weight, diet and other hydro biological factors of water like temperature, salinity, pH, dissolved oxygen etc., [17]. In another study, 24 and 48 h LC₅₀ value was 0.616 mg/L and 0.122 mg/L, respectively, in Sparus aurata [18], while, the LC₅₀ value of lindane for Etroplus maculatus was found to be 0.028 mg/L [19]. In contrast, the 96 h LC₅₀ of lindane to Jenynsia multidentata was found to be 119 mg/L and 98 mg/L for Corydorus paleatus [20]. Acute toxicity of lindane on Colisa lalia was studied by Ramalingam et al, [21]. In another investigation, 24 h LC₅₀ value of lindane to the fingerlings of Cyprinus carpio was found to be 0.38 mg/L indicating that lindane is toxic to fish, even at a low dose [22]. In guppy exposed to β-HCH for 48 hours the LC50 was 0.9 mg/L [23]. In a study [24], 48-h median lethal concentration of γ-HCH in 14 fish species, belonging to 6 families, ranged from 22 to 900 µg/L. Thus, lindane shows wide range of toxicity, in terms of median lethal concentrations.

Behavioural changes due to lindane
Fish exposed to lindane showed abnormal behaviour responses like erratic swimming, faster opercular activity, hyper excitability, loss of buoyancy and balance etc. Some of the early symptoms of lindane poisoning observed in this study are respiratory distress, increased physical activity, convulsions, difficulty in breathing, erratic swimming behaviour, swimming on lateral and ventral side and occasional darting up and down the water column [15]. These behavioural signs were reported in study of the acute toxicity of lindane to Clarias gariepinus [25]. The high toxicity and mortality of fish during acute treatment might have resulted from the neurotoxic behaviour of this organochlorine insecticide. When fishes were exposed to lindane the fish showed abnormal behaviour responses like erratic swimming, faster opercular activity, hyper excitability, loss of buoyancy and balance etc. The effects of sub-lethal concentrations of lindane on behavioural in Etroplus maculatus were investigated [19]. The frequency of observed behaviours had the same levels of significance throughout the period of study. There was a reduction in the frequency of occurrence between test concentrations, but remained significantly higher than the control. The study shows that lindane is harmful to E. maculatus at sub-lethal concentrations and that the application of this pesticide
close to bodies of water is a dangerous threat to aquatic life [19]. Behavioural manifestations in larvae of Sparus aurata exposed to lindane included weak swimming, incapacity to respond to external stimuli, uncoordinated movements, trembling, myoskeletal defects, opaque skin and exophthalmia [18]. In another study, acute bioassay tests were conducted with lindane on Colisa lalia. The fish were exposed to two selected concentrations viz., 0.1 and 0.37 mg 1\(^{-1}\) for 96 and 6 h respectively and their behaviour and bimodal respiration were studied. Erratic behavioural responses were noticed at 0.37 mg/L whereas the response at 0.1 mg/L were orderly and adaptive. Total oxygen consumption of fish increased but the dependence on the type of respiration differed in the two concentrations, and this selective dependence at 0.1 mg 1\(^{-1}\) is suggested to have survival value [21]. The β-HCH caused behavioural effect, loss of buoyancy and balance as well as uncoordinated movements below 56 µg/L to fertilized eggs and 32 µg/L to 1 month old medaka [26]. Bimodal [aquatic (V\(_{A}\)) and aerial (V\(_{A2}\))] oxygen consumption of the air-breathing fish, Anabas testudineus exposed to 0.075 and 0.59 mg 1\(^{-1}\) lindane was measured for 120 and 6 h respectively. In the controls 67.9% of O\(_2\) was obtained from air (V\(_{A2}\)) whereas only 32.1% was obtained from water (V\(_{A}\)) indicating that A. testudineus predominantly relies on aerial gas exchange. The fish held in both the concentrations of lindane showed a consistent increase in V\(_{A2}\), V\(_{A}\) and (V + V\(_{A2}\)) (total O\(_2\) consumption) in most of the periods of exposure; but the increases observed in mean O\(_2\) consumption at higher concentration were relatively lower than those in lower concentration. In 0.075 mg/L lindane, the stimulation seen in aerial respiration was more than that in aquatic respiration up to 24 h. The oxygen consumption returned to normal at 96 h in the lower concentration of lindane [27].

**Effects of lindane on Haematological parameters**

Blood is an excellent indicator of toxic stress and analysis of hematological parameters in fish are widely used to assess the toxic stress and functional status of the animal health [28]. Reduction in haemoglobin (Hb) and hematocrit (Hct) content in toxicant treated fish may be due to disorders in haemopoietic processes and accelerated disintegration of RBC membranes [29]. Lindane also causes negative effects in fish like anaemia, inhibition of ATPase activity and alterations in nervous functions [30]. The impact of acute and sublethal toxicity of lindane on some haematological parameters of a freshwater fish *Cyprinus carpio* was estimated [22]. During acute treatment (24 h), Hb, Hct and erythrocyte (RBC) values were decreased, whereas leucocyte (WBC) count increased in the pesticide treated fish. The hematological indices like mean cellular volume (MCV), mean cellular hemoglobin (MCH) and mean cellular hemoglobin concentration (MCHC) were decreased when compared to control group. In sublethal treatment (1/10th of LC50 24 h value, 0.038 ppm), RBC count was decreased whereas WBC count increased in the pesticide treated fish throughout the study period of exposure (25 days). Hb and Hct values were decreased up to 10th day and after that recovered showing a significant increase in the rest of the study period. Similarly, a biphasic response was observed in the value of MCV, MCH and MCHC. Plasma glucose level was significantly increased while plasma protein level decreased throughout the study period. In *Etroplus maculates*, lindane exposure showed marked changes in haematological parameters. The RBC, Hb, Hct, MCH, MCV and MCHC were significantly reduced in fish exposed to the toxicant concentrations compared to the control groups. However, the WBC was observed to be significantly higher [19].

**Effects of lindane on immune system**

In fish, it has been shown that exposure to environmental contaminants may lead to an increase in disease susceptibility and mortality due to immune suppression [31]. Lindane exposure either increased [32-35] or decreased antibody-secreting cells, lymphocyte mitogenesis, the number of NBTP cells, myeloperoxidase and lysozyme activity in vivo in rainbow trout, Oncorhynchus mykiss [36, 37, 38] but leaves unaffected the immune response in common carp [39]. Lindane decreases leucocyte counts in several haematopoietic tissues of tilapia but does not affect phagocytic functions such as phagocytosis and respiratory burst [40]. In rainbow trout, intraperitoneal injection of lindane (10-100 mg/kg bw) greatly depressed the number of antibody-secreting cells, serum lysozyme levels, respiratory burst activity and myeloperoxidase (contributes together with ROS and RNI to pathogen killing), proliferating capacity of B cells, but not of T cells, and its percentage in the head-kidney but at the same time increased the plasmatic ceruloplasmin, an acute phase protein [37,38]. The same group also demonstrated that oral administration of lindane (1mg/kg) for 30 days significantly decreased the respiratory burst activity of head-kidney leucocytes but unaffected the lymphocyte proliferation and number of circulating B lymphocytes in a similar way to the previous data in carp [38, 39]. Moreover, they have also demonstrated that these negative effects can be reversed by the in vitro addition of nitrogranulogen [36] or dietary intake of vitamin C [41]. Dietary intake of lindane (10-1000 ppm) failed to affect the spleen weight, serum and mucus antibody levels and phagocytosis in the common carp though most of the tissues reflected great contamination [42, 39]. Lindane bath of Nile tilapia also reduced the counts of circulating leucocytes, phagocytic activity and antibody levels [43]. Phagocytic cells are the main actors of the fish immune system. They secrete reactive oxygen species (ROS) involved in their bactericidal activity. In vitro, lindane (2.5-100 µM) treatment was able to increase ROS production in rainbow trout head-kidney phagocytes and MAF (macrophage activating factors) production by peripheral blood leucocytes, in both cases depending on the dose and with contradictory results [32, 34, 35]. These studies also demonstrated that low lindane concentrations
increase the cytoplasmatic cAMP but high doses increase the intracellular Ca²⁺, and these two factors contribute to the dual effects of induction/reduction of the leucocyte immune functions produced by lindane treatment in leucocytes [32, 34, 35]. In gilthead seabream, head kidney leucocyte incubation with lindane failed to significantly change the leucocyte viability (by necrosis and apoptosis) and innate cellular immune functions (phagocytosis, respiratory burst and cell-mediated cytotoxicity) but strikingly increased the expression of many immune-related genes (IL-1beta, TNFalpHa, MHCalpha, MHCIIalpha, Mx, TLR9, IgML and TCRApha) [44]. In order to assess the effect of lindane exposure on gene expression (specific for immune response) in tilapia (Oreochromis niloticus), fish were individually weighed and injected intraperitoneally with a single dose of 19.09 mg/kg bw lindane. The differential display (DD) technique was then used to identify differentially expressed cDNA fragments between treatment and control fish. Results showed that lindane exposure triggered the differential expression of these genes during the first 6, 18 and 24 h subsequent to treatment application, suggesting that lindane exposure can trigger a rapid immune system response in tilapias [45].

Effects of lindane on Biochemical profiles

Biochemical parameters of a freshwater fish Cyprinus carpio was studied by [22]. Fish exposed to lindane showed a significant increase in plasma glucose level, however, plasma protein level was found to be lower throughout the study period. The glycogen content in the muscle of lindane treated fish was decreased up to 15th day showing a percent decrease of 31.74, 80.84 and 86.88, respectively. After 15th day, it was increased up to the end. On the other hand, glycogen content in liver decreased up to 10th day after that it was gradually increased from day 15 to day 25 [22]. The effects of an acute lethal (0.59 mg/L) and a sublethal (0.075 mg/L) concentration of lindane on the levels of glycogen (liver and muscle), glucose (blood), and lactic acid (liver, muscle, and blood) of a freshwater fish, Anabas testudineus (Bloch) were determined. A marked decrease in the liver glycogen and significant increase in blood glucose and muscle glycogen at 1 hr of exposure to 0.075 mg/L lindane was noted. These results indicate extensive utilization of energy stores in the liver and simultaneous build up of fuel reserves in the muscle. After 3 hr of exposure, glycogenesis possibly becomes operative in liver through the Cori cycle. The activity of the cycle was observed in the fish in all the subsequent periods of exposure studied. At 120 hr, the decreases observed in muscle and liver glycogen indicate near exhaustion of glycogen stores. Though changes in glycogen content of both muscle and liver were similar in 0.59 mg/L lindane, they have less adaptive value since mortality of fish was significantly high at all three periods studied [46]. Measurement of carbohydrate metabolites in fish for 6 hr or longer could prove useful as a rapid method for evaluating the toxicity of pesticides and other toxicants [47]. Exposure of the European eel (Anguilla anguilla) to a high sublethal concentration of 0.335 ppm (0.50 of the 96-hr LC₅₀) of lindane for 6, 12, 24, 48, 72, and 96 hr affected carbohydrate metabolism. Muscle glycogen levels decreased significantly at 6, 12, 24, 48, 72, and 96 hr; liver glycogen content did not decline at any time. Muscle glucose levels in fish were elevated at 6, 12, 24, 48, 72, and 96 hr but in liver, the levels increased only at 96 hr. Mean values of muscle and liver pyruvate were elevated significantly at 6, 12, 24, 48 and 72 hr. Muscle lactate levels increased at 6, 12, 24, and 48 hr in pesticide-treated fish. Liver lactate levels were elevated only at 12, 24, and 48 hr of treatment. The observed effects of lindane on carbohydrate metabolism in fish are discussed in relation to acute stress syndrome [30].

A significant positive linear relationship was found between lipid content (% of wet weight) and the 48-h LC₅₀ of γ-HCH in fish species, revealing that the toxicity of γ-HCH in various fish species decrease with increasing total lipid content. If median lethal concentrations are normalized for 1% lipid content, then the range of 48-h LC₅₀ is reduced to between 18 and 32 µg/L. It is concluded that lipids of aquatic organisms can serve (among other functions) as a protective storage site against the toxic effects of γ-HCH and, possibly, of other lipophilic, persistent organic chemicals which are bioconcentrated in body lipids. Therefore, in organisms with higher lipid content, a smaller fraction of a lipophilic chemical will reach target organs (liver, lung, central and peripheral nerves, etc.) to cause adverse effects. Results suggest that this correlation can be used to extrapolate the acute toxicity (48-h LC₅₀) of γ-HCH to other fish species if their lipid content is known. These biomarkers are useful in the environmental risk assessment of freshwater and marine organisms [24].

Effects of lindane on Nervous system

Though the mechanism of action of lindane is yet to be clearly established, studies suggest that γ-aminobutyric acid (GABA) receptor ionophore complex is the primary target for lindane [47]. Lindane, a potent stimulant of the central nervous system upon entering into fish body brings out several physiological alterations [48]. Adult rainbow trout Oncorhynhus mykiss were exposed to 0.05 mg/l and 0.1 mg/L of lindane (γ-HCH) for two hours. The response was determined by measuring serotonin (5-HT) and 5-hydroxy indoleacetic acid (5-HIAA) levels in five brain regions (hypothalamus, medulla oblongata, telencephalon, tectum optic and remaining portion) and brain, seric and hepatic tryptophan concentrations. While hepatic tryptophan decreased significantly with both dose, significant increased levels of seric tryptophan appeared with the higher dosage. Tryptophan levels did not show significant variations in brain regions. Brain 5-HT increased significantly in hypothalamus but decreased in tectum optic. The
highest ratio 5-HT/5-HIAA appeared in hypothalamus and the lowest in telencephalon. This study demonstrates that the effect of lindane on serotoninergic metabolism is specific of determined brain structures [49].

Impact of lindane on Enzyme activity
In a study on *Carassius auratus gibelio*, effects of lindane on antioxidant defense enzymes, Catalase (CAT), Glutathione reductase (GR), Glucose-6-phosphate dehydrogenase (G6PD), Glutathione peroxidase (GPx) and Glutathione S-transferase (GST) were analysed [50]. The specific activity of CAT after 96h exposure of lindane was higher in liver, kidney and gill of *Carassius auratus gibelio*. In the same fish, the specific activity of GR decreased in liver, kidney and gill while the specific activity of G6PD increased in liver and kidney. The Specific activity of GPx decreased in liver and increased in kidney and gill and GST activity decreased in all the tissues studied [50]. Lindane is considered substrates for GST [51]. A study has been done on the effect of lindane on the activity of GST in different organs of fishes, *Jenynsia multidentata* and *Corydoras paleatus*. Exposure of *Jenynsia multidentata* above 6 mg/L caused activation a GST in liver and gills, followed by inhibition at 75 mg/L. *Corydoras paleatus* exposed to 6.0 mg/L lindane did not present significant changes in GST activity; however, enzymatic inhibition was observed above 25 mg/L [20].

Effects of lindane on Reproductive system
Literature survey demonstrated that the exposure of technical grades of γ-HCH at sublethal concentrations during pre-spawning phase increased significantly tissue bioconcentration and also decreased GSI and plasma levels of estradiol-17β thereby affected the reproductive physiology of *Heteropneustes fossilis*. The γ-HCH have varied effects in tissue bioconcentration for its isomers/metabolites which might have caused the disturbances at the receptor sites during pre-spawning phase in this species as reported by other authors [52]. The percentage of tissue bioconcentration of γ-HCH isomer was detected more frequently in this species. It seems quite possible that α-HCH was isomerized to γ-isomer which has the insecticidal property. This transformation could have been caused by bacterial activity and ultraviolet radiation in the water column and ultimately sink to the sediments and get bioconcentrated in fish tissues. The decrease in testosteronel and estradiol-17β sex steroid hormone production has also been reported by γ-HCH exposure as well as in vitro study in the catfish [53]. Estrogenic action were also found at 32 to 1000 μg/L of β-HCH which induced formation of phosphoproteins (vitellogenin) in guppy and medaka [54, 55].

Effects of lindane at Histopathological level
The effects of lindane on the liver of a teleost fish *Catla catla* were investigated after exposure to 1.2% lindane for 30 days. The severity of the hepatocytic alterations was prominent and these changes in hepatocyte ultrastructure could have a wider relevance for ecotoxicology, as they are correlated with the survival capacity of the fish [56]. Similar study was done to understand the effect of lindane in the kidney of *Catla catla*. The fish exposed to this pesticide, for 15 and 30 days, showed various causes of renal dysfunction in the fish [57]. In *Etroplus maculatus* lindane exposure had profound destructive effects on the gills, liver and kidney of the fish. The gills showed proliferation of the lamellar epithelium and lamellar fusion, the liver showed necrosis and the kidneys had constriction of the tubular lumen [19]. A study has been done on the effect of lindane on *Jenynsia multidentata* and *Corydoras paleatus* experimentally exposed to lindane and changes were observed in the tissues. Damage included fibrosis in liver and karyolitic nucleus in brain of both studied species [20]. In *Sparus aurata* exposed to lindane, mucous epithelium of the digestive tissue showed a severe alteration with hypertrophy and desquamation of mucous cells. A high cellular disorganization in the renal and hepatic tissue was observed [18]. Effect on growth, and histopathological lesions indicating an estrogentic effect, and lesions in liver (vacuolation), kidney (glomerular hyalinosis) and thyroid gland (hypertrophy) were also found in β-HCH exposure in *Poecilia reticulata* and *Oryzias latipes* [54, 55].

CONCLUSION

Lindane has been historically used as a broad spectrum pesticide in agricultural, livestock, forestry, veterinary and human health applications. Several factors have contributed to concern over the production and use of lindane. Lindane has been known to be persistent, toxic and bio accumulates besides having potential for long-range transport. The present review found a wide range of toxic effects of lindane to fish. As dirty practice of environmental contamination of lindane continues, a global ban of lindane is long overdue. Thus, the use of lindane should be strictly controlled and regulated by appropriate legislation in order to prevent the imminent disastrous consequences to the environment.

REFERENCES


