CHAPTER V
DISCUSSION
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ACUTE TOXICITY

Endosulfan

The 24, 48, 72 and 96 h median lethal concentrations (LC_{50}) of Hildan (active ingredient endosulfan) for *Channa punctatus* Bloch. was found to be 0.0182, 0.00245, 0.00105 and 0.0007 mg L^{-1}, respectively. The LC_{50} value of the pesticide decreased gradually with the increase in exposure period from 24 to 96 h. This reflects a time-dependent response of the fish to the toxicant.

In a previous study (Sastry and Siddiqui, 1982), the 96 h LC_{50} value for endosulfan was found to be 2 \mu g L^{-1} in *Channa punctatus*. The present study reveals that this value could dip much lower to 0.7 \mu g L^{-1}, indicating extreme sensitivity and susceptibility of this fish to endosulfan. The 96 h LC_{50} value of endosulfan sulphate, a persistent environmental metabolite of endosulfan for *Gambusia affinis*, *Heterandria formosa*, *Poecilia latipinna* and *Pimephales promelas* ranged from 0.0021 to 0.0035 mg L^{-1} (Carriger et al. 2011) indicating these species to be less sensitive to endosulfan than *Channa punctatus* Bloch. On the contrary, *Brachydenio rerio* and *Hyphessobrycon bifasciatus* were found to be more susceptible to the short-term effects of endosulfan with 24 h LC_{50} values of 2.6 and 1.6 \mu g L^{-1}, respectively (Jonsson et al., 1993).

When the sensitivity of *C. punctatus* to other pesticides was compared with that to endosulfan, it was found to be more tolerant to glyphosate and atrazine with a 96 h LC_{50} value of 32.540 and 42.380 mg L^{-1}, respectively (Nwani et al., 2010).

When compared to *Channa punctatus*, *Catla catla*, *Cirrhina mrigala* and *Labeo rohita* were found to be less susceptible to endosulfan as reported by Ilyas and Javed (2013). They calculated the 96 h LC_{50} values to be 0.98, 1.06
and 2.15 μg L⁻¹, respectively, although the value for Catla catla was somewhat close to that for C. punctatus obtained in the present study (0.7 μg L⁻¹).

**Lindane**

The 24, 48, 72 and 96 h median lethal concentrations (LC₅₀) of Kunahex (active ingredient lindane) for Channa punctatus Bloch were found to be 2.22833, 1.90645, 1.51619 and 1.40257 mg L⁻¹, respectively. The decreasing trend of the LC₅₀ values from 24 to 96 h was much more gradual when compared to that of endosulfan which exhibited a steeper slope from 24 h. However, C. punctatus was much less vulnerable to lindane than to endosulfan, although the former was also a persistent organochlorine pesticide (POP).

When comparisons were made with the toxicity of lindane to some other fish species, it was found that its 96 h LC₅₀ value was 0.36 mg L⁻¹ for Clarius gariepinus (Omitoyin et al., 2006) indicating this species to be more susceptible to lindane than Channa punctatus. Lawson et al. (2011) reported the 96h LC₅₀ value of lindane to be 1.29 ppm in a study conducted with Clarius gariepinus, which was somewhat comparable to the 96 h value for C. punctatus recorded in the present study. Pseudobagrus fulvidacro was also found to be very susceptible to lindane when compared with C. punctatus, since the 24, 48, 72 and 96 h LC50 values of lindane were 38.83, 33.84, 30.30 and 28.43 μg L⁻¹, respectively (Park et al., 2004).

**Malathion**

The 24, 48, 72 and 96 h median lethal concentrations (LC₅₀) of Kunamala (active ingredient malathion) for Channa punctatus Bloch. were found to be 5.5145, 2.2685, 1.595 and 0.9015 mg L⁻¹, respectively. Here also a gradual time-dependent response of the fish to the toxicant was observed with a more gradual decrease in LC₅₀ values when compared to that for endosulfan. The 96 h LC₅₀ value of malathion was found to be 0.8 ppm in a study conducted
on *Channa punctatus* (Magar and Afsar, 2012), which is comparable to that obtained in the present study.

Sandhya Rani and Venkataramana (2012) found the 96 h LC$_{50}$ value of malathion to be 1 ppm for *Glossogobius giuris* (Ham). Thus this species had comparable susceptibility to malathion as that exhibited by *C. punctatus*. The 96 h LC$_{50}$ value of malathion was found to be 9.14 and 15.3 mg L$^{-1}$ for two federally endangered Colorado squawfish *Ptychocheilus lucius* and *Gila elegans*, respectively (Beyers et al., 1994) indicating these species to be more tolerant to malathion toxicity. The grass carp *Ctenopharyngodon idella* was also found to be less susceptible to malathion as indicated by its 96 h LC$_{50}$ value of 2.138 (Salwa, 2008), while *Clarius batrachus* was found to be more susceptible to malathion with a 96 h LC$_{50}$ value of 0.25 ppm (Yogesha et al., 2009). *Esomus danricus*, which had a 96 h LC$_{50}$ value of 17.9 μg L$^{-1}$, was also found to be very susceptible to malathion (Das and Gupta, 2012). When the toxicity of malathion to *C. punctatus* was compared with those of other pesticides, profenofos, an organophosphate, was found to be more toxic with a 96 h LC$_{50}$ value of 2.68 μg L$^{-1}$ (Pandey et al., 2011).

**Carbaryl**

The 24, 48, 72 and 96 h median lethal concentrations (LC$_{50}$) of Sevin (active ingredient carbaryl) for *Channa punctatus* Bloch was found to be 10.916, 8.3385, 8.012 and 7.5155 mg L$^{-1}$, respectively. It is clear from the result that a LC$_{50}$ value of the pesticide decreased fairly gradually with increase of exposure period from 24 to 96 h. This reflects a time-dependent adaptability of the fish to the toxicant. Sastry et al., (2006) also found the 96 h LC$_{50}$ value for *Channa punctatus* exposed to Sevin close to our value, i.e., 10.5 mg L$^{-1}$. The 96 h LC$_{50}$ value of carbamate (50% WDP) for *Channa punctatus* was found to be 17.41 mg L$^{-1}$ in a study conducted by Singh et al. (2007), indicating that sevin was more toxic to this fish as shown by the present study. *Clarius batrachus* was found to be less susceptible to Sevin than *C. punctatus* with a higher 96 h LC$_{50}$ value of 15.3 mg L$^{-1}$ (Patnaik and Patra, 2006).
When compared to *C. punctatus*, *Catla catla*, *Anabas testudineus*, *Mystus cavasius* and *Mystus vittatus* were found to be more susceptible to carbaryl with lower 96 h LC$_{50}$ values of 6.4, 5.5, 4.6 and 1.4 ppm, respectively (Tilak et al., 1981). *Clarius batrachus* was also found to be very susceptible to carbaryl with a 96 h LC$_{50}$ value 0.25 ppm (Wasu et al. 2009) followed by *Ptychocheilus lucius* and *Gila elegans* with 96 h LC$_{50}$ values of 1.31 and 2.02 mg L$^{-1}$, respectively (Beyers et al., 1994). On the other hand, the hybrid catfish (*Clarius macrocephalus* × *Clarius gariepinus*) was very tolerant to carbaryl with a 96 h LC$_{50}$ value of 48 mg L$^{-1}$ (Somnuek et al., 2007).

Comparing the acute toxicities of other pesticides to *C. punctatus*, the 96 h LC$_{50}$ value of furadan, another carbamate, was found to be 0.05 mg L$^{-1}$ in a study with *Channa punctatus* (Shahi and Singh, 2010) indicating furadan to be more toxic than carbaryl. Carbosulfan was found to be more toxic to *C. punctatus* as well with a very low 96 h LC$_{50}$ value of 0.268 mg L$^{-1}$. On the contrary, *C. punctatus* was found to be more tolerant to glyphosate and atrazine having 96 h LC$_{50}$ values of 32.540 and 42.380 mg L$^{-1}$, respectively (Nwani et al., 2010). Cypermethrin and λ-cyhalothrin was found to be more toxic to *C. punctatus* than carbaryl (Kumar et al. 2007) with 96 h LC$_{50}$ values of 0.4 mg L$^{-1}$ and 7.92 μg L$^{-1}$, respectively.

From the present study it could be concluded that carbaryl is the least toxic pesticide to *C. punctatus* Bloch. followed by malathion and lindane. Endosulfan was found to be the most toxic pesticide to *Channa punctatus* Bloch. The different lethal concentration values among different pesticides and different species indicate the differential toxicity of a particular pesticide among different species. The difference in toxicity could also be due to differences in absorption pesticides, their accumulation, biotransformation and excretion (Wasu et al., 2009).
SUBLETHAL TOXIC EFFECTS ON GROWTH

Endosulfan

Endosulfan was found to cause reduction in the body weight of the fishes even at a very low concentration of 0.07 and 0.23 μg L⁻¹ indicating its high toxicity to the fish. The reduction was found to be more in the group exposed to the higher concentration of endosulfan.

Petri et al. (2006) observed a similar reduction in the body condition factor (weight/length³) of Atlantic Salmon fed with endosulfan-contaminated diet. Endosulfan was found to be acting as a growth suppressant in Danio rerio (Balasubramani and Pandian, 2008). Endocrine disrupting chemicals like endosulfan can suppress the growth of an organism by its accumulation in the muscle leading to impaired swimming speed, impaired foraging efficiency, lowered food intake, slowed digestion and adsorption, and finally resulting in stunted growth. Therefore, the accumulated endosulfan could be the root cause for the reduced growth in C. punctatus. In Cyprinion watsoni the body weight decreased at a concentration of 1ppb of endosulfan (Kalsoom et al., 2005). A significant reduction in body growth of endosulfan- exposed Cyprinus carpio was also observed (Salvo et al., 2008). The mechanism by which endosulfan could affect the growth of the fish was by acting on the central nervous system. Endosulfan was found to have deleterious effects on the nervous system of Danio rerio and also significantly impaired the animal’s exploratory performance (Pereira et al., 2012). Similar mechanisms were likely to be operative in C. punctatus in the present study.

Malathion

The reduction in the body weight of malathion-exposed fishes were statistically significant at both the exposure concentrations, although the reductions were proportionally higher in the higher concentration of malathion. Malathion was found to suppress growth and reduce survival in
the developing zebrafish *Danio rerio* (Cook *et al.*, 2005). Reduction in the growth of *Channa punctatus* exposed to malathion was also observed by Shukla *et al.* (1987). Wasu *et al.* (2009) also observed reduction in the body weight of malathion exposed *Clarius batrachus*. *Channa striata* exposed to malathion experienced a 30% reduction in growth along with reduction in the brain cholinesterase activity (Cong *et al.*, 2009). Since malathion is a known AChE inhibitor, inhibition of the enzyme could induce hypoactive behaviour in the fish resulting in reduced foraging and exploratory activity, which in turn could lead to reduced body growth of the fishes. Growth reduction was likely to be due to the expenditure of more energy on maintenance, thereby identifying malathion as a metabolic stressor.

**Carbaryl**

Carbaryl was also found to cause reduction in the growth of *C. punctatus*. The reduction was observed to be more in those exposed to the higher concentration of carbaryl, viz., 2.5 mg L⁻¹. The decrease in body weight could be due to excessive expenditure of energy on metabolism by the fishes that otherwise could have been utilized in fish growth. Growth decreased with increase in concentration of carbaryl in the air-breathing fish *Macropodus cupanus* (Arunachalam and Palanichamy, 1982). Similarly *Clarius batrachus* exposed to carbaryl also experienced a reduction in the body weight (Wasu *et al.*, 2009). Similar reduction in growth rate was also observed in *Mystus vittatus* (Bloch.) exposed to carbaryl (Arunachalam *et al.*, 1980). As carbaryl is also an acetylcholinesterase inhibitor, the decrease in growth could occur due to the inactivation of this enzyme, as was the possible reasons for malathion-induced growth suppression. The inactivation of enzyme could lead to reduced foraging ability of the fishes ultimately resulting in reduced food intake and consequent reduced growth of the fishes. The most important factors decreasing fish growth were found to comprise disorder in feeding behaviour, decrease in feeding rate, dysfunction of metabolic processes, and wastage of energy to overcome the stress caused by insecticide exposure (Tripathi *et al.*, 2003).
Acetylcholinesterase (AChE) is a serine hydrolase that hydrolyses the neurotransmitter acetylcholine (ACh). When a nerve impulse reaches a nerve ending, ACh stored there in vesicles is released and it interacts with the ACh receptor site on the postsynaptic membrane to stimulate the nerve fiber or muscle. AChE regulates nervous transmission by catalyzing hydrolysis of ACh and reducing its concentration in the junction (Fukuto, 1990). Organophosphates and carbamates are long known to inhibit AChE by attaching to a serine residue in the active site (Sussman et al., 1991). Inhibition of AChE leads to accumulation of ACh in the synaptic cleft, which results in impeded neurotransmission. AChE depression beyond a threshold has been shown to impair two types of swimming performance as well as swimming and feeding behaviour in chlorpyrifos-exposed coho salmon (Oncorhynchus kisutch) (Sandahl et al., 2005; Tierney et al., 2007). AChE assay is regarded as a good biomarker of pesticides, especially organophosphates and carbamates, and has been successfully used in aquatic animals including copepods, mollusks and fish to detect exposure to a range of neurotoxic compounds (Kirby et al., 2000; Forget et al., 2003; Roex et al., 2003; Eder et al., 2004; Lau et al., 2004; Linde-Arias et al., 2008). It is known that individual fishes may have different AChE composition due to difference in age and development, salinity, pH, and other intrinsic and extrinsic factors (Phillips et al.2002; Durieux et al. 2011). In spite of these, the three pesticides were observed to have marked effects on the acetylcholiesterase activity in C. punctatus.

Endosulfan

The evidence for anti-AChE activity of endosulfan is not unequivocal, although several studies have reported depression of AChE activity in fish species upon endosulfan exposure (Dutta and Arends, 2003; Ballesteros et al., 2009; Pereira et al., 2012). Again, some studies have reported reductions in muscle AChE activity, while that of brain tissue remained unaffected. In the
present study, endosulfan was found to cause reduction in both brain and muscle AChE activity in *C. punctatus*.

One of the early studies on the effects of endosulfan on AChE activity (Dutta and Arends, 2003) revealed that exposure to 1 µg L\(^{-1}\) endosulfan for a one-week period caused up to 23.11% reduction in brain AChE activity in *Lepomis macrochirus*. Sarma et al. (2010) showed that endosulfan exposure at 8.1 µg L\(^{-1}\) for 96 h led to a 59.9% reduction in brain AChE activity from that in the control in *Channa punctatus*. The present study shows that endosulfan could adversely affect AChE activity at a concentration more than two orders of magnitude lower than this (0.07 µg L\(^{-1}\)). Endosulfan inhibited the brain acetylcholinesterase activity (65.4%) in *Labeo rohita* (Kumari et al., 2010) which is almost comparable to the present study where brain AChE activity of *C. punctatus* was inhibited up to 62.33%. Significant reduction in brain AChE activity in *Oreochromis mossambicus* began at 3.3 µg L\(^{-1}\) and increased in a dose-dependent manner from 9.7 to 17.2% of the control at 5 µg L\(^{-1}\) (Kumar et al., 2011). On the contrary, muscle AChE activity in *Jenynsia multidentata* decreased significantly when exposed to endosulfan concentrations above 0.072 µg L\(^{-1}\), although brain AChE activity remained unaffected (Ballesteros et al., 2009). Da Cuià et al. (2011) also did not find any significant difference in brain AChE activity between control and endosulfan-exposed *Cichlasoma dimerus*. The present study shows that AChE reductions to the extent of c 57-65% of control occurred at considerably low endosulfan concentrations (0.23 and 0.07 µg L\(^{-1}\)) on longer exposure. The importance of exposure time was also emphasized in a study (Pereira et al., 2012) which showed that a 2.4 µg L\(^{-1}\) endosulfan exposure caused significant reduction in brain AChE activity in *Danio rerio* after a treatment time of 96 h but not after that of 24 h. Our study indicates that the threshold levels for brain and muscle AChE activity could dip much further with persistent exposure to extremely low levels of endosulfan. The findings that rain water in India could contain up to 3.02 µg L\(^{-1}\) of total endosulfan of which the more persistent yet toxic component endosulfan sulfate comprised a 0.4 µg L\(^{-1}\) fraction (Kumari et al., 2007), and that run off from agricultural fields in Tamil Nadu could contain 24 µg L\(^{-1}\) of endosulfan (Jayashree and Vasudevan, 2007), are especially important in this
regard. Tea growing areas of Assam and North Bengal in India have an undulating terrain and receive about 2500 mm rainfall annually, of which over 75-80% occur during June-September. *C. punctatus* populations inhabiting the low-lying rice paddies, marshes and swamps in such areas could be particularly vulnerable to endosulfan toxicity because of the transport and deposition of endosulfan in these ecosystems that could ideally serve as sinks for this pesticide.

**Malathion**

The present study reveals that malathion inhibited the brain and muscle AChE activity of *Channa punctatus* Bloch. Inhibition increased with increase in concentration in both brain (68.7-78.1%) and muscle (67.2-78.8%). The reductions in AChE activity in both the concentrations were statistically significant. *Heteropneustes fossilis* exposed to malathion also had a reduced AChE activity (Dutta *et al.*, 1995). Malathion caused significant reduction in the activity of AChE in *Onchorynchus mykiss* (Beauvais *et al.*, 2000), and in *Clarius macrocephalus* in a 96 h study (Sommuek *et al.*, 2007). Reduction in the brain and muscle acetylcholinesterase activity of *Tilapia mossambicus* (Peters) was observed after exposure to malathion in the study conducted by Sahib *et al.* (1980). A similar inhibition in the brain acetylcholinesterase activity of *Seriola dumerilli* after exposure to malathion was also reported (Jebali *et al.*, 2006). Thus the findings of the present study confirm the AChE inhibitory effects of malathion on fish brain and muscle, and shows this effect to be operative in *Channa punctatus* as well.

In such AChE inhibitory activity, the other organophosphates also play similar roles, as is evidenced by the inhibition of brain acetylcholinesterase with increase in concentration in *Clarius gariepinus* exposed to diazinon (Adedeji, 2011); reduced brain AChE activity of *Labeorohita* (Ham.) by 75.45% due to quinalphos exposure (Das and Mukherjee, 2000); and those in the Coho Salmon, *Onchorhyncus kisutch* (Sandahl *et al.*, 2005) and *Gambusia affinis* (Rao *et al.*, 2005).
After a recovery of 7 days, the differences between the brain and muscle AChE activity in control and the exposed fishes were not statistically significant, indicating that some recovery had occurred after transferring the fish to pesticide-free water could not come back to the normal condition. Similarly, exposure of Nile tilapia to monocrotophos, an organophosphate caused a significant reduction in the brain AChE activity which regained 95% of control AChE activity after transferring to a toxicant free media (Thangnipon et al., 1995). On the contrary, no significant recovery in the brain and muscle cholinesterase activity of juvenile rainbow trout (Onchorynchus mykiss) exposed to azinphos methyl, an organophosphate, was observed in a study conducted by Ferrari et al. (2007).

Carbaryl

Carbaryl is a known acetylcholinesterase inhibitor, which is also reflected in the present study. The reduction in the brain and muscle tissue AChE activity indicates the toxic effect of carbaryl on the enzyme. The present study found that the inhibition was more in those exposed to the higher concentration of carbaryl (2.5 mg L\(^{-1}\)). The increase in inhibition with increase in carbaryl concentration was more pronounced in the brain tissue (from 56.6% inhibition to 88.05% inhibition). This could lead to suggest that brain tissue AChE was more susceptible to carbaryl. A similar result was obtained in hybrid catfish (Clarius macrocephalus x Clarius gariepinus) exposed to carbaryl (Somnuek et al., 2007). On the other hand, the organophosphate chlorpyrifos, also an anticholinesterase compound, was more toxic to the muscle tissue of Gambusia affinis (Carr et al., 1997). Ghosh and Bhattacharya (1992) exposed Channa punctatus to 3.73 ppm of carbaryl for 7 days and observed significant inhibition in the bain acetylcholinesterase activity. The present study revealed that carbaryl could depress AChE activity at 0.75 mg L\(^{-1}\) on slightly longer exposure. Rao and Rao (1989) also observed inhibition in acetylcholinesterase activity in Channa punctatus after exposure to carbaryl. An increase in the cholinesterase inhibition with increase in carbaryl concentration was also observed in the study of Beauvais et al. (2001) in which they used larval Rainbow Trout (Oncorhynchus mykiss). Clarius
batrachus exposed to carbaryl experienced higher inhibition in longer exposure period of 15 days (Sharma et al., 1993).

Even after a recovery period of 7 days, the AChE activity was still lower than that of the control. This indicates that once the enzymes are inhibited by carbaryl in C. punctatus, it becomes difficult to revert back to the normal state. Somnuek et al. (2007) observed that carbaryl exposed Gambusia affinis could not completely recover its inhibition in AChE activity even after being transferred to pesticide free media, although slight recovery was observed. Kirby et al. (2000) also found that the main cause of muscle AChE reduction in Platichthys flesus in the UK estuaries was due to carbamate contamination. A slight recovery of 20% was observed in Golden Snail Apple (Pomacea canaliculata Lamarck) after transferring the snails to carbaryl free media but complete recovery was not achieved (Putkome et al., 2008). The results obtained in the present study indicate that while the AChE activity in C. punctatus exposed to malathion could recover to some extent, carbaryl’s impact on AChE activity was of a more irreversible nature.

MORPHOLOGICAL CHANGES

Caudal Bending

The most prominent morphological change observed in the malathion and carbaryl exposed fishes was caudal bending. The simultaneous and proportionate inhibition of AChE in the fishes exhibiting the phenomenon of caudal bending suggests that enzyme inhibition is the probable cause of this morphological change. Radiological studies did not reveal any bone deformation in the fishes that exhibited caudal bending. This further suggests that enzyme inhibition was the most likely cause of caudal bending. It is possible that inhibition of the enzyme leads to impaired signal transduction in the brain and muscle tissues resulting in a paralytic effect causing tail bending in the fishes.

Malathion
In case of malathion the phenomenon of caudal bending was observed only in the fishes exposed to the higher concentration of malathion i.e., 0.3 mg L\(^{-1}\). Caudal bending was the main morphological change observed in *Cyprinus carpio* exposed to chlorpyrifos, an organophosphate pesticide (Halappa and David 2009), while Patil and David (2010) observed the same effects with malathion and suggested that it could be due to brain and muscle AChE inhibition by malaoxon produced via malathion biotransformation. A similar morphological change of caudal bending in chlorpyrifos exposed *Channa punctatus* was reported by Devi and Mishra (2013). The extent of caudal bending was more pronounced in the higher concentrations.

**Carbaryl**

In the present study, carbaryl was observed to produce caudal bending in *C. punctatus* exposed to both lower and higher concentrations, i.e., 0.75 mg L\(^{-1}\) and 2.5 mg L\(^{-1}\). A similar neural paralytic effect was observed in *Clarius batrachus* poisoned with carbaryl (Patnaik and Patra 2006). The caudal region is more susceptible to bending because of its thin and flexible nature that results in the distortion of orientation due to paralysis of caudal musculature resulting from AChE inhibition. Bent tail and bent trunk were also observed in *Bombina orientalis* embryo exposed to carbaryl (Kang *et al.*, 2010). Morphological deformity, curvature in the tail region was also observed in zebrafish larvae exposed to Sevin (carbaryl). Similar morphological change of bent tail curvature was also observed in the embryo of *Danio rerio* with a corresponding inhibition of embryo AChE indicating a linking between the AChE inhibition and the morphological change (Schock *et al.*, 2012). The results obtained in the present study are in agreement with these findings.
Effects of Pesticides on Gill Ultrastructure as revealed by Scanning Electron Microscopy

Acute Exposure

No morphological lesion was seen in control gills as they were not under any stress.

Lesions in gill surface ultrastructure could lead to functional alterations and interferences in fundamental processes such as maintenance of osmoregulation and antioxidant defense of gills (Pandey et al., 2008). According to Arellano et al. (2001) and Biagini et al. (2009) the histological alterations observed in fish gills are acknowledged as a fast and valid method to determine the damages caused by exposition to different pollutants. The histopathological changes in the gill such as edema, epithelial lifting, fusion of secondary lamellae, haemorrhage in filaments, hypertrophy of epithelial cells and sloughing off of epithelial surface are the major effects reported in gills from the fish exposed to various types of pollutants (Mallatt, 1985). Many of these effects were seen in the present study. The gills which enable the fish to oxygenate their blood are highly suitable for this function in their orientation and morphology. They have large surface area which is being exposed to a constant flow of water that moves in the opposite direction to the flow of blood in that area. The secondary lamellae are in constant contact with water and are thus highly sensitive to any change in the water condition or composition (Roncero et al., 1990).

Endosulfan

The endosulfan exposed fishes had epithelial sloughing. Swollen tips of the gill filament were also observed in gammalin 20 exposed Clarius gariepinus (Ezemonye, 2010). Necrosis of epithelia, epithelial lifting and aneurism in the secondary lamellae were observed in gill tissues of Cirrhus mrigala exposed to dichlorvos (an organophosphate pesticide) for 10 days (Velmurugan et al., 2009). Gambusa affinis exposed to endosulfan for 30 days developed aneurism in the secondary lamellae (Cengiz and Unlu, 2002).
The formation of aneurysm has been described as a reaction of fish gills to pollutants (Mallatt, 1985). Aneurysm in the secondary lamellae was also observed in the present study. However, the secondary lamellae only showed partial fusion under endosulfan exposure.

**Malathion**

Malathion exposed fishes experienced epithelial disorganisation and curled lamellar tips. Epithelial disorganisations like epithelial necrosis observed in the present study are direct responses to the action of the pesticide. Lamellar fusion was also noticed in the present study. Lamellar fusion is often considered as a defensive mechanism because it diminishes the amount of vulnerable gill surface area (Mallatt, 1985). Similar alterations in the gill morphology of *Cirrhinus mrigala* were also observed by Roy and Munshi (1991) under malathion stress. Hyperplasia, desquamation, epithelial necrosis, epithelial lifting, oedema, lamellar fusion, collapsed secondary lamellae, curling of secondary lamellae and aneurism in the secondary lamellae were observed in gill tissues of *Cirrhinus mrigala* exposed to dichlorvos (an organophosphate pesticide) for 10 days (Velmurugan et al., 2009).

**Carbaryl**

Carbaryl exposed fishes experienced secondary lamellar fusion, and enlargement of the epithelial pavement cells. Contamination by pesticide produced some degenerative alterations in gills including hyperplasia of the epithelial lining of the secondary lamella (Zagarese and Williamson, 2001). Lamellar swelling may be related to a defense mechanism which reduces the water surface around the gill and increases the barrier distance of diffusion of toxicants from outside to the blood capillaries (Dutta et al., 1997).
Sublethal Exposure (21 Day Exposure to Sublethal Concentrations and 7 Day Recovery in Pesticide-Free Water)

No morphological change was seen in control gills as they were not under any stress.

Endosulfan

In the present study, the fish exposed to both sublethal concentrations of endosulfan had fused secondary lamellae, epithelial sloughing and thinning of microridges, and loss of distinct pavement cell boundary. It may be noted that fusion of secondary lamellae was only partial in acute exposure. Hence it appears that longer exposure could produce more sustained alterations such as fusion of secondary lamellae even if the exposure concentration was low. Fusion of secondary lamellae was likely to have interfered with gaseous exchange since it resulted in obstruction of water flow. A characteristic response of the fish at the lower concentration of 0.07 μg L⁻¹ was profuse secretion of mucous. As Mallatt (1985) had suggested, mucous secretion was a defensive mechanism. Hence it could be inferred that at this low concentration the fish was still trying to defend itself by producing mucous to protect its surface from the scouring and other effects of the pesticides. However, at the higher concentration of 0.23 μg L⁻¹, the effects were probably too drastic and severe to allow the fish to adopt any effective defensive measure, as a result of which the deleterious effects of epithelial sloughing was observed. Bufo bufo exposed to endosulfan also produced a thick mucous layer on its gills (Burnabo et al., 2008). The secretion of mucous could be a defensive mechanism to avoid contact with the toxicant media. Exposure of Clarias gariepinus to gamamlin 20, an organochlorine caused fusion of adjacent lamellae and mucous secretion (Ezemonye, 2010). Besides pesticides, any stress caused by variations in the environmental conditions induces the proliferation of mucous cells and increased mucous secretion (Fernandes et al., 2007).
**Malathion**

Some of the alterations observed in malathion exposed fishes were lamellar fusion, epithelial disintegration, mucous secretion, and swelling and enlargement of lamellar cells. Disintegration of the microridges and pavement boundary distortion were also seen. The freshwater fish *Metynnis roosevelti* exposed to methyl parathion was found to experience structural changes in gill lamellae, epithelial detachment, necrosis, hyperplasia, loss of microridges and altered cellular morphology (Machado and Fanta, 2003). Roy and Munshi (1991) also reported gill alterations like increase in gill area, inflammatory alterations in the gill epithelium and hyperplasia in malathion exposed *Cirrhinus mrigala*. Loss of microridges on the pavement cells appears to be a characteristic effect of malathion that indicates its surface-corrosive nature.

**Carbaryl**

Excessive mucous secretion was observed at the lower sublethal concentration of 0.75 mg L\(^{-1}\). Mucous secretion was not pronounced at the higher concentration of 2.5 mg L\(^{-1}\), presumably because the ability of the fish to activate its defense mechanism became severely impaired at the higher intensity of carbaryl exposure. Degeneration of microridges in the carbaryl exposed fishes was similar to those observed in monocrotophos exposed *Cyprinus carpio communis*. Monocrotophos induced shrunken epithelial gill filament resulting in the thinning and and degeneration of microridges (Johal et al., 2007). Carbaryl caused epithelial sloughing, fusion of the lamella and mucous secretion in goldfish *Carassius auratus* (Pfeiffer et al. 1997). Since microridge degeneration was also observed in malathion exposure in the present study, it seems that carbaryl also produces effects similar to those brought about by malathion and other organophosphates.
SUBLETHAL EFFECTS ON BEHAVIOUR

Opercular movements

Endosulfan

Opercular movement was found to be higher than that of the control in the higher sublethal concentration of endosulfan whereas it was lower than that of the control in the lower concentration of endosulfan. The lower opercular movements from the control could be an adaptive mechanism by the fish to avoid contact with the pesticide. However, at higher concentration the fish received a shock which overcame its defensive reactions as a result of which there was overstimulation of opercular movements. The accompanying damage on the gill surface ultrastructure might have necessitated hyperventilation in an attempt to counter respiratory distress. A similar increase in opercular movements was found in endosulfan exposed *Channa striatus* (Ganeshwade et al., 2012). *Cyprinus carpio* exposed to endosulfan also exhibited increased opercular beats (Rudainy and Kadhim, 2012).

Malathion

Malathion produced a response pattern that was the reverse of that produced by endosulfan. While exposure to the lower concentration of malathion (0.09 mg L⁻¹) resulted in an increase in opercular movement, a decrease in opercular movement was observed with an increase in the concentration of malathion. Thus this pesticide was stimulatory in its effects at the lower, but inhibitory at higher concentrations. It was found in the present study that malathion at its higher concentration significantly reduced brain and muscle AChE activity in *C. punctatus*, and caused caudal bending in several fishes. It is, therefore, possible that the reduction in opercular beats is a function of the inhibition of AChE in brain and muscle tissue caused by malathion. Opercular movements increased initially in malathion exposed *Labeo rohita* in all exposure periods of but decreased further steadily in lethal exposure compared to sublethal exposure periods (Patil and David, 2008) and similar
results were obtained in a study conducted by Wasu et al. (2009) in malathion exposed Clarius batrachus. However, an increase in the opercular movement was observed in Puntius stigma exposed to dimethoate, an organophosphate (Bhandare et al., 2011).

**Carbaryl**

In case of carbaryl exposed fish the opercular movement decreased with an increase in concentration. Wasu et al. (2009) observed reduction in opercular beats in Clarius batrachus exposed to carbaryl. On the contrary, Mystus vittatus exposed to carbaryl showed increased opercular beats (Arunachalam et al., 1980). Thus the evidence on increase or decrease in opercular beats appears to be somewhat contradictory with different species reacting differently to carbamates. It has been suggested that increased opercular activity may be due to the shock received by the fish in a new toxic environment. This provides the sensory stimulus to increase the opercular movement for proper ventilation of the gills, which in turn helps the fish to cope with hypoxia (Joseph et al., 1987, Lata et al., 2001). However, carbaryl was shown to be an effective AChE inhibitor in the present study, as it was observed to significantly reduce AChE activity at 0.75 mg L\(^{-1}\) and produce caudal bending effects. Hence, the reduction in opercular beats was also likely to be a function of the inhibition of AChE in brain and muscle tissue.

**Surfacing**

**Acute toxicity study**

During the 96 h acute toxicity study for determination of lethal concentrations, frequent surfacing phenomenon was observed in the fishes exposed to high concentrations of the three pesticides, viz., endosulfan, malathion, and carbaryl. The number of surfacing could be recorded for the first three hours only as at higher concentrations mortality began to start from the first few hours onwards. The patterns of surfacing of the three pesticides are described below.
Endosulfan

The surfacing frequency increased in all the concentrations (0.196–19.6 µg L⁻¹) of endosulfan with increase in exposure time. The phenomenon of surfacing might have been an expression of respiratory stress caused by gill damage forcing the fish to try to gulp more air. Similar behavioural patterns were observed in trout and *Labeo rohita* exposed to fenvalerate (Murthy, 1987; David *et al.*, 2002) and also in *Oreochromis mossambicus* exposed to endosulfan (Devi, 1991). Increase in surfacing could be linked with the increase in opercular movements observed in *C. punctatus* exposed to the higher concentration of endosulfan in the present study, lending evidence to its hyperexcitatory effects.

Malathion

Surfacing was also observed in malathion exposed fishes. The sharp increase in surfacing in the initial period of exposure could be due to the sudden shock received by the fishes. Goldfish was found to exhibit increased surfacing under stress when exposed to atrazine (Saglio and Trejasse, 1998). Patil and David (2008) also observed similar surfacing phenomenon in *Labeo rohita* exposed to malathion and suggested that the increase in surfacing was due to demand of higher amounts of oxygen under pesticide stress.

Chronic toxicity study (21 day exposure and 7 day recovery)

Endosulfan

During the chronic exposure surfacing was observed only in the sublethal concentration of 0.07 µg L⁻¹. Similar behavioural patterns were observed in *Cyprinus carpio* exposed to endosulfan (Rudainy and Kadhim, 2012) and also in *Oreochromis mossambicus* exposed to endosulfan (Devi, 1991). The surfacing phenomenon could be due to hypoxic condition (Devi, 1991).
Malathion

Exposure of fishes to 0.3 mg L\(^{-1}\) and 0.09 mg L\(^{-1}\) of malathion could not induce any surfacing in *Channa punctatus* Bloch.

Carbaryl

Only 2.5 mg L\(^{-1}\) of carbaryl could induce surfacing. Enhanced surfacing activity was observed as symptoms of overt toxicity in carbofuran exposed *Channa punctatus* (Gopal and Ram, 1995).

Based on the results obtained in the present study, surfacing appears to be a toxicant-induced response that occurs infrequently in *C. punctatus* and no clear pattern could be established with certainty. More detailed studies under varying conditions and different pesticide concentrations are needed before establishing surfacing as a behavioural endpoint in toxicity studies, especially those on sublethal toxicity.

Video Analysis of Locomotory Behaviour of Pesticide-Exposed Fishes

The six variables of movement assessed in the present study were distance travelled (DT), swimming velocity (SV), rotation angle of movement (RA), angular velocity (AV), momentum (M) and kinetic energy (KE). The responses of *C. punctatus* to the three pesticides in terms of these variables were characterized by certain differences.

Lindane

A longer (6-day) exposure to lindane elicited a positive, excitatory response in terms of all the movement parameters except angular velocity (AV). Responses in the 1-day exposure were similar, except that the effects on both rotation angle (RA) and AV were inhibitory. Increased locomotor activity of the fishes both during 6-day and 1-day exposure could be due to the hyperexcitability brought about by lindane exposure. Some major behavioural
manifestations like jerky behaviour, violent movements, hyperactivity, movement in short bursts, turbulent activity, respiratory distress or whip-like activity could be observed during the exposure of *Cyprinus carpiovar specularis* to endosulfan, which is also a persistent organic pesticide (POP) like lindane. It has also been found that violent behaviour appeared first in *Cyprinus carpiovar specularis*, followed by other behaviours (Rehman, 2006). Endosulfan exposure was found to increase opercular movement and induce surfacing in *C. punctatus* in the present study. The increase in distance covered, swimming velocity, kinetic energy and others in lindane exposed fishes could be associated with these observations on endosulfan, indicating that the modes of action of these two POP organochlorine pesticides on locomotory and ventilatory behaviour were similar. Lindane exposed fishes had larger rotation angle when exposed for 6 days which could recover after the recovery period. In contrast, those exposed for 1 day only had smaller rotation angle and could increase their rotation angle after a recovery period of 6 days. The change in the rotation angle might have been due to the neurotoxic effect of lindane. Shih et al. (2013) showed that zebrafish having deficiency of aromatic L-amino acid decarboxylase (AADC) suffered from impaired brain development and motor functions, which was reflected in the reduction in rotational movement and rotational angle of eye. Copper exposure was also found to affect the turning angle of goldfish (Kleerkoper et al., 1972; Kane et al., 2005).

**Malathion**

In contrast to lindane, malathion had an inhibitory effect on all parameters except kinetic energy (KE) during the 6-day exposure, while the reverse occurred during the short, 1-day exposure when malathion had a stimulatory effect on all the parameters. Thus it appears that malathion played a stimulatory, hyperexcitatory role on short exposure, but became inhibitory on longer exposure. Similar reductions in distance travelled, swimming velocity, and rotation angle was also observed by Brewer et al. (2001) in malathion exposed *Oncorhynchus mykis*. Malathion also inhibited the locomotor activity in wistar rats with production of a depression like effect (Brocardo et
In the present study, malathion exposure was found to lead to an increase in opercular movement at the lower, but decrease at the higher concentration of the pesticide in *C. punctatus*. Furthermore, malathion exposure also led to an initial increase in surfacing followed by a decrease with longer exposure. These observations, coupled with those made on locomotory behaviour, provide evidence for the stimulatory and hyperexcitatory action of malathion on exposure of shorter duration or at relatively low concentration. This action is reversed to become inhibitory and hypoexcitatory on longer exposure or at higher concentration.

**Carbaryl**

Carbaryl produced effects somewhat similar to that produced by lindane by stimulating all parameters except RA. Thus in spite of being an AChE inhibitor like malathion, the manifestations of carbaryl toxicity on locomotory behaviour were different. Carbaryl induced swimming activity in rainbow trout *Oncorhynchus mykiss* (Little *et al.*, 1990), although male bobwhites *Colinus virginianus*, exposed to carbofuran had reduced locomotor activity (Robel *et al.*, 1982). Carbaryl was also found to increase the swimming activity of *Daphnia pulex* (Dodson *et al.*, 1995). However, Dodson *et al.* (1994) also observed an inhibition in the turning angle in carbaryl exposed *Daphnia pulex* as was also found in the present study. The behavioural alterations could be also due to the neurotoxic effect of carbamate as carbofuran, which is also a carbamate, was found to induce alterations in the brain neurotransmitters (norepinephrine, dopamine and serotonin) levels in *Channa punctatus* (Gopal and Ram, 1995). Carbaryl also accelerated the swimming activity of *Mystus vittatus* (Bloch.) (Arunachalam *et al.*, 1980). However, unlike lindane and malathion, the effects of carbaryl on opercular movement and surfacing were the reverse of that on locomotory behaviour, since carbaryl was found to decrease both opercular movement and surfacing, although it increased almost all the locomotory behaviour endpoints on both short and long exposure.
Fractal Analysis of Path Tortuosity

In the present study, 6-day exposure to lindane induced *C. punctatus* to follow a swimming path of significantly higher tortuosity than that exhibited by the control fishes. The fractal dimension (FD) of the path followed by fishes exposed for 6 days to lindane was significantly higher than that of the control. These results indicate that the lindane exposed hyperactive fishes started moving in a more tortuous path than those in the control. This is also reflected in the longer distance covered with a greater swimming speed, and with a larger rotation angle but lower angular velocity, by the fishes exposed for 6 days to lindane. Such a movement path perhaps reflects the disorientation of the fishes on sustained exposure to lindane. After a recovery period of 6 days, the same fishes started following a path that was even less tortuous than that of the control (Fig. 172). Thus it appears that the fishes were not fully recovered and in an attempt to revert to their normal path tortuosity, they began moving with a jerky, linear movement.

In contrast, a shorter 1-day exposure to lindane resulted in a less tortuous path with significantly lower FD than that of the control (Fig. 175). Thus this pattern was similar to that followed by the fishes exposed for 6 days after their 6 day recovery period. When the 1-day exposed fishes were allowed to recover for 6 days, they still followed a less tortuous path than that of the control.

Patterns almost similar to that of lindane were also encountered in *C. punctatus* exposed to malathion and carbaryl. However, in contrast to lindane exposure, where the affected fishes showed signs of partial recovery, fishes exposed for 6 days to malathion continued following a highly tortuous path with significantly higher FD than that of the control, indicating a lack of recovery (Fig. 178). On the contrary, fishes exposed to malathion for 1 day started following a path more tortuous than that of the control after 6 day recovery (Fig. 181). Both 6-day and 1-day exposures to carbaryl produced a tortuosity pattern similar to that observed in the fishes exposed to lindane (Fig. 184, Fig. 187).
The lower fractal dimension in the short 1 day exposure could be due to the sudden impulse received by the fishes that made them unable to perform their normal exploratory movement. It was observed that under behavioural stress the fishes exhibited movement patterns with decreased fractal dimension (Kane et al., 2004). However, results obtained in the present study show that under certain conditions, it was also possible for stressed fishes to follow a more tortuous path, which depended upon the pesticide used.

Lindane exposed *Daphnia magna* also exhibited random migration from one area to another accompanied by less directed migration showing an uncoordinated movement (Goodrich et al., 1990). Malathion exposed fish was found to have irregular darting swimming movements and loss of equilibrium which could be due to inhibition of AChE activity leading to accumulation of acetylcholine in cholinergic synapse culminating in hyperstimulation (Patil and David, 2008). The highly tortuous path followed in the malathion exposed fish in the present study could be due to the same reason. Cabaryl also an AChE inhibitor as revealed in the present study could alter the behaviour leading to an uncoordinated movement describing a more complex tortuous path. Carbosulfan, also a carbamate induced erratic swimming pattern with vigorous jerky movement in *Channa punctatus* (Nwani et al., 2010).