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Effects Of Organophosphate Pesticides On The Olfactory Organ Of Fish: A Comprehensive Review

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Abstract

Olfaction results from stimulation of sensory receptor cells in the olfactory organs, which are innervated by the olfactory nerve (cranial nerve I) and transmit information to the brain. A paired olfactory organ in teleosts is located in the dorsal part of the snout in front of the eyes. Olfactory organ of teleost is composed of olfactory rosette (OR), olfactory bulb (OB) and olfactory nerve (ON). Pesticides such as Organophosphates are known to affect fundamental physiological systems (such as the enzyme acetylcholinesterase (AChE)), and have been shown to affect olfactory-mediated behaviours of fish. Organophosphate pesticides affected AChE activity levels in the OR and brain. organophosphate pesticide exposure significantly inhibited AChE activity in the OR, and both. These organophosphate pesticides affect the sensitivity of olfactory neurophysiology to pesticides acting not only potentially via AChE-inhibition, but also by other currently unknown modes of action.

Keywords: olfactory bulb, histopathology, olfactory receptor neurons, olfactory lamellae, water pollution, agrochemicals

1. Introduction

Chemoreception plays a major role in the lives of fishes. Nearly all aspects of life—feeding, prey detection, predator avoidance, species and sex recognition, sexual behaviour, parental behaviour, migration, etc.—are affected or mediated by the ability to detect water-born chemicals and to react appropriately to these stimuli (Hara 1993). Fish detect chemical stimuli through two different ways of chemoreception that are olfaction or smell and gestation or taste. The olfactory organ is developed from ectoderm (Hara and Zielinski 1989). Neurobiology of fish olfaction is extensively reviewed by Hara (1994), Laberge and Hara (2001), Zielinski and Hara (2001), Zielinski and Hara (2007), Hamdani and Doving (2007).

Olfaction results from stimulation of sensory receptor cells in the olfactory organs, which are innervated by the olfactory nerve (cranial nerve I) and transmit information to the brain. According to Zippel et al. (1997) the olfactory organ of fish shows suitable assortment with the degree of development and their ecological habitat. Cyclostomes contain a single olfactory organ with one external nasal opening. In case of elasmobranchs, they possess paired olfactory organ and the olfactory pits open ventrally whereas teleost opens their paired olfactory pits dorsally. Each nasal pit is generally connected through the external environment with anterior inlet and posterior outlet aperture, which are separated by a medial nasal flap. Water current enters into the anterior and leaves through the posterior nasal cavity by either through

locomotion of the fish or through actively by ciliary action or by pumping action of the nasal sac or olfactory chamber.

In elasmobranchs and eels, the olfactory organ is well developed (macrosmatic forms), and in the case of pikes, flying fish and pipe fish the organ is poorly developed (microsmotic forms). A paired olfactory organ in teleosts is located in the dorsal part of the snout in front of the eyes. Olfactory organ of teleost is composed of olfactory rosette (OR), olfactory bulb (OB) and olfactory nerve (ON). The olfactory rosette is composed of a series of olfactory lamelle, is raised from the floor of the olfactory cavity to increase the surface area for odor detection. The number of lamellae varies interspecifically as well as the rosette morphology. The olfactory lamellae mainly consist of two types of epithelia that is sensory and non-sensory epithelium. The sensory neuron extends a dendrite to the surface of the epithelium, bearing either cilia (ciliated olfactory neurons) or microvilli (microvillar olfactory neurons). The presence of these two neuron types is a distinguishing feature of teleost fish. The olfactory nerve is formed by the joining of the axon of millions of olfactory receptor neurons (ORN). The ORN replaced continuously following chemical or axonal lesions. In fish as well as other vertebrates, the ORN axon project directly to the olfactory bulb to synapse with second order olfactory neurons.

2. Anatomical features of the olfactory system

The olfactory organ composed of olfactory rosette, olfactory nerve and olfactory bulb.

2.1. Olfactory Rosette:

Olfactory rosette is made up of olfactory epithelium which is raised from the floor of the nasal sac and formed complicated series of folding. The arrangement, shape, number and degree of development of the olfactory rosette vary species to species (Hara 1975). The number of lamellae increases to some extent with the growth of the individual but remains relatively constant when the fish reaches certain developmental stage (Yamamoto 1982). According to Yamamoto (1982), sometimes the arrangement of olfactory lamellae uses to identify the genera of the fish. In some species secondary folding in lamellae also found. Secondary folding in rainbow trout is first observed by Teichmann (1954). Pfeiffer (1963) also reported the presence of secondary lamellae in pacific salmon and in rainbow trout. Secondary folding also found in sharks, dipnoi and garfish. According to Hara (1975), Bertmar (1972), Pfeiffer (1963), Bashor et al. (1974), secondary folding of the lamellae is the mechanism to increase the surface area of the lamellae, which in turn increase the olfactory capacity nothing else.

Table 1: Number of olfactory lamellae in different fishes

Name of the fish	Number of lamellae	Reference
Epalzeorhynchos bicolor	45-48	Mokhtar and Abd-Elhafeez 2014
Labeo bata	46-48	Samajdar and Mandal 2016
Labeo rohita	32-38	Ghosh and Mandal 2014
Macrognathus aculeatus	16-18	Chakrabarti and Guin 2011
Oreochromis nilotica	19-20	Chakrabarti and Ghosh 2011
Puntius javanicus	25-26	Chakrabarti and Ghosh 2010
Etroplus suratensis	12	Ghosh and Chakrabarti 2010
Cyprinus carpio	25-26	Chakrabarti et al. 2006
Channa punctatus	18-20	Mandal et al. 2005
Acanthopagrus schlegeli	51	Ralph et al. 2002
Lampris guttatus	14-16	Mana and Kawamura 2002
Coryphaena hippurus	61-64	Mana and Kawamura 2002
Ictalurus punctatus	25-32	Yasuhiro et al. 1998
Alburnus alburnus	23	Hernadi 1993
Salvelinus alpinus	12-16	Hara et al.1992
Salvelinus fontinalis	12-17	Hara et al.1992
Salvelinus narnaycush	13-17	Hara et al.1992
Plotosus lineatus	40-160	Theisen et al. 1991
Siluiris glanis	109	Jakubowski and Mikrosk 1981
Anabas testudineus	7-10	Rahmani and Khan 1980
Salvelinus fontanalis	12-14	Hara et al. 1973
Coregonus clupeaformis	12-16	Hara et al. 1973
Thymallus articus	10-15	Watling and Hillemann 1964

Haplopagrus guentheri	230	Pfeiffer 1964
Channa argus	80-90	Shibuya 1960
Poxinus poxinus	11-19	Wunder 1957
Gasterosteus aculeatus	2	Wunder 1957
Salmo gairdneri	14-18	Wunder 1957
Lota lota	30-32	Wunder 1957
Anguilla anguilla	60-90	Wunder 1957

2.1.1. Olfactory epithelium:

Olfactory epithelium positioned in two rows separated by a central core to form the olfactory lamellae. The epithelium is separated into two regions sensory and nonsensory. All classes of vertebrates olfactory epithelium almost share similar pattern of histology and ultrastructure (Bannister 1965, Frisch 1967, Kerjaschki 1977, Muller and Marc 1984, Morrison and Costanzo 1990). Sensory and nonsensory epithelium distribution pattern are categorised into different types by Yamamoto and Uede (1979). In category I, the sensory epithelium distributed except for the edge of each lamellae. In category II, the sensory epithelium is partitioned into large areas by nonsensory epithelium. In category III, the sensory epithelium is dispersed into distinct islets and in category IV, the sensory epithelium is interfering regularly with the nonsensory epithelium. The category V is described by Bandyopadhyay and Dutta (1998), which is sensory epithelium is confined to the proximal region where the middle and distal region are occupied by nonsensory region. Farbman (1992), Zeiske et al. (1992) solve the matter because the epithelium called pseudostratified; in histological sections it is seen that the nucleus scattered in different layers of the epithelial cells as a result is seems to look like stratified epithelium though it is not true.

2.1.1.1. Sensory epithelium:

The sensory epithelium composed of the following cell types:

2.1.1.1.1. Receptor cells:

The olfactory receptor cell is a bipolar primary neuron with a cylindrical dendrite. Dendrite terminates at the free surface of the epithelium or to the external environment. The distal end of dendrite forms a swelling (olfactory knob) producing slightly above the epithelial surface.

Teleost possess two types of receptor cells that are ciliated and microvillar (Zielinski and Hara 1988). Lampreys possess only ciliated receptor cells (Thronhill 1967) while hagfish have both ciliated and microvillar receptor cells (Theisen 1973, 1976) and cartilaginous fish possess only microvillar receptor cells (Theinsen et al. 1986, Zeiske et al. 1992). In some species there is a third type of receptor cell found with rod-like process (Bannister 1965, Ichikawa and Ueda 1977, Muller and Marc 1984). Crypt cells mainly present in actinipterygian fishes including freshwater (*Xiphophorus helleri, Carassius auratus*) and marine fishes (*Plotosus lineatus*) (Hansen and Finger 2000). According to Yamamoto 1982, an average teleost contains 5 to 10 million olfactory receptor cells in each side of the nasal cavity.

Number of cilia and microvilli vary in the receptor cells in a species specific way. TEM study of cilia shows 9+2 axonemal process but the microvilli do not content any skeletal structure. Third type of receptor cells are crypt cells, the cell body is oval and are present at the upper third of the olfactory epithelium, and these cells bears both cilia and microvilli.

Olfactory Receptor Cells (ORC) are columnar (14.12-24.37 µm) and extended from the epithelium surface to basal lamina. The bipolar ORC has a round cell body, a long dendrite and an axonal process. Apical surface of the dendrite possesses either 10-12 cilia or microvilli. TEM shows rich mitochondrial population in the dendrite part. The receptor cells form a part of the olfactory transduction mechanism, which is stimulated by odour-bearing substances and detection of food (Zeiske et al. 2003). Hansen et al. (2003) shows that olfactory epithelium of channel catfish contains three intermingled types of olfactory receptor neurons: ciliated, microvillus, and crypt, which are responsible for the detection of bile salt and amino acid odorants.

2.1.1.1.1.1. Ciliated receptor cells:

The cilia of the ciliated receptor cell emerge from the knob of the cell, which is a convex distal end of the cell. The cell body is situated at the lower third region of the epithelium. The number and length of the cilia vary considerably according to the species, size and age. In Zebra fish the number of cilia varies from 3-10 and length 2-3 µm (Hansen and Zeiske 1998). In *Danio rerio* the cell body as well as the nuclei of microvillous receptor cells generally are less basophilic than those of the neurons with ciliated receptor cells (Hansen and Zeiske 1998).

2.1.1.1.1.2. Microvillous receptor cells:
Microvillous receptor cells contains short olfactory knob with microvilli projected on the surface of the epithelium. The cell body located in the mid region of the epithelium. Number of microvilli varies according to species; 30-60 in *Anguilla anguilla* (Schulte 1972), 40-70 in *Carassius auratus* (Hansen et al.1999) in *Danio rerio* 10-30 short microvilli 0.5-0.8 µm, in diameter (Hansen and Zeiske 1998). The cells are often more electron-dense than the neighbouring supporting cells, the receptor cells are rich in polyribosomes, RER and mitochondria (Hansen and Zeiske 1998).

2.1.1.1.1.3. Crypt receptor cells:
The cell body is situated at the upper third region of the epithelium, the cell body is submerged, apical part contains microvilli and the bottom contains short cilia. Hansen and Finger (2000) show crypt supporting cells, which bears numerous microvilli at their apical end. In loach fish, *Triplophysa dalaica* crypt cells were present in the lamellae (Waryani et al. 2013). Crypt cells were also found in catfish, sword tail, and needle fish (Hansen et al. 1997). Hansen & Zeiske (1998) reported that the crypt cell is a receptor neuron in the peripheral olfactory organ of the zebra fish, *Danio rerio*. The crypt cells of *Danio rerio* are surrounded by one or two specialized supporting cells which are particularly electron-lucent and bear microvilli-like apices (Hansen & Zeiske 1998).

2.1.1.1.2. Supporting cells (SC):
These are broad and columnar in shape (10.25-12.55 µm), alternatively arranged with receptor cells. Apical surface possesses microvilli and the cell body is more or less cylindrical. Prominent nucleus located in more superficial layer in the epithelium in a single row. Ciliated supporting cell are present in *Salmo trutta trutta* (Bertmar 1973) and *Anguila anguilla* (Schulte 1972). These cells are located in close association to the ORCs. Many secretory vesicles are found in the apical portion of cytoplasm.

2.1.1.1.3. Basal cells (BC):
The basal cells are small and undifferentiated and found adjacent to the basal cell membrane. It is thought that these cells are the precursors of the receptor or supporting cells (Hara 1994). Basal are small, round with prominent central nucleus. Basal cells (BC) are of two types, have been seen through electron microscopy- a flattened cell with dark cytoplasm, globular basal (GBC) cells and round shaped with light cytoplasm, horizontal basal cells (HBC) (Graziadei and Monti Graziadei 1979). These cells are situated at the basal part of the epithelium. According to Yamamoto (1982), Zeiske et al. (1992) basal cells may act as stem cells for regeneration of the olfactory epithelium, which characterized by relatively short life span and may be replaced throughout the life by these progenitor basal cells. This view is supported by presence of rough endoplasmic reticulum in the cytoplasm of red-tail shark, *Epalzeorhynchos bicolour* basal cells (Mokhtar and Abd-Elhafeez 2014).

2.1.1.2. Non-sensory epithelium:
The non-sensory epithelium consists of ciliated non-sensory cells, epidermal cells, basal cells and secretory cells like mucous cells also found in the non-sensory epithelium.

2.1.1.2.1. Ciliated non-sensory cells:
Ciliated non-sensory cells are present mainly at the non-sensory epithelium in teleost (Hansen and Zielinski 2005). These cells present as islets in the sensory areas of goldfish (Hansen et al. 1999). The cilia protruded from the apical surface of these cells vary species to species, in *Danio rario* up to 60 (Hansen and Zeiske 1998) and *Anguilla anguilla* up to 140 cilia (Schulte 1972). The broad apical surface of these cells involved in mucous and water across the epithelium (Zeiske et al. 1992).

2.1.1.2.2. Mucous cells (MC):
Mucous cells are large and oval in shape (6.85-7.12 µm), contains many secretory granules in the cytoplasm with various electron densities. Found throughout the epithelium, especially at the non-sensory regions. The nucleus situated at the basal part of the cell. Secrete both acid and neutral mucin (PAS-AB positive) and their secretion create a suitable medium for diffusion of odorants and help in smooth flow of water and prevent damage caused by infectious agents. In salmonids, additional mucous cells are present in the non-sensory epithelium in the neuroepithelium (Hara 2011).

2.1.1.2.3. Epidermal cells:
This cell is the basic component of the nonsensory epithelium. Median raphae covered with epidermal cells, which is a part of nonsensory areas. This type of cells has fingerprint like microridges patterns (Yamamoto 1982; Zeiske et al. 1992).

2.2. Olfactory nerve:
The olfactory nerves made up of unmyelinated axons, arise from the olfactory epithelium and terminate in the olfactory bulb. The axons of the olfactory nerve united to forms the olfactory nerve fascicle, according to Farbman (1992) the axons are very fine and their diameter is about 0.1-0.3 µm. The length of the olfactory nerve which connects the olfactory epithelium and olfactory bulb is varies depending upon the position of the olfactory bulb in a variety of species. Depending upon the position of the olfactory bulb, these can be of three types.

1. Position of the olfactory bulb close to nose: in this type the olfactory nerve is very short; therefore, a long olfactory tract is present, pedunculate form. All elasmobranchs' possess this type.
2. The olfactory bulb present close association to hemisphere of forebrain: as a result long olfactory nerve is present, sessile form. Most of the teleost posses this type, *Anguilla*, *Esox*, Salmon.
3. The bulb situated between nasal aperture and forebrain: so olfactory nerve in this category is not so long. This type found in *Raniceps raninus* (Døving 1967).

2.3. Olfactory bulb:
Though the position of olfactory bulb varies species to species, the basic structure and function are same. The main function of the bulb is to synaptic contact between the primary olfactory nerve fibres and dendrites of the secondary neurons that are called mitral cell and satellite cells (Hara 1975). Olfactory bulb is divisible into four distinct layers from outer to the inner regions, which are olfactory nerve layer, glomerulus layer, mitral cell layer and granular layer respectively.

3. Neurogenesis in the olfactory epithelium:

Olfactory sensory neurons can be replaced; initiation of the renovation is started in by the mitosis of the basal cells (Nagahara 1940). Olfactory neurogenesis is an ongoing process and the period of degeneration and regeneration is varying species to species. Coggings and Zwiers (1991) showed that olfactory neurogenesis an ongoing process, and is supported by different proteins associated with growth and maturation of the olfactory neurons. Such two neuron specific phosphoproteins are B-50 and GAP 43 which helps in growing, developing and regeneration (Coggings and Zwiers 1991). Verhaagen et al. (1989) showed the expression of these proteins by immunohistochemistry in maturing sensory neurons, though olfactory sensory neurons continuously produced throughout adult life.

4. Organophosphate pesticides and its effects on the olfactory organ of fish:

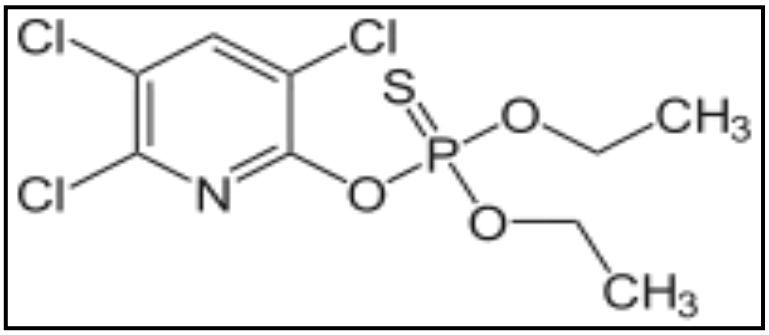
An organophosphate (OP) or phosphate ester is the general name for esters of phosphoric acid. The insecticidal properties of organophosphorus (OP) were first discovered in the 1930s, and the compounds were developed for pesticide use in the 1940s and in the beginning of 1980's chlorinated pesticides have been fully replaced by organophosphate pesticides (Svoboda et al. 2001). Organophosphate pesticides are one of the most potentially harmful toxic chemicals introduced into the environment. Though they have contributed considerably to human welfare, their adverse effects on non-target organisms such as fish are quite significant. According to Kumar et al. (2010) in India, pesticides constitute an important element in agriculture development and safety of public health since the tropical climate is very favourable to pest breeding. According to Ling et al. (2011) aquatic ecosystem may be contaminated by OP pesticides by runoff and ground water leaching, which is a serious problem and fishes are more frequently exposed to these pollutants and may be taken in through gills, skin and contaminated foods. These OP pesticides may cause different levels of environmental contamination often affecting non target organisms (Silva et al. 1993, Rodrigues and Fanta 1998).
The toxicity of OP pesticides is due to the disruption of the nervous system of an invertebrate or a vertebrate through the inhibition of cholinesterase (ChE) enzymes. These enzymes are involved in transmitting normal nerve impulses acetylcholine throughout the nervous system. An acute pesticide dose reduces the activity of ChEs, and nerve impulses cannot be transmitted normally. This can paralyze the nervous system, and it may lead to death, usually from respiratory failure (Amdur et al. 1991, Grue et al. 1983).

4.1. Chlorpyrifos:

4.1.1. Chemical Class and Type:

Chlorpyrifos is one of the broad-spectrum, chlorinated organophosphate (OP) pesticides. Chemical name of chlorpyrifos is O, O-Diethyl O-3, 5, 6-trichloropyridin-2-yl phosphorothioate. The Chemical Abstracts Service (CAS) registry number is 2921-88-2.

4.1.2. Molecular Structure -



4.1.3. Physical and Chemical Properties:

Chlorpyrifos is a colorless to white crystalline solid (Tomlin 2006). It has a faint mercaptan (thiol) odor, like the smell of sulfur compounds found in rotten eggs, onions, garlic and skunks (Lewis 1998).Vapour pressure: 1.87 x 10^{-5} mmHg at 25 °C; Octanol-Water Partition Coefficient (K_{ow}) (Tomlin 2006): 4.70; Molecular weight: 350.6 g M^{-1}; Solubility (water): 0.0014 g/L (1.4 mg L^{-1}) at 25 °C; and Soil Sorption Coefficient (K_{oc}): 360 to 31,000 depending on soil type and environmental conditions (Smegal 2000).

4.1.4. Effects of chlorpyrifos on fish:

Chlorpyrifos is the widely used organophosphate pesticide, second largest retailing in India and used for more than a decade to control pests on cotton, paddy fields, pasture and vegetable crops (Rao 2003). The non-target organism, fish are adversely effected because of its increased toxic load to aquatic environment. Acute and chronic toxic effects of chlorpyrifos in different fish species were extensively studied (Anita et al. 2016, Padmanabha et al. 2015, Díaz and Girón 2014, Banaee et al 2013, Ali and Kumar 2012, Reddy et al. 2011, Oruç 2010, Halappa and David 2009, Ramesh and Saravanan 2008, Chawanrat et al. 2007, Girón et al. 2006).

4.1.5. Availability in different water sources:

Chlorpyrifos measured several different concentrations in water, sediments and suspended particles collected from Horqueta and Brown streams of Argentina (Jergentz et al. 2005), Laguna de Terminos, Mexico (Carvalho et al. 2009), Caspian Sea, Iran (Rahmanikhah et al. 2011), Lake Naivasha, Kenya (Otieno et al. 2012), Paddy field water samples, Bangladesh (Bhattacharjee et al. 2012), New Dqamietta drainage canal and EI-Embaby drain, Egypt (Malhat and Nasr 2011). Fish were killed in an incident in association with CPF in water reaching several hundred ppb was also reported earlier (Abdel Halim et al. 2006).

In India CPF residues were also detected in water samples (0.003-0.006 µl L^{-1}) collected from Kaithal and Pant Nagar areas (Mukherjee and Arora 2011), in 16% and 20% of the made tea samples of Dooars and Hill regions, West Bengal respectively (Bishnu et al. 2009) and in tissues of fish (88.6 µg/g) collected from Kolleru Lake in Andhra Pradesh (Amaraneni and Pillala 2001).

Table 2: Acute toxicity of CPF

Name of the fish	96h LC_{50} value (µg L^{-1})	References
Heteropneustes fossilis	174	Misha and Verma 2016
Labeo bata	109.64	Samajdar and Mandal 2015
Channa punctatus	0.253	Devi and Mishra 2013
Poecila reticulata	176	Sharbidre et al. 2011a
Oreochromis niloticus	154.01	Oruç 2010
Cyprinus carpio (common carp)	160	Halappa and David 2009

Gambusia affinis (mosquito fish)	297	Kavitha and Venkateswara 2008
Notemigonus crysoleucas golden shiner)	35	Barron and Woodburn 1995
Anguilla Anguilla (European eel)	540	Ferrando et al. 1991
Rutilus rutilus (Roach)	250	Douglas and Bell. 1990
Salvelinus namaycush (Lake trout)	419	Mayer and Ellersieck 1986
Lepomis macrochirus (Bluegill sun fish)	4.2	Mayer and Ellesieck 1986
Oncorhynchus mykiss (rainbow trout)	24	Mayer and Ellersieck 1986
Cyprinodon variegates (sheepshead minnow)	136	Clark et al. 1985
Carrassius auratus (Gold fish)	524	Holcombe and Phipps 1985
Pimephales promelas (fathead minnow)	203	Holcombe et al. 1982
Ictalurus punctatus (channel catfish)	280	Johnson and Finley 1980
Onchorhincus clarki (Cut throat trout)	18	Johnson and Finley 1980

4.1.6. Effects on development:

A variety of studies have shown that CPF exposure during development even at a low doses and low exposure period to fish can cause persisting neurobehavioural dysfunction. Levin et al. (2004) studied on the effects of CPF on zebrafish hatchling's swimming behaviour, and he found that behavioural impairment was persists till adulthood. It was found that CPF exposure to the embryonic zebrafish causes significant impairment in discrimination learning and swimming speed (Levin et al. 2003). In another experiment it was found that when zebrafish larvae from 0 to 7 days post fertilization exposed of sub-chronic dose of 1 µM CPF shows significantly impaired body morphology (Richendrfer et al. 2012).

4.1.7. Neurotoxic effects:

CPF can produce neurotoxic effects. In mammalian models numerous studies have assessed the cognitive alterations after acute or chronic exposure to CPF (Braquenier et al. 2010, Middlemore-Risher et al. 2010). In fish the data available are sparse. In zebrafish, CPF causes persistent neurobehavioral impairment tests were done on sensorimotor response, stress response and learning, this study demonstrated that CPF caused selective longterm neurobehavioral alterations in zebrafish (Sledge et al. 2011). Eddins et al. (2010) show a significant decrease in the activity of whole brain is occurs in the zebrafish after exposure to CPF.

4.1.8. Genotoxicity:

Most of the pesticides are genotoxic and having the possibility of causing DNA damage, increased incidences of neoplasia and adverse effect on vitality and progeny of aquatic animals, as a result which may reduce the productivity of aquaculture (Kaushik and Kaushik 2007). Anita et al. (2016) used micronucleus (MN) assay to analyzed the incidence of nuclear anomalies in the blood cells of freshwater fish *Cirrhinus mrigala*, in the experiment they found that MN induction was peak on day 14 at 0.08mgL^{-1} concentration of chlorpyrifos. Effects of CPF on the nucleus were also clearly evident from their study as alteration in cell morphology, presence of nuclear anomalies as broken egg and large size micronuclei. Palanikumar et al. (2014) reported increased micronuclei in *Chanos chanos* with increasing doses of chlorpyrifos. Ali et al. (2009) studied the MN induction in *Channa punctatus* on exposure of chlorpyrifos (203µgL^{-1}) on days 1, 3, 5, 7, 14, 21, 28, and 35 and observed maximum MN induction (1.62%) on day 14, highest DNA damage was observed on day 5, followed by a gradual nonlinear decline in the lymphocytes and gill cells.

4.1.9. Behavioural toxicity:
Behaviour is also measured a potential means in ecotoxicology which is an integrated result of endogenous and exogenous processes and low level of exposures have been implicated in various behavioural and physiological impairments (Taylor and Brown 1999, Cohn and MacPhail 1997). Sledge et al. 2011, Levin et al. 2004 studied on zebrafish, and CPF exposure cause persisting developmental behavioral dysfunction. Swimming behaviour of fish is frequently observed as a response in toxicity investigations because changed locomotor activity can point to effects to the nervous system. In the experiment of Sharbidre et al. (2011a) on *Poecila reticulata* when the fish exposed to sublethal concentration of CPF showed less activity, loss of equilibrium, motionlessness, erratic swimming and loss of appetite. The fish stood motionless in the aquarium bottom, hanging vertically.

4.1.10. Effects on histopathological architecture:
Histological information, along with biochemical and physiological data may provide a more complete and accurate explanation of the activity leading to death of the organisms due to a chemical agent (Mehrle and Mayer 1985). Fish livers and gills are useful biomarkers, as their morphological alterations to indicate prior exposure to environmental stressors or toxicants specially CPF. Due to their lipophilicity liver is the main organ of detoxification, CPF having also a high rate of gill absorption; this could be a contributing factor in the sensitivity of the fish to this pesticide exposure (Ali et al. 2009). Hypertrophy; hyperplasia and lifting of epithelial cells and fusion of secondary lamellae in gill were pronounced in all treatments.
In a study on common carp, CPF altered the structure of the gills and liver. In the liver tissue of common carp hydropic degeneration, vacuolisation, pyknotic nuclei, and fatty infiltration were revealed while the gills of common carp displayed varied degrees of epithelial hypertrophy, oedema with epithelial separation from basement membranes, general necrosis, and epithelial desquamation (Xing et al. 2012). Manjunatha and Philip (2015) observed vacuolization and presence of sinusoid spaces in liver tissue of *Danio rerio* exposed to 200 μgL^{-1} of chlorpyrifos for 24, 48, 72 and 96h. Topal et al. (2014) observed degenerative changes and hyperaemia in liver and lamellar hyperaemia, lamellar oedemas, clumping, cellular degeneration, hyperplasia, and lamellar atrophy in gill of rainbow trout. Devi and Mishra (2013) reported that sublethal concentrations of chlorpyrifos (1.46 μlL^{-1} and 0.538 μlL^{-1}) for 3 and 7 days caused histopathological changes in liver and gill tissues of *Channa punctatus*. Aniladevi et al. (2008) observed similar histopathological alterations in gill and liver tissues of *Oreochromis mossambicus* exposed to sublethal concentration of CPF. De Silva and Samayawardhena (2002) observed similar effect on gill of *Poecilia reticulata* exposed to CPF.

4.1.11. Effect on endocrine function:
CPF is suspected as triggers for harmful effects on the reproductive system in fish. Chlorpyrifos can interfere with steroid hormone production. Oruç (2010) reported that in *Oreochromis niloticus*, CPF exposure decreased serum estrogenic and testosterone levels, estradiol level after 15 days of exposure decreased by chlorpyrifos treatments. Cortisol level in *Oreochromis niloticus* was also found to be lower than that of control level after CPF treatments (Oruç 2010).

4.1.12. Induction of biochemical alterations:
According to Banaee et al. (2008) pesticides can cause serious impairment to physiological and health status of fish, so biochemical tests are routine laboratory tests useful in recognizing acute or chronic toxicity of insecticides. Padmini and Rajaram (2016) studied the effect of different concentrations of chlorpyrifos on protein, glycogen and lipid in liver and kidney of *Channa gachua* for 96h and reported decreased levels in both the tissues in comparison with control. Khatun and Mahanta (2014) reported that Sub-lethal dose of chlorpyrifos on *Heteropneustes fossilis* causes significant decrease in serum T3, T4 and TSH levels. Khan and Sharma (2014) reported an increase in ACP, ALP, LDH and decrease in ATPase at various concentration of CFP for different exposure periods. Similar result was found in *Gambusia affinis* due to sublethal exposure of CPF (Khan and Sharma 2014). Topal et al. (2014) observed CPF exposure in rainbow trout causes a decreased CA enzyme activity in the gills and decrease in CA activity in liver. Protein profiles in liver tissue of *Carassius auratus* exposed mixed response exposed to CPF (Vaidehi et al. 2013). Banaee et al. (2013) reported hyperglycemia, increased blood triglyceride, and increased levels of AST, LDH in *Cyprinus carpio* exposed to CPF for varied exposure period. Reddy et al. (2011) studied the effect of sublethal concentrations of chlorpyrifos on protein metabolism in gills, kidney, liver, and muscle of *Clarias batrachus*.

4.1.13. Immunological alterations:
According to Banaee (2012) pesecticides can alter the immune functions of the body and result in uncontrolled cell proliferation, immune-depression, and alterations of the host protection mechanism against pathogens. Wang et al. (2011) reported the effects of chlorpyrifos on the immune factors of fish such as mRNA levels of IL-1β and IFN- γ2b in immune organs of common carp. Díaz and Girón (2014) studied the immune response parameters of *Oreochromis niloticus* in which IgM concentration in plasma is decreased after exposure of chlorpyrifos. On the other hand, organisms which are exposed to high concentration of the pesticide showed an increase in the lysozyme activity. Girón et al. (2006) reported that *O. Niloticus* exposed to CPF during 96h caused significant decline in the phagocytic capacity and in the percentage of phagocytic cells present in blood.

4.1.14. Oxidative stress:
Oxidative stress has also become a very popular of toxicological research as a possible mechanism of toxicity (Abdollahi et al. 2004, Sharma et al. 2005). It occurs due to the imbalance between the productions of reactive oxygen species (ROS) and antioxidant defences in living organisms (Nishida et al. 2011). According to Sevcikova et al. (2011) the main endogenous source of ROS is mitochondrial respiration. ROS are produced by living organism as a result of normal cellular metabolism by electron transport chains of mitochondria and endoplasmic reticulum. ROS can be also generated by several oxidative enzymes during catalysis, including xanthine oxidase, tryptophan dioxygenase, diamine oxidase, guanyl cyclase and glucose oxidase (Halliwell and Gutteridge 1999, Fridovich 1978).
An antioxidant defense system (ADS) is needed to protect biomolecules from the harmful effects of reactive oxygen species (ROS). Fish are endowed with defensive mechanisms to neutralize the impact of reactive oxygen species (ROS) resulting from the metabolism of various chemicals. These include various antioxidant defense enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPOx), glutathione S-transferase (GST), and glutathione reductase (GR). Low molecular weight antioxidants such as glutathione (GSH), ascorbate (vitamin C), and vitamin A are also reported to contribute in the quenching of oxyradicals (Orbea et al. 2002). Membrane lipids are one of the most important targets of ROS, which undergo peroxidation (LPO). Thus, LPO estimation has also been successfully employed to signify oxidative stress induced in aquatic animals by such chemicals (Favari et al. 2002, Monserrat et al. 2003).

4.1.14.1. Lipid peroxidation (LPO):
Lipid peroxidation (LPO) is one of the molecular mechanisms involved in pesticide toxicity (Kehrer 1993). According to Kavithaa 2008, an elevated lipid peroxidation level was observed in *Gambusia affinis* after 96 h exposure to the lethal concentration of CPF. An altered result was found in the same study found with decreased levels of antioxidant enzyme (SOD, CAT and GR) activities in the exposed fish can also be effectively used for better assessment of CPF toxicity in biomonitoring of aquatic environment. Lipid peroxidation is the most frequently used technique to analyse the effects of pollutants (Livingstone 2001, Lushchak 2011). Ferreira et al. (2005), Farombi et al. (2007), shown elevated LPO in many fishes collected from heavily polluted sites. An increase in MDA levels and a decrease in non-enzymatic antioxidant levels were reported in different tissues of *Poecilia reticulata* exposed to chlorpyrifos (Sharbidre et al. 2011a).

4.1.14.2. Glutathione (GSH):
Glutathione is the main non protein thiol which plays the major function in the antioxidant cellular defence. The main source of glutathione is liver and it is composed of glycine, cysteine and glutamic acid. Free glutathione is found as reduced form (GSH) and can be converted to oxidised form under stress. Enzyme glutathione reductase can revert the GSH into reduced form (Pastore et al. 2003).

4.1.14.3. Glutathione *S*- transferase (GST):
GST is one of the major enzymatic antioxidants, reported as the biomarker of oxidative stress of fish (Viarengo et al. 2007). GST-mediated conjugation may be an important mechanism for detoxifying peroxidized lipid breakdown products but at higher amounts it has adverse biological effects (Leaver and George 1998). The main function of GST activity is protection against the toxicity of xenobiotic-induced lipid peroxidation. Elevated GST activity may reflect the possibility of better protection against pesticide toxicity. *Oreochromis niloticus* increase GST activity with the treatment of chlorpyrifos (Oruc 2010). GST activity in *Salmo trutta* has been observed inductive effects exposed to propiconazole (Egaas et al. 1999). Guppy, *Poecila reticulate* shows oxidative stress-induction potential in brain, liver and gill tissues exposed for 96 h to different sublethal concentrations of CPF.

4.1.14.4. Catalase (CAT):
All aerobic organisms contain enzyme catalase (CAT), which protects the cell from toxic effects of H_2O_2 caused by various metabolic actions, by converting water and oxygen. The structure of CAT is a tetramer of four polypeptide chains and used four porphyrine heame groups for the reaction. CAT, GST, GR and SOD levels fluctuated in all treatment groups relative to the control (Deb and Das 2013).

4.1.14.5. Superoxide dismutase (SOD):
Superoxide dismutase is an important antioxidant enzyme found in all aerobic organisms inside and outside of the cell membrane. SOD represents a class of closely related enzymes that catalyze the breakdown of the superoxide anion into oxygen and hydrogen peroxide (H_2O_2) (Zelko et al. 2002). SOD together with GPx and CAT form the main enzyme defence mechanism against harmful effects of free radicals.

4.1.15. Acetylcholinesterase (AChE) activity
CPF is well known chemical to inhibit enzyme Acetylcholinesterase (AChE) activity (Taylor and Brown 1999). Symptoms of high-level exposure to OPs include muscle twitching, hyperactivity, paralysis, loss of stability and eventually death, whereas low level exposures have been implicated in various behavioural and physiological impairments (Sandahl et al. 2005, Kavithaa et al. 2008, Leticia and Derardo 2008). Two main specific biomarkers for pesticides are AChE and carboxylesterase (CbE). AChE in fish is mainly cholinergic and its activity is essential for normal behaviour and muscular function (Kirby et al. 2000). The acute systemic toxicity of CPF is caused by inhibition of cholinesterase through the active metabolite chlorpyrifos oxon, CPF is more toxic in immature animals despite their ability to recover rapidly from cholinesterase inhibition. According to Behra et al. (2002), AChE is critical to the normal expansion of the zebrafish nervous system so zebrafish studies of the neurobehavioral teratology of acetylcholinesterase inhibitors like CPF are predominantly significant. CPF was an effective inhibitor of AChE activity in fingerling channel catfish (Straus and Chambers 1995). In a study with *Gambusia affinis* shows an inhibition of AChE activity exposed to lethal concentration of CPF for 96 h, (Kavithaa et al. 2008). The inhibition of AChE leading to the accumulation of ACh at synaptic junctions might have been altered the locomotor behaviour of exposed fish. A positive relationship was found between the recovery pattern of AChE activity and locomotor activities. In ecotoxicology, it is clearly illustrated that the locomotor behaviour of test organism as a promising tool to assess the recovery status of test organism after adverse affects. *Poecila reticulate*, fish showed in the brain AChE dose-dependent inhibition. Fish exposed to the higher concentrations of CPF showed upto 66% inhibition of AChE (Samajdar et al. 2023)

5. Conclusion

In conclusion, it has been demonstrated that a brief exposure of the olfactory rosette to any of the organophosphate pesticides is sufficient to elicit changes in not only the rosette's olfactory neurophysiology, but also in AChE activity in the primary olfactory system and brain. It is obvious that the impairments caused by the brief exposure times used in this investigation have the potential to dramatically affect olfactory activities in wild fish. This study contributes to a growing body of evidence indicating that the contamination of freshwater habitats with neurotoxic chemicals that affect salmonid olfaction may be deleterious to fish populations' ecological fitness and long-term survival.

References:

1. Amdur MO, Doull J, Klaassen DC (1991) eds., Casarett and Doull's Toxicology, The basic science of poisons, (4th ed.): Elmsford, N.Y., Pergamon Press, 1,033 p.
2. Anita B, Yadav AS, Cheema N (2016) Genotoxic effects of chlorpyrifos in freshwater fish Cirrhinus mrigala using micronucleus assay. Advances in Biology 1-6.
3. Bandyopadhyay SK, Datta NC (1998) Surface ultrastructure o the olfactory rosette of an air-breathing catfish, Heteropneustes fossilis (Bloch). Journal of Biosis 23: 617–622.
4. Bannister LH (1965) The fine structure of the olfactory surface of teleostean fishes. Quarterly Journal of Microscopical Science 106: 333-342.
5. Bannister LH (1965) The fine structure of the olfactory surface of teleostean fishes. Quarterly Journal of Microscopical Science 106: 333-342.
6. Bashor D P, Beuerman RW, Easton DM (1974) Ciliary Action and Normal Movement of odorant wavefronts in garfish nasal capsule of Lepisosteus asseus. Experientia 30: 777–779.

7. Bertmar G (1972) Secondary Folding of Olfactory Organ in Young and Adult Sea Trout. Acta Zoologica 53: 113-120.
8. Cogging PJ, Zwiers H (1991) B-50 (GAP-43): biochemistry and functional neurochemistry of an neuron-specific phosphoprotein. Journal of Neurochemistry 56: 1095-1106.
9. Farbman AI (1992) Cell biology of olfaction. In development and cell biology series (eds) Barlow PW, Bray D, Green PB, Slack JMW. Cambridge University Press, U.K. pp 167-206.
10. Frisch D (1967) Ultrastructure of the mouse olfactory mucosa. American Journal of Anatomy 121: 87-120.
11. Ghosh D, Mandal DK (2014) Mercuric chloride induced toxicity responses in the olfactory epithelium of Labeo rohita (HAMILTON): a light and electron microscopy study. Fish Physiology and Biochemistry 40(1): 83-92.
12. Grue CE, Fleming WJ, Busby DG, Hill EF (1983) Assessing hazards of organophosphate pesticides in wildlife, in Transactions of the 48th North American Wildlife & Natural Resources Conference: Washington, D.C., The Wildlife Management Institute, p 200–220.
13. Hamdani EH, Døving KB (2007) The functional organization of the fish olfactory system. Progress in Neurobiology 82: 80-86.
14. Hansen A, Eller P, Finger TE, Zeiske E (1997). The crypt cell; a microvillous ciliated olfactory receptor cell in teleost fishes. Chemical Senses 22: 694–695.
15. Hansen A, Finger TE (2000) Phyletic distribution of crypt- Type Olfactory receptor neuron in fishes. Brain Behaviour and Research 55: 100-110.
16. Hansen A, Finger TE (2000) Phyletic distribution of crypt- Type Olfactory receptor neuron in fishes. Brain Behaviour and Research 55: 100-110.
17. Hansen A, Rolen SH, Anderson K, Morita Y, Caprio J, Finger TE (2003) Correlation between olfactory receptor cell type and function in the channel catfish. Journal of Neuroscience 23: 9328–9339.
18. Hansen A, Zeiske E (1998) The peripheral olfactory organ of the Zebrafish, Danio rario: An ultrastructural study. Chemical senses 23: 39-48.
19. Hansen A, Zippel HP, Sorensen PW, Caprio J (1999) Ultrastructure of the olfactory epithelium in intact, axotomised and bulbeotomized goldfish, Carassius auratus. Microswcopy Research and Technique. 45: 325-338.
20. Hara TJ (1975) Olfaction in fish. Progress in Neurobiology 5: 271-335.
21. Hara TJ (1993) Chemoreception. In: The Physiology of Fishes. (Ed. Evans, D.H.). CRC Press, Boca Raton FL, pp. 191-218.
22. Hara TJ (2011) Gustatory detection of tetrodotoxin and saxitoxin, and its competitive inhibition by quinine and strychnine in freshwater fishes. Marine drugs 9: 2283-2290.
23. Hara TJ, Zielinski B (1989) Structural and functional development of the olfactory organ in teleosts. Transactions of the American Microscopical Society 118: 183-194.
24. Ichikawa M, Ueda K (1977) Fine structure of the olfactory epithelium in the goldfish, Carassius auratus. A study of retrograde degeneration. Cel and Tissue Research 183: 445-455.
25. Kerjaschki D (1977) Some freeze-etching data on the olfactory epithelium. In Olfaction and Taste VI. Eds. Magnen J. Le and MacLeod P. Information Retrieval, Ltd. London pp 75-85.
26. Kumar M, Prasad MR, Srivastva K, Tripathi S, Srivastva AK (2010) Branchial histopathological study of Catfish Heteropneustes fossilis following exposure to purified neem extract, Azadirachtin. World Journal of Zoology 5(4): 239-243.
27. Laberge F, Hara TJ (2001) Neurobiology of fish olfaction: A review. Brain Research Reviews 36: 46-59.
28. Ling XP, Zhang YH, Lu YH, Huang HQ (2011) Superoxide dismutase, catalase and acetyl cholinesterase: biomarkers for the joint effects of cadmium, zinc and methyl parathion contamination in water. Environmental Technology 32(13): 1463-1470.
29. Morrison E E, Costanzo R M (1990) Morphology of the human olfactory epithelium. The Journal of Comparative Neurology 297: 482-95.
30. Muller JF, Marc RE (1984) Three distinct morphological classes of receptors in fish olfactory organs. Journal of Comparative Neurology 222: 482-495.
31. Muller JF, Marc RE (1984) Three distinct morphological classes of receptors in fish olfactory organs. Journal of Comparative Neurology 222: 482-495.
32. Padmanabha A, Reddy HRV, Khavi M, Prabhudeva KN, Rajanna KB, Chethan N (2015) Acute effects of chlorpyrifos on oxygen consumption and food consumption of freshwater fish, Oreochromis mossambicus (Peters). International Journal of Recent Scientific Research 6(4): 3380-3384.

33. Pfeiffer W (1963) The morphology of the olfactory organ of the Pacific salmon (Oncorhynchus), Canadian Journal of Zoology 41: 1233-1236.
34. Rao JV, Rani CHS, Kavitha P, Rao RN, Madhavendra SS (2003) Toxicity of chlorpyrifos to the fish, Oreochromis mossambicus. Bulletin of Environmental Contamination and Toxicology 70: 985-992.
35. Rodrigues EL, Fanta E (1998) Liver histopathology of the fish Brachydanio rerio after acute exposure to sublethal levels of the organophosphate Dimetoato 500. Revista Brasileira de Zoologia 15: 441–450.
36. Samajdar I, Saha S and Mandal DK (2023) Chlorpyrifos induced oxidative stress responses and alteration of acetylcholinesterase activity in the olfactory organ of freshwater minor carp, *Labeo bata*. Iranian Journal of Ichthyology. 10(4): 248-263.
37. Samajdar I, Mandal DK (2015) Acute toxicity and impact of an organophosphate pesticide, chlorpyrifos on some haematological parameters of an Indian minor carp, *Labeo bata* (Hamilton 1822). International Journal of Environmental Sciences. 6(1): 106-113.
38. Samajdar I, Mandal DK (2016) Histological organization and ultra-structure of the apical surface of the olfactory epithelium of a carp, *Labeo bata* (Hamilton). International Journal of Pure and Applied Zoology. 4(2): 134-141.
39. Silva HC, Medina HGS, Fanta E, Bacila M (1993) Sub-lethal effects of the organophosphate Folidol 600 (methyl parathion) on Callichthys callichthys (Pisces, Teleostei). Comperative Biochemistry and Physiology 105: 197–201.
40. Smegal DC (2000) Human Health Risk Assessment Chlorpyrifos; U.S. Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Office of Pesticide Programs, Health Effects Division, U.S. Government Printing Office: Washington, DC.
41. Svoboda M, Luskova V, Drastichova J, Žlábek V. (2001) The effect of diazinon on haematological indices of common carp (Cyprinus carpio L). Acta Veterinaria Brno 70: 457-465.
42. Teichmann H (1954) Vergleichende untersuchungen an der nase der fische. Zeitschrift fur Morphologie und Okologie der Tiere 43: 171-212.
43. Theisen B (1973) The olfactory system in the hagfish Myxine glutiosa. I. Fine structure of the apical part of the olfactory epithelium. Acta Zoologica (Stockholm) 54: 271-84.
44. Theisen B (1976) The olfactory system in the Pacific hagfishes Eptatretus stoutii, Eptatretus deani, and Myxine circifons. Acta Zoologica (Stockholm) 54: 167-173.
45. Theisen B, Zeiske E, Breucker H (1986) Functional morphology of the olfactory organs in the spiny dogfish (Squalus acanthias L.) and the small spotted catshark (Scyliorhinus canicula L.). Acta Zoologica (Stockholm) 67: 73-86.
46. Thornhill RA (1967) The ultrastructure of the olfactory epithelium of the lamprey Lampetra fluviatilis. Journal of cell science 2: 591-602.
47. Tomlin C D S (2006) The Pesticide Manual, A World Compendium, 14th ed.; British Crop Protection Council: Alton, Hampshire, UK; p 186-187.
48. Verhaagen J, Oestreicher AB, Gispen WH, Margolis FL (1989) The expression of the growth associated protein B50/ GAP 43 in the olfactory system of neonatal and adult rats. Journal of Neurosciences 9: 683-691.
49. Yamamoto M (1982) Comparative morphology of the peripheral olfactory organ in teleosts. Fish Chemoreception, Hara TJ (Ed) Amsterdam: Elsevier pp. 39-59.
50. Yamamoto M (1982) Comparative morphology of the peripheral olfactory organ in teleosts. Fish Chemoreception, Hara TJ (Ed) Amsterdam: Elsevier pp. 39-59.
51. Yamamoto M, Ueda K (1979) Comparative morphology of fish olfactory epithelium. X. Perciformes, Beryciformes, Scorpaeniformes, and Pleuronectiformes. Journal of the Faculty of Science, the University of Tokyo (Section 4 Zoology) 14: 273-297.
52. Zeiske E, Kasumyan A, Bartsch P, Hansen A (2003) Early development of the olfactory organ in sturgeons of the genus Acipenser: A comparative and electron microscopic study. Anatomy and Embryology 206: 357–372.
53. Zeiske E, Theisen B, Breucker H (1992) Structure Development and Evolutionary aspects of the peripheral olfactory system, Fish chemoreception, Hara T.J, Ed London: Chapman and Hall. Pp. 13-39.
54. Zeiske E, Theisen B, Breucker H (1992) Structure Development and Evolutionary aspects of the peripheral olfactory system, Fish chemoreception, Hara T.J, Ed London: Chapman and Hall. Pp. 13-39.
55. Zielinski B, Hara TJ (1988) Morphological and physiological development of olfactory receptor cell in rainbow trout (Salmo gairdneri) embryos. Journal of Comparative Neurology 271: 300-311.
56. Zielinski B, Hara TJ (2001) The neurobiology of fish olfaction. In: Kapoor BG, Hara TJ (eds). Sensory Biology of Jawed Fishes: New Insights. Science Publishers. Enfield's. UK. Pp 347-366.

57. Zielinski B, Hara TJ (2007) Olfaction. In: Sensory Systems Neuroscience, Elsevier Academic Press, Oxford, pp 1-43.
58. Zippel HP, Hansen A, Caprio J (1997) Renewing olfactory receptor neurons in goldfish do not require contact with the olfactory bulb to develop normal chemical responsiveness. Journal of Comperative Physiology 181 (5): 425–437.