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Studies of the toxicity of pesticides methylamine on cat fish species

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Abstract
In the present study the toxicity of methylamine on fresh water teleosts i.e. Clarias batrachus and Heteropneustes fossilis has been noticed. Lethal and sublethal levels of the pesticide methylamine have been studied for histomorphology and histopathology in various organs viz. gill. Striking injury is seen in gill epithelium due to extreme levels of pesticide in Clarias batrachus and Heteropneustes fossilis. Fusion of gill lamellae, the separation of basement membrane, hyperplasia and severe erosion of secondary gill lamellae, thereby reducing the respiratory surface of gill were observed when treated with methylamine. Increased mucus secretion affected respiratory behavior, serious hypoxic conditions, reduced oxidative metabolism and ion regulation, the histopathological changes and the extent of damage were clearly dependent on the dose and duration.

Keywords: Pesticides, Methylamine, Cat fish

Introduction

Fish is one of the most important food for human health a good source of protein, fat, minerals, and vitamins. Fish liver oil is rich in many vitamins like A, D, and B-complexes. The fish, therefore can solve the problem of malnutrition in poor and developing countries. The pesticidal pollution is a global burning problem due to its extensive use. Pesticides are the chemicals used to control pests. Their residue reaches plants and animals through air, water and soil. In the environment, human beings and domestic animals (cows, pigs and pets) ingest these pesticides through food and fodder respectively. By consuming milk and meat products exposed to pesticides animals, further endanger human health. Pollution through air also deposits pesticide residue on crops. Pesticides may find their way into the overground water reservoirs, streams and thus producing an adverse impact on the aquatic biota including fishes. The concentration of various pesticides is increasing day by day in the ponds, streams and rivers, since they are used for insect control. These synthetic chemical compounds have altered the chemical nature of our environment. These chemicals reach in fresh water ecosystem due to spraying by public authorities, forest management and in agricultural fields. In some cases the industries directly discharge its effluent in lentic and lotic water system. Thus day by day increased use of pesticides is posing great threat to fresh water ecosystem especially fish. Fishes are highly susceptible to pesticides. Fresh water animals like fishes is one of the main source of food for rapidly increasing human populations, but the discharge of agricultural, municipal and industrial wastes into the aquatic environment causes high mortality of fishes. The environmental stress may be chemical, physical or radio-active in nature. Chemical pollution caused by different types of chemical substances viz., elements and their compounds, different organic substances such as insecticides, pesticides, solvents, fertilizers and variety of drugs to which we are normally exposed. Even if present in minute quantities, their variety, toxicity and persistence have an adverse effect on ecological systems of land and water, from which one can not separate the animal life, it's health and environment. Today about 1,000 or even more chemical formulations are used as pesticides around the world of which about 250 are commonly used in agriculture, including about 100 insecticides, 50 herbicides, 50 fungicides, 20 pesticides and 30 other chemicals.

Material and method

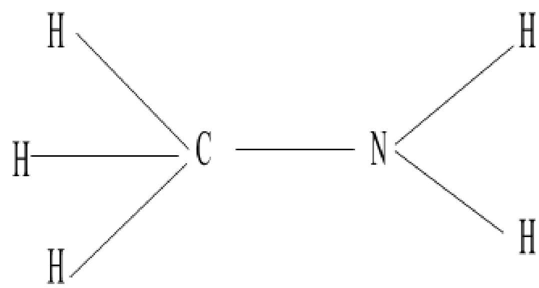
Experimental animals

This sub part deals with material and method employed for carrying out the present investigations. The present work is designed to observe the toxicity of a carbamate pesticide methylamine on two fresh water food fishes *Clarias batrachus* (Mangur) Subphylum- Gnathostomata, Series- Pisces, Class- Teleostomi, Subclass- Actinopterygii, Order-Cypriniformis, Division- Siluri and *Heteropneustes fossilis* (Singhi) Subphylum-Gnathostomata, Series- Pisces, Class-Teleostomi, Subclass- Actinopterygii, Order- Cypriniformis, Division- Siluri, with reference to histopathological aspects.

Toxicant used

Different types of insecticide carbamate pesticides are commonly used in agriculture operations and in controlling insects pests in the western Uttar Pradesh, including the vicinity of Khurja, district Bulandshahr. A carbamate pesticide has been selected for present investigation. Its structural formula is:

Formula:



Its chemical name is methylamine (9 C I): with molecular formula CH3NH2, molecular weight 31.06, soluble in water, boiling point $63C^0$ and melting point: $93.5C^0$ (Lid 1993). Methylamine is used to control a wide spectrum of chewing and sucking insects and mites in a large variety of crops, especially rice, cotton and wheat etc. It is toxic to body and central nervous system of the pests. It is an inhibitor of acetylcolinesterase. The chemical is of technical grade.

Test fishes

The test fishes selected are fresh water fish *Clarias batrachus* (Linn) commonly known as Mangur in Vernacular Hindi language by local fisher man and *Heteropneustes fossilis* (Block) commonly known as Singhi by local fisher man. Reason behind selecting these fishes is that they are well known for their food value and easily available in fish market at much lesser price as compared to carps (Azmi 1990). One more reason to select these fishes *Clarias batrachus* and *Heteropneustes fossilis* is that they are able to survive for longer period under laboratory conditions due to their air breathing nature. Due to the presence of accessory respiratory organs, they do not suffer much from asphyxiation, when kept out of water for short periods for experimental purpose.

External characteristics of *Clarias batrachus* are Dorsal fin, anal fins but separate from caudal, pectoral fins with a pungent poisons spine, scales absent, four pairs of barbels present, dendritic accessory respiratory organs dorsal to gills. External characteristics of *Heteropneustes fossilis* fish, skin without scales, body elongated and laterally compressed, barbels long and four pairs, dorsal fin is short without spine, pectoral fins long with a pungent poisonous spine, ventral fin situated at the level of dorsal fin, accessory breathing organs present, air bladder also present. This fish is highly nourshing and esteemed as food. The specimens of size 17±2 cms and weight 100 ± 5 gms were selected for the purpose.

Methodology

For experimentation following methods have been adapted for one type of studies

Histopathological studies

Fresh and healthy specimens of *Clarias batrchus* and *Heteropneustes fossilis* were procured from local fish dealers, which were approximately same in size and weight, are used in bioassay experiments. After disinfecting them with 0.1% potassium permanganate ($KMnO_4$) solution they were acclimatized for a duration of 15 days in the laboratory conditions. During this period fishes were provided no food. The series of experiments were set up to determine the effect of the pesticide methylamine on the tissue under study i.e. gill, liver and kidney. Median tolerance limit for 96 hours (in which 50% of the experimental fishes died within 96 hours) was treated as the lethal concentration which was 0.027 ml/litre. Five fishes were kept in the first series of lethal concentration. Sub lethal concentration (chronic treatment) i.e. in which no mortality occurred for a longer period was taken in second set of experiment. The sublethal concentrations selected was 0.009 ml/litre which were 1/3 of the median tolerance limit for 96 hours. Five fishes each were exposed in this concentration i.e.0.009 ml/ litre for a period of 30 and 60 days. In chronic exposure the solution was changed regularly after 24 hours interval. Control experiments were also conducted side by side for comparison. In control the fishes approximately of same size and equal in number were kept and both experimental and controlled fishes were not fed with diets. The temperature was maintained at 21± 30C^0 for these experiments. After definite period of exposure fishes of both series, control and treated (acute and chronic) were sacrificed and the required tissues i.e. gill, liver and kidney were removed and after washing with water they were fixed in 10% neutral formaline, dehydrated in different grads of alcohols, cleared with xylene and embeded in paraffin wax (58C^0–60C^0) prescribed for histopathological studies. After completing this phase, the sections of 5-6 µ thickness were cut stained with ehrichs haematoxylene and counter stained with alcoholic eosine and then mounted with DPX (Humason 1972).

Observation

Sudden death of aquatic fauna or a particular animal species is generally due to the acute toxicity of pollutants. But when the animals are exposed to lower concentrations or sublethal concentrations, although no sudden deaths results for longer periods even then several pathological alterations in different tissues are brought about which may lead to the complete disturbances in the life processes of animals. Not only is this, their nutritive value, also adversely affected. The significance of histopathological assessment of the toxicological effects is of great importance that occurred in the tissue as a result of toxicant exposure. In India enormous amount of different chemicals are being used for various purposes out of which 70% chemical formulations instead of controlling crop pest, affect- many non-target organisms. They find their way in to fresh water bodies through air and run off of rains and ultimately pollute them. Thus the aquatic life, of fish in particular which constitutes the major part of the diet, is affected. The pesticides due to their toxic actions cause histopathological alterations or changes in different tissues fish. Histopathological studies may thus be useful to evaluate the pollution potential of pesticides because the trace levels of pesticides, which do not cause animal mortality over a given period, can be capable of producing considerable damage in various organs of the animals. Thus microscopic examination or histopathological studies of the different fish tissues before and after the treatment by toxic substances can give an indication of water pollution or water contamination by toxicants. Histopathological changes in tissues of fishes and other organisms have been reported by many investigators. Some of them are: Mathur (1962), Eller (1971), Mathur (1972), Verma et al., (1975), Mukherjee and Bhattacharya (1975), Dubale and Punita (1979), Dubale and Punita (1979), Manda and Kulshrestha (1980), Kumaraguru et al., (1982),Chetri(1984), Joshi and Dubey (1984), Anthony et al., (1986), Ram and Sathyanesan (1987b), Ramamurthy et al., (1987), Srivastava et al., (1989), Richmonds et al., (1989), Chauhan et al., (1989), Hinton et al., (1990), Srivastava et al., (1990), Banerjee and Paul (1993), Bana et al., (1993), Shammi (1993), Dutta et al., (1993), Pandey et al., (1993), Satyadeven (1994), Arora et al., (1994), Neskovik et al.,(1996), Schwaiger et al., (1997), Rodrigues et al., (1998), Erkmen et al., (2000), Sharma et al., (2001), Jiraungkoorskul et al.,(2002), Veiga et al., (2002), Cengiz and Unlu (2002), Baruch and Das (2002), Jiraungkoorskul et al.,(2003), Cengiz and Unlu (2003), Otriz et al., (2003), Thophon et al., (2003), Fanta et al., (2003), Elnemaki and Abuzinadah (2003), Iqbal et al.,(2004), Cengiz (2006), Nagarajan and Aruna Dei (2006), Olurin et al., (2006), Sharma et al., (2006), Sindhe et al., (2006), Camargo and Martinez (2007), Joshi et al.,(2007) Altino et al., (2007), velanurugan et al., (2007), Jayachandran and Pugazhendy(2009), Mlambo et al., (2009), Mohamed (2009).

Keeping in mind the importance of histopathological studies, author in the present study has included few important body organs viz, gill, liver and kidney to study the toxic effects of methylamine carbamate pesticide. For this purpose effect of sublethal concentrations of the pesticide have been observed in detail.

Histomorphology of gill

Fishes are the first vertebrate where gills evolved for gaseous exchange and are essentially composed of a highly complex vasculature, surrounded by a large surface area epithelium that provides a thin barrier between fishes blood and aquatic environment. The gill of *Clarias batrachus* and *Heteropneustes fossilis* are located near the head region and are composed of five paired gill arches on both lateral sides of the pharynx. Anchored to the gill arches is a complex arrangement of epithelium, circulatory, and neural tissues. Gill filaments are the basic functional unit of gill tissue and long and narrow projections lateral to the gill arch that taper at their distal end .Each filament is supplied with an afferent filamental artery that extends along the filament. Blood in this vessel also travels across the filaments breadth through numerous folds on the dorsal and ventral surfaces of the filament-termed lamellae, lying perpendicular to the filaments long axis. Blood that crosses the lamellae drains into an efferent filamental artery that runs along the length of the filament and carries blood in the opposite direction to that in the afferent filamental artery. The region of the filament that contains the afferent blood supply is commonly referred to as the afferent edge, whereas the region that collects efferent blood is referred to as the efferent edge. These two terms are synonymous with trailing edge and leading edge, respectively, relative to water flow across the filament. Gill filaments contain three distinct vascular systems: (1) the respiratory circulation which receives the entire cardiac output and perfuses the secondary lamellae: (2) a nutrient system that arises from the postlamellar circulation and perfuses filamental tissues: (3) a network consisting of subepithelial sinusoids surrounding afferent and efferent margins of the filament and traversing the filament beneath the interlamellar epithelium.

Lamellae are evenly distributed along a filaments length, and the spaces between lamellae are channels through which water flows. Each individual lamella reveals that it is essentially composed of two epithelial sheets, held apart by a series of individual cells, termed pillar cells. The spaces around the pillar cells and between the two epithelial layers are perfused with blood, flowing as a sheet, not through vessels. Lamellae dramatically increase the surface area of the gill filament epithelium and result in a small diffusion distance between the blood that perfuses each lamella and the respiratory water. Moreover, blood flow through the lamellae is countercurrent to water flow between them.

Histopathological stress in gill due to methylamine

Sublethal treatment

Gill shows severe eosin and fusion of secondary gill lamella, space in between basement membrane and primary lamellae increased gradually. Vacuolization followed with lacunae formation was observed in secondary lamellae. Clumping of blood cells were of common occurrence. The general appearance of gill becomes altogether changed due to pesticide exposure within 30 days. Most prominent and severe changes were observed after 60 days exposure to methylamine. Secondary gill lamellae were completely lost. Basement membrane separated completely from primary gill lamella forming a continuous space. Furthermore erosion of basement membrane was observed at places. The shape of gills appeared as if they had been hypertrophied. The cells of cartilaginous axis were found to be enlarged at places. Hyperamia was also observed.

Discussion

The present investigations on histopathological studies have been done in gill, liver and kidney

Gills: Most striking injury is seen in epithelium due to extreme levels of environmental pollutants. This has also been discussed by Dalela et al., (1979), Jauhar and Kulshrestha (1985), Gill et al., (1986), and Shrivastava et al., (1990), for pesticides, Baker (1969), Garine and Yevich (1979). and Temmink et al., (1983). In the present study the injury caused to the gills in the form of necrosis and desquamation of lamellar epithelium that simultaneously exposed the supporting pilaster cells and capillaries to the ambient medium were noticed. Similar epithelial sloughing of gill lamellae have also been reported by Bhatnagar (1979). Dalela et al., (1979) reported most conspicuous histopathological changes on acute exposure of *Channa gachua* to thiodon and rogor, were the separation of respiratory gill epithelium from the basement membrane, pronounced hypermia, necrosis, fusion of adjacent gill lamellae, erosion at the distal end of gill filaments and loss of cell membrane. Verma et al., (1975) observed the presence of vacuolation in the gills of *Colisa fasiatus* after chronic exposure to sub lethal concentration of lindane which support the observed in the gills of *Clarias batrachus* exposed to sublethal concentration of thiodon and malathion. These findings confirm the authors observations in *Clarias batrachus* and *Heteropneustes fossilis* in which the fusion of gill lamellae, the separation of basement membrane, hyperplasia and severe erosion of secondary gill lamellae, there by reducing the respiratory surface of gill when treated with methylamine. Increased mucus secretion affected respiratory behaviour, the histopathological changes and the extent of damage were clearly increasing when the dose and duration were increased.

Increased mucus secretion over delicate gills impair the respiratory efficiency. Mucus secretion may lead to a thin film over delicate gills and may also provide lubrication to them. The same time this may result in the increase in permeability of gill tissue allowing more toxicant, which as a result caused the fusion of gill lamellae. Gill et al., (1988) studied the effect of carbaryl and dimethoate on gills of *Puntius conchonius* (Ham) and reported in gills wilting of pilar system, separation of lamellar epithelium, lamellar thrombosis, curling and oedematous of secondary lamellae hypertrophy in choride cells. Hypertrophy of respiratory cells as well as curling of secondary lamellae in the gills of *Clarias batrachus* and *Heteropneustes fossilis* has been reported in the present study when exposed to methylamine as also reported by Gill et al., (1988). In the present study the secondary gill lamellae of test fish were found to become short due to their severe erosion.

Author in the present study observed separation of respiratory epithelium in gills of *Clarias batrachus* and *Heteropneustes fossilis* which resulted in to the increase in diffusion gap. It was followed by necrosis of lamellar epithelium cells and damage in the gill, there by leading to serious hypoxic conditions that adversely effecting oxidative metabolism and ion regulation. Srivastava and Srivastava (1984) observed haemorrhage, shortening of secondary gill lamellae, pycnotic nuclei, hyperplasia and hypertrophy in *Channa gauchua* when exposed to malathion and chlordane. Cases of hypertrophy and hyperplasia were also noticed in the present study in *Clarias batrachus* and *Heteropneustes fossilis* when exposed to methylamine. Hyperemia in the gills of *Clarias batrachus* and *Heteropneustes fossilis* was observed after sublethal treatment by methylamine which is in agreement with the findings of Mishra et al., (1989) in the gills of *Puntius ticto* after the treatment of paraquat. Remarkable pathological changes were also observed under methylamine treatment. These prominent changes include necrosis in respiratory lamellae, degenerative changes in inter lamellar spaces, building of lips of respiratory lamellae, separation of epithelial layer of respiratory lamellae and atrophy of respiratory lamellae during 30 and 60 days exposure of methylamine to the test fish *Clarias batrachus* and *Heteropneustes fossilis*. These results are in agreement with Santhamma et al., (1999) who studied the histopathology of few tissues like, gills, intestine and liver of fish *Tilapia mossambica* exposed to monocrotophos. Reports are available indicating towards the mass mortality of fishes at a time from different parts of our country and abroad. This sudden death of fish in large number in a very short time reflects towards mixing of toxicants from one or the other source in acute or lethal concentration. These cases of mass mortality would always have been of temporary nature because of immediate availability of protective measures in that particular area. The impaired respiration followed by gill damage seems to be the cause of this type of mass mortality of fish in water bodies.

In the present findings also the lethal exposure of fish resulted in their death within very short period due to impaired respiration because of damaged gills. After sublethal exposure the death may be the result of more than one mode of action, the protein degeneration, alteration of membrane permeability and transport may be the most probable causes.

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