Toxic effects of meothrin on the gill ultrastructure in grass carp, *Ctenopharyngodon idellus*

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**Abstract** — The fingerling Grass carp, *Ctenopharyngodon idellus*, was exposed in different concentrations of meothrin nearby 24h LC$_{50}$, and its gill morphology was examined by light and electron microscopy. The results indicate that meothrin caused fish convulsions, cough, ataxia, intermittent paralysis and overturned. Morphological examination revealed severe alterations to compare with control fish. These initially consisted in hypertrophy of the secondary lamellae, and finally resulted in local telangiectasia, fusion between secondary lamellae. Ultrastructural changes in the gills showed the pillar cell system collapse and resulted in large non-tissue space, which were invaded by leucocytes. The cytoplasm of the epithelial cells appeared many myelin figures, electron dense deposits. Chloride cells were severely damaged with a degenerated mitochondria and nuclear membrane. Higher concentration of meothrin caused a loss of adhesion between the epithelial cells, accompanied by a collapsing of the structural integrity of the primary lamellae and degenerating of epithelial and chloride cells.

**Keywords**: meothrin; *Ctenopharyngodon idellus*; gill ultrastructure.

1 Introduction

Meothrin or fenpropathrin ((s)-α-cyan-3-phenoxybenzyl (s)-α-(4-chlorophenyl)-3-methylbutylate), a synthetic pyrethroidal insecticide, is used to control insects and mite on grain and cotton crops, fruits trees, vegetables, flowers and other crops. Thus meothrin is widely distributed in the surrounding environment. There are many reports on cypermethrin uptake, depuration, metabolism and toxicology in fish and aquatic animals (Coats, 1979; Mclease, 1980). Although some reports of meothrin hydrolysis in water and its metabolism in plants were published (Mikami, 1985), there are only few systematic descriptions of the toxic syndromes associating with exposure to the pesticide, such descriptions are helpful for understanding the mechanism of action of the pesticide. It indicates that meothrin is a strong poisonous pesticide to fish, its 24h LC$_{50}$ is 2.2 ppb. No detailed histological studies of the effects of meothrin on aquatic animal are available and the exact mechanism of meothrin poisoning in fish is difficult to ascertain. The gill which serves as major organ for
respiration and osmotic regulation, is easily damaged by even low concentrations of numerous pollutants (Skidmore, 1972; Papathanassion, 1983; Lars, 1983; Karlsson, 1985; Waster, 1988) and pesticides (Drewett, 1983). Zinc, copper, cadmium, sodium bromide (Waster, 1988), as well as lindane and DDT, have been reported to cause histopathological changes in fish gill and other organ structures (Mathur, 1962). Some literatures indicated that the severely damaged gill impairs oxygen uptake (Lars, 1983; Waster, 1988). The purpose of this paper is to show histological effects of meothrin on the gill structure of the grass carp and to evaluate the toxicity to fish using light and electron microscopy, and to attempt identifying the mechanisms in the reaction of this tissue to meothrin.

2 Materials and methods

2.1 Exposure of fish to meothrin

Seventy individuals of grass carp, with an average wet weight 9.5–10.5g, total length 10–11.5cm, were obtained from the fish pond of the institute. The fish were acclimated for 1 week at 23±1°C in aquaria with tap water filtered by active carbon. Meothrin of 99.9% purity from Sumitomo, Japan was used. Test solutions were prepared by adding the appropriate quantity of meothrin acetone solution into dilution water. The exposures took place in two sets of glass aquaria, each with the volume of 10L, containing meothrin 1pppb and 3pppb, respectively, near the concentration of meothrin 24h LC₃₀ (2.2pppb), and each control solution contained the same concentration of acetone as that in the test group. The test solutions were replenished by siphoning out 10L solution and replacing it with a fresh test solution at every 12h in 1pppb test group and at every 2h in 3pppb group.

2.2 Preparation of the sample for LM and TEM

Fish gills were removed from experimental animals after 6, 12, 24, 48, 72h exposure in 1pppb test group, and 1, 2, 4, 6, 8, 10h for 3pppb test group. Gill filaments were dissected out, and fixed in 0.1mol/L PBS buffered (pH 7.2) 2.5% glutaraldehyde for at least 4h, and postfixed with osmium tetroxide (1% in the same buffer) for 2h. After acetone dehydration, specimens were embedded in Epon 812 resin, polymerization in 60°C oven, semithin sections 1–2 μm were obtained with a LKB ultratome and stained with 1% toluidine blue in 1% borax. A number of these sections were used for light photomicrography. Ultrathin section were cut out with glass knives, stained with saturated solution of uranyl acetate in 50% alcohol, post stained with lead citrate, and examined under a JEM-100CX electron microscope.
3 Results

3.1 Toxicity

Symptoms of toxic reactions were observed among the grass carp after immerse in meothrin. The affected fish in 1ppb meothrin appeared frequently to burst out with rapid swimming or attempts to burrow into the bottom of the tank after exposure at 6h, some fish appeared to suffer cough and convulsions. The affected fish in 3ppb meothrin after immersion at 2h suffered convulsions and cough; subsequently these fish showed symptoms of paralysis, ataxia and overturned. No mortality was recorded up to 10h in the remaining 3ppb meothrin groups. No abnormal signs and mortality were recorded in the control group.

3.2 Light microscope examination

In 1ppb meothrin test group, light microscopical examinations of the gill revealed secondary lamellae disarray and a slight curl-over as compared with the control fish gill (Fig.1), so that the maximum length from the lamellar margin to the gill filaments was reduced. The lesions of the secondary lamellae include the hypertrophy of the secondary lamellar epithelium, and collapse of the pillar cell system (Fig.2). Some of the secondary lamellae fused; sometimes even a group of secondary lamellae fused completely, without conspicuous interlamellar space.

Fig.1 Control fish, section through gill filament, showing the longer secondary lamellae, × 360

Fig.2 Fish exposed to 1ppb meothrin for 6h, section through gill filament, the secondary lamellae with hypertrophy can be seen, × 250
In 3ppb meothrin test group, the secondary lamellae was severely impaired as compared with the 1ppb test group. At 2h period, about half of the secondary lamellae were grossly curled over. At 4h period, the secondary lamellar epithelium was completely separated from the pillar cell system to form a large non-tissue space. Some secondary lamellae are clogged with a large accumulation of blood cells at the distal ends as result of telangiectasia (Fig.3). After a period of 8h, the secondary lamellae may show increase in the number of chloride cells and hypertrophy of the epithelial cells. The apical epithelial tissue of the secondary lamellae becomes more than one layer in thickness.

3.3 TEM examination

The normally epithelium of secondary lamellae of the grass carp gill is simple consisting of a thin single or double cell sheet separated by the pillar cell system and blood lacunae (Fig.4). The epithelia of the primary lamellae showed epithelial cells, chloride cells, mucous cells and accessory cells interposed between the epithelial cells. After immerse in 1ppb meothrin, the apical part of the secondary lamellae become enlarged. Lesions of the secondary lamellae were mainly characterized by an increased diffusion distance, due to the swelling of the epithelia. In the spaces, necrotic cells, pillar cells, myelin figures and leucocytes were frequently found (Fig.5). Furthermore, degenerating and necrotic epithelial cells were often seen to be torn off from the epithelium; the epithelial cells lost their flat shape and became rounded off. The epithelial cell membrane became more irregular in shape and protruded into the ambient water (Fig.6). The cytoplasm of the epithelial cells was vacuolated, containing many myelin figures (Fig.7). Cytoplasmic accumulation with organelles and electron-dense deposits were also seen in the epithelial cells (Fig.8). The pillar cell, which is dominated by the nucleus and thin cell flanges, became irregular in shape and microridge protruded into the blood vessels. Some secondary lamellar pillar cell system had broken down, thus formed a large extensive clubbing with blood cells. The most pronounced change was a hypertrophy of the secondary lamellae,
accompanied by increased number of epithelial and chloride cells in these part of the gill as compared with the unexposed epithelia. The central lamellar blood vessels were markedly reduced in size and in some cases were not easily identified (Fig.6). Some tips of the secondary lamellae were more heterogeneous than the normal, containing an increased fraction of chloride and mucous cells (Fig.5). Degeneration was observed in the chloride cells, which showed a loss of cytoplasm, and they lost their contact with neighboring epithelial cells, resulting in large intercellular gaps. Furthermore, mitochondria of the chloride cells were severely damaged with fewer cristate (Fig.9), the nuclear membrane became swollen, and in some cases broken down (Fig.10).

Fig. 4 The tip of the normal secondary lamellae from a grass carp gill. The double layer of epithelial cells and a pillar cell are seen. ×4550

Fig. 5 The tip of a secondary lamellae of a grass carp gill after exposure to 1 ppb methrin for 12h. A necrotic epithelial cell is also seen (arrow) ×2440

The results of 3ppb test group are similar to that 1 ppb test group, the most pronounced change in the secondary lamellae was an increase in the number of chloride cells and mucous cells, which become rounded, vacuolated, accompanied with cytoplasm protrusion (Fig.11). The other change was the loss of adhesion between the epithelial cells and chloride cells, and the rupture of their basement membrane had resulted in the dispersion and degeneration of cell contents and deprivation of staining capacity (Fig. 12). Sometimes these cells were almost completely disconnected from the lamellae. The surface extensions of the rounded cell were longer and more numerous than those of the plated epithelial cells of unexposed gills.
Fig. 6 The center part of a secondary lamellae after exposure to 1ppb meothrin for 12h, the epithelial cells had become rounded, and hypertrophy of the epithelial cells, irregular erythrocytes can also be seen. ×3500

Fig. 7 An epithelial cell contained many myelin figures and cell inclusions (arrow) which were composed of degenerating mitochondria after exposure to 1ppb meothrin for 12h. ×8450

Fig. 8 A degenerating epithelial cell contained electron-dense inclusions after exposure 1ppb meothrin for 12h, ×7200

Fig. 9 A chloride cell in the primary epithelium of a grass carp after exposure to 1ppb meothrin for 12h, note all mitochondria were damaged, many of them no longer have cristae, ×8450
4 Discussion

In order to avoid the changes of the gill filaments before chemical fixation, in-situ fixation with glutaraldehyde was used to minimize shrinkage and then the gills were removed and fixed with the same glutaraldehyde.

The secondary lamellae are the sensitive part of the respiratory system, where the major part of the gas exchange takes place (Karlsson, 1985). The signs of meothrin poisoning to fish, hypertrophy and telangiectasia, are similar to different fish species was demonstrated in the exposure to some other pollutants in gill tissue (Smart, 1976; Temmink, 1983; Stoker, 1985). The results suggest that hypertrophy and telangiectasia of secondary lamellae
should be a primary reaction to pollutants. The external epithelium is responsible for the variation in thickness. The barrier is usually thinnest adjacent to the blood vessels. The nuclei of the epithelial cells frequently align with the pillar cell and therefore minimize the gas exchange distance. Sometimes the secondary lamellar epithelium is double layered. The epithelial cells are sometimes separated by an intercellular space, which has been suggested to contain lymph (Hughes, 1973). Such a lymphatic space may have an important function in the regulation of the water-blood pathway. Since swelling of these spaces together with infiltration of leucocytes is a common reaction after exposure to pollutants, the increased non-tissue spaces filled with fluid could result in inadequate gas exchange, consequently, in a reduced diffusion capacity. The epithelial cells lost their normal shape, microridges and cytoplasmic protrusions. This phenomenon may be due to osmotic regulation changes and swelling of the secondary lamellae. The number of degenerating epithelial cells and necrotic cells are increased, indicating a reduced life span of the epithelial cells. The gill lesion with an increased number of myelin bodies in secondary lamellar epithelium cells may affect blood circulation and be responsible for respiratory impairment. The plasma membrane of the epithelial cells is normally forming microvilli-like structure. This structure may increase the functional lamellar area and produce microturbulence, enhancing the effectiveness of the exchange processes over the surface epithelia (Karlsson, 1985). The decrease of microvilli-like structure and changes of epithelial cell surface may diminish the capacity for gas exchange. Following exposure to meothrin, leucocytes were present in non-tissue spaces, indicating an action on the immunological defense (Temmink, 1983).

The most severe lesion, telangiectasia of gill, was observed in all test fish. This type of structural damage caused by meothrin shows a close similarity to lesion caused by other environmental pollutants such as Cd (Karlsson, 1985), Zn (Skidmore, 1972), and ammonia (Smart, 1976). Another type of lesion in gills, hypertrophy, was also observed in all test fish; this type of structural damage show a close similarity to those caused by pollutants such as tributyltin compounds (Holm, 1991) and lindane (Drewett, 1983). May be fusion of the secondary lamellae is the result of hypertrophy, this phenomenon was also reported in some cases by pollutants such as chromate (Temmink, 1983).

The chloride cells are found in the stratified epithelium, separating from the bases of successive lamellae, some chloride cells aslo found in the secondary lamellae. Such a great extent of chloride cell distribution in the secondary lamellae was detected only following experimental acute heavy metal contamination and environmental changes. This result implies that the increased chloride cells in the secondary lamellae are a re-
action to the changes of environment. Chloride cells owe their identity in part to the dense population of mitochondria, and a characteristic of chloride cells is the abundance of the agranular endoplasmic reticulum. The granular endoplasmic reticulum is not so well presented in the ground substance of chloride cells as is the agranular ER (Karnaky, 1976). The structure and function of chloride cells were studied by many authors (Karlsson, 1983; Cioni, 1991). Recently, the basal lateral cell surface was shown to be the site of the k-dependent, ouabain-sensitive phosphatase component of the Na-K ATPase enzyme complex (Karnaky, 1976). Many reports suggest that chloride cells are involved in the osmoregulatory mechanism of fish (Karlsson, 1983; Karnaky, 1986). The most pronounced lesion of meothrin to chloride cells is the degenerating mitochondria which was seldom reported in other poisoning test in fish gills. Since mitochondria are very sensitive organelle to poisoning and to changing of osmoregulation; the damage of mitochondria can severely affect the function of chloride cells. Furthermore, swelling and degeneration of chloride cell nuclear membrane, losing of cytoplasm may be the result of changes of osmolarity. The lesions of mitochondria caused severe inhibition of Na-K-ATPase, enzymes which are vital for energetic process such as oxidative phosphorylation (Cionic, 1991). Chloride cell mitochondria are the first organelle which showed alteration and signs of degeneration indicating dysfunction. Several models of action of pollutants such as tributyltin toxicity (Holm, 1991) and cadmium (Papathanasiou, 1983) have been proposed, including inhibition of mitochondrial oxidative phosphorylation. The present study affirms the usefulness of morphology in the identification of gill damage. Possible effects on energy metabolism and on the brain and nervous system should be investigated through biochemical, histological and physiological studies.

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**Abbreviations**

SL = secondary lamella  
PC = pillar cell  
EC = epithelial cell  
NT = non-tissue space  
MC = mucous cell  
N = nucleus  
ED = electron-dense  
PL = primary lamella  
L = leucocyte  
CC = chloride cell  
E = erythrocyte  
M = mitochondria  
MF = myelin figures  
SER = smooth endoplasmic reticulum

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