Study on Some Neural and Behavioral Changes Induced by Carbamate (Mancozeb) Fungicide on Freshwater Fish *Clarias batrachus*

**Pallavi Srivastava and Ajay Singh**

Department of Zoology, D.D.U. University Gorakhpur

**Abstract:** The subclasses of carbamate pesticides, including dithiocarbamates, are being used in pest control programs because of their low toxicity. The effects of fungicide exposure on nervous and muscles tissues of catfish *Clarias batrachus*, were examined. The LC₅₀ values were estimated on fish, which were dose as well as time dependent. The exposures, 40% & 80% of 24 h LC₅₀ (10.86 mg/l) caused significant (p<0.05) inhibition in acetylcholinesterase activity. The bioassay study has performed after 24h and 72h. Based on the obtained data from the present study, the central theory that environmentally relevant concentrations of Mancozeb affect acetylcholinesterase (AChE) activity in fish. The conclusion also holds that the theories such as metabolites of Mancozeb convert the enzyme activities also. Inhibition was observed in AChE in nerve tissue of fish and behavioral changes are also observed, very little changes in activity of AChE are observed in muscles tissues of fish. Therefore, the findings of present investigation show that Mancozeb has potential to damage enzymatic pathways in fish. Therefore, it is suggested that appropriate toxicological risk assessment should be made in the areas where Mancozeb is proposed to be used in pest control activities.

**Key words:** Mancozeb · Acetylcholinesterase (ACHE) · Dithiocarbamates

**INTRODUCTION**

Pollution of pesticides is a serious problem facing the world [1]. Recent evidence indicates that fish which is the most important fauna are quickly becoming scarce. Pesticides are entered in body of organisms via absorption and cause metabolic changes. However, these chemicals may reach other ecological communities through rains and wind, affecting many other organisms. Only 0.1% reaches the specific target [2]. The range of pesticides toxicity is large in aquatic organisms, among different fish species it varies and the variation depends upon the age, sex, size, physiology and biological conditions of individual fish and also the environmental factors [3]. Indian fishery contributing about one third of total fish production of the country and share about 95% of total aquaculture production. Therefore, aquaculture as an enterprise has some innate advantages, such as high returns, high productivity, high feed conversion ratio, utilization of agriculture and animal wastes, high employment generation etc [4].

Mancozeb [[1,2- ethanediy l bis- [carbamodithioate]] (2-)] manganese is a fungicide, subclass of carbamate pesticides called dithiocarbamates. They have a similar action to carbamate insecticides they affect the nervous system through their main metabolite, carbon disulfide. Dithiocarbamates used as fungicides, being effective against a broad spectrum of fungi and plant diseases caused by fungi. In industry, they used as slimicides in water-cooling systems, in sugar, pulp and paper manufacturing and as vulcanization accelerators and antioxidants in rubber. Because of their chelating properties, they used as scavengers in wastewater treatment. The general formula of dithiocarbamates characterized by the presence of double bond sulfur as:

\[
R1 \quad S \\
\quad || \\
N-C-S-R3 \\
\quad / \\
R2
\]
Mancozeb is marketed by the trade names Dithane, Manzeb, Nemispot and Manzane. Mancozeb, if applied to soil, will have a low mobility based on its high adsorption coefficient. If it is released into water, it will tend to adsorb to sediment and suspended solids. It has low soil persistence with a reported half-life of 1-7 days. Again, the primary concern with Mancozeb is its spontaneous degradation into a number of compounds, such as sulfur, 5, 6-dihydro-3 H-imidazol [2,1-C]-1, 2, 4-dithiazole-3-thione, ethylenethiourea (ETU) and ethylenediamine (EDA) in the presence of water and oxygen. ETU has a persistence of 5-10 weeks. While Mancozeb is practically insoluble in water making it unlikely to contaminate groundwater, its metabolite, ETU, has the potential to be mobile in soils [5]. ETU stable, has high water solubility and is of particular importance because of its specific toxicity. For this reason, toxicological information on this compound is included in this study. The chemical structure of ethylenethiourea (ETU) is:

\[
\begin{align*}
\text{CH}_2-\text{NH} & \\
\text{C} = \text{S} & \\
\text{CH}_2-\text{NH}
\end{align*}
\]

ETU is one of the important residues in plants and in the environment following the agricultural use of ethylene bisdithiocarbamates (EBDCs). It is also a metabolite formed when EBDCs ingested by animals and man. ETU is a stable compound with respect to hydrolytic reactions but easily oxidized to ethyleneurea (EU) which is considerably more stable than ETU and can be consider major breakdown products. In animals ETU also degrade into urea, 2-imidazoline, glycine and oxalic acid. It also degrades into natural substances, which affect protein and fat.

Effect of toxicants on enzymatic activity is one of the most important biochemical parameters, which affected under stress. When an organ is diseased due to the effect of a toxicant, enzyme activity appears to be increased or it may be inhibited due to the active site being either denatured or distorted. Acetylcholinesterase, or acetyl-hydrolase, is a serine protease that hydrolyses the neurotransmitter acetylcholine. AChE is found at mainly neuromuscular junctions and cholinergic brain synapse, where its activity serves to terminate synaptic transmission. It belongs to carboxylesterase family of enzymes. Inhibition in AChE may lead to muscular paralysis, bronchial constriction and death by asphyxiation in fish.

However, the toxic effects of Mancozeb on fishes have not been adequately researched. The aim of present study is to evaluate the toxicity and the effect of sub-lethal doses of Mancozeb to analyze the enzymatic responses is freshwater fish *Clarias batrachus*, is an important fish of Indian capture fishery.

**MATERIALS AND METHODS**

**Chemical:** Mancozeb (CAS No. 8018-01-7) has purchased from Syngenta Ltd. from India, a technical grade pesticide. Other chemicals such as Giemsa stain has purchased from local Indian market.

**Experimented Animals:** The freshwater fish *Clarias batrachus* obtained from local hatchery (Chappy Hatchery). Fish had an average weight of 40.01±1.50g and average length 17.26±0.10cm for adult and for fingerlings, the average weight had 10.20±1.45g and average length 8±0.10cm. Fish were fed with commercial fish food and acclimatized under laboratory conditions for 2 weeks, containing de-chlorinated tap water (pH= 7.6, alkalinity 150 mg/l CaCO$_3$, DO= 7.03 mg/l, Temperature=22°C). The photoperiod used 12/12 dark/light. The aquaria used in study was made of glass had constant aeration. Physical dimensions of aquarium had 100×40×40 cm and a 120L capacity.

**Toxicity Test:** Toxicity test has performed by the method of Singh and Agarwal, [6]. Five fishes kept in glass aquaria containing 10 L de-chlorinated tap water. This experiment has replicated six times. Fish exposed for 24h to 96h to four different concentrations (10 mg/l, 15 mg/l, 20 mg/l and 25 mg/l for Mancozeb) of pesticides in laboratory. Control fish kept in similar conditions without any treatment. Each group of fish replicated three times. Mortality recorded after every 24h. Dead animals removed to prevent the decomposition of body in experimental aquarium. The effective doses ($LC_{50}$ values, upper and lower confidence limits and slope value) calculated by probit log method of Russel *et al.*, [7]. $LC_{50}$ values calculated by POLO programmed. Product moment correlation co-efficient was applied in between exposure time and lethal concentration [8] and AChE measurement has performed by Ellman *et al.*, [13].

**RESULTS**

**Acute Toxicity:** After treatment, all the experimented fishes immediately settled down at the bottom of aquarium. Within 5-10 min, the breathing of fishes affected and they came to the water air interface for air
Table 1: Pisciical activity of fungicide Mancozeb against freshwater fish *Clarias batrachus* at different time intervals

<table>
<thead>
<tr>
<th>Exposure periods</th>
<th>Effective doses (mg/L)</th>
<th>Slope</th>
<th>Heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td>24h</td>
<td>(LC_{50} = 11.42, (3.05-16.9))</td>
<td>3.21±0.94</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>(LC_{24} = 28.59, (21.16-38.3))</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(LC_{48} = 71.54, (48.5-263.3))</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(LC_{72} = 7.64, (1.18-12.58))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>48h</td>
<td>(LC_{50} = 28.59, (21.16-38.3))</td>
<td>3.23±0.99</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>(LC_{24} = 19.00, (10.47-24.5))</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(LC_{48} = 47.28, (34.6-127.6))</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(LC_{72} = 7.62, (1.56-11.9))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>72h</td>
<td>(LC_{50} = 71.54, (48.5-263.3))</td>
<td>4.01±1.19</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>(LC_{24} = 33.23, (26.1-61.8))</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(LC_{48} = 7.33, (1.26-11.4))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>96h</td>
<td>(LC_{50} = 15.91, (8.54-20.3))</td>
<td>4.38±1.37</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>(LC_{24} = 14.36, (6.89-18.4))</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Batches of fifteen fishes were exposed to four different concentrations of the fungicides. Concentrations given are the final concentration (v/v) in the aquarium water containing de-chlorinated tap water. Values given in parenthesis are Lower and Upper confidence of LC\(_{50}\) values.

Table 2: Acetylcholinesterase (AChE) activity (µM ‘SH’ hydrolyzed/min/mg tissue) in muscle and liver tissues of freshwater fish *Clarias batrachus* after 24h and 72h of exposure to 40% and 80% of LC\(_{50}\) of Mancozeb (Carbamate)

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Tissue</th>
<th>Exposure Period</th>
<th>Control</th>
<th>40% of LC(_{50})</th>
<th>80% of LC(_{50})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mancozeb (Carbamate)</td>
<td>Nervous</td>
<td>24h</td>
<td>0.049±0.021 (100)</td>
<td>0.036±0.016* (74)</td>
<td>0.024±0.011* (51)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>72h</td>
<td>0.049±0.021 (100)</td>
<td>0.032±0.014* (67)</td>
<td>0.019±0.008* (39)</td>
</tr>
<tr>
<td></td>
<td>Muscles</td>
<td>24h</td>
<td>0.087±0.016 (100)</td>
<td>0.075±0.033* (87)</td>
<td>0.073±0.032* (85)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>72h</td>
<td>0.084±0.018 (100)</td>
<td>0.070±0.031* (84)</td>
<td>0.068±0.030* (82)</td>
</tr>
</tbody>
</table>

* All these assay replicates six times and the values are mean ±SE of all the replicates.
* Values given in the parenthesis are percent values with control taken as 100%.
* Significant (P<0.05) when Student’s ‘t’ test was applied between treated and control groups.

Fig. 1: Percent Acetylcholinesterase (AChE) activity (µM ‘SH’ hydrolyzed/min/mg tissue) in muscles and liver tissues of the freshwater fish *Clarias batrachus* after exposure to 40% and 80% of LC\(_{50}\) (24h and 72h) of Mancozeb (Carbamate).

breathing, the respiratory impairment, probably due to the effect of the pesticides on gills and general metabolisms. After 30-60 min, their swimming activity is also slow down. During exposure the loss of equilibrium, hypo and hyperactive activity and vertical position observed after 48h. Finally, their activity ceases and fishes died. LC\(_{50}\) values of Mancozeb for period ranging from 24-96h on fish *Clarias batrachus* presented in (Table 1). The toxicity in both the cases was time as well as dose dependent. There was a significant negative correlation between LC\(_{10}\) values and exposure periods. Thus with an increase in exposure period the values of LC\(_{50}\) of Mancozeb decreased.

**Acetylcholinesterase Activity:** After 24h exposures to 40% and 80% of 24 h LC\(_{50}\) of Mancozeb (Carbamate), Acetylcholinesterase (AChE) activity was decreased to 74% and 51% in nervous tissue and 87% and 85% in muscles tissue. After 72h, decrement in AChE activity reached up to 67% and 39% in nervous tissue and 84% and 82% in muscles tissue at 40% and 80% of 24 h LC\(_{50}\), respectively (Table 2 and Figure 1).
DISCUSSION

It suggested that the metabolites of ETU in the fish produced primarily by fragmentation of the imidazoline ring and decarboxylation of the fourth and fifth carbon atoms. These fragments may incorporate with different metabolic pathways in body of fish and showed maximum effects.

In present study Acetylcholinesterase activity was decreased in nervous tissues of the fish *Clarias batrachus* after exposure to different sub-lethal doses of Mancozeb. During neurotransmission ACh is released from the nerve into the synaptic cleft and binds to ACh receptors on the post-synaptic membrane, relaying the signal from nerve. AChE, also located on the post-synaptic membrane, terminate the signal transmission by hydrolyzing ACh. The liberated choline is taken up again and ACh is synthesized by combining with acetyl-CoA through the action of choline acetyl-transferase. To receive another impulse, ACh must be released from the ACh receptor. This occurs when the concentration of ACh in the synaptic cleft is very low. Inhibition of AChE leads to accumulation of ACh in the synaptic cleft and results in impeded neurotransmission. AChE-inhibitors block the normal breakdown of the neurotransmitter, acetylcholine into acetic acid and choline. They do it by blocking the site where acetylcholine attaches the enzymes. In normal condition, ACh attaches to the serine hydroxyl group on AChE and Mancozeb may have high affinity to conjugate with serine and form enzyme-inhibitor complex. This prevents acetylcholine from interacting with cholinesterases enzyme and being break down. This leads to the buildup of excessive levels of neurotransmitter ACh at the neuromuscular junctions. Due to this nerves collapse in brain of fish that leads behavioral changes. In the present study, Mancozeb shows significant behavioral changes in fish (hyperactive movement, hypo movement, vertical position and loss of equilibrium). Behavior provides a unique perspective linking the physiology and ecology of an organism and its environment [9]. Behavioral action is a sequence of quantifiable actions, which operated through the central and peripheral nervous systems [10] and the cumulative manifestation of genetic, biochemical and physiologic processes essential to life such as feeding, reproduction and predator avoidance. For the best meet of the challenge of surviving in a changing environment, behavior allows an organism to adjust to external and internal stimuli in order to adapted environmental variables. Since behavior is not a random process, but instead of it, is a highly structured and predictable sequence of activities designed to ensure maximal fitness and survival of the individual. Behavior is subject of vast study and all aspects of behavior are not well established. It has been found that at every movement, animal showed a varied behavioral response, for its own survival and perhaps for evolutionary adaptation [11]. Alterations in fish behavior, particularly in non-migratory species, also provide important indices for ecosystem assessment. Hindrance in acetylcholinesterase activity during fungicide exposure caused disruption in the learned behavior in fish that affect the survival of fish and ultimately lead to mortality. Nagaraju *et al.* [3] analyzed the disruption in schooling behavior in fish *Labeo rohita*. Torre *et al.* [12] reported Cyprinus carpio and *Cnesterodon decemmaculatus* were highly sensitive to pollutant and showed reduced level of acetylcholinesterase. Hindrance in acetylcholinesterase activity due to plant extract also observed by Pratap *et al.* [4].

REFERENCES