

# Toxicological Effects of Organophosphate (Melathion) On Acetylcholinesterase Enzyme Activity Earthworm, *Eisenia Foetida*

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**Abstract**—Fertilizers and pesticides causes a significant effect on the biochemical physiology of non-target organisms in agriculture. Malathion, an organophosphate pesticide, has been reported to adversely affect growth, reproduction, and other physiological functions in Earthworm, *Eisenia foetida*, indicating its potential ecological risk. As enzymes catalyze specific biochemical reactions and collectively regulate metabolic processes, alterations in metabolic enzymes and protein profiles of earthworms can serve as reliable biomarkers in ecotoxicological assessments. Owing to its broad-spectrum activity against insects and mites, malathion is extensively used in agricultural practices especially in the Vidarbha region of Maharashtra.

The present study was undertaken to evaluate the chronic effects of pesticide exposure on acetylcholinesterase (AChE) activity in earthworm *Eisenia foetida*, an enzyme of critical physiological importance in neural transmission. AChE inhibition was observed to be concentration-dependent. After 28 days of exposure, AChE activity declined to 45%, 55%, and 68% at doses of 0, 1, 2, and 3 mg/kg of Melathion, respectively. These findings suggest that prolonged exposure to pesticides may induce significant and potentially irreversible disruptions in the biochemical mechanisms of earthworms, thereby posing ecological concerns.

**Index Terms**—Earthworm *Eisenia Foetida*, Acetylcholinesterase enzyme, Melathion, Pesticide, Biomarker, Cholinesterase, Phosphorylation, Physiology, Biochemical. Cellular metabolism

## I. INTRODUCTION

Biological sensitivity to environmental contaminants varies considerably among species, and toxicants often exert highly selective modes of action that disrupt specific physiological and biochemical functions. In aquatic invertebrates, pesticide exposure has been shown to impair essential physiological processes, including respiration, metabolism, feeding, and reproduction.

Earthworms are widely recognized as valuable biomarker of soil quality, land use practices, and overall soil ecosystem health. Numerous studies have demonstrated their capacity to accumulate substantial concentrations of pesticides and heavy metals, thereby supporting their application as biomarker of soil pollution. The ecological diversity in earthworm life-history strategies provides a suitable framework for investigating the interrelationship between ecological traits and physiological responses at the biochemical level.

Pesticides can induce significant alterations in biochemical parameters, particularly in enzymatic systems. Enzymes regulate specific metabolic pathways and collectively maintain cellular metabolism, the assessment of metabolic enzymes and protein profiles in earthworms represents a sensitive approach for Eco toxicological bio monitoring. Among these, acetylcholinesterase activity is extensively used as a biomarker of pesticide exposure. Previous studies indicate that factors such as earthworm age and soil type (loamy, sandy, and clay soils) do not significantly influence enzyme activity;

however, soil temperature exerts a pronounced effect. Additionally, earthworms exhibit relatively slow recovery rates—often extending over several months—from organophosphate-induced phosphorylation of cholinesterase enzymes. This prolonged inhibition enhances the reliability of cholinesterase suppression as a biomarker of pesticide contamination.

Farrukh et al. investigated the effects of endosulfan, an organochlorine pesticide, on the growth, reproduction, and avoidance behavior of *Eisenia foetida* and reported significant adverse effects on these biological parameters. Owing to its broad-spectrum efficacy against insects and mites in agricultural applications, Melathion has been extensively used in the agricultural regions of Madhya Pradesh, Central India. The present study was therefore undertaken to evaluate the chronic effects of this pesticide on acetylcholinesterase activity in *Eisenia foetida*, an enzyme of critical importance in earthworm neurophysiology and metabolic regulation

## II. MATERIAL AND METHODS

Culture, acclimatization and calculation of LC 50 was done as per the method of Farrukh et al. After calculation of LC50 which was found to be 4.57mg/kg dry weight of soil the sub lethal doses of Melathion were selected as 1, 2 and 3 mg/kg dry weight of soil and were coded as E0(control), E1(1 mg/kg), E2(1mg/kg) and E3(3mg/kg). The experiment was conducted at regular 7 days intervals i.e., 7, 14, 21 and 28 days on earthworms exposed to Melathion.

### Extraction of enzymes

Following the method of Mishra et al. with slight modifications earthworms taken for enzyme estimation were kept in moist filter paper for 24hrs to void their gut contents and then homogenized (10% w/v) in 0.1M, pH 7.5 phosphate buffer. For Acetyl Cholinesterase activity, the homogenates cooled up to 4°C were centrifuged at 10,000rpm for 10 min and the resultant supernatant was recentrifuged at

10,000rpm for 10 min (name of the centrifuge model) and supernatant was stored in ice for AchE activity.

### Determination of acetyl cholinesterase activity

Acetyl Cholinesterase activity was determined following the method of Ellman, et al., (1961). The assay consists of 2.8 ml of 0.1M phosphate buffer pH7.2, 50µl of 0.16mM DTNB, 50µl of protein and 100µl of 0.2 ACT iodide as substrate. The reactions were performed at 37°C and were initiated by adding the substrate (ACT iodide). The measurement of rate of production of thiocholine was accomplished by measurement of the continuous reaction of the thiol with DTNB to produce the yellow anion of 5-thio-2-nitrobenzoic acid. The rate of color production was recorded for 6 min at 412nm in a spectrophotometer. The activity was calculated as µmol/mg protein/min.

### Statistical analysis

Statistical significance of all the values observed during the experiments were analyzed by comparing the values with their relevant controls at 95% confidence interval at significance of  $p < (0.05)$  and  $p < (0.001)$  using NCSS, (2007) version 7.1.14 statistical software.

## III. RESULTS

Acetyl cholinesterase activity (µ mol/mg of protein/min)

As evident from the below given table Melathion caused a significant decreased in AchE activity of earthworms of all the three groups i.e., E1, E2 and E3 except the control group E0. The dose of 1mg/kg caused decrease in AchE activity up to 45% i.e. ( $44.16 \pm 0.65$  µ mol/mg of protein/min) in comparison to control value ( $80.19 \pm 0.27$  µ mol/mg of protein/min) over the 28 days. The next dose of 2mg/kg caused a decrease in AchE activity up to 55% i.e. ( $36.56 \pm 3.53$ ) and the highest dose of 3mg/kg brought about a maximum decrease in AchE activity up to 68% ( $26.97 \pm 0.89$ ) during the entire period of 28 days. All the values were found to be significantly different from their relevant controls at ( $p < 0.001$ ).

Table 1 Showing the AchE activity ( $\mu$  mol/mg of protein/min.) of earthworms during 7, 14, 21 and 28 days of exposure to different concentrations of Melathion (mean $\pm$ S.E where n=9)

( $p < 0.001$ )

Dosage	7 Days	14 Days	21 Days	28 Days
Control	80.2 $\pm$ 0.34	80.15 $\pm$ 0.66	80.40 $\pm$ 0.81	80.19 $\pm$ 0.27
E1(1mg/kg)	50.18 $\pm$ 0.43	48.29 $\pm$ 2.36	46.37 $\pm$ 2.00	44.16 $\pm$ 1.07
E2(2mg/kg)	44.85 $\pm$ 0.39	40.22 $\pm$ 1.25	38.78 $\pm$ 1.310	36.56 $\pm$ 3.53
E3(3mg/kg)	34.11 $\pm$ 1.57	31.66 $\pm$ 0.50	28.18 $\pm$ 1.3.	26.97 $\pm$ 0.89

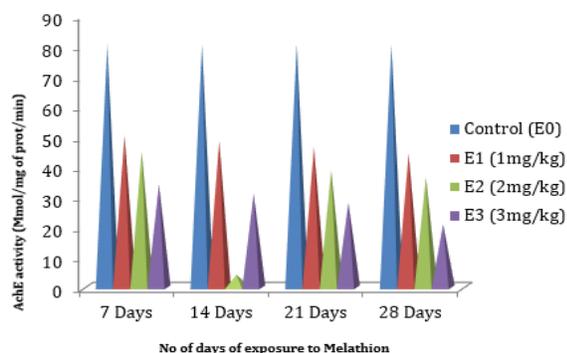


Figure 1 Showing the change AchE enzyme activity ( $\mu$ mol/mg of protein/min.) of earthworms during 7, 14, 21 and 28 days of exposure to different concentrations of melathion with well-matched control earthworms.

#### IV. DISCUSSION

The present study demonstrates that exposure to different sublethal concentrations of melathion resulted in a significant inhibition of acetylcholinesterase (AChE) activity ( $p < 0.001$ ) in *Eisenia foetida* over a 28-day experimental period (7, 14, 21, and 28 days). The inhibition of AChE activity was concentration-dependent. After 28 days of exposure, AChE activity found to be 55%, 45%, and 32% at doses of 1, 2, and 3 mg/kg melathion, respectively. Furthermore, no significant recovery of enzyme activity was observed after 30 days, indicating persistent toxic effects.

Previous investigations have established that inhibition of AChE activity by organophosphate pesticides such as melathion serves as a reliable in vivo biomarker of toxicity across diverse species. Organophosphate compounds disrupt nerve cell membrane cation transport, leading to neural hyperexcitability and central nervous system stimulation. This ultimately impairs synaptic transmission through suppression of AChE activity.

Importantly, toxic effects on enzyme activity are often mediated not only by the parent compound but also by its bioactive metabolites.

The findings of the present study indicate that melathion exerts prolonged toxic effects with substantial potential to induce neurotoxicity in earthworms. Significant reductions in AChE activity were observed at all tested sublethal concentrations. Additionally, both evaluated pesticides significantly altered the avoidance behavior of *Eisenia foetida*. These observations are consistent with previous reports indicating that AChE inhibition functions as a biomarker directly associated with behavioral disturbances. Earthworm body wall muscles possess vertebrate-like cholinergic neuromuscular junctions containing AChE, which plays a critical role in regulating synaptic transmission. Although there is a clear mechanistic basis linking AChE inhibition by anticholinesterase pesticides to impaired locomotor activity, relatively few studies have explicitly demonstrated this relationship in earthworms.

#### V. CONCLUSION

Therefore, from the present findings it can be concluded that long term exposure to organophosphate pesticides could lead to severe and irreparable effects on biochemical mechanisms of earthworms which is not a good sign for the growth and development of earthworms in pesticide contaminated field conditions. And therefore, infers a greater risk to cause decrease in earthworm population.

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