Bull. Environ. Contam. Toxicol. (1998) 60:904-908


Bulletin of Environmental Contamination and Toxicology

# Inhibition and Recovery Kinetics of Acetylcholinesterase Activity in *Drawida calebi* and *Octochaetona surensis*, the Tropical Earthworms, Exposed to Carbaryl Insecticide

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Received: 7 October 1997/Accepted: 31 March 1998

Acetylcholinesterase activity has widely been accepted as a reliable parameter for assessing toxicity due to heavy metals and pesticides present in the environment. There are reports on its manifold response to heavy metals ( Mercury, Cadmium, Lead) and synthetic chemicals (Slate *et al.* 1987 ; Patnaik and Dash 1992a; Reddy and Venugopal 1993 ; Devi and Fingerman 1995 ). Insecticides inhibit cholinesterase (ChE) activity and lead to accumulation of acetylcholine (ACh) at the synapse with consequent disruption of nervous activity in different animals(O'Brien 1967 ;Patnaik and Dash 1992a).Earthworms play important roles in soil fertility, organic matter decomposition and nutrient recycling in the soil sub-system (Senapati and Dash 1984 ;Lee 1985 ; Spiers *et al.* 1986; Mishra and Sahoo 1997) for which it has become a choice organism to monitor the impact of pollution load due to increasing use of synthetic chemicals. This paper reports on the effect of carbaryl, a carbamate insecticide on acetylcholinesterase (AChE) activity in two tropical earthworms *Drawida calebi* (Gates) and *Octochaetona surensis* (Michaelsen).

## MATERIALS AND METHODS

Carbaryl (1-napthyl N - methylcarbamate) 50 % WDP was obtained from Bhopal Pesticides Pvt. Ltd., Bhopal. Healthy species of *Drawida calebi* and *Octochaetona surensis* were collected from grasslands and crop fields and cultured in earthen pots in the laboratory containing air dried, ground and sieved soil free from pesticide treatment and amended with 5% organic matter ( air-dried and powdered cowdung and leaf litter in equal proportion), moisture content of soil was maintained at 20 g% and temperature of 25° C. Earthworms were acclimatized in the laboratory pot culture for ten days. Earthen pots containing 10 kg of the processed soil were taken in five sets with five replicates for each species.Optimum soil moisture (20 g%) and temperature (25°C) were maintained. Healthy worms of *Drawida calebi* and *Octochaetona surensis* were taken from the pot culture and were released into four experimental sets each containing eight worms per pot. The pots of two sets for each species were sprayed with 10 ml of 9 ppm *( approximately* $LC_{20}$ *equivalent as evident from our pilot experiment* ) carbaryl suspension.

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The other three sets were sprayed with 10 ml distilled water of which two sets were taken as control, for each species. To study the recovery kinetics, the insecticide treated worms of one set for each species were taken out after 24 hours of exposure to the insecticide and were reintroduced into the pots of the fifth set; the former set was discarded.

Five worms from each of the sets (one from each replicate) selected randomly for both the species were sacrificed at an interval of 1 day, 2 days, 3 days, 6 days and 9 days. The whole body tissue was taken for estimation of AChE activity by spectrophotometric method (Glick 1957) and the enzyme activity was expressed in micromoles of ACh hydrolysed per mg of body protein per hour. The protein content of the body tissue homogenate was determined according to Lowry *et al.* (1951) using bovine serum albumin as standard. The difference in the rate of AChE activity between control and treated sets was tested statistically by student 't' test.

## RESULTS AND DISCUSSION

Figure **1** shows the AChE activity of the worms under continuous exposure to carbaryl for a period of 9 days. Inhibition in the enzyme activity was marked in both the species after the first check at 24 hr. The activity in *Drawida calebi* was 11.81 and 7.39 in control and treated pots whereas in *O.surensis* it was 30.97 and 25.43 respectively, with a percent inhibition of 37.45 in *Drawida calebi* and 17.89 in *O.surensis* over the control. The rate of inhibition increased with time of exposure with maximum inhibition i.e. 68.43 and 61.82 in *Drawida calebi* and *Octochaetona surensis* respectively at ninth day. It is clear from Figure **2** that *D.calebi* is more susceptible to carbaryl toxicity with higher rate of inhibition in AChE activity as compared to *O.surensis.* The difference in the enzyme activity between control and treated worms is statistically significant ( $t = 7.43$, $p < 0.05$ for *D. calebi,* $t = 4.51$, $p < 0.05$ for *O.surensis* ).

Figure **3** shows the rate of recovery in AChE activity after 24 hr exposure to carbaryl treatment and reintroduction of the worms into normal untreated soil. *D.calebi* recovered from AChE inhibition in six days and *O.surensis* in nine days. Though half-life of carbaryl is 3 days, the delayed recovery in both the species was probably due to the delay in the repair of the damage caused to its physiological and biochemical mechanism by the insecticide stress. It is evident from Figure **3** that percent inhibition recorded after 24 hr of reintroduction was 45.38 and 19.09 in *D.calebi* and *O.surensis* respectively . However, the rate of recovery from the insecticide stress was rapid in *D.calebi* as compared to *O.surensis.* Patnaik and Dash (199213) observed more rapid recovery in *D.calebi* than *L.mauritii* after fenitrothion treatment . Thus, carbaryl has differential inhibition of AChE activity in tropical earthworms which are non target organisms. Patnaik and Dash (1992a,b) also reported significant inhibition in AChE activity in *D.calebi* and *L.mauritii* continually exposed to organophosphate insecticides. Pree *et al.* (1987) reported that carbamates impart more inhibitory effect in AChE activity of nematodes than organophosphates. Therefore , monitoring of AChE activity in earthworms has the potential to serve as a bioindicator to pesticide pollution in the soil subsystem.

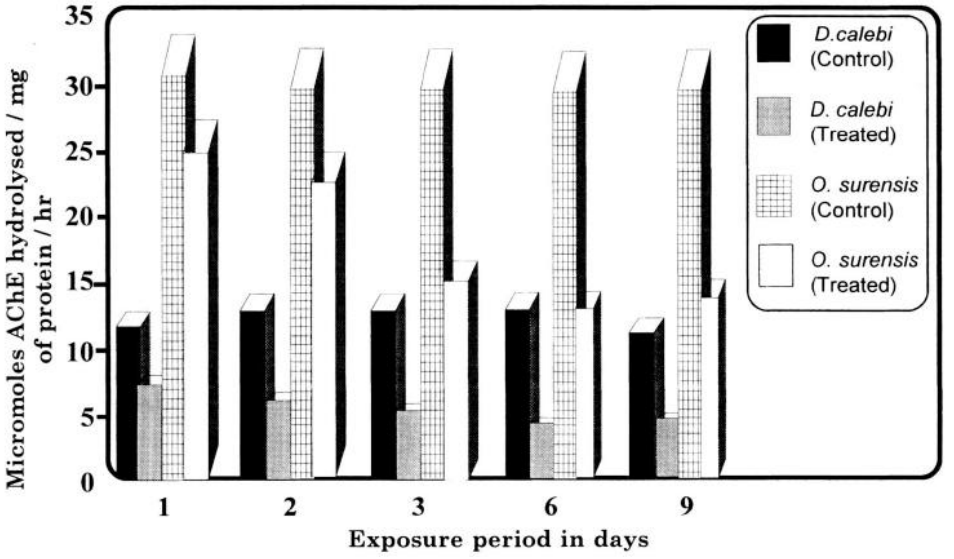


**Figure 1** . AChE activity in earthworms under continuous exposure to carbaryl.

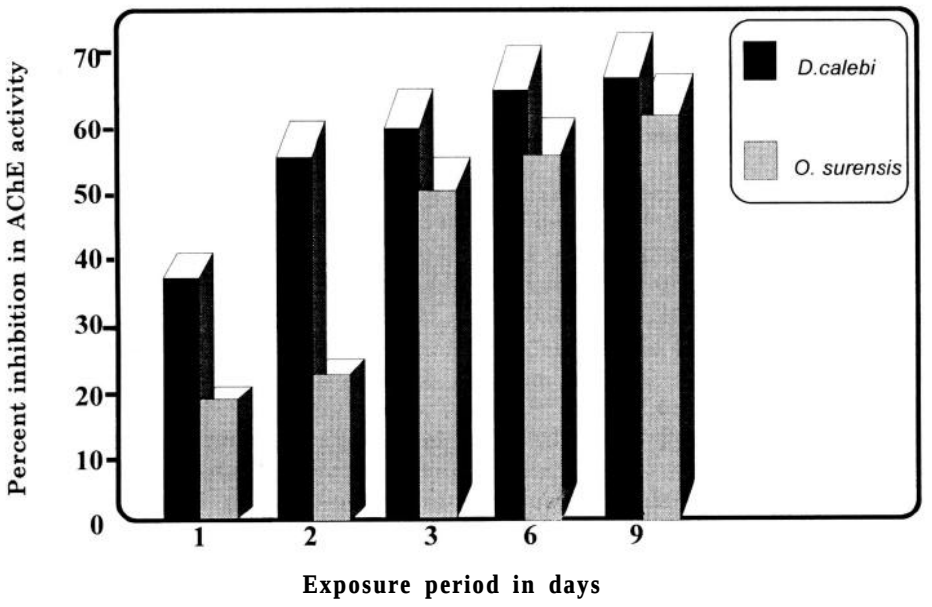


**Figure 2.** Percent inhibition of AChE activity in earthworms under continuous exposure to carbaryl.

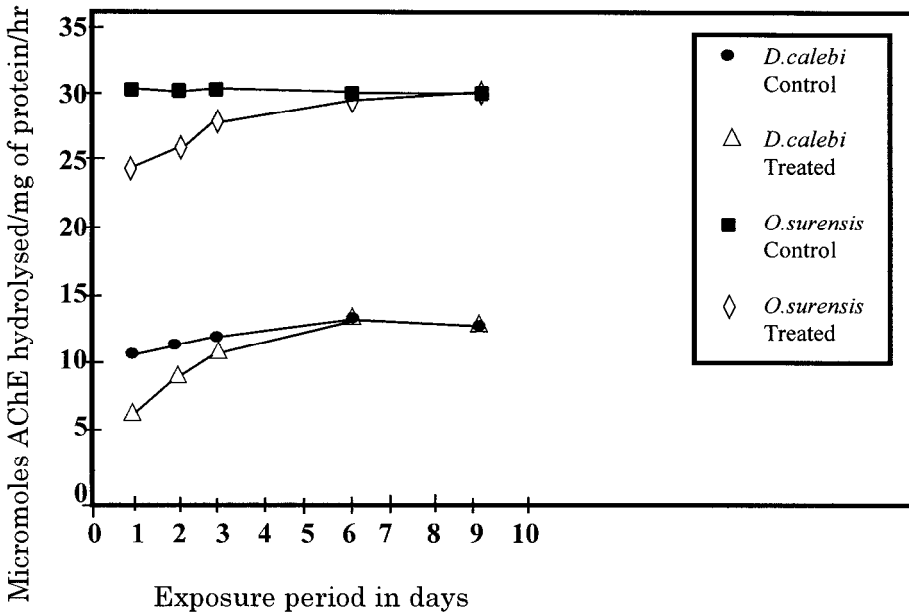


**Figure 3.** Recovery of AChE activity in earthworms after 24 hr exposure to carbarly.

*Acknowledgement.* S.C.Pradhan thanks UGC, New Delhi for providing teacher fellowship to him.

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