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Rakesh Savara
Department of Zoology, Andhra University, Visakhapatnam, India

Ch. Bharathi
Department of Zoology, Andhra University, Visakhapatnam, India.

Correspondence:
Rakesh Savara
Department of Zoology, Andhra University, Visakhapatnam, India.

Mortality Rate of carbofuran to a brackish water Oligochaete, *Pontodrilus bermudensis* Beddard

Rakesh Savara and Ch. Bharathi

Abstract
Carbamate compound pesticide (Carbofuran) is found toxic to brackish water Oligochaete *Pontodrilus bermudensis* at different percent of seawater (25, 50, 75 100%) at different time of exposure (24, 48, 72, 96 hours and 10 days). The pesticide is highly toxicity in high salinity and long term of exposure. Carbofuran exerted more influence at long term period and in high salinity. The results showed that the toxicity of the pesticide as synergistic at high concentration of sea water.

Keywords: Toxicity, Carbofuran, seawater, *Pontodrilus bermudensis.*

Introduction
Large scale of applications of pesticides and other chemicals on agricultural crops, forests and marsh lands, in public health and agricultural practices in significant quantities is resulting in an imbalance in the ecosystems (Ernst *et al.* 1976) [3]. Many of these substances due to their characteristics persistence accumulate in biological systems subsequently magnify in quantum in food chain (Mc Leese and Metcalfe, 1980) [5]. There is an increase concern over indiscriminate use of pesticides in the environments and a number of studies are made to understand the toxic effects on several non-target organisms (Edwards 1973) [1]. Several investigations have proved that endosulfan, organochlorine cyclodiene insecticides as toxic to a number of non target organisms including marine and estuarine ones (O'Brien, 1967) [9]. However voluminous information on the toxicity of a number of organochlorine and Carbamate pesticides to various aquatic and terrestrial organisms is valuable on such knowledge exists on marine brackish water oligochaete worms, which are important in the food chain in brackish water littoral habitats. In the present study, the toxicity of Carbofuran to brackish water oligochaete, *Pontodrilus bermudensis* are reported. There is paucity of information on the toxicity of Carbofuran to marine/brackish water organisms of lower trophic levels. *Pontodrilus bermudensis*, a littoral oligochaete occurs in large numbers in the Southern Lighter Channel (SLC) in the Visakhapatnam horbour, where the salinity fluctuates from 6 to 33% (Ganapati *et al.* 1958) [4].

Materials and Methods
The worms collected from (Southern Lighter Channel, Visakhapatnam harbor) their natural habitat and transported to the laboratory along with the soil collected *in situ* to avoid desiccation. They washed with filtered sea water similar in strength to that of brackish water (salinity 20%) from habitat and acclimated to the laboratory conditions for a period of 5 to 7 days (temp. 30±2 ^{0}C). Acclimated worms of equal size (90mm) used for experimentation. A modified method of renewal technique (EPA, 1975) [2] adopted to test the toxicity of different test concentrations to *P.bermudensis.* Standard solution of commercial grade (35%) Carbofuran (W/V) prepared in acetone. This solution added to the aqueous media (20% sea water) in different ratios to yield the final desired concentration. Control warms conducted with acetone. The acetone concentration never exceeded more than 0.1ml/L water in any experiment. Worms in batches of 10 each were used for each concentration. All bioassay experiments conducted for 24, 48, 72, 96h and 10days exposure at different seawater concentrations (100, 75, 50 and 25% salinity). The experiments repeated until the percentage mortality in each of the experimental concentration was constant. Pilot experiments were conducted to choose concentrations which resulted in mortality range of 5-100%. Mortality was scored at different exposure periods.

Results

Reviewing the experimental results (Table: 1), it is seen that *P. bermudensis* exhibits tolerance over a wide range of experimental salinities (5 to 25%) under laboratory conditions, which is comparable to the fluctuations of the salinity in their natural habitat (6 to 33%). In 25% seawater media observations of different concentrations of the pesticide were chosen for this experiment. In 24hrs exposure, the kills scored at 10 to 18ppb of the pesticide were 5 to 100% respectively, while mortality was absent in the controls with acetone. The range of concentrations used for 48hrs exposure was lower than those of 24hrs exposure experiments. The pesticide concentrations ranged from 10ppb to17ppb in the test media in which the worms were tested. The mortality rates after 48hrs exposure were 5 to 100% respectively. Similarly in 72hrs test experiments, the test concentrations ranged from 9ppb to 16ppb, the mortality rates were 5 to 100% respectively. The test concentrations of Carbofuran employed for 96hrs exposure ranged from 9ppb to 15ppb, the mortality rates 5 to 100%. Similarly in 10days experiments the concentrations ranged from 7ppb to 13ppb, the mortality rates 5 to 100% (Table-1 and Fig-1).

Similar to the earlier experiments the 24hrs exposed worms in 50% sea water medium, the concentration range of the pesticide varied from 9 to 16ppb that result the mortalities between 5 to 100%. The range concentration of Carbofuran varied from 9 to 15ppb for 48hrs exposure periods, which result the mortalities between 5-100%. In 72hrs exposure experiments the range concentration tested varied from 9 to 14ppm that result the mortality rates between 5 to 100%. The 96hrs exposure experiments the range concentration tested varied from 8 to 13ppb those results the mortality rates between 5 to 100%. And in 10days exposure experiments the range concentration tested varied from 5 to 11ppb that result the mortality rates between 5 to 100% (Table- 1 and Fig- 2).

In 75% sea water medium the percentage mortalities between 5-100% occurred in the Carbofuran concentrations ranging from 8 to 15ppb for 24hrs period of exposure. In 48hrs exposure experiments in this sea water medium, the toxicant concentrations ranged from 7 to 14ppb and the mortality rates 5 to 100%. The toxicant concentrations range varied from 7 to 13ppb that showed the percentage kills 5 to 100% for 72hrs exposure experiments. Similarly the concentration range of the pesticide varied from 6 to 12ppb in 96hrs exposure experiments, the resulted the mortality rates between 5 to 100%. In10days of concentration range of the pesticide varied from 3 to 9ppb and the mortality rates of 5 to 100 % (Table-1 and Fig- 3).

In medium of 100% seawater, 5 to 100% kills were recorded in the pesticide Carbofuran concentrations ranged from 6 to 14ppb for 24hrs exposure experiments, which the results of mortality rates between 5 to 100%. The ranged concentration of the pesticide from 5 to 13ppb, which the results mortality rates 5 to 100% for 48hrs exposure period. Similarly the concentration range of pesticide for 72hrs exposure varied from 5 to 12ppb and the mortality rates between 5 to 100% in the seawater media. For 96hrs or 4days period of the concentration range of Carbofuran tested is from 4 to 11ppb, which the results of mortality rates of 5 to 100%. Similarly in 10days concentration ranged of pesticide from 1 to 7ppb and the results of the mortality rates 5 to 100%. The mortality rate was increasing with the period of exposure and concentration of seawater media (Table-1 and Fig- 4). Perhaps, the rate of mortality in *P. bermudensis*, as in many polychaetes, is a function of the salinity gradient the worms are exposed.

Table 1: Dose mortality of Carbofuran to *P. bermudensis* for 24, 48, 72, 96hrs and 10days exposure in different salinity seawater media

% of seawater	Duration exposure	Conc. of Carbofuran (ppb)	% of Mortality
25%	24 hrs	10 - 18	5 – 100
	48 hrs	10 - 17	5 – 100
	72hrs	09 - 16	5 – 100
	96 hrs	09 - 15	5 – 100
	10days	07 - 13	5 – 100
50%	24 hrs	09 - 16	5 – 100
	48 hrs	09 - 15	5 – 100
	72 hrs	09 - 14	5 – 100
	96 hrs	08 - 13	5 – 100
	10days	05 - 11	5 – 100
75%	24 hrs	08 - 15	5 – 100
	48 hrs	07 - 14	5 – 100
	72 hrs	07 - 13	5 – 100
	96 hrs	06 - 12	5 – 100
	10days	03 - 09	5 – 100
100%	24 hrs	06 - 14	5 – 100
	48 hrs	05 - 13	5 – 100
	72 hrs	05 - 12	5 – 100
	96 hrs	04 - 11	5 – 100
	10days	01 - 07	5 – 100

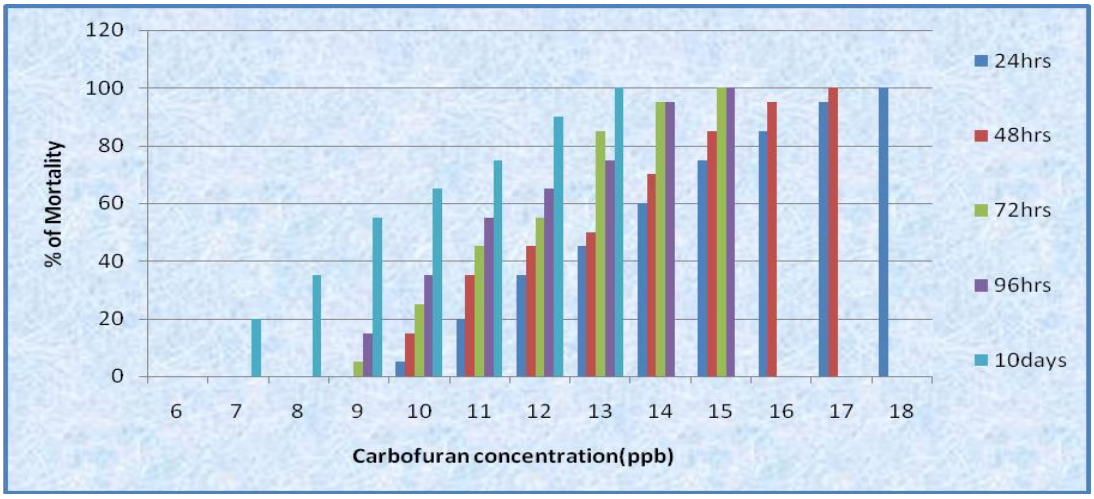

Fig 1: Mortality rates in *P. bermudensis* exposed to different concentrations of Carbofuran for 24, 48, 72, 96hrs and 10days at 25% seawater media.

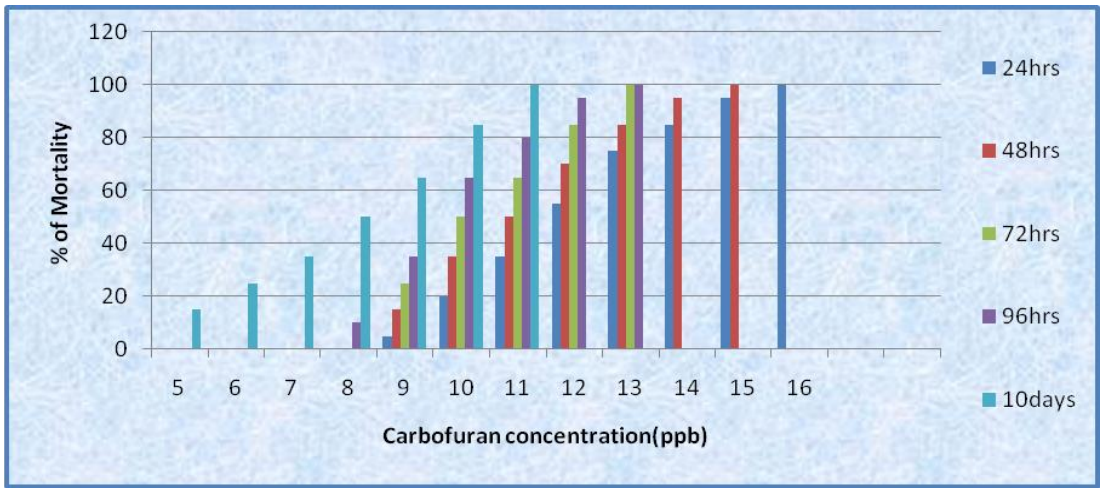

Fig 2: Mortality rates in *P. bermudensis* exposed to different concentrations of Carbofuran for 24, 48, 72, 96hrs and 10days at 50% seawater media.

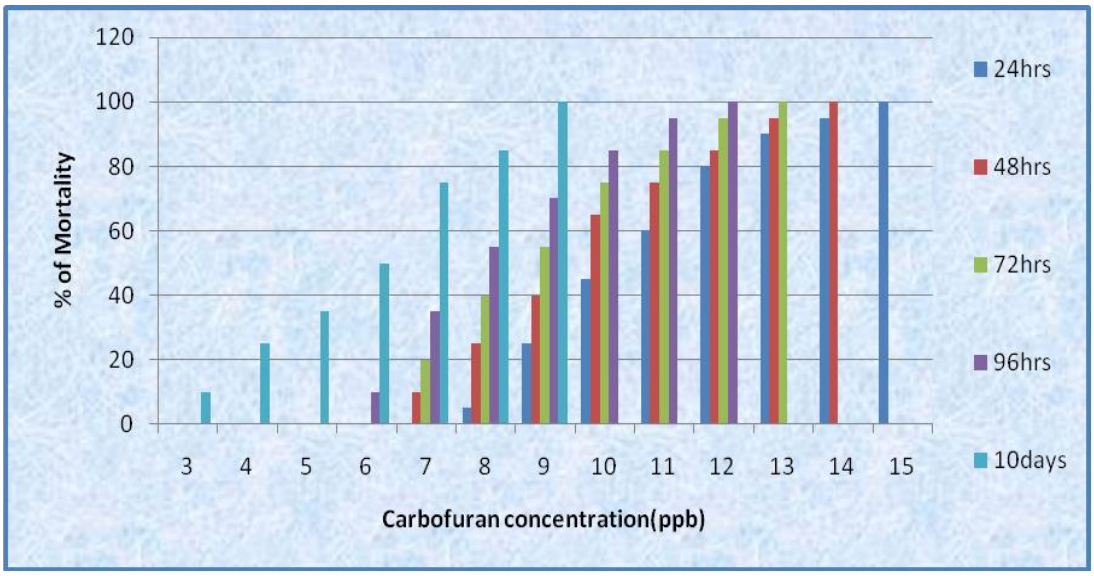

Fig 3: Mortality rates in *P. bermudensis* exposed to different concentrations of Carbofuran for 24, 48, 72, 96hrs and 10days at 75% seawater media.

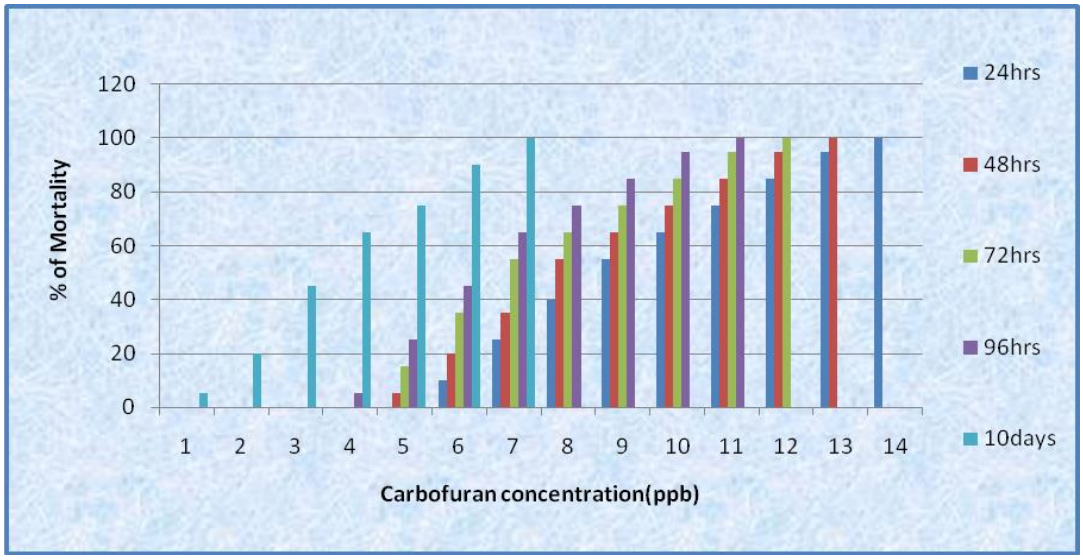

Fig 4: Mortality rates in *P. bermudensis* exposed to different concentrations of Carbofuran for 24, 48, 72, 96hrs and 10days at 100% seawater media.

Discussion

The Results are presented in table -1 and figures 1 to 4. The difference between observed and calculated percentage mortalities were tested for significance at the end of 24, 48, 72, 96hours and 10days by using 'Chi-square test'. The results clearly indicate that the dose percentage of mortality increase with increasing salinity water, exposure periods, and concentration of Carbofuran, indicating that the even very low concentration of this pesticide is toxic to the test species on long term exposure. Very few insecticides have been tested with the terrestrial and aquatic oligochaete annelid worms in the laboratory and the majorities have been tested in the field (Edwards 1973) [1]. Carbamate insecticides, carbaryl and carbofuran are reported as highly toxic to *Lumbricus terrestris* (Stenersen *et al.* 1973) [11]. Increase in toxicity of DDT with increasing saltiness also has been reported in mosquito fish (Murphy 1970) [6]. Increased toxicity of phosphomidon with increase in salinities was reported in *Metapenaeus Monoceros* (Vijayalakshmi *et al.* 1986) [8]. Nandini *et al.* (1985) [8] also found that the salinity dependent naphthalene toxicity increased in high salinities to speckled prawn *Metapenaeus Monoceros*. Nagavalli (1984) [7] found that the different salinities with PCP showed surprisingly a changed toxicity pattern i.e. the toxicity of PCP increased with increasing time of exposure and increasing salinities, above and below the optimum seawater media to *Pontodrilus bermudensis*. Satti babu *et al.* (2013) [10] found that the different salinities with Monocrotophos insecticide showed that the toxicity of Monocrotophos mortality rate increases with the increasing of the salinities and time of exposure in *Pontodrilus bermudensis*. *P. bermudensis* considered to be atrophic indicators were the most tolerant to sewage sludge, and oligotrophic species were the least tolerant. However, this was not true for pulp mill effluent or the chemical pollutants. The results confirmed the use of present oligochaete assemblages to indicate the degree of trophy in natural systems, but also suggested new assemblages for indicating the presence of particular chemical contaminants. The presence of sediments resulted in increased tolerances for all species, demonstrating the role of sediments as important modifiers of toxic effects on oligochaetes.

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