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Research Article

Cytogenetic damage induced by thiodicarb, a carbamate insecticide in the bone marrow of *Calotes versicolor*

Nisha Shrivastava, Anisha and Tumul Singh*
Department of Zoology, Udai Pratap College (Autonomous), Varanasi-221002, India
*Corresponding author; Email: tumultapan@yahoo.co.in

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Abstract: The present work is designed to investigate *in vivo* cytogenetic effects of thiodicarb a carbamate insecticide, by evaluating its capability to induce clastogenic effects in the bone marrow cells of garden lizard *Calotes versicolor*. Adult male garden lizards were acclimatized for one week in the laboratory and then treated daily with an intraperitoneal dose of 1/4th LD_{50} (40mg/kg body weight) of thiodicarb. The bone marrow was used for Micronucleus Assay. The obtained data showed significant increase of MN frequency in Polychromatic Erythrocytes of the bone marrow ($P \leq 0.005$). This study also reveals that significant level of induction is time dependent as the significant increase in MN was seen after 21 and 28 days of treatment when compared to control.

Keywords: Carbamate, Thiodicarb, Micronucleus, Clastogenic, Polychromatic Erythrocytes.

Abbreviations: Micronucleus (MN), Polychromatic Erythrocyte (PCE), Lethal Dose 50% (LD_{50}), Phosphate Buffer Saline: PBS, Bovine Calf Serum: BCS, Roswell Park Memorial Institute medium: RPMI medium.

INTRODUCTION

Carbamates are a part of a large group of synthetic pesticides that have developed in the last 40 years (Essay and Sobbhy 1998) and includes a versatile class of compounds used as insecticides, fungicides, nematocides, acaracides, molluscicides, sprout inhibitors or herbicides (Paíga *et. al.*, 2009). Carbamates are used in homes, gardens and agriculture. It has been reported to inhibit cellular metabolism including energy, protein, and nucleic acid metabolism, thereby, causing cell regression, and death (Amanullah and Hari 2011, Kumari *et. al.*, 2014). Many investigations have shown that some of the carbamate pesticides are cytotoxic, mutagenic, clastogenic and carcinogenic (Priya *et. al.*, 2014, Srivastava and Singh 2013). Wei *et. al.*, (1997) reported that three carbamate insecticides Propoxur, methomyl

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and aldicarb induce significant increase in micronucleated cells, and chromosomal aberrations in mammalian cells.

Reptiles have previously been shown to be excellent indicators of the potential association between contaminants and genetic damage (Hall and Clark 1982, Clark *et. al.,* 2000, Talent *et. al.,* 2002, Matson *et. al.,* 2009, Strunjak-Perovic *et. al.,* 2010). In addition, they bioaccumulate and biomagnify them to levels equal to or greater than those described in birds and mammals (Bryan *et. al.,* 1987, Hall and Henry 1992). Lizards inhabiting the agricultural farms, gardens and buildings are an important component of food chain, and these are constantly being exposed to various pesticides as a non target species either directly or through the food chain in a biomagnified manner.

Thiodicarb (Chemical Name- 3,7,9,13-tetramethyl-5,11-dioxa-2,8,14-trithia-4,7,9,12 tetraazapentadeca-3,12-diene-6,10-dione, Trade Names: Larvin, Nivral and Semevin), is a highly effective oxime carbamate insecticide used to protect agricultural crops against Beet Armyworm, Corn Earworm, Black Cutworm, Bollworms and Budworms. Thiodicarb causes metabolic disturbances in insects and warm blooded animals by inhibiting the acetylcholinesterase. Methomyl (S-methyl N-(methylcarbamoyloxy) thioacetimidate) and methomyl oxime (N-hydroxyethanimidothioic acid methyl ester) are the degradation products of thiodicarb in the soil.

Moreover, there is little information regarding genotoxic effects of thiodicarb on lizards. Hence, interest aroused, and the present work was undertaken to investigate *in vivo* cytogenetic effects of thiodicarb (carbamate insecticide) in the bone marrow cells of the garden lizard, *Calotes versicolor* by using Micronucleus Assay.

MATERIALS AND METHODS

Experimental Design- Adult male garden lizards, *Calotes versicolor* were caught locally in suburbs of Varanasi. They measured snout - vent length 10 ± 2 cm and average body weight 30 ± 2g. They were immediately brought to laboratory, housed in vivarium (wire net cages of size 18 x12x 10 inch). They were provided with food (crickets, maggots, flies) and water *ad libitum.* The lizards were acclimatized for one week prior to experimentation. The guidelines of the Committee for the Purpose of Control and Supervision of Experiment on Animals, Ministry of Statistics & Programme Implementation, Government of India, were followed in maintenance and sacrifice of animals.

LD_{50} value for thiodicarb (wettable powder, Sigma Aldrich) was determined according to the methods described by Miller and Tainter (1944) and modified by Randhawa (2009). The acute intraperitoneal dose showing 50% mortality during the treatment period of 96 hrs was selected as LD_{50} (40 mg/kg body weight). $1/4^{th}$ of LD_{50} value was selected for further study. A total of 40 lizards were taken for experimentation. The animals were divided into two groups of 20 lizards each. The first group was kept as control and the lizards of second group were injected daily with $1/4^{th}$ LD_{50} (40 mg/kg body weight) of thiodiocarb intraperitoneally. Five lizards from each group were sacrificed after 7 days, 14 days, 21 days and 28 days, of treatment.

Micronucleus (MN) Assay- The femur bones were dissected out from each animal, and used for MN assay. The bone-marrow was flushed out from femur bones with 1 ml of RPMI medium (Sigma Chemicals) and PBS (3:2) and centrifuged at 3000 rpm for 30 minutes. The supernatant was discarded and the bone-marrow pellet was re-suspended in 20µl of BCS (Bovine Calf

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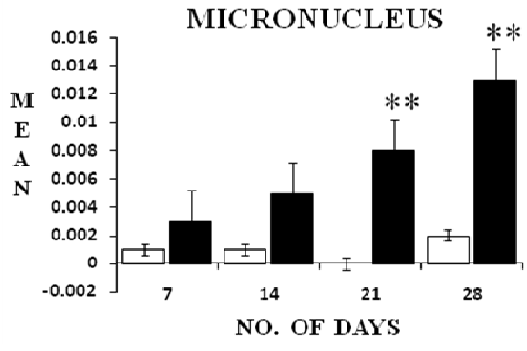
Serum, (Sigma Chemicals). The evenly spread bone-marrow smear was stained with Giemsa (Qualigen India). Slides were scored under oil immersion at a magnification of 1000x. 1000 Polychromatic Erythrocytes (PCE) were examined for control and treated groups separately, to score the number of PCE (MN) frequencies. (Schmid 1976, Hayashi 1994).

Statistical Analysis: Data was expressed as arithmetic mean ± standard error of mean. Significance of the data was analyzed using the test criterion, Student's t- test.

RESULTS AND DISCUSSION

C. versicolor was selected for this study because reptiles have been shown as valuable models for ecotoxicological studies and risk assessment both *in vivo* and *in vitro* (Talent *et. al.*, 2002, Matson *et. al.*, 2009, Martinez-Lopez *et. al.*, 2010, Strunjak-Perovic *et. al.*, 2010). The results are illustrated in figure.

Figure: Graph showing Micronucleated Polychromatic Erythrocytes in the bone marrow cells of *Calotes versicolor*, following intraperitoneal treatment with 1/4th LD_{50} of thiodicarb for 7, 14, 21 and 28 days.



**significant at P≤ 0.005 ☐ Control ■ Treated

Significant changes were observed in MN frequencies after 7 and 14 days of treatment. MN frequencies increased significantly (P≤ 0.005) in the treated group as compared to control group after 21 and 28 days of treatment.

MN is composed of small chromatin fragments which arise from chromosome breaks after clastogenic action or whole chromosomes that do not migrate during anaphase as a result of aneugenic effects (Cavas *et. al.*, 2003). MN assay has been used to estimate the clastogenic potential of chemical compounds. Clastogenic activities induce MN, which may be due to chromatid or chromosome fragments or lagging chromosome induced by clastogenic chemicals or spindle poisons. Induction of MN in the PCE of bone marrow cells has been regarded as one of the most sensitive bioassays for monitoring the mutagenic and genotoxic effects of a compound (Heddle *et. al.*, 1983). The efficacy of the MN assay as an indicator of structural genomic damage has been proven and the assay has been successfully used as a measure of genotoxic stress under both laboratory and field conditions (Cavas and Ergene-Gozukara, 2003).

Concerning cytogenetic effects of thiodicarb, our results are indicative of potential clastogenicity of thiodicarb, the induced MN cells in *Calotes* may indirectly reflect chromosome breakage or impairment of the mitotic apparatus suggesting that thiodicarb acts as a clastogen or influences the mitotic apparatus as a spindle poison in bone marrow cells of *Calotes*. This study reveals that chronic exposure of thiodicarb for an extended period of 21 and 28 days significantly increased the level of MN induction suggesting that it is time dependent. It may be suggested that thiodicarb has a tendency to accumulate and concentrate in the animal body or it may be degraded into harmful xenobiotic agents which may be clastogenic or genotoxic.

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However, the exact mechanism of cytogenetic damage induced by thiodicarb in *Calotes* needs to be further investigated.

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