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# BENDIOCARB - A CARBAMATE INSECTICIDE INDUCED CHROMOSOMAL ABERRATIONS IN BONE MARROW CELLS OF *CALOTES VERSICOLOR*

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**ABSTRACT**

We investigated the *in vivo* cytogenetic effects of bendiocarb, a carbamate insecticide, by evaluating its capability to induce chromosomal aberrations in the metaphase chromosomes from bone marrow cells of *Calotes versicolor* (males). Following daily intraperitoneal (IP) injections of 4 mg/kg body weight ($1/4^{th}$ $LD_{50}$) of bendiocarb, the animals were sacrificed after 7, 14, 21 and 28 days to prepare metaphase plates. The karyotype with chromosome number $2n = 34 = 12V + 22m$ validated the experimental animals as male *Calotes versicolor*. Treated group showed numerical changes in the form of aneuploidy with a general pattern of monosomy and structural changes in the form of gaps, breaks, additions and deletions. Gap frequencies increased significantly after 14 days ($p \leq 0.005$) and after 21 and 28 days ($p \leq 0.0005$). Breaks increased significantly after 14 and 21 days ($p \leq 0.005$ and 0.05). Deletions were significant after 21 and 28 days ($p \leq 0.05$). These results indicate that the frequency of karyological and cytogenetic damage increased with exposure time suggesting that chronic exposure of bendiocarb induces genotoxicity in male *Calotes versicolor* and Chromosomal aberration assay is a sensitive biomarker of *in vivo* genotoxicity testing in the reptilian model selected in this study.

## INTRODUCTION

Synthetic pesticides are complex mixture of chemicals and acts as environmental contaminants or pollutants. There are three major groups of synthetic pesticides, carbamates are the recent additions. Cytogenetic analysis have shown that carbamate pesticides are cytotoxic, mutagenic, clastogenic and carcinogenic (Priya *et al.*, 2014; Srivastava and Singh, 2013; Shrivastava *et al.*, 2014; 2015; Anisha *et al.*, 2014; Krishnappa and Venkateshwarlu, 2007). Several cytogenetic markers such as chromosomal aberrations (CA), micronuclei (MN), single cell gel electrophoresis (SCGE) and mitotic index have extensively been used for detection of early biological effects of DNA-damaging agents (Wei *et al.*, 1997; Kumar, *et al.*, 2013; Kusum Lata, *et al.*, 2010; Choudhury, 2013; Poletta *et al.*, 2011). Tracing many reasons the excessive use of synthetic pesticides has gained momentum for the rapid decline in the population of herpetofauna in the last few decades (Lajmanovich, *et al.*, 2005; Capriglione, *et al.*, 2011; Cakici, *et al.*, 2012; Sparling, *et al.*, 2010; Gibbons *et al.*, 2000; Gibbons *et al.*, 2015). Reptiles have shown to be excellent models for studies of association between chemical or physical agents and genetic damage however; they remain to be the group of vertebrates less studied in genetic toxicology (Poletta *et al.*, 2013).

Bendiocarb (Chemical Name- 2, 3-isopropylidenedioxyphenyl methylcarbamate), a carbamate insecticide, is extensively used against mosquitoes, flies, wasps, ants, fleas, cockroaches, silverfish and ticks. Holeckova *et al.* (2009) reported its genotoxic effects in cultured peripheral bovine lymphocytes by FISH and WCP (Whole chromosome painting) technique. Anisha *et al.* (2014) reported that bendiocarb induces micronuclei in bone marrow cells of *Calotes versicolor*. However, there is no report on bendiocarb induced chromosomal aberration in *Calotes versicolor*. Therefore, in the present work we have tried to investigate the *in vivo* cytogenetic effects of bendiocarb by evaluating its capability to induce karyological and cytogenetic damage in the form of numerical and structural aberrations in the metaphase chromosome prepared from bone marrow cells of *Calotes versicolor*. The objective was to test for the sensitivity of chromosomal aberration assay as a biomarker of pesticide induced genotoxicity testing in the reptilian model *Calotes versicolor*.

## MATERIALS AND METHODS

### Location and duration of study

This study was conducted in the Department of Zoology Udai Pratap College Varanasi, India. The preliminary studies standardization of experimental procedures, animal acclimatization, actual animal experiment and evaluation of results lasted for a period of four months. However, the actual administration of bendiocarb to the test animals lasted for 28 days. Adult male garden lizards, *Calotes versicolor* (average

snout - vent length 10 ± 2 cm and body weight 30 ± 2g) were housed in vivarium (wire net cages of size 18 x12x 10 inch). These were provided with food (crickets, maggots, flies) and water *ad libitum*. These 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.

**$LD_{50}$**

According to the method described by Randhawa (2009) the $LD_{50}$ value for (IP) dose of bendiocarb was determined to be 16 mg/kg body weight. Wettable powder of bendiocarb purchased from SIGMA-ALDRICH, India was used for this study.

**Experimental Design**

A total of 32 lizards were taken for experimentation and the animals were divided into two groups of 16 each. The first group was kept as control and received vehicle and the lizards of second group were injected daily with 4 mg/kg body weight ($1/4^{th}$ $LD_{50}$) of bendiocarb intraperitoneally. Four lizards from each group were selected to prepare metaphase plates after 7 days, 14 days, 21 days and 28 days, post treatment.

**Preparation of Metaphase Plates**

Control and treated groups were colchicinised (Colchicine purchased from SRL) at the rate of 0.005 mg/g body weight after 24 hours of the last dose of bendiocarb administered. Lizards were sacrificed after 2 hours of colchicine treatment and bone-marrow from femur bone was flushed in hypotonic solution (0.56 % KCl) and then incubated at 30°C for 10 min. The cell suspension was centrifuged at 1800 rpm for 30 min, fixed in acetomethanol (acetic acid: methanol, 1:3, v/v). Centrifugation and fixation was repeated three times at 10-min intervals. The material was re-suspended in a small volume of fixative, dropped onto chilled slides, flame-dried, and stained in 5% buffered Giemsa (pH 6.8).

**Karyotyping**

20 good quality metaphase plates of control group were used to prepare karyotype to ascertain the species and sex of the experimental animal. The chromosomes were classified according to the method described by Levan *et al*., (1964).

**Chromosomal Aberration (CA) Assay**

One hundred good-quality metaphases were examined under oil immersion for control and treated groups separately to observe CA (Brusick, 1980).

**Statistical Analysis**

Data was expressed as arithmetic Mean ± SEM. Significance of the data was analyzed using the test criterion, Student's t-test.

## RESULTS

Metaphase plate prepared from bone marrow of control group is shown in Fig. 1. In most of the metaphase plates, the nuclear centre was clustered with microchromosomes and the macrochromosomes were evenly distributed and occupied the peripheral region of the nucleus. The centromeres of macrochromosomes encircled the microchromosome cluster forming a ring on the inner side with the chromosome arms pointing outward at the equatorial plane. Each macrochromosome, thus, acquired a 'V' shaped configuration with side by side arrangement. Side-by-side arrangement of macrochromosomes varied from cell to cell.

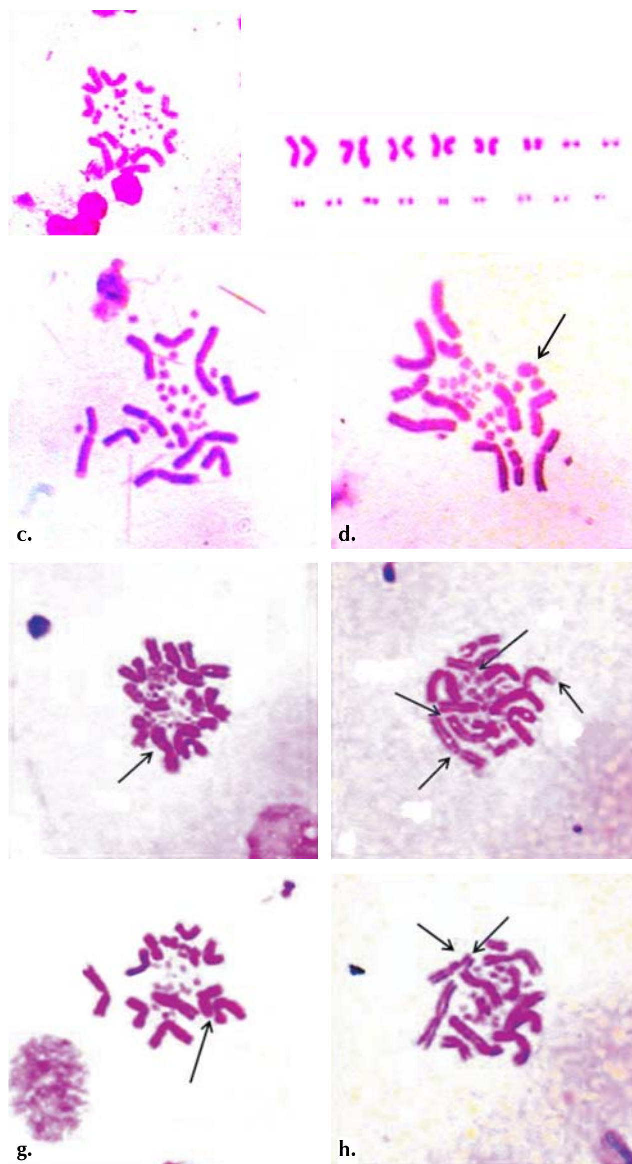


**Figure 1: Photomicrographs of Metaphase plates of Calotes versicolor showing a. Normal metaphase plates, b. Karyotype of *C.versicolor* (male). c-h. Showing chromosomal aberrations after bendiocarb treatment. Monosomy of chromosome 6, an aneuploidy. d-h. (chb- chromosome break, cg- chromatid gap, cb- chromatid break, ca- chromatid addition and cd- chromatid deletion)**

Fig. 2, shows karyotype of normal metaphase chromosomes from control group. The chromosome number comprised of 2n = 34 = 12V + 22m chromosomes, 6 pairs were macrochromosomes and 11 pairs were spot microchromosomes. Chromosome pair number 1, 3, 4, 5, 6 were metacentric, $2^{nd}$ pair was submetacentric and carried

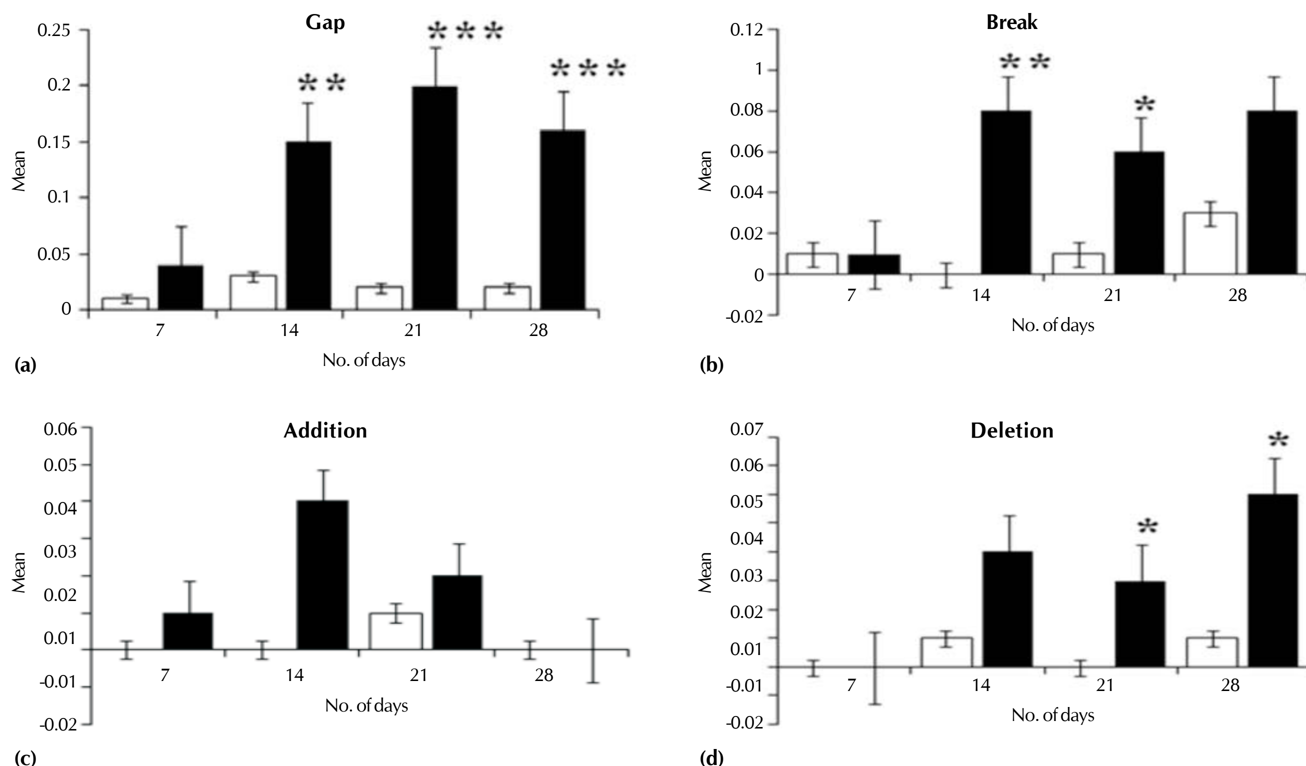


**Figure 9: Time dependent induction in different types of chromosomal aberrations (A-Gap, B- Break, C-Addition and D- Deletion) in the bone marrow cells of *Calotes versicolor*, following treatment with ¼ $LD_{50}$ of Bendiocarb. *$p \leq 0.05$, **$p \leq 0.005$ and ***$p \leq 0.0005$.**
**☐ Control ■ Treated**

satellite (SAT) at both of its long arms. Heteromorphic sex chromosomes were not found, males are homogametic with regards to the sex chromosome as found in most of the karyotype. The observation on karyotype confirmed that the experimental animal selected for this study were male *Calotes versicolor*.

On the basis of observation on metaphase chromosomes of the treated group it was concluded that 'V' shaped configuration with side by side arrangement of macrochromosomes were lost (Fig. 3-8). Numerical aberrations in the form of aneuploidy were observed consistently in several metaphase plates following bendiocarb injection in the treated group. Fig. 3, shows loss of one chromosome of $6^{th}$ pair. The numerical aberrations increased following chronic treatment for extended period as compared to control with a general pattern of loss of one chromosome (monosomy). Structural aberrations in the form of chromosome break (Fig. 4); chromatid gaps, breaks, additions and deletions were observed in the bone marrow cells of treated group (Fig. 5-8). The results illustrated in Fig. 9, are indicating a time dependent increase in the level of induction of structural aberrations following chronic exposure for an extended period. After 7 days of treatment, there was no significant change in frequencies of CA. Frequencies of CA in the form of gaps increased in the treated group significantly at 14 days ($p \leq 0.005$) and at 21 days and 28 days ($p \leq 0.0005$). Breaks increased significantly ($p \leq 0.005$) and ($p \leq 0.05$) after 14 and 21 days of treatment, respectively. Deletions increased significantly ($p \leq 0.05$) after 21 and 28 days of treatment. However, no significant change was found for CA showing addition.

## DISCUSSION

Pesticides are useful to control pest outbreaks, but excessive use leads to severe environmental pollution as many pesticides are not easily degradable, these persist in soil, leach to groundwater and surface water and causes environmental contamination, degradation and pollution. Hazards caused by pesticides on the animals are due to their accidental exposure to pesticides either by ingestion or inhalation (Hernandez *et al*., 2006; Gokhan *et al*., 2008). Depending on their chemical properties they can enter the organism, bioaccumulate in food chains and consequently lead to severe health hazards. Repeated application poses a great threat to biodiversity. Overall, intensive pesticide application results in several negative effects in the environment that cannot be ignored and needs to be biomonitored.

The male garden lizard, *C. versicolor was* selected for this study, because reptiles have been shown to be valuable models for ecotoxicological studies and risk assessment both *in vivo* and *in vitro* (Talent *et al*., 2002; Matson *et al*., 2005; 2009; Martinez-Lopez *et al*., 2010). In our investigation the karyotype and pattern of chromosomal arrangement in metaphase and location of SAT at the ends of the long arm of the homologous pair of 2nd chromosome of control lizards

were highly consistent and very similar to earlier reports by, Makino and Asana (1948); Sharma *et al*., (1980); Kritpetcharat *et al*., (1999) and Thounaojam, *et al*., (2004). Location difference of SAT exists between homologous pair of 2nd chromosome; one is at the middle of long arm, the other at the end of the long arm, as reported earlier by Zou *et al*., (2008).

As the determination of acute toxicity is usually an initial step that provides information on health hazards likely to arise from a short term exposure in the assessment and evaluation of the toxic characteristics of a substance, an intraperitoneal median lethal dose ($LD_{50}$) of the bendiocarb formulation was determined before actual experimental design. A very low concentration (1/4 $LD_{50}$) of bendiocarb was used in this investigation to evaluate the cytogenetic effects of bendiocarb. The evaluation of CA is a fully accepted method to reveal genotoxicity, as it is indicative of real genetic effects (Tompa *et al*., 1992). So we evaluated numerical and structural changes in metaphase chromosomes in the treated group.

The production of Chromosomal Aberration is a complex cellular process with mechanisms of chromosome breakage and re-joining that are not yet completely understood. Aneuploidy may result from non-disjunction of chromosomes at anaphase; chromosome loss during cell division so that one daughter cell becomes monosomic. Aneuploidy may be detected by counting chromosomes at metaphase stage (Parry *et al*., 2002). According to the prevailing theories, structural CA results from: (1) direct DNA breakage, (2) replication on a damage DNA template, and (3) inhibition of DNA synthesis (Assayed *et al*., 2010).

Numerical aberrations in the form of aneuploidy in the metaphase plates observed in treated group suggest a clastogenic or aneugenic effect of bendiocarb. Structural aberrations in the form of chromosome break, chromatid gaps, breaks, additions and deletions were observed in the bone marrow cells of *Calotes* following treatment with bendiocarb. It may be surmised that bendiocarb acts as a spindle poison leading to disorientation of spindle apparatus, impairment of mitotic apparatus and chromatid breakage as a result of which the 'V' shaped configuration of metaphase chromosomes may have been lost and numerical and structural aberrations were created in the bone marrow cells of *Calotes*. The repeated dose for an extended period might have also lead to aberrant segregation of one or more chromosome during mitosis producing monosomic cells (aneuploidy). Our results are in accordance with the reports on numerical and structural aberrations in *in vitro* bovine peripheral lymphocytes exposed to bendiocarb (Holeckova *et al*., 2009). Our findings on structural aberrations are also similar to the findings of Chauhan *et al*., (2000) and Giri *et al*., (2002) on mouse bone marrow cells exposed to carbofuran and carbosulfan, respectively. Similar results have also been reported on cultured human lymphocytes exposed to mancozeb by Srivastava *et al*., (2012). Sarangi, (2000) reported that carbaryl is a potent inducer of chromosomal aberrations in kidney cells of fish *Channa punctatus*. Present observation concurrent with earlier reports on several carbamates discussed above substantiates the genotoxic potential of bendiocarb.

Our investigation on genotoxic effects of bendiocarb further indicated a time dependent increase in the level of induction in CA after chronic exposure of the tested dose for an extended period and suggests that bendiocarb either 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 mutagenic. Time dependent induction in CA also suggests that long term exposure of bendiocarb may lead to generation of active electrophiles capable of interacting with the biological macromolecules DNA, RNA and proteins in reptilian cells *in vivo*. This may lead to altered enzyme activity causing abnormal DNA repair response. On the other hand, there is increasing evidence of pesticide induced oxidative stress through the generation of reactive oxygen radicals, leading to lipid peroxidation and DNA damage (Leomanni *et al*., 2015). Bendiocarb may have the potential to induce oxidative stress leading to generation of free radicals and alterations in antioxidant status or the oxygen free- radical (OFR) scavenging system.

It may be concluded that bendiocarb exposure under controlled laboratory conditions induced genotoxicity as revealed by chromosomal aberration assay. Further, this work also indicates that the frequency of karyological and cytogenetic damage in the form of numerical and structural aberrations is significantly correlated with the exposure time. The test system proved to be highly sensitive for genotoxicity assessment and the reptilian model *Calotes versicolor* may act as a good and interesting model for genotoxicity testing and ecological risk assessments.

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