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Research Paper

Time dependent induction of genotoxicity and cytotoxicity by thiodicarb in *Calotes versicolor*

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Abstract: In the present work three very sensitive biomarkers chromosomal aberrations (CA), micronucleus (MN) and mitotic index (MI) has been used to test the genotoxic and cytotoxic potentials of thiodicarb, a carbamate insecticide in the bone marrow of *Calotes versicolor*. Adult male garden lizards were acclimatized for one week in the laboratory and then injected intraperitoneally with a daily dose of 13.33 mg/kg body weight ($1/3^{rd}$ LD_{50}) of thiodicarb. The bone marrow was used for scoring (CA), (MN) and (MI). Chromosomal aberrations in the form of numerical and structural changes were observed in the treated group. Numerical changes included aneuploidy with a general pattern of monosomy and the structural changes included gaps, breaks, additions and deletions. Frequencies of CA in the form of gaps increased significantly after 14^{th} ($P\leq 0.05$), 21^{st} ($P\leq 0.005$) and 28^{th} day ($P\leq 0.0005$) of treatment. Breaks increased significantly ($P\leq 0.05$) after 21^{st} day and deletions increased significantly ($P\leq 0.05$) after 28^{th} day of treatment. MN frequencies increased significantly after 21^{st} and 28^{th} day $P\leq 0.005$ and 0.0005 respectively. No significant change in frequencies of MI was observed till 14^{th} day. However, after 21^{st} day MI frequencies decreased significantly ($P\leq 0.05$) in the treated group. This study thus, reveals that thiodicarb induces genotoxicity and cytotoxicity in a time dependent manner in the bone marrow cells of *Calotes versicolor*.

Keywords: Thiodicarb, Chromosomal aberration, Micronucleus, Mitotic Index, *Calotes*

Abbreviations: Bovine Calf Serum (BCS), Chromosomal Aberration (CA), Deoxyribo Nucleic Acid (DNA), Fluorescence in situ hybridization (FISH), Lethal Dose 50% (LD50), Microlitre (µl), Micronucleus (MN), Mitotic Index (MI), Phosphate Buffer Saline: PBS, Polychromatic Erythrocyte (PCE), Potassium Chloride (KCl), Potential of Hydrogen (pH), Probability (P), Roswell Park Memorial Institute medium: RPMI medium, Rotations per minute (rpm), and Whole chromosome painting (WCP).

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INTRODUCTION

Reptiles as a vertebrate are interesting and unusual and are an integral part of natural ecosystem. Reptiles were first to make transition from aquatic to terrestrial life and from them the birds and mammals evolved. In the wake of a changing global environment, reptile populations, like those of other vertebrates, appear to be declining. Vulnerability of reptiles within the context of the factors known or suspected to be associated with their decline are habitat loss and degradation, introduction of invasive species, environmental pollution, disease and parasitism, unsustainable use, and global climate change. Decline of reptilian population may often be a cumulative effect of more than one of the potential causes. In general the reptiles are correspondingly defenceless against the global threats of deforestation, draining of wetlands, and pollution from agricultural runoff and climate change. Reptilian decline problem is a serious threat as reptiles appear to be in a greater danger of extinction worldwide.

Pesticide pollution may be one of the causes of population decline and mass extinction of several reptilian species contributing to a loss in biodiversity. In recent years environmental awareness all over the world has led to several biomonitoring studies done on reptiles. Such studies have gained momentum and have established the fact that out of many reasons the excessive use of synthetic pesticides may be one of the several causes for the rapid decline in the population of reptiles in the last few decades (Gibbons *et al.*, 2015). Several published reports have suggested that pesticide exposure either directly or indirectly through food chain in a biomagnified manner influences the animal behavior, morphology, physiology, biochemistry, metabolism, immune response and DNA integrity. These altered conditions affect the organisms and their ability to acquire resources, grow, fight against diseases and reproduce. Pesticide pollution has thus, become a serious threat for the modern society, and is potentially dreadful for a myriad of non target organisms including reptiles. Reptiles have been shown as valuable models for ecotoxicological studies and risk assessment both *in vivo* and *in vitro* (Matson *et al.*, 2009; Strunjak- Perovic *et al.*, 2010).

Genetic ecotoxicology is the study of pollutant induced genetic changes in organisms. Reptiles have been shown to be excellent indicators of the potential association between contaminants and genetic damage (Matson *et. al.*, 2009, Strunjak-Perovic *et. al.* 2010). Several techniques are being used by researchers all over the world to assess pesticide induced genotoxicity and cytotoxicity on both aquatic and terrestrial organisms. Some of these techniques include chromosomal aberration assay, micronucleus assay, mitotic index and comet assay, FISH and WCP (Whole chromosome painting) technique. Published information reveals the use of such techniques in biomonitoring studies to detect DNA damage in different species of reptiles (Poletta *et al*., 2009, 2011; Capriglione, *et al*., 2011; Anisha *et al*, 2014; Shrivastava *et al*., 2014; 2015; Anisha and Singh, 2015). These investigations have demonstrated a high sensitivity to detect the effects of genotoxic and cytotoxic agents.

In order to protect agricultural, household and industrial products from various plant and animal pests, several synthetic pesticides are being used in India and elsewhere in the world. Most of the synthetic pesticides are complex mixtures of a variety of chemicals and majority of them pose threat to our environment. Most pesticides fall in one of three broad categories the organophosphates, organochlorines and carbamates. Their use is rather inevitable in modern agricultural practices. However, lack of proper

guidelines and regulations by industries and thus unaware public, these pesticides are generally being indiscriminately used. The degradation product of such hazardous chemicals and their accumulation in water bodies and soil leads to further deterioration of the natural habitat of several organisms. This aspect is of great concern all over the world.

Carbamates are a part of a large group of synthetic pesticides that have developed in the last 40 years and include a versatile class of compounds used as insecticides, fungicides, nematocides, acaracides, molluscicides, sprout inhibitors or herbicides. Carbamates are used in homes, gardens and agriculture. Many investigations have shown that some of the carbamate pesticides are cytotoxic, mutagenic, clastogenic and carcinogenic (Wei *et al*., 1997; Srivastava and Singh, 2013; Shrivastava *et al*., 2014; 2015; Anisha *et al*., 2014).

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. Methomyl (S-methyl N-(methylcarbamoyloxy) thioacetimidate) and methomyl oxime (N-hydroxyethanimidothioic acid methyl ester) are the degradation products of thiodicarb in the soil (EFSA, 2005).

There appears to be dearth of information pertaining to the genotoxic effects of carbamates on lizards. 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. So the garden lizard *Calotes versicolor* was selected as a model organism to assess pesticide induced cytogenetic effects. Our aim was to investigate the genotoxic and cytotoxic potentials of the carbamate insecticide, thiodicarb on the bone marrow cells of the males of *Calotes versicolor* following chronic exposure, under laboratory conditions. The genotoxic and cytotoxic effects were assessed by three very sensitive biomarkers of cytogenetic risk assessment; the chromosomal aberration assay, micronucleus assay and mitotic index. The present study will help in understanding the effects of pesticide on the genetic material of this lizard and establishment of standard methods and techniques for bio monitoring studies. The work also aimed at adding to the contrasting evidences collected on the genotoxicity of carbamate in general.

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 Randhawa (2009). The evaluated value showing 50% mortality during the treatment period of 96 hours following acute intraperitoneal dose of thiodicarb was 40

mg/kg body weight. 1/3rd LD_{50} value (13.33 mg/kg body weight) was selected for further study. A total of 64 lizards were taken for experimentation. The animals were divided into two groups of 32 lizards each. The first group was kept as control and the lizards of second group were injected daily with 1/3rd LD_{50} (13.33 mg/kg body weight) of thiodiocarb intraperitoneally.

The lizards of control and treated groups were used for Micronucleus (MN) Assay Mitotic index (MI) and Chromosomal Aberration Assay. Four lizards from each group were sacrificed after 7th day, 14th day, 21st day and 28th day, of treatment; the femur bones were dissected and used for MN Assay and determination of MI. Four lizards from 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 thiodicarb administered (7th day, 14th day, 21st day and 28th day). These were sacrificed after 2 hours of colchicine treatment to prepare metaphase plates for Chromosomal Aberration Assay.

Micronucleus (MN) Assay- The bone-marrow was flushed out from femur bones with 1 ml solution of RPMI (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 Serum, SIGMA-CHEMICALS). The evenly spread bone-marrow smear was stained with Giemsa (QUALIGEN INDIA). Slides were scored under oil emersion 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 (Hayashi 1994).

Mitotic Index

The bone-marrow was flushed in hypotonic solution (0.56 % KCl), incubated at 30°C for 10 min to make single cell suspension. 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 (pH 6.8) Giemsa. 1000 cells were examined in each preparation. Mitotic activity were estimated according to the following formula: Mitotic index (MI) = [no. dividing cells/total no. of cells] x 100 (Mansour *et al.*, 2008).

Chromosomal Aberration (CA) Assay- Bone-marrow from femur bones of colchicinized lizards was flushed in hypotonic solution (0.56 % KCl) and then incubated at 30°C for 10 minutes. 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). One hundred good-quality metaphases were examined under oil emersion at a magnification of 1000x for control and treated groups separately (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.

RESULT

Figure 1 shows the results of MN assay. After 7th and 14th day of treatment no significant change was observed for frequencies of MN. Frequencies of MN increased significantly ($P \leq 0.005$) in treated group (0.009±0.002) as compared to control group (0.001±0.0009) after 21st day of treatment. After 28th day MN (0.014±0.003) increased significantly ($P \leq 0.0005$) in the

treated group as compared to control group (0.001±0.0009).

Figure 2 shows the results of MI assay. Increase in frequencies of MI was observed after 7th and 14th day of treatment but the result was insignificant. Frequencies of MI decreased significantly (P≤ 0.05) in treated group (5.2±0.097) and (4.7±0.225) as compared to control group (6.04±0.297) and (6.02±0.344) after 21st and 28th day of treatment respectively.

Figure 3 shows the results of CA assay. No significant change was observed for frequencies of gaps, breaks, additions and deletions after 7th day of treatment. Frequencies of CA in the form gaps increased significantly (P≤ 0.05) in treated group (0.08±0.027) as compared to control group (0.01±0.009) after 14th day of treatment while changes in frequencies for breaks, additions and deletions were insignificant. After 21st day gaps and breaks increased significantly with mean frequencies 0.14±0.034 and 0.06±0.023 in the treated group at P≤ 0.005 and 0.05 respectively, as compared to control group (0.02±0.014 and 0.01±0.009). However, changes in frequencies for additions and deletions were insignificant after 21th day of treatment. After 28th day gaps increased significantly (0.2±0.04; P≤ 0.0005) in the treated group as compared to control (0.02±0.014). Breaks (0.08±0.027) and deletions (0.06±0.023) increased significantly (P≤ 0.05) in the treated group as compared to control group (0.01±0.009) and (0.00±0.00) after 28th day, while frequency for additions remained insignificant.

DISCUSSION

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 thiodicarb formulation was determined before actual experimental design. A very low concentration (1/3rd LD50) of thiodicarb was used in this investigation to evaluate the cytogenetic effects of thiodicarb. In the present work we have used three very sensitive biomarkers MN, MI and CA to investigate the genotoxic and cytotoxic potentials of thiodicarb, a carbamate insecticide. Comparison of these methods is useful, since it allows estimation of the amount and progression of DNA breakage, which translates into chromosome and/or genome mutation. Genetic damages have been classified into three groups as follows: 1. Premutagenic damage like DNA strand breaks, DNA adduct or unscheduled DNA synthesis; 2. Gene mutation which means insertion or deletion of a couple of base pairs; 3. Chromosomal aberrations, including loss or gain of whole chromosome (aneuploidy), deletion or breaks (clastogenicity), and chromosomal segments or rearrangements. Premutagenic damages may be repaired prior to cell division while the damages in the second and third groups are permanent and have the ability of transmission to daughter cells after cell division.

MN is formed either from acentric chromosome fragments or whole chromosomes left behind during mitotic cell division it 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. The primary mechanism of action of aneugenic chemicals is the induction of damage to centromeric DNA, which results in a failure of attachment of such damaged chromosomes to the mitotic spindle. MN provides a measure of both chromosome breakage and chromosome loss and it has

been shown to be a sensitive indicator of chromosome damage as compared to classical metaphase chromosome analysis. MN assay has been used to estimate the clastogenic potential of chemical compounds. The efficacy of MN assay as an indicator of structural genomic damage has been proved, 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 under both laboratory and field conditions. Both clastogenicity (chromosome breakage) and aneugenicity (chromosome lagging due to a dysfunction of the mitotic apparatus) may be detected by the MN assay. The key advantage of the MN assay is the relative ease of scoring and the statistical power obtained from scoring larger number of cells than are typically used for metaphase analysis. The micronucleated PCE in bone marrow of *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*.

The results illustrated in Figure 1 shows time dependent increase in MN frequency following intraperitoneal thiodicarb treatment. No significant induction in MN frequencies was observed in the PCE of bone marrow after 7 and 14 days, however, MN frequencies increased significantly after 21st and 28th day P≤ 0.005 and 0.0005 respectively in the treated group. These findings are similar to the earlier reports on thiodicarb and bendiocarb induced cytogenetic damage (MN) in bone marrow cells of *Calotes versicolor* (Shrivastava *et al*., 2015; Anisha *et al*., 2014). Reports on effects of carbamate in reptiles are meagerly available however; good deal of information

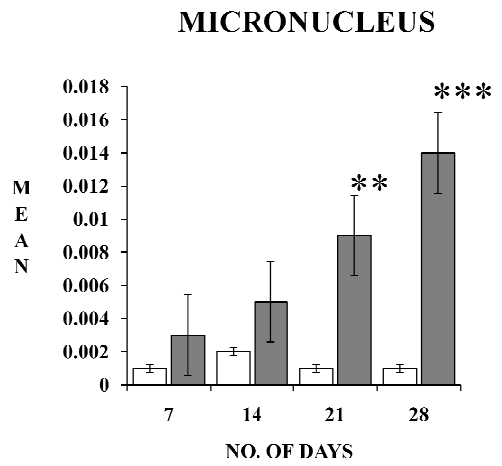

Figure 1- Graph showing time (in days) dependent induction of Micronucleated Polychromatic Erythrocytes in the bone marrow cells of *Calotes versicolor*, following intraperitoneal treatment with 1/3rd LD_{50} of thiodicarb for 7th, 14th, 21st and 28th day.

** P≤ 0.005 and *** P≤ 0.0005.

□ Control ■ Treated

is available regarding its influence in other vertebrates. Hence, we compared the concerned results with our findings. Genotoxic potential of carbaryl has been reported in erythrocytes of *Anabas testudineus* and *L. limnocharis* by Bhunya and Sahoo (2004) and Krishnappa and Venkteshwarlu (2007) respectively. Carbosulfan induced genotoxiciy in fish, mice and chicks have been reported by Nwani *et al*., 2010; Giri *et al*., 2002; 2011. *In vivo* cytogenetic activity in the mouse bone marrow induced by carbamate insecticides benfuracarb, carbosulfan and furathiocarb has been assessed by Stehrer-Schmid and Wolf, (1995). Propoxur, methomyl and aldicarb is reported to induce micronucleated cells significantly in mice (Wei *et al*., 1997). Most of these results are in accordance with our findings thus, concerning genotoxic effects of thiodicarb,

our results are indicative of potential clastogenicity of thiodicarb, as this insecticide induced micronuclei formation in the PCE of bone marrow cells of *C. versicolor*.

Evaluation of the mitotic index (MI) is an additional check on the potential of any chemical agent. Mitotic index is the percentage of cells in a culture which are in the process of division. It is proved to be a good and an easy marker to evaluate and compare cell proliferation rate. Normal cell proliferation can be affected by exposure to physical, chemical and biological agents which may lead to cytotoxicity. Level of cytotoxicity can be determined by the decreased rate of MI which may be due to decrease in cell proliferation in culture or decrease in the mitotic rate under *in vivo* conditions. The reduction in MI suggests a cytotoxic/cytostatic effect of chemicals or delay in cell cycle, as a result of decreased DNA synthesis or decreased cell viability. MI is an acceptable measure of cytotoxicity for all living organisms. The mitotic index assay is also being used as a good indicator to study the effects of chemical and physical agents on genetic material as it provides a useful index of accumulated genetic damage during the lifespan of the cells. In the present study MI increased after 7^{th} and 14^{th} days of treatment however the results were insignificant. The increase in MI may result from shortening the duration of mitotic cycle and allowing the interphase cells to enter the subsequent division stages. However, MI frequencies decreased significantly after 21 days ($P \leq 0.05$) in the treated group as compared to control (Figure 2).

MI can be disrupted in several ways including inhibiting the process of cell division, by disturbing the normal functioning of mitotic spindle, and by producing chromosomal abnormalities which lead to MI reduction or due to the inhibition of DNA synthesis which may further lead to increased number of interphase or dead cells.

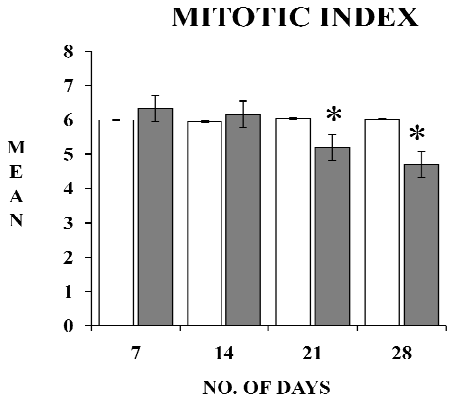

Figure 2. Graph showing time (in days) dependent analysis of thiodicarb treatment on mitotic index in bone marrow cells of *Calotes versicolor* following intraperitoneal treatment with $1/3^{rd}$ LD_{50} of thiodicarb for 7^{th}, 14^{th}, 21^{st} and 28^{th} day.

* $P \leq 0.05$. ☐ Control ■ Treated

Reduction in MI could also be due to the inhibition of DNA synthesis or blocking the cell from entering mitosis (Tulay and Ozlem, 2010). These results are in accordance with earlier reports on carbamate induced cytotoxicity. Bendiocarb induces inhibition of MI in cultured peripheral lymphocytes of cattle (Holeckova *et al*., 2009). Carbofuron induces cytotoxicity and inhibition of MI in Chinese hamster (Soloneski *et al*., 2008). The MI significantly ($p<0.05$) decreased in kidney tissue of fish after mancozeb exposure (Srivastava and Singh, 2013). Carbosulfan causes reduction in MI in bone marrow cells of rats Topaktas *et al*., (1996). Dioxacarb, a phenyl methyl carbamate causes cytotoxicity on human peripheral blood lymphocytes and *Allium* root meristematic cells by mitotic inhibition (Eren *et al*., 2014). Carbamates are known to inhibit proteins by binding to

them in the present study, the same mechanism might have occurred with thiodicarb causing inhibition of some enzymes leading to DNA damage, and thus to clastogenic and physiological events.

CA can provide both qualitative and quantitative data on the effects of exposure to a mutagen and it is a fully accepted method to reveal genotoxicity as it is indicative of real genetic effects (Tompa *et. al.*, 1992). The production of CA is a complex cellular process with mechanisms of chromosome breakage and re-joining that are not yet completely understood. 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 **c**lastogenic or aneugenic effect of thiodicarb. 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 thiodicarb. CA in the form of chromatid gap may be due to fragile sites with regions of genomic instability. Although the mechanisms underlying gap formation are unclear, multiple factors are known to be involved, including environmental agents such as radiation, cellular response to stress, and DNA damaging chemicals (Bradshaw and McEntee, 1989). It may be surmised that thiodicarb 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*.

In the present study frequencies of CA in the form of gaps increased significantly after 14^{th} ($P \leq 0.05$), 21^{st} ($P \leq 0.005$) and 28^{th} day ($P \leq 0.0005$) of treatment. Breaks increased significantly ($P \leq 0.05$) after 21^{st} day and deletions increased significantly ($P \leq 0.05$) after 28^{th} day of treatment, however, no significant change was observed in frequency of additions. Concerning genotoxic effects of thiodicarb, our results indicate its potential to interact with and cause alterations in the cellular DNA and damage its structure. The significant increase in CA was seen after 14, 21 and 28 days (Fig. 3) of treatment suggesting 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 mutagenic.

Time dependent induction in CA also suggests that long term exposure of thiodicarb 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 free oxygen radicals, leading to lipid peroxidation and DNA damage (Leomanni *et al.,* 2015). Thiodicarb, 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. Our findings are in accordance with the reports on thiodicarb and bendiocarb induced genotoxicity in *Calotes versicolor* (Shrivastava *et. al*., 2014; Anisha et. al. 2015). These findings are also in accordance with those of Giri *et. al*., (2002) on mouse bone marrow cells exposed to carbosulfan. Similar results have also been reported on cultured human

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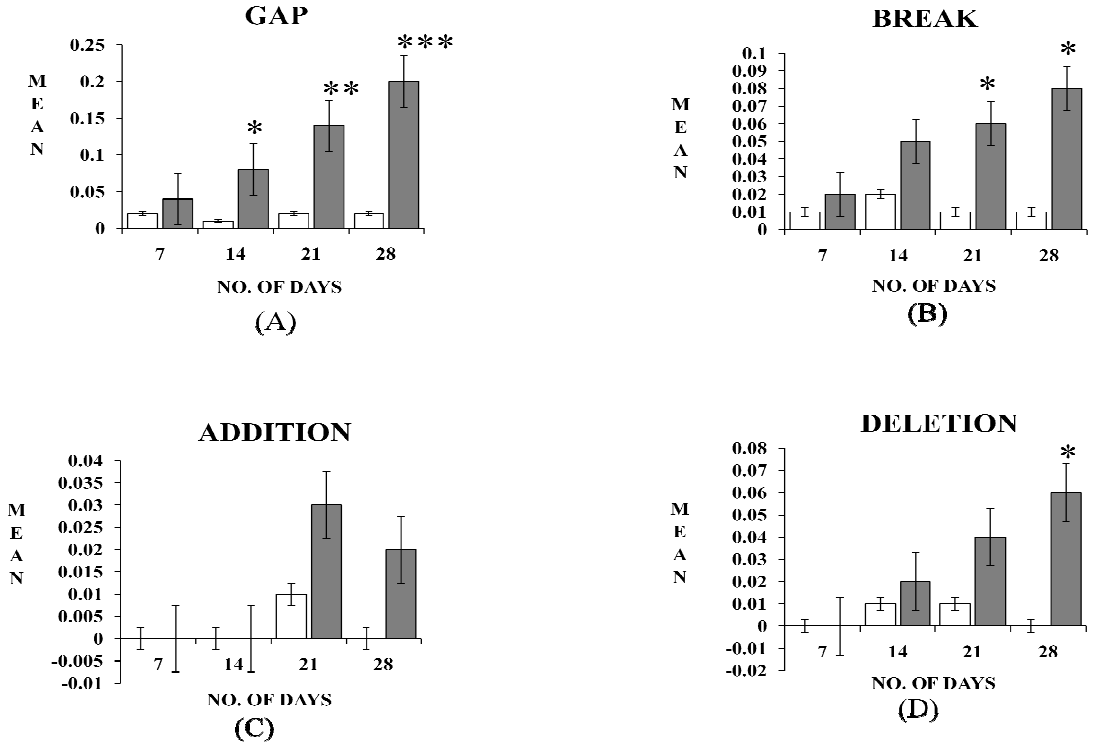

* P≤ 0.05, ** P≤ 0.005 and *** ≤ 0.0005. Control Treated

Figure 3. Graph showing time (in days) 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 intraperitoneal treatment with 1/3rd LD_{50} of thiodicarb for 7th, 14th, 21st and 28th day.

lymphocytes exposed to mancozeb by Srivastava *et. al*., (2012). 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 Giri *et al*., (2002) on mouse bone marrow cells exposed to carbosulfan. Present observation concurrent with earlier reports on several carbamates discussed above substantiates the genotoxic potential of carbamate and it may be surmised that thiodicarb is a genotoxic agent in *Calotes.*

The study thus reveals that chronic exposure of thiodicarb for an extended period significantly increased the level of MN frequencies in PCE, decreased the mitotic activity in bone marrow cells and increased the frequency of chromosomal aberrations suggesting its influence as time dependent. It may be surmised that thiodicarb has a tendency to accumulate and concentrate in the animal body or degraded into harmful xenobiotic agents which may be clastogenic, genotoxic and cytotoxic. This study further indicates the degree to which the tested dose causes chromosomal damage of developing RBC, WBC and

thrombocytes in the bone marrow of lizard studied.

Conclusion: It may be concluded that thiodicarb exposure under controlled laboratory conditions induced genotoxicity and cytotoxicity as revealed by MN, MI, and CA the frequency of these markers and exposure time is also significantly correlated in the present work. The test systems proved equally sensitive for genotoxicity and cytotoxicity assessment. If such severe cytogenetic effects arise in the poikilotherm vertebrate investigated in the present work, its prolonged exposure to thiodicarb in natural conditions may be considered as a cytogenetic hazard. Nevertheless, the exact mechanism of genotoxicity and cytotoxicity induced by bendiocarb in *Calotes* needs to be further investigated. This study will be useful as an endeavour to understand the toxic effects of these potential factors and will help conservation biologists in assessing it as a cause of population decline of reptiles. Long-term monitoring of reptile populations aided by the standard methods and techniques will help in ameliorating their impact on natural populations of reptiles and their habitats. However, the exact mechanism of genotoxicity induced by thiodicarb in *Calotes* needs to be further investigated.

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