# *Cytotoxic and genotoxic effects of dioxacarb by human peripheral blood lymphocytes CAs and Allium test*

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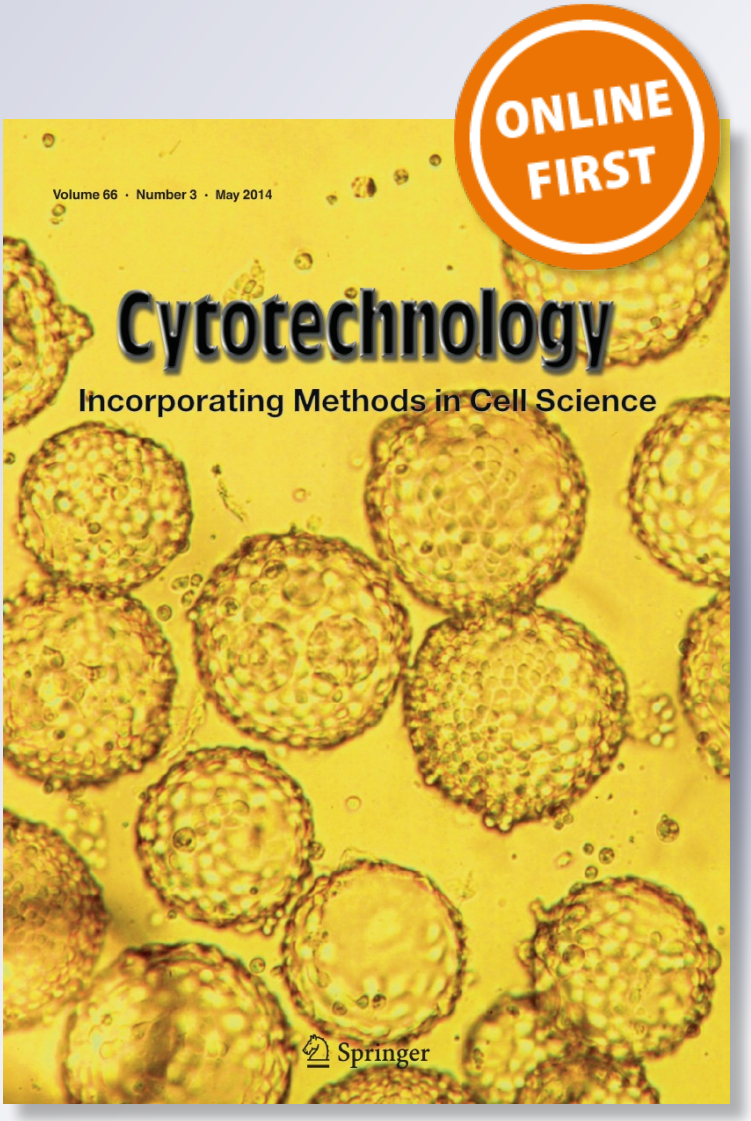


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ORIGINAL RESEARCH

# Cytotoxic and genotoxic effects of dioxacarb by human peripheral blood lymphocytes CAs and Allium test

**Yasin Eren · Sevim Feyza Erdoğmuş · Dilek Akyıl · Arzu Özkara · Muhsin Konuk · Esra Sağlam**

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**Abstract** Dioxacarb (Elecron, Famid) is a phenyl methylcarbamate insecticide and in vitro cytotoxic and genotoxic effects of this pesticide on human peripheral blood lymphocytes and Allium root meristematic cells were investigated by chromosomal aberrations (CAs) and Allium test. Human lymphocytes were treated with 62.5, 125, 250 and 500 ppm doses of dioxacarb for CAs. CA/cell, abnormal cell % and mitotic index % (MI %) data were obtained from these concentrations in 24 and 48 h treatment periods. Dioxacarb did not increase the CA/cell frequency significantly, so this insecticide was not identified as genotoxic. But it was found cytotoxic especially at 250 and 500 ppm concentrations because of the reduced the MI % and increased the abnormal cell %. In Allium test, 25 ppm (EC50/2), 50 ppm (EC50) and 100 ppm (EC50 × 2) concentrations were used for root growth inhibition (EC50 determination) and Allium mitotic index (MI) determination tests. The used concentrations of dioxacarb induced dose-dependent inhibition of MI and root growth on root meristems. Mitotic inhibition of dioxacarb was found significantly higher than for the positive control. These Allium results indicated the high cytotoxicity of dioxacarb. The present study is the first research on cytotoxicity and genotoxicity of dioxacarb by human lymphocyte CAs and Allium test.

**Keywords** Dioxacarb · CAs · Allium test · Cytotoxicity · Genotoxicity

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## Introduction

Pesticides are of vital importance in the fight against diseases, for the production and storage of food being widely used for pest control in agriculture, gardening, homes and soil treatment (Crespo-Corral et al. 2008; Janssen 1997). In spite of their extensive use, an average of 35 % of the produce is lost worldwide (Janssen 1997). Carbamates represent one of the main

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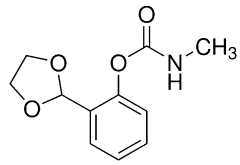

**Fig. 1** Chemical structure of dioxacarb insecticide

category of synthetic organic pesticides since their introduction into the agrochemical market in the 1950s (Tomlin 1997) and are used annually on a large scale worldwide (Paiga et al. 2009). They constitute a versatile class of compounds used as insecticides, fungicides, nematocides, acaracides, molluscicides, sprout inhibitors or herbicides.

Dioxacarb (Elecron, Famid) is a phenyl methylcarbamate insecticide. It is the inhibitor of cholinesterase like the organophosphates (Boskovic et al. 1976). Dioxacarb was found toxic to aquatic animals. Possible symptoms of dioxacarb were anorexia, nausea/vomiting, abdominal cramps, diarrhea, chest tightness, increased salivation and lacrimation, miosis/blurred vision, sweating, bradycardia, bowel/urinary incontinence, muscle twitching, hypertension, hyperglycema, tachycardia (Sigma-Aldrich 2013). This insecticide behaves like the other carbamates. The chemical structure of dioxacarb is given in Fig. 1.

Carbamates are generally toxic and have potential for adversely affecting non-target organisms. Although the toxicity of carbamate pesticides is primarily through blockade of neural transmission via inhibition of acetylcholinesterase activity, studies revealed histopathological alterations in other mammalian organs (Yarsan et al. 1999; Seth et al. 2001; Schmuck and Mihail 2004). Due to the potential risk of carbamates on human health, the evaluation of their toxicity is routinely required for their safe use (Weyermann et al. 2005).

Although carbamates present low bioaccumulation potentials and short-term toxicity, they are considered hazardous to the environment and human health being included in the priority list released by the United States Environmental Protection Agency (EPA 1992). The acute toxicity of the different carbamates ranges from highly toxic to only slightly toxic or practically non-toxic (IPCS 1986).

Concerning the main carbamate insecticides in use, it was estimated for humans that toxicity potential of these insecticides, vary from high toxicity (LD50 <50 mg/kg; for aldicarb, aldoxycarb, aminocarb, bendiocarb, carbofuran, dimetan, dimetilan, dioxacarb formetanate, methiocarb, methomyl, oxamyl and propoxur) to moderate toxicity (LD50 = 50–200 mg/kg; bufencarb, carbosulfan, pirimicarb, promecarb, thiodicarb, trimethacarb) and to low toxicity (LD50 >200 mg/kg; fenocarb, carbaryl, isoprocarb) (Erdman 2003).

Epidemiologic studies of pesticide exposure and cancer incidence were recently reviewed by Weichenthal et al. (2010). This survey illustrates that carbamates may induce different types of cancer at occupational levels.

Carbamate compounds are often called anticholinesterases. Acetylcholinesterase is the enzyme responsible for the hydrolysis of acetylcholine into choline and acetic acid. Acetylcholine (ACh) is a substance that transmits a nerve impulse from a nerve cell to a specific receptor such as another nerve cell or a muscle cell. In the presence of inhibitors, acetylcholinesterase becomes progressively inhibted and is not further capable of hydrolyzing ACh to choline and acetic acid (Jokanovic 2009; Jokanovic and Maksimovic 1997).

The potential genetic hazard from pesticides suggests that evaluation of their genotoxicity should be extended using various approaches and test systems. Cytogenetic markers like chromosomal aberrations (CA), micronuclei (MN), and sister chromatid exchanges (SCEs), are extensively used in genotoxic screening (Yadav and Kaushik 2002; Zeljezic and Garaj-Vrhovac 2004; Dimitrov et al. 2006).

Allium test aimed to test the two targets: Toxicity and mutagenicity. Toxicity is measured by observation of growth inhibition, and mutagenicity is correlated to the rate of chromosome breaks. Root tip cells of *Allium cepa* are suitable for such cytological tests. The Allium test is a sensitive test, indicating excellent correlation to other test systems (Fiskesjö 1985).

Several researchers have used cytotoxicity and genotoxicity assays with the aim of evaluating the potential genotoxicity of carbamates in different test systems. But no data are available on the effect of dioxacarb on cultured human lymphocytes by human peripheral lymphocyte CAs, and Allium test. For these reasons, it was aimed to obtain additional genotoxicity and cytotoxicity data for dioxacarb (carbamate insecticide) by using CAs in human lymphocytes and the Allium test.

## Materials and methods

### Materials

Dioxacarb, [IUPAC name 2-(1,3-dioxolan-2-yl) phenyl methylcarbamate], CAS No: [6988–21–2] was purchased from Sigma-Aldrich (St. Louis, MO, USA). Oninons were purchased from a local market for Allium test.

### *Allium* test

#### *Root growth inhibition test (EC50 determination)*

The protocol of the root growth inhibition test was carried out as described by Fiskesjö (1985). The onions were grown in freshly made distilled water for 24 h and then exposed for 4 day to the five different pesticide concentrations (6.25, 12.5, 25, 50 and 100 ppm). In order to determine the efficient concentration (EC50) values, ten roots from each onion were cut off at the end of the treatment period and length of each root was measured. It was accepted as "EC50 value" when one of the concentration decreased the root growth by about 50 % (compared with the negative control group "1 % DMSO"). To determine the possible toxic effects on roots, 25 ppm (EC50/2), 50 ppm (EC50) and 100 ppm (EC50x2) concentrations of dioxacarb were tested by the Allium MI test.

#### *Mitotic index (MI) determination*

Onions (*Allium cepa*, 2n = 16) were used in the *Allium* test system. The *Allium* test was performed according to Fiskesjö (1985). Five onion bulbs were treated with Methyl methanesulfonate (MMS) (10 ppm) (Sigma-Aldrich), DMSO at 1% and 6, 25, 12.5, 25, 50, 100 ppm concentrations of dioxacarb for 72 h. At the end of 24, 48 and 72 h, root tips were cut and fixed in ethanol: glacial acetic acid (3:1) then were hydrolyzed in 1 N HCL at 60 °C for 7 mins. Root tips from each concentration were stained with Feulgen dye for 1 h. Five slides were prepared for each concentration and 1,000 cells/per slide were counted. Totally about 5,000 cells were evaluated for each concentration. Obtained data were evaluated with One-Way ANOVA, Dunnett's t test (2-sided). In the mitotic index (MI) study, about 5,000 cells were counted, and MI % was determined with the following formulation.

$$\text{MI}\,\% = \text{Divided cell number/Total cell number} \times 100\,(\text{Fiskesjo1985}).$$

### CA assay with human lymphocytes

Blood samples were collected from four healthy non-smoking (age, 18–24 years) donors, who were free of any known exposure to genotoxic agents. Whole blood was cultured in chromosome medium B (Biochrome, Berlin, Germany) supplemented with 10 ppm of bromodeoxyuridine (Sigma-Aldrich). The cultures were incubated at 37 °C for 72 h. Duplicate cultures were used at each concentration. Test substances were added after 24 and 48 h of culture initiation, and colchicine (0.06 ppm) (Sigma-Aldrich) was added to each culture at 2 h before harvesting. Human lymphocytes were treated with four concentrations of dioxacarb (62.5, 125, 250, and 500 ppm). A negative (1 % DMSO) and a positive control (mytomycin C, (Sigma-Aldrich), 0.25 ppm) were also used for testing the accuracy of the assays.

The CA test was performed as described by Evans (1984). One hundred metaphases were analyzed for the CA assay per donor (totally 400 metaphases per concentration). The mean frequency of abnormal cells and the number of CAs per cell (CA/cell) were calculated. The MI (MI: number of metaphases/total interphases and metaphases) was scored by recording the number of metaphases in 1,000 cells from each donor. MI was calculated according to the OECD Guideline (1997).

### Statistical analysis

The SPSS computer program was used to analyze the mean frequency of abnormal cells (structural and numerical CAs), the number of CAs per cell (CA/cell), the MI and EC50 value. The one-way analysis of variance (ANOVA) by Dunnnett's t test (2-sided) was used for the determination of statistically significant data. The level of significance chosen was 0.05.

## Results

The present study evaluated the cytotoxic capacity of dioxacarb using the human peripheral lymphocyte chromosome aberrations assay (CA) and the Allium

**Table 1** Chromosomal aberrations in human lymphocytes treated with dioxacarb

| Test substances | Treatment | | Chromosome aberrations | | | | | | | | CA/ Cell ± SD | Abnormal Cell % ± SD | MI % ± SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Time | Concentration (ppm) | B′ | B″ | F | SU | DC | T | CE | P | | | |
| Control | 24 | – | 4 | 1 | – | – | – | – | – | – | 0.05 ± 0.02 | 4.00 ± 1.21 | 7.04 ± 1.94 |
| DMSO 1 % (Negative control) | 24 | – | 5 | –1 | – | – | – | – | – | – | 0.05 ± 0.02 | 4.70 ± 1.14 | 6.85 ± 0.98 |
| MMC (Positive control) | 24 | 0.25 | 20 | 6 | 5 | – | – | – | 3 | – | 0.34 ± 0.12 | 33.20 ± 4.77* | 3.47 ± 0.74* |
| Dioxacarb | 24 | 62.5 | 3 | 1 | – | – | – | – | – | – | 0.04 ± 0.01 | 5.12 ± 1.18 | 6.24 ± 1.54 |
| | 24 | 125 | 4 | 1 | 1 | – | – | – | – | – | 0.06 ± 0.02 | 5.20 ± 2.10 | 5.36 ± 1.12* |
| | 24 | 250 | 4 | 1 | 1 | – | – | – | – | – | 0.06 ± 0.03 | 6.00 ± 1.95* | 4.05 ± 0.88* |
| | 24 | 500 | 3 | 2 | 2 | – | 1 | – | 1 | 1 | 0.1 ± 0.22 | 9.00 ± 2.78* | 3.42 ± 0.53* |
| Control | 48 | – | 3 | 1 | 1 | – | – | – | – | – | 0.05 ± 0.02 | 4.20 ± 1.45 | 6.88 ± 1.67 |
| DMSO 1 % (Negative control) | 48 | – | 5 | 1 | 1 | – | – | – | – | – | 0.07 ± 0.02 | 5.40 ± 1.78 | 6.64 ± 1.41 |
| MMC (Positive control) | 48 | 0.25 | 29 | 13 | 9 | – | – | – | – | – | 0.51 ± 0.16 | 48.60 ± 5.36* | 3.35 ± 0.65* |
| Dioxacarb | 48 | 62.5 | 3 | 1 | – | – | – | – | – | – | 0.04 ± 0.01 | 5.80 ± 0.94 | 6.35 ± 1.39 |
| | 48 | 125 | 4 | 1 | 1 | – | – | – | – | – | 0.06 ± 0.02 | 7.70 ± 1.99* | 5.12 ± 1.24* |
| | 48 | 250 | 4 | 2 | – | – | – | – | – | – | 0.06 ± 0.03 | 8.20 ± 2.05* | 3.97 ± 0.77* |
| | 48 | 500 | 5 | 2 | 1 | 1 | – | – | – | 1 | 0.1 ± 0.28 | 10.40 ± 3.14* | 3.30 ± 0.71* |

*MMC* mitomycin C, *B′* chromatid break, *B″* chromosome break, *F* fragment, *SU* sister union, *DC* dicentric, *T* translocation, *CE* chromatid exchange, *P* polyploidy, *SD* standard deviation, *MI* mitotic index, *DMSO* dimethyl sulfoxide

* Significantly different from the negative control: $p \leq 0.05$

test. Different concentrations of dioxacarb demonstrated a dose-dependent cytotoxic effect in both CA and Allium assays.

## Human lymphocyte chromosome aberrations results

CA results indicated that human lymphocytes treated with dioxacarb at concentrations of 62.5, 125, 250 and 500 ppm did not increase the CAs frequency significantly, compared with the negative control (Table 1). The solvent-control cultures did not show any difference, compared with the controls, this result suggested that 1 % dose of DMSO has no important genotoxic effects. Mitomycin C (MMC; positive control), showed a significant increase of CAs when compared to the negative control. Chromatid breaks, chromosome breaks, fragments, sister unions, dicentric chromosomes, chromatid exchanges, and polyploidies were investigated in this study and some of these aberrations are given in Fig. 2. No translocation was found at any concentration. On the other hand, the results showed that dioxacarb decreased the MI % at 125, 250 and 500 ppm concentrations in 24 and 48 h treatments. On the other hand, 62.5 ppm concentration did not affect the MI signicantly. MI % results of 500 ppm concentration (3.42 % for 24 h; 3.30 % for 48 h) were found closer to the positive control results. So these data indicated that 500 ppm concentration of dioxacarb was considerably cytotoxic to human lymphocytes.

## Allium test results

The activity of dioxacarb on root growth inhibiton was tried to analyzed with *Allium cepa* root tips that were treated for about 96 h. 50 ppm concentration of dioxacarb was decreased the root length by about 50 % (2.31 %), compared with the negative control group (DMSO at 1 %). The results also indicated that there is a dose-dependent decrease. So, EC50 determination showed the cytotoxic effects of dioxacarb. These

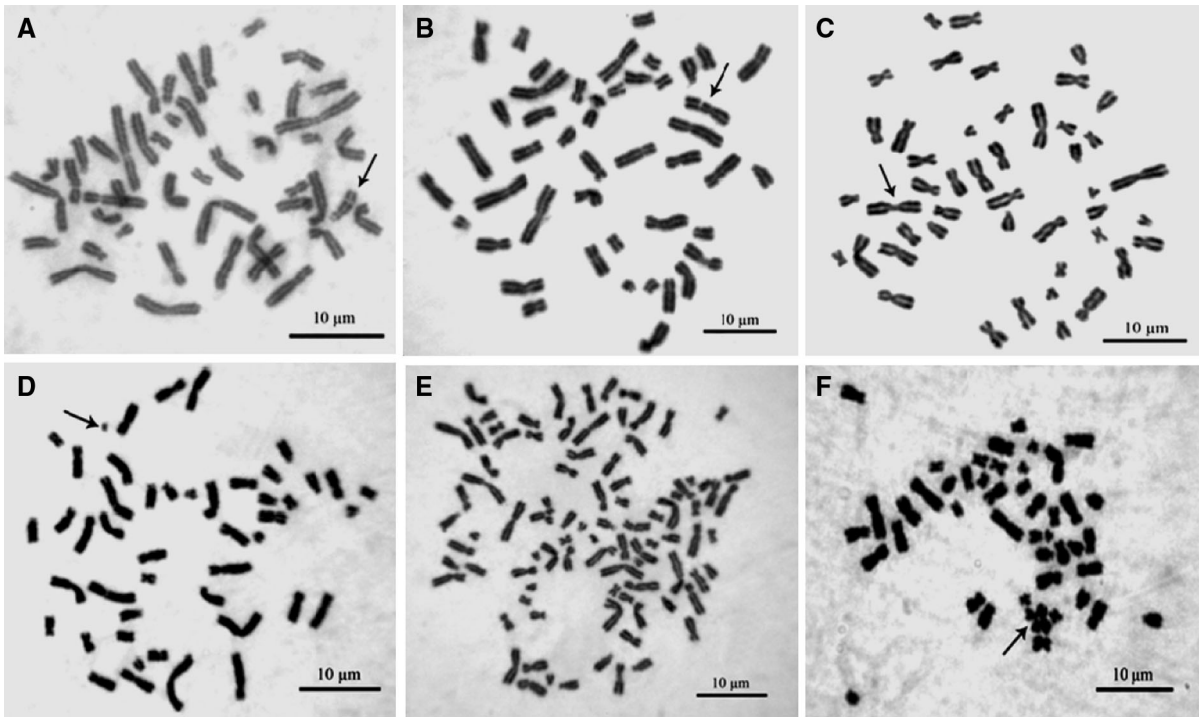


**Fig. 2** Some photos of choromosome aberrations obtained from dioxacarb treatment. **a** Chromosome break, **b** chromatid break, **c** dicentric chromosome, **d** Fragment, **e** Polyploidy, **f** chromatid exchange; Note: Chromosome aberrations are indicated by *arrows*

**Table 2** Root growth inhibition test (EC50 determination) results of dioxacarb

| Test substance | Concentration (ppm) | Treatment period | Mean of root length (cm) ± SD | % inhibition |
|---|---|---|---|---|
| Negative control (1% DMSO) | – | 96 h | 4.40 ± 0.21 | – |
| Dioxacarb | 100 | | 2.02 ± 0.17* | 54.09 |
| | 50 | | 2.31 ± 0.19* | 47.50 |
| | 25 | | 2.51 ± 0.30* | 42.95 |
| | 12.5 | | 2.88 ± 0.12* | 34.55 |
| | 6.25 | | 3.55 ± 0.27* | 19.32 |

*SD* standard deviation

* Means difference versus negative control was significant at a 0.05 level (Dunnet *t* test 2sided)

results were also found significant by statistical analysis (Table 2).

MI and mitotic phase determination studies were carried out for 24, 48 and 72 h. All concentrations of dioxacarb decreased the MI compared to negative control at each exposure time. There was an inverse ratio between the mitotic index (%) and the concentrations. MI % of dioxacarb were found lower than the positive control values for all concentrations and periods of dioxacarb treatment. As a result this insecticide showed high cytotoxic effect to *Allium cepa* root tips. All concentrations of dioxacarb changed the percentage of mitotic phases. Generally, dioxacarb decreased the percentages of prophase, metaphase, anaphase and telophase when compared with the negative control group for all treatment periods. The results of mitotic phase determination were similar to the MI. Percentages of mitotic phases at different concentrations of dioxacarb were below the positive control (10 ppm MMS) values, like for the

**Table 3** Effects of dioxacarb pesticide on mitotic index and mitotic phases of *Allium cepa* root meristematic cells

| Concentration (ppm) | Treatment time (h) | Counted cell number | Mitotic Index ± SD | Mitotic phases (%) ± SD | | | |
|---|---|---|---|---|---|---|---|
| | | | | Prophase | Metaphase | Anaphase | Telophase |
| Negative control (%1 DMSO) | 24 | 5,039 | 83.80 ± 10.83 | 78.24 ± 10.67 | 1.87 ± 0.28 | 1.75 ± 0.57 | 1.93 ± 0.48 |
| Positive control (10 ppm MMS) | | 4,439 | 39.86 ± 5.57* | 37.97 ± 5.36* | 0.75 ± 0.27* | 0.58 ± 0.24* | 0.55 ± 0.38* |
| 25 | | 5,126 | 29.05 ± 1.95* | 26.28 ± 4.25* | 0.74 ± 0.12* | 1.34 ± 0.37* | 0.69 ± 0.31* |
| 50 | | 5,062 | 29.02 ± 3.04* | 26.65 ± 5.12* | 0.69 ± 0.16* | 1.01 ± 0.32* | 0.67 ± 0.27* |
| 100 | | 5,080 | 24.23 ± 6.42* | 22.11 ± 3.14* | 0.69 ± 0.13* | 0.83 ± 0.22* | 0.60 ± 0.21* |
| Negative control (%1 DMSO) | 48 | 5,092 | 77.21 ± 1.78 | 71.97 ± 2.50 | 1.74 ± 0.27 | 1.57 ± 0.36 | 1.92 ± 0.28 |
| Positive control (10 ppm MMS) | | 5,073 | 35.36 ± 2.02* | 33.45 ± 2.12* | 0.66 ± 0.29* | 0.67 ± 0.40* | 0.57 ± 0.40* |
| 25 | | 5,048 | 25.34 ± 6.64* | 23.12 ± 3.12* | 0.77 ± 0.33* | 0.99 ± 0.34* | 0.46 ± 0.19* |
| 50 | | 5,083 | 18.95 ± 4.38* | 17.37 ± 2.15* | 0.63 ± 0.17* | 0.51 ± 0.22* | 0.43 ± 0.15* |
| 100 | | 5,066 | 17.05 ± 3.04* | 15.71 ± 1.98* | 0.57 ± 0.12* | 0.47 ± 0.18* | 0.30 ± 0.08* |
| Negative control (%1 DMSO) | 72 | 5,200 | 74.68 ± 2.26 | 69.64 ± 2.77 | 1.57 ± 0.42 | 1.48 ± 0.35 | 1.99 ± 0.45 |
| Positive control (10 ppm MMS) | | 5,233 | 30.91 ± 4.02* | 29.26 ± 3.79* | 0.55 ± 0.48* | 0.60 ± 0.25* | 0.51 ± 0.35* |
| 25 | | 5,108 | 16.01 ± 4.53* | 13.88 ± 1.12* | 0.72 ± 0.27* | 0.92 ± 0.24* | 0.48 ± 0.14* |
| 50 | | 5,065 | 14.08 ± 1.64* | 12.63 ± 1.57* | 0.67 ± 0.19* | 0.44 ± 0.17* | 0.35 ± 0.09* |
| 100 | | 5,094 | 11.78 ± 4.58* | 10.53 ± 0.97* | 0.58 ± 0.21* | 0.38 ± 0.13* | 0.29 ± 0.11* |

*SD* Standard deviation, DMSO dimethyl sulfoxide, MMS Methyl methanesulfonate

* Means difference versus negative control was significant at a 0.05 level, Dunnet *t* test (2sided)

values of MI %. There were statistically significant differences between negative control and the other groups (Table 3).

## Discussion

Dioxacarb (Elecron, Famid) is a phenyl methylcarbamate insecticide. Dioxacarb has anticholinesterase activity that hydrolysis acetylcholine to choline and acetic acid (Boskovic et al. 1976). So, this insecticide behaves like the other carbamates. This study aimed to establish if dioxacarb has cytotoxic and/or genotoxic activity or not. No data on cytotoxicity and genotoxicity of dioxacarb and its metabolites are known via human lymphocyte CAs assay and Allium test. This investigation adds further information to clarify the possible risk derived from dioxacarb cytotoxicity and genotoxicity, confirming that CAs and Allium test are a reliable screen for toxicity of dioxacarb.

Dioxacarb produced dose-dependent cytotoxic effects on both onion root cells and human lymphocytes. As a result of human peripheral lymphocyte chromosome aberration study (CAs), 62.5 125, 250 and 500 ppm of dioxacarb has cytotoxic effects. Especially 125, 250 and 500 ppm concentrations decreased the MI significantly. The EC50 of dioxacarb was found to be about 500 ppm from the human lympocyte MI study. But EC50 of dioxacarb was found 50 ppm in Allium test that is a plantal test system. Both human lympocyte MI and Allium test EC50 determination results are in vitro. On the other hand as a result of in vivo LC50 determination studies, the LC50 (lethal concentrations) of dioxacarb for carp, rainbow trout and *Poecilia reticulata* were found 25.5, 2.7 and 36.0 ppm respectively in 48 h. The minimum lethal concentration (LCs) was found to be 17.5 ppm for the carp, 0.35 ppm for the rainbow trout and 16.0 ppm for *Poecilia reticulata* (Heiduk and Svobodova 1980).

Other experiments on the effect of dioxacarb on simian kidney cells in culture have shown a threshold concentration for cytotoxic effects of 10 ppm. However, no correlation for several carbamates was found between this cytotoxic effect and the LD of warm blood animals (Dura et al. 1975). Previous studies determined the LD (lethal dose) values of dioxacarb in rats, rabbits and mouse they were 115, 1,950, 68 mg/kg, respectively (Boskovic et al. 1976; Kuhr and Dorough 1976). These in vivo and in vitro results showed that toxic concentrations of dioxacarb were mutable for different organisms.

A previous study about the mutagenicity of dioxacarb was performed by Ames test with 100, 10, 1, 0.1 and 0.01 μg/plate concentrations. But mutagenic activity was not found with *Salmonella typhimurium* TA100 and TA98 strains with and without metabolic activation (Konuk et al. 2008).

The Allium genus, specialy *A. cepa* species has shown to be an efficient species for such studies, mainly when it is used for assays of chromosome aberrations and for genetic tests (Fernandes et al. 2007; Saxena et al. 2010). So in this research, Allium test was used for the determination of cytotoxic effects of dioxacarb.

In this study, 25 (EC50/2), 50 (EC50) and 100 (EC50x2) ppm concentrations of dioxacarb were used. The used concentrations of dioxacarb induced dose-dependent inhibition of MI and root growth on root meristems. The maximum root growth inhibition was observed in 100 ppm concentration (54.09 %, 96 h) paralell to the MI study (11.78 %, 72 h). Also mitotic activity decreased depending to the treatment period. Mitotic inhibition of dioxacarb was found significantly higher than for the positive control (10 ppm MMS). As a result of these data, dioxacarb can be called a mitotoxic agent that prevented entry into the cell cycle. Previous studies reported that several pesticides inhibited mitosis (Pandey 2008; Yıldız and Arıkan 2008). Inhibition of these pesticides may be due to inhibition of microtubule formation, DNA synthesis blocking and G2-phase blocking activities (Hidalgo et al. 1989; Majewska et al. 2003). Some chemicals act as premetaphase inhibitiors that can delay or stop the cell at metaphase stage, owing to the chemical-microtubule interactions (Scolnic and Halazonetis 2000; Pandey 2008). In this study a small number of prophase, metaphase, anaphase and telophase were observed numerously. So dioxacarb could not act as a premetaphase inhibitor, but it could be identified as mitotic inhibitor.

The CAs assay is one of the most sensitive, useful methods to test the genotoxic potential of environmental mutagenic agents on human cells and the CAs analysis reveals alterations in the chromosome structure only. Also, increased levels of CAs have been associated with cancer risk (Bolognesi 2003).

In CAs, 62.5, 125, 250 and 500 ppm doses were used and all concentrations were found cytotoxic, except 62.5 ppm. This result showed that concentrations above 500 ppm may stop the mitotic activity completely. Chromatid and chromosome breaks have been widely observed. But significant results were not obtained in CA/cell frequency. Chromosome aberrations, especially breaks probably act at the G2 phase of the cell cycle (Biswas et al. 2004). Clastogenic agents may induce DNA double-strand breaks and lead to either cell cycle arrest or apoptosis (Kastan et al. 1991; Bunz et al. 1998; Morrison et al. 2000; Obe and Durante 2010). The results of this research showed that dioxacarb did not induce a significant increase in CAs compared to the control group and so dioxacarb was not identified as a clastogenic agent. But abnormal cell % and MI % data indicated that this insecticide was cytotoxic, especially at concentrations of 250 and 500 ppm concentrations.

Consequently, root growth inhibition test and Allium MI study showed that dioxacarb was cytotoxic at the used concentrations and it was more efficient than the positive control on root growth and MI. Also, this insecticide showed cytotoxic activity on human peripheral lympocyte as a result of the abnormal cell % and MI % data.

**Conflict of interest** The authors declare that there are no conflict of interest.

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