Mutagenicity and genotoxicity of dicapthion insecticide

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Abstract Mutagenic and genotoxic effects of dicapthion were investigated by using the bacterial reverse mutation assay in *Salmonella typhimurium* TA97, TA98, TA100 and TA102 strains with or without metabolic activation system (S9 mix), and chromosome aberrations (CAs), sister chromatid exchanges (SCEs), and micronucleus (MN) tests in human peripheral blood lymphocytes in vitro. Dicapthion was dissolved in dimethyl sulfoxide for all test systems. 0.1, 1, 10 and 100 µg/plate doses of dicapthion were found to be weakly mutagenic on *S. typhimurium* TA 98 without S9 mix. The human peripheral lymphocytes were treated with four experimental concentrations of dicapthion (25, 50, 100, and 200 µg/mL) for 24 and 48 h. Dicapthion increased the frequency of SCE only at the 100 µg/mL concentration for the 24 and 48 h applications. Dicapthion also induced abnormal cell frequency, CA/cell ratio and frequency of MN dose dependently for 24 and 48 h. Dicapthion showed a statistically significant cytotoxic effect by decreasing the mitotic index in all concentrations and a cytostatic effect by decreasing nuclear division index in 100 and 200 µg/mL concentrations for both treatment periods when compared with both

untreated and solvent controls. These values decreased also in a dose dependent manner.

Keywords Ames · Sister chromatid exchanges · Chromosome aberrations · Micronucleus · Mitotic index

Introduction

Organophosphorus pesticides (OPs), strong inhibitors of cholinesterase enzymes, were developed to replace organohalide pesticides in the late 1950's because OPs are relatively easier to degrade via microbial or environmental processes (Obare et al. 2010). About 70 % of the insecticides in current use in the US are OPs (US EPA 2010). However, these chemicals or their derivatives can accumulate in the organisms and cause risk of mutagenicity, carcinogenicity, teratogenicity and immunotoxicity. Dicapthion is an OP compound which inhibits cholinesterase, and is also known as; Isomeric Chlorthion, Isochlorthion, Dicaptan(R), Dicapthion, O,O-Dimethyl-O-(2-Chloro-4-Nitro-phenyl) Phosphorothioate, O-(2-Chloro-4-Nitrophenyl)-O,O-Dimethyl Phosphorothioate. It is used as insecticide and miticide. It was introduced over 40 years ago, but its use has not become very expensive and it is described only in a few publications. Acute and dermal LD₅₀ values of dicapthion for male and female rats were found 400, 790 and 330,

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125 mg/kg, respectively (Auld Hon and Biggs 1972). Observed toxicity of dicaphton to *Chlorella vulgaris* was found 1.36 pC (Lv and Zhang 2010). 2-Chloro-4-nitrophenol is the active ingredient of dicaphton and it is highly toxic to human beings (Muangsiri and Werawatganone 2006; Arora and Jain 2011). It was also shown that it inhibits P-glycoprotein activity (Bain and LeBlanc 1996).

To our knowledge, there is no report on the genotoxicity and mutagenicity of dicaphton except in the present paper. On the other hand, some of the OPs had not a mutagenic effect, i.e. chlorpyrifos (Gollapudi et al. 1995), baygon, mobile, mortein and total (Akintonwa et al. 2008), and some of the them had a mutagenic effect in the Ames test, i.e. acephate (Carver et al. 1985), chloracetophone (Kappas et al. 1990), miral (Sierra-Tores et al. 1998), methamidophos (Karabay and Oguz 2005), dicrotophos (only 5,000 µg/plate) (Wu et al. 2010). Some of the OPs were also found genotoxic in human lymphocytes with different test systems i.e. chlorpyrifos (Sandal and Yilmaz 2011), afugan (Yüzbaşıoğlu et al. 2006), acephate (Özkan et al. 2009), dimethoate and methyl parathion (Undeğer and Başaran 2002) and profenofos (Prabhavathy et al. 2006).

The objective of this study was to investigate both the mutagenic and genotoxic effects of dicaphton by the bacterial reverse mutation assay in *S. typhimurium* TA 97, TA98, TA100 and TA102 strains with or without S9 mix and CAs, SCEs, and MN tests in human peripheral lymphocytes in vitro, respectively.

Materials

Organisms

The LT-2 TA97, TA98, TA100 and TA102 histidine-demanding auxotrophs of *S. typhimurium* were kindly obtained from Prof N Diril, Hacettepe University, Ankara Turkey. TA97 and TA98 were used for determining the frame shift, TA100 was used to determine the base pair exchange, and TA102 was used for determining oxidative damage and Ochre type of mutations.

Chemicals

Dicaphton (CAS No. 2463-84-5, purity 99.0 %) was purchased from Chemservice (Bodrum, Turkey). Some

chemical properties of the dicaphton are given in Table 1. S9 from Liver from rat (Sprague–Dawley), bacto agar, nutrient broth no: 2 oxid, 2-aminoanthracene (2AA, CAS No. 613-13-8), bromodeoxyuridine (BrdUrd, CAS No. 59-14-3), colchicine (CAS No. 64-86-8) were purchased from Sigma Aldrich (St. Louis, MO, USA) and mitomycin C (MMC, CAS No. 5-7-7) was purchased from Calbiochem (Merck KGaA, Darmstadt, Germany). 4-Nitro-o-phenylendiamine (NPD, CAS No. 99-56-9), 2-aminofluorene (2AF, CAS No. 153-78-6), L-histidin HCl, D-biotin, ampicillin trihydrate, D-glucose 6-phosphate and β-nicotinamide adenine dinucleotide phosphate were purchased from Fluka. Citric acid monohydrate, sodium hydroxide, sodium azide (SA, CAS No. 26628-22-8), potassium chloride, sodium chloride and DMSO were purchased from Riedel. Chromosome medium B was obtained from Biochrom (Merck KGaA).

Lymphocyte cultures

Peripheral venous blood was obtained from four healthy donors (two male and two female, nonsmokers, age: 22–23 years) not exposed to known genotoxicants. The same subjects were used for all performed assays. This study was performed according to the IPCS guidelines (Albertini et al. 2000).

Methods

Ames plate incorporation test

Preparation of the stocks of *S. typhimurium* TA97, TA98, TA100 and TA102 strains, the histidine requirement, presence of the *rfa* and *uvrB* mutations, and R-factor genetics of these strains were determined according to the method of Maron and Ames (1983). The stocks were kept at −80 °C. Cytotoxic doses of dicaphton (10,000, 1,000, 100, 10, 1 and 0.1 µg/plate) were determined by the method of Dean et al. (1985).

The Ames test was performed as a standard plate incorporation assay with *S. typhimurium* strains TA97, TA98, TA100 and TA102 with or without S9 mix (Maron and Ames 1983). Selection of the strains was based on the testing and strain selection strategies of Mortelmans and Zeiger (2000). For each tester strain, a specific positive control was always used to test the experimental flaws, if any. While NPD for TA97 and

Table 1 Some chemical properties of the dicaphton

Common name	IUPAC name	Formula	Chemical structure	Using areas
Dicaphton	(2-Chloro-4-nitro-phenoxy)-dimethoxy-sulfanylidene-phosphorane	C ₈ H ₉ ClNO ₅ PS		Insecticide

TA 98, SA for TA100, MMC for TA102 was used without metabolic activation, 2AF for TA97 and TA 98, 2AA for TA100 and TA 102 was used with metabolic activation as positive controls.

500 µl of S9 mix (or 500 µl phosphate buffer), 100 µl of the test solution for each concentration and 100 µl of a cell suspension from an overnight culture ($1\text{--}2 \times 10^9$ cells/mL) were added to 2 mL top agar (kept at 45 °C) and vortexed for 3 s. The entire mixture was overlaid on the minimal agar plate. The plates were incubated at 37 °C for 72 h and then the revertant bacterial colonies on each plate were counted. Positive controls and solvent control (DMSO) were concurrently maintained. Samples were tested on triplicate plates in two independent parallel experiments.

The interpretation of the Ames test results for genotoxicity testing of chemicals United States Environmental Protection Agency (1996) methods were followed. According to the guideline, a mutagenic potential is assumed, if the revertant frequency is 2.0 or higher over the solvent control or a dose-related increase in the number of revertant colonies in one or more strains, and a weak mutagenic potential is assumed, if the number of revertants is below the double of the background number of colonies (Mortelmans and Zeiger 2000).

Chromosomal aberrations and sister-chromatid exchange assay

Heparinized whole-blood sample (0.2 mL) was added to 2.5 mL Chromosome Medium B supplemented with 10 µg/mL BrdUrd for CAs and SCEs and incubated in the dark at 37 °C for 72 h and treated with 25, 50, 100 and 200 µg/mL concentrations of dicaphton dissolved in DMSO for 24 h (Dicaphton was added 48 h after initiating the culture) and 48 h (Dicaphton was added 24 h after initiating the culture). An untreated control, a solvent control (DMSO, 9 µl/mL) and a positive control (MMC, 0.25 µg/mL)

were also maintained in every experiment. The cells were exposed to colchicine (0.06 µg/mL) 2 h before harvesting to arrest the cell cycle at metaphase stage. The cultured peripheral blood lymphocytes were harvested by treating with 0.075 M KCl 15 min at 37 °C and then fixed three times with freshly prepared cold methanol:glacial acetic acid (3:1 v/v) at room temperature. Slides were made by dropping of the centrifuged cells. The staining of air-dried slides for CAs and SCEs was performed following the standard methods using 5 % Giemsa stain and modified fluorescence plus Giemsa method, respectively (Speit and Haupter 1985).

One hundred well-spread metaphase showing structural and/or numerical alterations per donor were examined to obtain the required number of CAs (a total of 400 metaphase per concentration). The CA was classified according to the International System for Human Cytogenetic Nomenclature (Mitelman 1995). Gaps were not considered as CA according to Mace et al. (1978). MI was also determined by scoring 3,000 cells from each donor. The scoring of SCE was performed according to the IPCS guidelines (Albertini et al. 2000). For the observation of SCEs per cell, twenty-five second division metaphases were scored for each concentration (totally 100 s division metaphases per concentration) to calculate the mean number of SCE (SCE/cell). In order to determine replication index (RI) or proliferation index, a total of 400 cells (100 cells from each donor) were scored and RI is calculated as follows: $RI = (M1 \% \times 1) + (M2 \% \times 2) + (M3 \% \times 3)/N$. M1, M2, and M3 represent the number of cells undergoing first, second and third mitosis, respectively, and N is the total number of viable cells scored.

Micronucleus assay

Whole blood cultures were prepared as described for the SCE and CA assay except for 5-bomodeoxyuridine. Peripheral lymphocytes were incubated at 37 °C for

68 h. Cytochalasin B (6 µg/mL) was added to each culture 44 h after starting cultures, in order to block cytokinesis and obtain binucleated cells. The same concentrations of dicapthon were used in SCE and CA for 24 and 48 h. Cells were harvested by centrifugation and processed for MN test in peripheral lymphocytes (Fenech 2000; Kirsch-Volders et al. 2003). 8,000 binucleated lymphocytes were scored from each donor per concentration. The effect of dicapthon on cell proliferation was evaluated using the NDI. A total of 8,000 cells (2,000 cells from each donor) was scored and NDI is calculated using the formula: $NDI = (M1) + (2 \times M2) + (3 \times M3) + (4 \times M4)/N$; where M1–M4 represent the number of cells with 1–4 nuclei and N is the total number of viable cells scored (Fenech 2000).

Statistical analysis

Data are presented as mean $\pm$ SD. The test results were analyzed statistically by using the SPSS 15.0 version for Windows software; Mann–Whitney test was performed for Ames test. The statistical significance was evaluated with the Student's *t* test for the percentage of CA, SCE, MN, RI, MI, and NDI. Dose response relationships were determined by using the correlation analysis.

Results

The results from the Ames test are shown in Table 2. Non-cytotoxic doses of the dicapthon were determined firstly. According to the results obtained, over 100 µg/plate of dicapthon was determined as cytotoxic. Therefore, the doses below these values were employed in the tests. The average revertant colony numbers in solvent control were 87.5 ± 4.9 for TA97, 32.33 ± 6.5 for TA98, 106.5 ± 4.8 for TA100 and 313 ± 25.91 for TA102 in the absence of S9 mix and 115.33 ± 9.9 , 24.5 ± 2.7 , 156.5 ± 13.4 and 342 ± 9.4 in the presence of S9 mix, respectively. Spontaneous revertants were within the normal values for the four strains examined. Some concentrations of dicapthon lowered the spontaneous reversion slightly. On the contrary, the plates with the positive control mutagens (SA, 2AF, NPD, 2AA, and MMC) showed significant increases relative to the spontaneous mutation rate in the four tested strains. While the

highest value observed was in TA102 without S9 mix at 100 µg/plate concentration of dicapthon (375.16 ± 17.5), and the lowest value was in TA98 with S9 mix at 100 µg/plate concentration of dicapthon (20.66 ± 3.3). Most of the results were increasing or decreasing relative to the solvent control group, statistically significant at $p < 0.05$ (Mann–Whitney test) in examined strains. While the application of S9 mix in TA98 and TA102 led to decreased revertant colony numbers, the application of S9 mix in TA97 and TA100 led to increased revertant colony numbers. After applying 5 different concentrations of dicapthon, there were no induced revertants along the dose range tested in either with or without S9 mix with the four strains tested. The results of the present study showed that 0.1, 1, 10 and 100 µg/plate doses of dicapthon were found to be weak mutagenic *S. typhimurium* TA98 without S9 mix.

Table 3 shows the effect of dicapthon on SCE and RI in human lymphocyte cells. Dicapthon increased the frequency of SCE only at the 100 µg/mL concentration at 24 and 48 h. Decreased values for 24 h were found to be statistically significant when compared with the untreated, solvent and positive controls. The highest increase in SCE frequency was observed after treatment with the positive control (55.3 ± 17.62) for 48 h. Not only the frequency of SCEs, but also minimum–maximum numbers of SCEs did not change with increasing dicapthon concentration. Dicapthon decreased the RI significantly at all concentrations for 24 h, especially at the 100 and 200 µg/mL concentration, and significantly increased the RI at all concentrations for 48 h except for the 200 µg/mL concentration. Dicapthon increased abnormal cell frequency and CA/cell ratio in a dose dependent manner for 24 h ($r = 0.944$ $p < 0.01$ and $r = 0.908$ $p < 0.01$, respectively) and 48 h ($r = 0.938$ $p < 0.01$ and $r = 0.875$ $p < 0.01$, respectively). This increase was statistically significant at the 100 and 200 µg/mL concentrations for 24 and 48 h when compared with untreated and solvent controls (Table 4). Six types of structural aberrations (chromatid and chromosome breaks, chromatid exchanges, fragments, sister chromatid union and dicentric chromosome) and only one type of numerical aberration (polyploidy) were observed. Chromatid breaks and fragments were observed to be the most common abnormalities. The aberrations were significantly lower than those of the positive control. The results show that all experimental

Table 2 Mutagenicity analysis of dicapthon using *S. typhimurium* assay with TA97, TA98, TA100 and TA102 strains with or without S9 mix

Test materials	Doses (µg/plate)	Arithmetic mean $\pm$ SD*					
		TA97		TA98		TA100	
		S9(–)	S9(+)	S9(–)	S9(+)	S9(–)	S9(+)
Dicapthon	100	85.66 $\pm$ 6.5	134.66 $\pm$ 10.1*	48.33 $\pm$ 7.5**	20.66 $\pm$ 3.3	123.33 $\pm$ 8.1*	130.66 $\pm$ 9*
	10	92.66 $\pm$ 5.3*	112.83 $\pm$ 5.3	45 $\pm$ 7.4**	25 $\pm$ 4.7	104.5 $\pm$ 7.6*	141.66 $\pm$ 3.7*
	1	89.83 $\pm$ 6.3*	109 $\pm$ 5.6*	48.66 $\pm$ 5.4**	27.66 $\pm$ 4.2*	107.16 $\pm$ 11.5	136.16 $\pm$ 4.1*
	0.1	83.16 $\pm$ 4.1*	124 $\pm$ 6.9*	46.5 $\pm$ 5.7**	21.5 $\pm$ 2.8*	100 $\pm$ 5.2*	148.5 $\pm$ 7.5*
	0.01	99.83 $\pm$ 14*	116.33 $\pm$ 12.7	34.66 $\pm$ 4*	21.66 $\pm$ 2.8*	97.5 $\pm$ 9.2*	125.16 $\pm$ 7.6*
Control		91.66 $\pm$ 5.7*	124.66 $\pm$ 5.9*	32.16 $\pm$ 3.1	29.5 $\pm$ 1.5*	118.66 $\pm$ 12.8*	149.33 $\pm$ 6.2
DMSO		87.5 $\pm$ 4.9	115.33 $\pm$ 9.9	32.33 $\pm$ 6.5	24.5 $\pm$ 2.7	106.5 $\pm$ 4.8	156.5 $\pm$ 13.4
SA	10					2,743 $\pm$ 202.8*	
2AF	200		1,365.33 $\pm$ 100.8*		971.16 $\pm$ 32.2*		
NPD	200	1,193.33 $\pm$ 99.5*		1,495 $\pm$ 178.4*			
2AA	5						1,986 $\pm$ 210.5*
MMC	0.5						2,207 $\pm$ 197*

m weak mutagen, *SA* sodium azide, *2AF* 2-aminofluorene, *NPD* 4-nitro-o-phenylenediamine, *2AA* 2-aminoanthracene, *MMC* mitomycin-C

* Statistically significant at $p < 0.05$ (Mann–Whitney test)

Table 3 The effects of dicaphton on SCE frequency and RI in human peripheral blood lymphocytes

Test substance	Treatment		M1	M2	M3	Mean RI $\pm$ SD	Min–Max SCE	Mean SCE/Cell $\pm$ SD
	Time (h)	Doses ($\mu\text{g/mL}$)						
Control	24	–	166	118	116	$1.87 \pm 0.06c_3$	0–7	$4.13 \pm 0.08c_3$
DMSO		9 μl	223	83	94	$1.67 \pm 0.12a_1c_3$	2–8	$4.49 \pm 0.44c_3$
MMC		0.25	282	55	63	$1.44 \pm 0.04a_3b_1$	8–70	$35.3 \pm 13.48a_3b_3$
Dicaphton		25	261	54	85	$1.56 \pm 0.06a_3c_1$	0–8	$3.05 \pm 0.11a_3b_3c_3$
		50	246	81	73	$1.57 \pm 0.08a_3c_1$	0–8	$3.46 \pm 0.22a_3b_2c_3$
		100	303	42	55	$1.38 \pm 0.07a_3b_2$	3–7	$4.99 \pm 0.32a_2c_3$
		200	314	32	54	$1.35 \pm 0.01a_3b_2c_2$	0–5	$2.5 \pm 0.17a_3b_3c_3$
Control	48	–	262	57	81	$1.54 \pm 0.08c_1$	0–7	$2.85 \pm 0.57c_3$
DMSO		9 μl	279	68	53	1.43 ± 0.05	0–8	$2.99 \pm 0.03c_3$
MMC		0.25	298	60	42	$1.36 \pm 0.07a_1$	30–110	$55.3 \pm 17.62a_3b_3$
Dicaphton		25	249	63	88	$1.59 \pm 0.05b_2c_2$	0–10	$2.73 \pm 0.24c_3$
		50	217	55	128	$1.77 \pm 0.06a_2b_3c_3$	0–7	$2.71 \pm 0.26c_3$
		100	231	63	106	$1.68 \pm 0.05a_1b_3c_3$	0–6	$3.33 \pm 0.23c_3$
		200	287	53	60	1.43 ± 0.11	0–4	$2.43 \pm 0.17b_1c_3$

SD standard deviation, M1 first mitotic division, M2 second mitotic division, M3 third mitotic division

a: significant from untreated control, b: significant from solvent control, c: significant from positive control. 1: $p \leq 0.05$, 2: $p \leq 0.01$, 3: $p \leq 0.001$

doses of dicaphton significantly decreased MI compared to the untreated and solvent controls at each exposure time (Table 4). The MI decreased in a dose dependent manner at 24 h ($r = -0.929$ $p < 0.01$) and 48 h ($r = -0.987$ $p < 0.01$). The highest value was obtained for the 24 h examination at 25 $\mu\text{g/mL}$ (5.58 ± 0.31), and the lowest one in 48 h applications of 200 $\mu\text{g/mL}$ (3.42 ± 0.16) concentrations of dicaphton. The results of MN test are given in Table 5. Dicaphton significantly increased the frequency of MN in human lymphocytes at all concentrations except 25 $\mu\text{g/mL}$ for the 24 h application when compared to both untreated and solvent controls. This increase was dose dependent for 24 h ($r = 0.958$ $p < 0.01$) and 48 h ($r = 0.894$ $p < 0.01$). Dicaphton significantly decreased the NDI at the two highest concentrations (100 and 200 $\mu\text{g/mL}$) for the 24 h application, and also significantly decreased at all concentrations except 25 $\mu\text{g/mL}$ for the 48 h application when compared to the untreated and solvent controls. NDI was lower than for the positive control for the 24 h application at 200 $\mu\text{g/mL}$ and for the 48 h application at 100 and 200 $\mu\text{g/mL}$. This decrease was dose dependent for the 24 h ($r = -0.956$ $p < 0.01$) and 48 h applications ($r = -0.964$ $p < 0.01$).

Discussion

There is no sufficient evidence in relation to dicaphton genotoxicity or mutagenicity and toxicity. This study is the first report on detecting the genotoxic and/or mutagenic effects of dicaphton using Ames test in *S. typhimurium* strains, and SCE, CAs and MN tests in human lymphocytes in vitro.

Ames test, or so-called Salmonella/microsome test, is not only one of the most reliable short-term bacterial test systems but it is also cheap and gives results very rapidly. It has been widely used through out the world to detect mutagenic activity of pesticides via the detection of various types of gene mutations such as base-pair substitutions (TA100 and TA1535), frame-shift mutations (TA98 and TA97) and transition or transversion events (TA102) (Konuk et al. 2008; Liman et al. 2010, 2011). The results of the Ames test showed that 0.1, 1, 10 and 100 $\mu\text{g/plate}$ doses of dicaphton were found to be weakly mutagenic in *S. typhimurium* TA98 without S9 mix. Revertant colony numbers in TA102 without S9 mix were also increased by all doses of dicaphton when compared to the negative control but it was not found mutagenic. Dicaphton may induce frame shift mutations because

Table 4 Total chromosomal aberrations and MI in cultured human lymphocytes treated with dicaphon

Test substance	Treatment		Structural aberrations						NA	Abnormal cell $\pm$ SD (%)		CA/cell $\pm$ SD	MI $\pm$ SD
	Time (h)	Doses (μ g/mL)	ctb	cte	csb	f	scu	dc					
Control	24	–	2	1	1	2	2		1	5.00 $\pm$ 2c ₃		0.06 $\pm$ 0.01b ₁ c ₃	7.46 $\pm$ 0.21b ₁ c ₃
DMSO		9 μ l	3		2	4				8.00 $\pm$ 2c ₃		0.09 $\pm$ 0.01a ₁ c ₃	6.97 $\pm$ 0.2a ₁ c ₃
MMC		0.25	25	2	8	13	2	2	2	28.33 $\pm$ 1.52a ₃ b ₃		0.54 $\pm$ 0.03a ₃ b ₃	3.48 $\pm$ 0.35a ₃ b ₃
Dicaphon		25	4		2	2				7.00 $\pm$ 1c ₃		0.08 $\pm$ 0.02c ₃	5.58 $\pm$ 0.31a ₃ b ₃ c ₃
		50	5		1	3		2		10.33 $\pm$ 1.15a ₂ c ₃		0.11 $\pm$ 0.02a ₁ c ₃	5.43 $\pm$ 0.41a ₃ b ₃ c ₃
		100	5		1	7	1			13.67 $\pm$ 0.57a ₂ b ₂ c ₃		0.14 $\pm$ 0.02a ₂ b ₁ c ₃	4.7 $\pm$ 0.32a ₃ b ₃ c ₂
		200	9		3	6				18.00 $\pm$ 2a ₃ b ₂ c ₂		0.18 $\pm$ 0.01a ₃ b ₃ c ₃	3.51 $\pm$ 0.25a ₃ b ₃
Control	48	–	2		2	1	1			3.66 $\pm$ 1.15b ₁ c ₃		0.06 $\pm$ 0.02c ₃	7.18 $\pm$ 0.1b ₃ c ₃
DMSO		9 μ l	5		2	1		1		6.66 $\pm$ 1.15a ₁ c ₃		0.09 $\pm$ 0.02c ₃	6.5 $\pm$ 0.2a ₃ c ₃
MMC		0.25	35	5	9	9	1		3	36 $\pm$ 2a ₃ b ₃		0.62 $\pm$ 0.02a ₃ b ₃	3.21 $\pm$ 0.4a ₃ b ₃
Dicaphon		25	5			3				7.33 $\pm$ 0.57a ₂ c ₃		0.08 $\pm$ 0.01c ₃	5.54 $\pm$ 0.11a ₃ b ₃ c ₃
		50	5		1	3				8 $\pm$ 1a ₂ c ₃		0.09 $\pm$ 0.02c ₃	5.32 $\pm$ 0.13a ₃ b ₃ c ₃
		100	5		1	5		1	1	11.33 $\pm$ 1.15a ₃ b ₂ c ₃		0.13 $\pm$ 0.01a ₂ b ₁ c ₃	4.64 $\pm$ 0.19a ₃ b ₃ c ₃
		200	4		2	6		2	2	13.66 $\pm$ 0.57a ₃ b ₃ c ₃		0.16 $\pm$ 0.03a ₂ b ₁ c ₃	3.42 $\pm$ 0.16a ₃ b ₃

NA numerical aberrations, *ctb* chromatid break, *cte* chromatid exchange, *csb* chromosome break, *f* fragment, *scu* sister chromatid union, *dc* dicentric chromosome, *p* polyploidy, *a*: significant from untreated control, *b*: significant from solvent control, *c*: significant from positive control 1: $p \leq 0.05$, 2: $p \leq 0.01$, 3: $p \leq 0.001$, *SD* standard deviation

Table 5 The effects of dicaphton on MN and NDI in human peripheral blood lymphocyte cultures

Test substance	Treatment	Distribution of binuclear cells according to the number of MN				Distribution of cells according to the number of nuclei				NDI $\pm$ SD
		Time (h)	Doses (μ g/mL)	0	1	2	3	4	>4	BN (%)
Control	24	–	–	7,989	11					
DMSO	9 μ l			7,984	16					
MMC	0.25			7,771	218	10	1	1		
Dicaphton	25			7,979	21					
	50			7,976	19	5				
	100			7,920	80					
	200			7,888	108	4				
Control	48	–	–	7,987	13					
DMSO	9 μ l			7,979	21					
MMC	0.25			7,357	565	70	7	1		
Dicaphton	25			7,960	38	2				
	50			7,932	65	2	1			
	100			7,886	110	4				
	200			7,868	130	2				

SD standard deviation

a: significant from untreated control, b: significant from solvent control, c: significant from positive control. 1: $p \leq 0.05$, 2: $p \leq 0.01$, 3: $p \leq 0.001$

TA97 and TA98 are used for determining these mutations. Revertant colony numbers in TA97 and TA100 increased when S9 mix was added. These result suggested that dicapthion's metabolites appeared after metabolic reactions in the living body, enhanced DNA interactions. In order to establish a dose–response relationship, 5 different concentrations of dicapthion were tested, and no induced revertants were observed along the dose range tested in either with or without S9 mix with four strains. Some of the OPs also showed a mutagenic effect in Ames test (Carver et al. 1985; Kappas et al. 1990; Sierra-Tores et al. 1998; Karabay and Oguz 2005; Wu et al. 2010).

Since CAs are characterized by changes in either chromosomal structure or in the total number of chromosomes (Russel 2002), SCEs represent recombinogenic events arising at DNA lesions (Conrad et al. 2011), and MN are expressed in cells when either acentric chromosome fragments of whole chromosomes fail to be segregated to the daughter nuclei during mitosis (Fenech and Bonassi 2011), they are one of the best-established cytogenetic methods for the determination of the genotoxic effects of pesticides in human peripheral blood lymphocytes (Yılmaz et al. 2008; Ennaceur et al. 2008; Kocaman and Topaktaş 2010; Ünal et al. 2011). Dicapthion did not induce SCEs in human peripheral blood lymphocytes except 100 µg/mL concentration. Inhibition of RI in the 24 h application at 100 and 200 µg/mL concentrations indicates that cell proliferation was inhibited by dicapthion, resulting in a 17.36 and 19.16 % reduction of proliferation, respectively. Dicapthion significantly increased the CAs at the 100 and 200 µg/mL concentrations in 24 and 48 h applications when compared with the untreated and solvent controls. There was a dose dependent effect on induction of structural CA but not a numerical effect. Dicapthion induced six types of structural aberrations. The incidence of CAs was found as chromatid break > fragment > chromosome break > dicentric chromosome > polyploidy. Chromatid breaks could be due to dicapthion acting mostly in the late S or G₂ phase of the cell cycle (Natarajan 2002). Aberrations induced by dicapthion may be the results of clastogenic action of the chemical by breaking the phosphodiester backbone of DNA. Some studies concerning malathion (Balaji and Sasikala 1993), methyl parathion (Kumar et al. 1993), baytex-1,000 (Yadav and Kaushik 2002), atrazine (Kligerman et al. 2000), cypermethrin

(Undeğer and Başaran 2005), afugan (Yüzbaşıoğlu et al. 2006), profenofos (Prabhavathy et al. 2006), acephate (Özkan et al. 2009) and thiacloprid (Kocaman et al. 2012) showed that pesticides may have genotoxic potential in human lymphocytes. Dicapthion significantly decreased MI in a dose dependent manner. Decreasing of the MI could be due to decreasing ATP level and the pressure from the functioning of the energy production center (Epel 1963; Jain and Andsorbhoy 1988), blocking of G₂ preventing the cell from entering mitosis (Van't Hof 1968), inhibition of certain cell cycle specific enzymes such as DNA polymerase, which is necessary for the synthesis of DNA precursors as well as other enzymes more directly involved with spindle production, assembly or orientation (Hidalgo et al. 1989). The clastogenic response of various compounds is often associated with high toxicity (Müller and Sofuni 2000). A decrease in mitotic index has also been reported for many other pesticides such as etoxazole (Rencüzoğulları et al. 2004), afugan (Yüzbaşıoğlu et al. 2006), conan 5FL (Yılmaz et al. 2008), cyfluthrin (İla et al. 2008), dichlorvos (Eroğlu 2009), acephate (Özkan et al. 2009) and thiacloprid (Timoroğlu et al. 2012) in cultured human lymphocytes. Dicapthion significantly increased micronuclei frequency at all concentrations (except at the 25 µg/mL concentration in the 24 h application) in a dose dependent manner compared to untreated and solvent controls. Micronuclei are formed from acentric chromosomal fragments which arise as a result of chromosome breaks after clastogenic effect or whole chromosomes that do not migrate during anaphase as a result of aneugenic affects (Mace et al. 1978; Fenech 2007; Heddle et al. 1991). Dicapthion may induce MN because of its clastogenic effects. Various pesticides such as malathion (Giri et al. 2002), atrazine (Kligerman et al. 2000), cypermethrin (Undeğer and Başaran 2005), afugan (Yüzbaşıoğlu et al. 2006), hexachlorobenzene (Ennaceur et al. 2008), cyfluthrin (İla et al. 2008), dichlorvos (Eroğlu, 2009), acephate (Özkan et al. 2009) and thiacloprid (Timoroğlu et al. 2012) have also caused the formation of micronuclei in different test systems. The reduction in the NDI observed for lymphocytes cultured with 100 and 200 µg/mL indicates that the dicapthion, at high concentrations, has a cytostatic effect via inhibition of cell cycle progression. Dicapthion's cytostatic effect was higher than that of MMC at 200 µg/mL for the 24 h and 100 and

200 µg/mL for the 48 h applications. According to Eke and Çelik (2008), the results of the CA and MN assays should be considered of higher significance than the results of an SCE test, because the SCE test is generally used for genotoxic exposure and the biological significance of an increase in SCE in the processes of mutagenesis and carcinogenesis is not clear but CA and MN assay are used for the detection of chromosome mutations.

As a result, dicapthon was found to be weakly mutagenic in the Ames test. It has a genotoxic effect by increasing CAs and MN formation and it also has a cytotoxic/cytostatic effect by decreasing the MI and NDI in human peripheral blood lymphocytes. This insecticide can cause harm to humans and the environment when not used properly. Further studies should be conducted especially under in vivo test systems to better understand its potential mutagenicity and genotoxicity.

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