Mutagenesis vol. 20 no. 2 pp. 101–104, 2004
Advance Access publication 8 March 2005
doi:10.1093/mutage/gei013

In vitro effect of karathane LC (dinocap) on human lymphocytes

Mustafa Çelik[1], Fatma Ünal[2,*], Deniz Yüzbaşıoğlu[2], Mehmet Ali Ergün[3], Orhan Arslan[4] and Reşat Kasap[5]

[1]Sütçü İmam Üniversitesi, Fen-Edebiyat Fakültesi, Biyoloji Bölümü, Kahramanmaraş-Turkey, [2]Gazi Üniversitesi, Fen-Edebiyat Fakültesi, Biyoloji Bölümü, Teknikokullar, 06500 Ankara-Turkey, [3]Gazi Üniversitesi, Tıp Fakültesi, Tibbi Genetik Bölümü, Ankara-Turkey, [4]Gazi Üniversitesi, Gazi Eğitim Fakültesi, Biyoloji Eğitimi Bölümü, 06500 Ankara-Turkey and [5]Gazi Üniversitesi, Fen-Edebiyat Fakültesi, İstatistik Bölümü, 06500 Ankara-Turkey

Karathane LC (active ingredient dinocap), a contact fungicide and a non-systemic acaricide was investigated for its ability to induce chromosome aberrations (CAs) and sister chromatid exchanges (SCEs) in cultured human lymphocytes of peripheral blood. In addition to the cytogenetic analysis, the effect of karathane LC on the cell proliferation kinetics (CPK) by the replication index (RI) was studied. The mitotic index (MI) was also determined to detect the cytotoxic effect. Lymphocytes were treated with four different concentrations (5, 10, 15 and 20 μg/ml) of karathane LC for 24 and 48 h. Significant differences between exposed and non-exposed groups found in CAs, SCEs and MI demonstrate the mutagenic, clastogenic and also the cytotoxic effect of karathane LC.

Introduction

Despite the valuable contributions associated with the use of pesticides, many of these biologically active chemicals represent a potential hazard to humans and to nature. Many of these chemicals are carcinogenic (1–3) and mutagenic, inducing gene mutations, chromosomal alterations and DNA damage (4,5). In epidemiological studies, a significant increase was found in the risk of leukemia and multiple myeloma and also stomach, liver, pancreatic and bladder cancer associated with pesticide exposure (3,6–8). Owing to increasing evidence of carcinogenic, mutagenic and teratogenic effects in exposed humans and experimental animals, interest in pesticide toxicity is increasing every day.

A number of *in vitro* and *in vivo* test systems have been developed to study the effects of chemicals on cellular DNA and chromosomes. The analysis of chromosomal aberrations (CAs) and sister chromatid exchanges (SCEs) in peripheral blood lymphocytes are the widely used biomarkers of genotoxic, carcinogenic and/or mutagenic effects (9–12). CAs can trigger the development of cancer (13–15) and an increased frequency of SCEs could be an indicator of persistent DNA damage (11,16).

In this study, cultured human peripheral lymphocytes were used for the evaluation of genotoxic and mutagenic activity of the commercial fungicide karathane LC with the active ingredient dinocap. Dinocap is a contact fungicide and a non-systemic acaricide. It is registered for use on powdery mildews in pome fruit, stone fruit, citrus fruit, soft fruit, vines, cucurbits, ornamentals, tobacco, hops and some vegetables. It is also used in the control of mites (*Panonycus*, *Tetranycus* and *Aculus* sp.) in fruit trees and vines. EPA has classified dinocap as a Group E 'not likely' carcinogen (17). The results on the genotoxicity of dinocap covering a large number of systems and organisms *in vivo* and *in vitro* have taken into account several different genetic endpoints, including DNA damage, gene mutations and chromosomal damage, and are negative (18). However, contrasting results have been reported using the Ames test and the Allium test, wherein positive results were obtained by Moriya *et al*. (19) and Çelik (20). The present study was undertaken to examine the ability of karathane LC (containing 475 g/l dinocap) to induce CAs and SCEs in cultured human peripheral lymphocytes. Changes in cell proliferation kinetics (CPK) by the replication index (RI) and cytotoxic effect by the mitotic index (MI) were also evaluated.

Materials and methods

Human peripheral blood cells were used as the test system, and the commercial fungicide karathane LC as the test substance. The chemical structure of dinocap, the active component of karathane LC, is shown in Figure 1.

Peripheral blood was obtained from healthy non-smoking male (aged 25 years) and female (aged 25 years) donors, free of any known exposure to genotoxic agents. A whole blood sample of 0.2 ml was added to 2.5 ml Chromosome Medium B (Biochrom) supplemented with 10 μg/ml bromodeoxyuridine. The cultures were incubated at 37°C for 72 h. The cells were treated with 5, 10, 15 and 20 μg/ml concentrations of karathane LC (obtained from Agro-San, Turkey) on the basis of active ingredient dinocap for 24 and 48 h. In addition, a negative and a positive control (mitomycin-C (MMC), 0.10 μg/ml) were also used for each experiment to ensure the validity of the assay. The test substance karathane LC and positive control MMC were dissolved in distilled water. Colchicine (0.06 μg/ml) was added during the last 2 h of culture. To collect the cells, the cultures were centrifuged (1200 r.p.m., 10 min) and treated with hypotonic solution (0.075 M KCl) for 30 min at 37°C. Then the cells were fixed in cold methanol:acetic acid (3:1) for 20 min at room temperature. The treatment with the fixative was repeated three times. The cells were spread on glass slides and air dried.

For chromosomal aberrations, 1-day-old slides were stained with 5% Giemsa (pH 6.8) prepared in Sorensen buffer solution, for 15–20 min, washed in distilled water, dried at room temperature and mounted with depex. For the SCEs, slides were stained with Giemsa according to the Speit and Houpter's method (21) with some modifications.

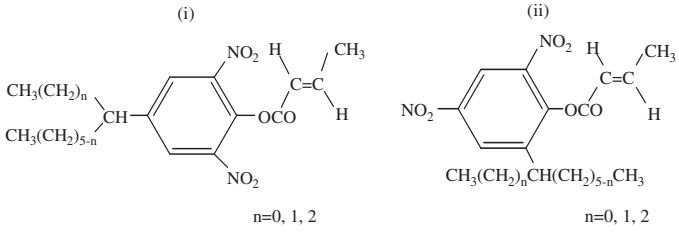


Fig. 1. Chemical structure of dinocap, the active component of karathane LC. Compounds of type (i) are more effective as a fungicide, and those of type (ii) as an acaricide.

*To whom correspondence should be addressed. Tel: +90 312 212 60 30/3061; Fax: +90 312 212 22 79; Email: funal@gazi.edu.tr

© The Author 2005. Published by Oxford University Press on behalf of the UK Environmental Mutagen Society.
All rights reserved. For permissions, please email: journals.permissions@oupjournals.org

Downloaded from https://academic.oup.com/mutage/article/20/2/101/1081754 by Gazi University user on 10 December 2020

A total of 200 well spread metaphases/concentration (100 metaphase/donor) were analysed for the CA assays. Numerical and structural chromosomal abnormalities within each metaphase were recorded. A total of 50 second mitosis was analysed in each treatment for the SCE assays. The results were recorded as the frequency of SCEs/metaphase. The MI was determined by scoring 1000 cells from each donor.

In the SCE study, a total of 200 cells (100 cells from each donor) were also scored to obtain the Proliferative Rate Index (PRI), calculated according to the formula M1 + 2M2 + 3M3/N, where M1, M2 and M3 represent the number of cells undergoing first, second and third mitosis and N the number of observed cells (22,23).

The significance between the percentage of abnormal cells, CA/cell, PRI and MI in treated cultures and their controls were determined using the *z*-test. The significance between mean SCE in treated cultures and their controls were determined using the *t*-test. Regression analysis was carried out to find out the dose–response relationship.

Results

Analysis of the cultured human lymphocytes revealed that karathane LC induced various types of chromosomal aberrations compared with the control (Table I). The data obtained from two donors are equivalent and are pooled together. Chromatid breaks, fragments, sister chromatid union (except 5 μg/ml), dicentric chromosomes and gaps were observed with all concentrations of karathane LC at both treatment times. Ring chromosomes, chromatid exchanges and polyploidies were also observed at some treatments. In addition, contraction and morphological defect of chromosomes were also determined. Concentrations of 10, 15 and 20 μg/ml of karathane LC induced a significant amount of abnormal cells (%) over negative control at both 24 and 48 h when gaps were included. Concentrations of 5 μg/ml also induced higher aberrations at both the 24- and 48-h durations; however, they are not significant over the control. When gaps were excluded, 15 and 20 μg/ml concentrations at 24 h and 10, 15 and 20 μg/ml concentrations at 48 h showed significant increase in the percentage of abnormal cells. Similarly, chromosomal aberrations including gaps/cell are significantly increased at 10, 15 and 20 μg/ml concentrations over the negative control at both 24- and 48-h treatments. CA/cell values without a gap at 10, 15 and 20 μg/ml concentrations were also significantly different from the negative control at both 24- and 48-h periods. The regression analyses revealed that there is a significant correlation between the dose and the percentage of abnormal cells at 24 h ($r = 0.98$, +G/−G) and 48 h ($r = 0.97$, +G and $r = 0.99$, −G). The regression analyses between the concentration and CA/cell was also correlated significantly at 24 h ($r = 0.99$, +G and $r = 0.98$, −G) and 48 h ($r = 0.99$, +G/−G). The 48-h treatment period induced more abnormalities and higher frequency of CA/cell than the 24-h treatment in all concentrations. The potency of karathane LC for the induction of abnormal cells (%) and CA/cell (+G/−G) was lower than those of the positive control, MMC, at both the 24-and 48-h treatments.

It is apparent from Table II that karathane LC also induced SCEs. SCE frequency was significantly higher than in the negative control with all concentrations at 48 h, but only with 20 μg/ml at the 24-h treatment. The 48-h period induced more SCE/cell than the 24-h treatment at all concentrations. Minimum and maximum numbers of SCEs are also given in Table II. The increase of SCEs was dose-dependent at both the 24-h ($r = 0.90$) and 48-h ($r = 0.98$) treatment periods. The potency of karathane LC for the induction of SCEs was lower than those of the positive control, MMC at both the 24- and 48-h treatments.

Karathane LC, in general, decreased the RI at both treatment periods with increasing concentrations ($r = -0.95$ at both 24 and 48 h) but this decrease was not significantly different from the negative control value (Table II). RI was lower than those of the positive control at the 20 μg/ml concentration at both the 24- and 48-h treatments. Karathane LC also decreased MI significantly in a progressive dose-related manner at both 24- ($r = -0.99$) and 48-h ($r = -0.98$) treatment groups compared with the negative control. Reduction of the MI value was more with the test chemical than with the positive control MMC at 20 μg/ml for 24 h and at 15 and 20 μg/ml concentrations for 48 h. Decrease in MI was not significant only at 5 μg/ml for the 24-h treatment.

Discussion

The results obtained in this *in vitro* study demonstrate that karathane LC induced CAs as well as SCEs in cultured

Table I. Induction of chromosomal aberrations in cultured human lymphocytes treated with karathane LC

Treatment	Dose (μg/ml)	Aberrations								Abnormal cell (%) ± SE (+G)	Abnormal cell (%) ± SE (−G)	CA/cell ± SE (+G)	CA/cell ± SE (−G)
		B	F	SU	DC	G	R	CE	P				
24 h													
Negative control		2	1	1	1	—	—	—	—	2.50 ± 1.00	2.50 ± 1.00	0.03 ± 0.01	0.03 ± 0.01
MMC	0.1	17	9	3	2	1	2	1	—	15.00 ± 2.50	14.50 ± 2.48	0.18 ± 0.03	0.17 ± 0.03
Karathane LC	5	3	1	—	1	1	1	—	—	3.50 ± 1.30	3.00 ± 1.20	0.04 ± 0.01	0.03 ± 0.01
	10	4	3	2	2	1	1	1	—	7.00 ± 1.80[a]	6.50 ± 1.74	0.07 ± 0.02[a]	0.07 ± 0.02[a]
	15	5	6	2	4	2	—	1	1	9.50 ± 2.00[b]	8.50 ± 1.97[b]	0.11 ± 0.02[b]	0.10 ± 0.02[b]
	20	7	5	4	3	3	1	—	1	11.00 ± 2.20[c]	9.50 ± 2.00[b]	0.12 ± 0.02[c]	0.11 ± 0.02[b]
48 h													
Negative control		3	2	2	—	—	—	—	—	3.50 ± 1.30	3.50 ± 1.30	0.04 ± 0.01	0.04 ± 0.01
MMC	0.1	23	12	4	2	1	1	1	1	19.50 ± 2.80	19.00 ± 2.77	0.23 ± 0.03	0.23 ± 0.03
Karathane LC	5	5	3	3	1	1	—	—	—	6.50 ± 1.74	6.00 ± 1.68	0.07 ± 0.02	0.07 ± 0.02
	10	8	4	4	2	3	—	—	—	10.00 ± 2.12[b]	8.50 ± 1.97[a]	0.11 ± 0.02[b]	0.11 ± 0.02[b]
	15	13	4	2	2	4	—	1	1	11.50 ± 2.25[b]	9.50 ± 2.00[b]	0.14 ± 0.02[c]	0.14 ± 0.02[c]
	20	14	5	5	1	3	1	2	1	13.00 ± 2.37[c]	11.50 ± 2.25[b]	0.16 ± 0.02[c]	0.16 ± 0.02[c]

B, chromatid break; F, fragment; SU, sister chromatid union; DC, dicentric; G, gap; R, ring chromosome; CE, chromatid exchange; P, polyploidy.
[a]Significantly different from the control $P < 0.05$ (*z*-test).
[b]Significantly different from the control $P < 0.01$ (*z*-test).
[c]Significantly different from the control $P < 0.001$ (*z*-test).

Downloaded from https://academic.oup.com/mutage/article/20/2/101/1081754 by Gazi University user on 10 December 2020

Table II. The frequency of the SCE, RI and the MI in cultured human lymphocytes treated with karathane LC

Treatment	Dose (μg/ml)	Minimum–maximum SCE	SCE/cell ± SE	M1	M2	M3	RI ± SE	MI ± SE
24 h								
Negative control	—	2–8	5.12 ± 0.286	44	70	86	2.21 ± 0.06	6.25 ± 0.54
MMC	0.1	10–33	20.74 ± 0.882	76	64	60	1.92 ± 0.05	3.70 ± 0.42
Karathane LC	5	1–10	5.18 ± 0.380	46	94	60	2.07 ± 0.05	5.70 ± 0.51
	10	1–12	5.38 ± 0.316	64	82	54	1.96 ± 0.05	4.80 ± 0.47[a]
	15	1–10	5.46 ± 0.283	70	74	56	1.93 ± 0.06	4.15 ± 0.44[b]
	20	2–11	6.32 ± 0.314**	72	78	50	1.89 ± 0.05	3.60 ± 0.41[c]
48 h								
Negative control	—	1–11	5.16 ± 0.297	45	72	83	2.19 ± 0.06	5.95 ± 0.52
MMC	0.1	11–38	24.26 ± 0.945	93	72	35	1.71 ± 0.07	3.20 ± 0.39
Karathane LC	5	1–14	6.68 ± 0.368**	58	96	46	1.94 ± 0.05	4.55 ± 0.46[a]
	10	2–16	7.32 ± 0.450***	74	72	54	1.90 ± 0.06	3.55 ± 0.41[c]
	15	3–15	7.76 ± 0.402***	78	74	48	1.85 ± 0.06	2.85 ± 0.37[c]
	20	3–16	8.96 ± 0.430***	110	56	34	1.62 ± 0.07	2.40 ± 0.34[c]

[a]Significantly different from the control $P < 0.05$ (*z*-test).
[b]Significantly different from the control $P < 0.01$ (*z*-test).
[c]Significantly different from the control $P < 0.001$ (*z*-test).
**Significantly different from the control $P < 0.01$ (*t*-test).
***Significantly different from the control $P < 0.001$ (*t*-test).

human peripheral lymphocytes in a dose-dependent manner. The seven types of aberrations recorded were chromatid break, fragment, sister chromatid union, dicentric chromosome, chromatid exchange, ring chromosome and polyploidy as observed in other studies (4,5,9,10,24). This fungicide has also induced chromosome breaks, sister chromatid union, bridges, laggards, c-mitosis, stickiness, multipolarity and polyploidy in *Allium cepa* (20). In this study, karathane LC significantly increased CAs and CA/cell frequency both with and without a gap. Several studies on the clastogenic action of different compounds have suggested that gaps may indeed be associated with a mutagenic action and may be considered as aberrations (11,25,26). However, the gaps were not elevated as CA by some authors (9,27). Chromosomal aberrations are very important biomarkers for DNA damage induced by chemical agents with or without gaps (26).

In this study, the most pronounced aberration was the chromatid break, suggesting that the chemical acts mostly in the late S or G_2 phase of the cell cycle (28). Fragments were the second common aberrations in the karathane LC-treated lymphocytes. Presence of fragments reveals the increased mutagenic potential of the test compound (29). Fragments and sister chromatid union observed in this study may result from the terminal deletion (30). In this study, quadriradial and triradial type of chromatid exchanges were also observed. Quadriradial chromosomes can be formed from homologous or non-homologous chromosomes (31).

Karathane LC has also caused polyploidy in some cells, indicating that the spindle apparatus made of proteins seems to be affected (9,27,32). Dicentric and ring chromosomes resulting from breakage–fusion–bridge cycle (28) were also induced by karathane. In this investigation, chromosome contractions were also evident in the cultures treated with the highest concentration of karathane LC. The contraction may have arisen from the effects of the chemical on the histone proteins (9).

Karathane LC has not induced a significant effect on the PRI, indicating that it does not influence the kinetics of cell growth. Similar results were obtained by some other pesticides (33–35). However, karathane LC significantly decreased the MI in a dose-related manner. Karathane LC may possibly decrease the MI by affecting G_2 and prophase or synthesis and assembly and/or formation of the mitotic spindle (24). Karathane LC has also decreased MI in *Allium cepa* (20).

Karathane LC induced the formation of SCEs in 20 μg/ml concentration at 24 h and all concentrations at 48-h treatments compared with the control. SCE analysis is one of the most sensitive genotoxic assays and has been widely used to detect the mutagenic and carcinogenic potential of chemicals (26,36). SCEs arise from the reciprocal exchange of DNA at apparently identical loci of the sister chromatids of a duplicated chromosome in response to a damaged DNA template (36–38). The SCEs were induced by substances, which form covalent adducts with DNA or by those that interfere in the metabolism or in the DNA repair reactions (39). Our results are in agreement with those obtained after treating the human peripheral lymphocytes with different pesticides (4,9,24).

In conclusion, the results obtained in this research suggest that karathane LC should be considered as cytotoxic, clastogenic and mutagenic *in vitro* and as posing a genotoxic hazard to humans in all probability. Pesticides that are genotoxic–mutagenic to somatic cells could affect germinal cells as well (40,41). Hence, human exposure to karathane LC should be restricted.

Acknowledgement

The authors thank the Gazi University for the financial support under grant No. FEF 05/2002-12.

References

1. Forastiere,F., Quercia,A., Miceli,M., Setimi,L., Terenzoni,B., Rapiti,E., Faustini,A., Borgia,P., Calvariani,F. and Perucci,C.A. (1993) Cancer among farmers in central Italy. *Scand. J. Work Environ. Health*, **19**, 382–389.
2. Wesseling,C., Antich,D., Hogstedt,C., Rodriguez,A.C. and Ahlbom,A. (1999) Geographical difference of cancer incidence in Costa Rica in relation to environmental and occupational pesticide exposure. *Int. J. Epidemiol.*, **28**, 365–374.

Downloaded from https://academic.oup.com/mutage/article/20/2/101/1081754 by Gazi University user on 10 December 2020

3. Clary,T. and Ritz,B. (2003) Pancreatic cancer mortality and organochlorine pesticide exposure in California 1989-1996. *Am. J. Ind. Med.*, **43**, 306–313.
4. Sinha,S., Panneerselwam,N. and Shanmugam,G. (1995) Genotoxicity of the herbicide butachlor in cultured human lymphocytes. *Mutat. Res.*, **344**, 63–67.
5. Rencüzoğulları,E. and Topaktaş,M. (2000) Chromosomal aberrations in cultured human lymphocytes treated with the mixtures of carbosulfan, ethyl carbamate and ethyl methanesulfonate. *Cytologia*, **65**, 83–92.
6. Brown,L.M., Blair,A., Gibson,R. *et al*. (1990) Pesticide exposures and other agricultural risk factors for leukemia among men in Iowa and Minnesota. *Cancer Res.,* **50**, 6585–6591.
7. Khuder,S.A. and Mutgi,A.B. (1997) Meta-analysis of multiple myeloma and farming. *Am. J. Ind. Med.*, **32**, 510–516.
8. Webster,L.R., McKenzie,G.H. and Moriarty,H.T. (2002) Organophosphate-based pesticides and genetic damage implicated in bladder cancer. *Cancer Genet. Cytogenet.*, **133**, 112–117.
9. Topaktaş,M. and Rencüzoğulları,E. (1993) Chromosomal aberrations in cultured human lymphocytes treated with marshal and its active ingredients carbosulfan. *Mutat. Res.*, **319**, 103–111.
10. Ribas,G., Surrales,J., Carbonell,E., Creus,A., Xamena,N. and Marcos,R. (1998) Lack of genotoxicity of the herbicide atrazine in cultured human lymphocytes. *Mutat. Res.*, **416**, 93–99.
11. Cardoso,R.S., Takahashi-Hyodo,S., Peitl,P.,Jr, Ghilardi-Neto,T. and Sakamoto-Hojo,E.T. (2001) Evaluation of chromosomal aberrations, micronuclei, and sister chromatid exchanges in hospital workers chronically exposed to ionizing radiation. *Teratogen. Carcin. Mut.*, **21**, 431–439.
12. Gómez-Arroyo,S., Díaz-Sánchez,Y., Meneses-Pérez,M.A., Villalobos-Pietrini,R. and De León-Rodriguez,J. (2000) Cytogenetic biomonitoring in a Mexican floriculture worker group exposed to pesticides. *Mutat. Res.*, **446**, 117–124.
13. Sandberg,A.A. (1991) Chromosome abnormalities in human cancer and leukemia. *Mutat. Res.*, **247**, 231–240.
14. Bonassi,S., Abbondandolo,A., Camurri,L., Dal Pra,L., De Ferrari,M., Degrassi,F., Forni,A., Lamberti,L., Lando,C., Padovani,P., Sbrana,I., Vecchio,D. and Puntoni,R. (1995) Are chromosome aberrations in circulating lymphocytes predictive of future cancer onset in humans? *Cancer Genet. Cytogenet.*, **79**, 133–135.
15. Hagmar,L., Bonassi,S., Stromberg,U., Brogger,A., Knudsen,L.E., Norppa,H. and Reuterwall,C. (1998) Chromosomal aberrations in lymphocytes predict human cancer: a report from the European Study Group on cytogenetic biomarkers and health (ESCH). *Cancer Res.*, **58**, 4117–4121.
16. Palitti,F.C., Tanzarella,R., Cozzi,R., Ricondy,E., Vitagliana,A. and Fiori,M. (1982) Comparison of frequencies of SCEs induced by chemical mutagens in bone-marrow spleen and spermatogoneal cells of mice. *Mutat. Res.*, **103**, 191–195.
17. EPA R.E.D. Facts (2003) www.epa.gov
18. World Health Organization (WHO) (1996) The WHO Recommended Classification of Pesticides by Hazard and Guidelines to Classification 1996–1997. International Programme on Chemical Safety (IPCS), WHO/PCS/96.3. Geneva: WHO.
19. Moriya,M., Ohta,T., Watanabe,K., Miyazawa,T., Kato,K. and Shirasu,Y. (1983) Further mutagenicity studies on pesticides in bacterial reversion assay systems. *Mutat. Res.*, **116**, 185–216.
20. Çelik,M. (2003) Ph.D. Thesis. Dinocap fungisitinin *Allium cepa* L. kök ucu hücreleri ve insan periferal lenfositlerinde sitogenetik etkileri. Gazi Üniversitesi, Fen Bilimleri Enstitüsü.
21. Speit,G. and Houpter,S. (1985) On the mechanism of differential giemsa staining of bromodeoxyuridine substitute chromosomes. II. Differences between the demonstration of sister chromatid differentiation and replication patterns. *Hum. Genet.*, **70**, 126–129.
22. Schneider,E.L. and Lewis,J. (1981) Aging and sister chromatid exchange: VIII. Effect of the aging environment on sister chromatid exchange induction and cell cycle kinetics in Ehrlich ascites tumor cells. A brief note. *Mech. Ageing Dev.*, **17**, 327–330.
23. Lin,M.F., Wu,C.L. and Wang,T.C. (1987) Pesticide clastogenicity in Chinese hamster ovary cells. *Mutat. Res.*, **188**, 241–250.
24. Soloneski,S., Gonzales,M., Piaggio,E., Apezteguía,M., Reigosa,M.A. and Larramendy,M.L. (2001) Effect of the dithiocarbamate pesticide zineb and its commercial formulation azzurro. I. Genotoxic evaluation on cultured human lymphocytes exposed *in vitro*. *Mutagenesis*, **16**, 487–493.
25. Anderson,D. and Richardson,C.R. (1981) Issues relevant to the assessment of chemically induced chromosome damage *in vivo* and their relationship to chemical mutagenesis. *Mutat. Res.*, **90**, 261–272.
26. Burim,R.V., Canalle,R., Lopes,J.L.C., Vichnewski,W. and Takahashi,C.S. (2001) Genotoxic action of the sesquiterpene lactone centratherin on mammalian cells *in vitro* and *in vivo*. *Teratogen. Carcin. Mut.*, **21**, 383–393.
27. Rencüzoğulları,E., İla,H.B., Kayraldiz,A. and Topaktaş,M. (2001) Chromosome aberrations and sister chromatid exchange in cultured human lymphocytes treated with sodium metabisulfite, a food preservative. *Mutat. Res.*, **490**, 107–112.
28. Natarajan,A.T. (2002) Chromosome aberrations: past, present and future. *Mutat. Res.*, **504**, 3–16.
29. Rani,M.V.U. and Rao,M.S. (1991) *In vitro* effect of fenthion on human lymphocytes. *Bull. Environ. Contam. Toxicol.*, **47**, 316–320.
30. Kayraldiz,A. and Topaktaş,M. (2001) Indirect genotoxic effect of gamma rays in human peripheral lymphocytes. *Cytologia*, **66**, 25–31.
31. Therman,E. and Kuhn,E.M. (1985) Incidence and origin of symmetric and asymmetric dicentrics in Bloom's syndrome. *Cancer Genet. Cytogen.*, **15**, 293–301.
32. Hemavathy,K.C. and Krishnamurthy,N.B. (1988) Cytogenetic effects of Cuman L, a dithiocarbamate fungicide. *Mutat. Res.*, **208**, 57–60.
33. Dolara,P., Salvadori,M., Capobianco,T. and Toricelli,F. (1992) Sister chromatid exchanges in human lymphocytes induced by dimethoate, omethoate, deltamethrin, benomyl and their mixture. *Mutat. Res. Lett.*, **283**, 113–118.
34. Rencüzoğulları,E. and Topaktaş,M. (1996) The effects of Marshal and its effective ingredient carbosulfan on SCE, MI and RI in cultured human lymphocytes. *Turk. J. Biol.*, **20**, 1–12.
35. Ardito,G., Bigatti,P. and Lamberti,L. (1997) Increased frequencies of sister chromatid exchanges and micronuclei in *in vitro* lymphocyte cultures treated with the fungicides thiram and ziram. *Boll. Soc. Ital. Biol. Sper.*, **73**, 1–7.
36. Tucker,J.D., Auletta,A., Cimino,M.C., Dearfield,K.L., Jacobson-Kram,D., Tice,R.R. and Carrano,A.V. (1993) Sister-chromatid exchange: second report of the Gene-Tox program. *Mutat.Res.*, **297**, 101–180.
37. Tice,R.R. and Hollaender,A. (1984a) Sister chromatid exchanges: twenty-five years of experimental research. In *The Nature of Sister Chromatid Exchanges*. Vol. A, Plenum Press, New York, NY.
38. Tice,R.R. and Hollaender,A. (1984b) Sister chromatid exchanges: twenty-five years of experimental research. In *Genetic Toxicology and Human Studies*, Vol. B, Plenum Press, New York, NY.
39. Carrano,A.V. and Thompson,L.H. (1982) Sister chromatid exchange and gene mutation. *Cytogenet. Cell. Genet.*, **33**, 57–61.
40. Wyrobek,A.J., Gordon,L.A., Burkhart,J.G., Francis,M.W., Kapp,R.W., Letz,G., Malling,H.V., Topham,J.C. and Whorton,M.D. (1983) An evaluation of the mouse sperm morphology test and other sperm tests in non-human mammals. *Mutat. Res.*, **115**, 1–72.
41. Fahrig,R. (1990) Allgemeine Strategie für Genotoxizitätsprüfungen. *Z. Gesellsch. Umwelt-Mutationsforschung (GUM)*, **3–4**, 22–23.

Received on November 30, 2004; revised on January 31, 2005; accepted on February 1, 2005

Downloaded from https://academic.oup.com/mutage/article/20/2/101/1081754 by Gazi University user on 10 December 2020