J. Plant Protection and Pathology, Mansoura University,Vol.1(9): 681 - 696, 2010

Genotoxic and probable mutagenic effects of some pesticides on mice bone marrow cells

El-Bendary, H. M.*; Salwa E. Negm; A. A. Saleh**; M. M. Kady** and F. A. Hosam Eldeen****

*** Plant Protection Dept., Fac. Agric., Fayoum University**

**** Pesticides Dept., Fac. Agric., Mansoura University**

ABSTRACT

Lambda-cyhalothrin, Profenofos and Chlorpyrifos are a broad-spectrum pesticides extensively used to control pests for agricultural and household purposes. In the present study an attempt has been made to evaluate its toxicity profile, the cytotoxic, genotoxic and gene mutations effects in-vitro using structural chromosome aberration (SCA) and micronucleus (MN) test systems in erythrocytes assays in mice bone marrow cells. All doses of tested pesticides increased the number of structural chromosomal aberrations and the frequency of micronucleated erythrocytes compared with the control group. While, the results observed that tested pesticides caused a significant increase in the number of structural chromosome aberration and the frequency of micronucleus formation of the metaphase plates of the samples treated with the higher two concentration treatments of 1/10 and 1/40 LD_{50} of all tested pesticides for 24 hour. In the case of micronucleus test the mice administered for 30, 60, and 90 days, the data revealed satellite associations, chromatid breaks and gaps indicating its effect on chromosomes compared with the control group. The acceptable daily intake (ADI) doses not induce any significant effect. It was also observed that, all tested pesticides induced significant increase in the frequency of chromosome aberration in the bone marrow cells which showed a significant dose-response correlation. Hence, its may be proposed that in-vitro assays like micronucleus and chromosomal aberrations test which indicate genetic damage could be used to study the toxic effect of organophosphorus and pyrethroid pesticides poisoning in humans.

Keywords*:* Chromosomal aberrations (CA), micronucleus (MN), cytotoxicity, lambda-cyhalothrin (LCT), profenofos, chlorpyrifos, mice bone marrow.

INTRODUCTION

The agricultural chemicals commonly labeled as pesticides are perhaps the largest group of poisonous substances being intentionally disseminated throughout the environment. For some pesticides neither health nor environmental risk evaluations are available. Therefore, at the moment the prevention of occupational and environmental consequences of pesticide use may only be achieved if methodologies and threshold environmental values are developed for the assessment of risk due to handling pesticides. Pre-marketing preventive actions are the primary responsibility of industry and the public health and governmental authorities.

These include discovering the toxicological properties of each pesticide (hazard identification); determine the dose-response relationship [No Observed Effect Level, NOEL, identification], assessing or predicting the exposure level in the various exposure and characterizing the risk. Post-

marketing preventive activities consist of the promotion of proper risk management at the workplace.

This part of the present study aims to evaluate the mutagenic effects of the lambda-cyhalothrin, profenofos and chlorpyrifos, reflected by the production of chromosomal aberrations in maternal bone marrow cells (*in vivo*) compared with control group.

MATERIALS AND METHODS

Animals: 180 male albino mice were used in this investigation aged 4-5 weeks and of mean weight 20 gram. The animals were randomly housed in appropriate stainless cages in group of 5 animals/cage. The animals were rearranged to 4 classes (1 control + 3 for tested pesticides) and 10 subclasses (1 control + 3 treatment x 3 pesticides) they were also monitored daily for abnormal symptom.

Chemicals: Lambda-cyhalothrin**:** is a restricted use synthetic pyrethroid insecticide, acute oral LD_{50} for rats = 95 mg/kg. b.wt. and (ADI) = 0.005 mg/kg. b.wt. per day. Profenofos**:** is an organophosphorus insecticide, cholinesterase inhibitor**,** acute oral LD_{50} for rats = 358 mg/kg. b.wt. and (ADI) = 0.01 mg/kg. b.wt. per day. Chlorpyrifos**:** is organophosphorus insecticide, acute oral LD_{50} for rats 150 mg/ kg. b.wt. and (ADI) 0.01 mg/ kg. b.wt. per day.

Animal treatment schedule: Randomized groups of rats housed in cages containing saw dust as bedding and were allocated into 4 groups each group contained 45 males, the first group used as a control while the other groups were treated with tested pesticides at doses of 1/10 LD_{50}, 1/40 LD_{50} and daily acceptable intake (ADI) through the oral administration for 24 hour. For investigate micronucleus the other groups were treated with tested pesticides at doses 1/10 LD_{50}, 1/40 LD_{50} and daily acceptable intake (ADI) for 30, 60 and 90 days. Pesticides were given in twice dose weekly through the oral administration.

Sampling:

Chromosomal aberrations test: According to the method described by **Alder and El-Tarras (1989)**:-

Pesticides were injected separately at sublethal level as mentioned above; animals were injected intraperitoneally with a colchicine solution (4 mg/ kg b.wt.) 1-1.5 hour prior to collect tissue sampling.

Animals were killed at 24 hr after treatment. The bone marrow from all animals was transfer to individual centrifuge tubes, and then the cells were centrifuged for 5 minutes at 1000 r.p.m. After centrifugation, the supernatant fluid was discarded completely. Hypotonic solution (kcl 0.56 %) was added slowly, while agitating the tubes to disperse the pellet, and then the tubes were incubated for 17 min. at room temperature.

At the end of hypotonic treatment, the tubes were centrifuged again at 1000 r.p.m. for 5 min. and the supernatant fluids was discarded of freshly prepared cold fixative (methanol + glacial acetic acid 3:1)**.**

After 10 min the cell were centrifuged again and the supernatant was discarded, then the fixation process was repeated. The third fixation step should last 1 hr refrigerate and can be extended to the next day.

Staining of the slides: The slides were stained for 30 min., in orcein, the staining was carried out using 2 % orcein in 50 % acetic acid, (2 g. orcein powder were boiled for 1 hr in 100 ml of 50 % acetic acid, filtered when still warm for 30 min.).

The stained slides were then transferred to 70 % ethanol for 10 seconds (twice), 90 % for 1 min, and 100 % ethanol for 25 min. After that, the slides were covered with cover slide, left to dry and examined under oil immersion lens.

Micronucleus test: The monitoring of micronucleated polychromatic erythrocytes in mice bone marrow were done according to the procedure described by Schmid (1975) with some modifications according to Brusick (1980 b) and Alder (1984).

Staining: The preparations were stained in ordinary vertical staining jar according to method described by Gallapudiand and Kamara (1979).

The slides were fixed in absolute methanol for 5 min., rinsed twice in deionized distilled water staining for 10 min., in Giemsa rinsed again thoroughly in deionized distilled water air-dried cleaned in xylene for 3 min., and mounted.

Screening of slides: In this study only polychromatic erythrocytes were scored according to Brusick (1980). Micronuclei were identified as dark-blue staining bodies in the cytoplasm of polychromatic erythrocytes.

RESULTS AND DISCUSSION

Analysis of chromosomal aberrations in rat bone marrow cells.

Since several studies have shown that, the exposure to pesticides may induce genotoxic effects in occupationally exposed human population**.** This part of the present study aims to evaluate the mutagenic effects of the lambda-cyhalothrin, profenofos and chlorpyrifos, reflected by the production of chromosomal aberration in maternal bone marrow cells (*in vivo*) compared with control group, 150 cells were examined and the number of cells with either one or more than one aberration was counted, as well as the structural and numerical aberrations were examined.

Table (1) and Fig (1-12) summarize some chromosomal aberration types that are observed in maternal bone marrow cells after treatment by different doses with tested pesticides. The tested pesticides induced highly significant increase of chromosomal aberration within both high dose compared with the control group and also the data showed dose response relationship that, at high dose 1/10 $LD_{50,}$ the total chromosomal aberration were more than at low dose 1/40 LD_{50}. The results showed the potent mutagenic effect of this pesticides that clear from the data which indicate the significant increase of aberrant cells in high dose, it was mean mutagenic effect of these pesticides only at high dose but low dose (ADI) did not induced any significant effect.

Table (1): Chromatid and Chromosomal aberrations induced by lambda-cyhalothrin, profenofos, and chloropyrifos at (1/10, 1/40 from LD_{50} and ADI) for 24 hours.

Treatments Dose (mg/kg b.wt)		Total cells scored	Poly-ploidy	Type of chromosomal aberration								Total	% CA cells
				Chromatid type					Chromosome type				
				Tg	Tb	Td	F	aF	R	Min	Dic		
con.	Non	150	0	2.0	0.0	0.0	1.0	1.0	2.0	1.0	0.0	7.0	4.6 %
Lambdacyhalothrn	1/10	150	0	5.0	6.0	5.0	4.0	3.0	12.0	3.0	1.0	39.0	26.0 %
	1/40	150	0	4.0	2.0	3.0	3.0	2.0	12.0	2.0	1.0	29.0	19.3 %
	ADI	150	0	2.0	1.0	1.0	2.0	1.0	3.0	1.0	0.0	12.0	8.0 %
Profenofos	1/10	150	0	9.0	6.0	5.0	8.0	4.0	14.0	4.0	2.0	52.0	34.6 %
	1/40	150	0	7.0	5.0	4.0	6.0	3.0	12.0	2.0	1.0	40.0	26.6 %
	ADI	150	0	3.0	1.0	1.0	3.0	2.0	5.0	1.0	0.0	16.0	10.6 %
Chlorpyrifos	1/10	150	0	14.0	8.0	9.0	11.0	5.0	15.0	6.0	2.0	70.0	46.6 %
	1/40	150	0	12.0	6.0	8.0	9.0	4.0	14.0	5.0	1.0	59.0	39.3 %
	ADI	150	0	3.0	2.0	1.0	2.0	1.0	7.0	2.0	1.0	19.0	12.6 %

Tg = gap / Tb = break / Td =deletion / F =fragment / aF = acentric fragment / R = ring / Min = minute / Dic = dicentric

On the other hand, it is prominent that most frequent aberration was the ring followed by chromatide gaps, while the chromosome dicentric aberration was the lowest. The most frequent aberrations were induced by chlorpyrifos, followed by profenofos while, lambda-cyhalothrin was the lowest. The obtained results revealed that lambda-cyhalothrin had the lowest mutagenic potential in bone marrow cells in comparison to the other pesticides tested.

Generally, tested pesticides were able to show significant results of chromosome aberration within both high doses levels when compared to control group. Some studies have also shown a positive association between genotoxicity and occupational exposure to pesticides.

The previous mentioned data was agreed with (International Programme on Chemical Safety, (1990) noted that lambda-cyhalothrin induced negative results in a range of in vivo and in vitro assays designed to detect gene mutations, chromosomal damage, and other genotoxic effects. De Ferrari *et al.,* (1991) indicated that organophosphorus insecticides showed significant increase in the incidence chromosome aberrations in lymphocytes. Significant increase in the frequency of chromosomes aberration in peripheral blood lymphocytes of workers occupationally exposed to a mixture of pesticides, was observed by Kourakis *et al.,* (1996).

Also, Stachetti Rodrigues G. *et al.,* (1997) stated that, chlorpyrifos showed clastogenic potency at doses between 10 and 50 ppm, Giri S. *et al.,* (2002) Study the genotoxic effects of fenvalerate which caused a significant increase in (CA) and Rahman *et al.,* (2002) confirmed the ability of the organophosphorus pesticides to induce in vivo genoyoxic effect in leucocytes of Swiss albino mice.

A significant increase in chromosomal aberration was reported in a rural population exposed to dimethoate as organophoshoru pesticides, and the total number of gaps and breaks on human chromosomes was significantly increased with exposure to organophosphate, was reported by Nehez *et al*. (2006), and Lucy R*. et al.,* (2002) respectively.

On the other hand the previous mentioned data was disagree with, Bhaskar Gollapudi B., *et al.* (1995) who noted that cytogenetic abnormalities in mammalian cells both in vitro (rat lymphocyte chromosomal aberration test) and in vivo, and (mouse bone marrow micronucleus test) there was no indication of genotoxic activity for chlorpyrifos in any of these assays. Also, a single i.p. injection of organophosphorus compounds at the highest tolerated dose received by male mice did not produce chromosome damage Noël Degraeve *et al.,* (2002).

Bhunya S. P. and Jena G. B. (2003) stated that a significant induction of chromosome aberrations was observed only after 24 h of exposure with the highest dose (5 mg/kg) of an organophosphate pesticide, monocrotophos.

Also, Ayla Çelik, *et al.,* (2005) stated that cytotoxic and genotoxic effects of lambda-cyhalothrin (LCT) increased the number of the structural chromosomal aberration. Similar results were reported by other investigator, Donbak Y. and Kenan Daglioglu, (2008) who showed that, cyfluthrin increased significantly chromosomal aberration (induce gene mutation).

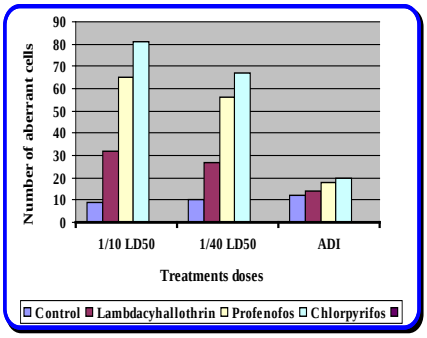

Fig. (1): Comparison between the scored chromatid and chrosomal aberrations induced by tested pesticides with control

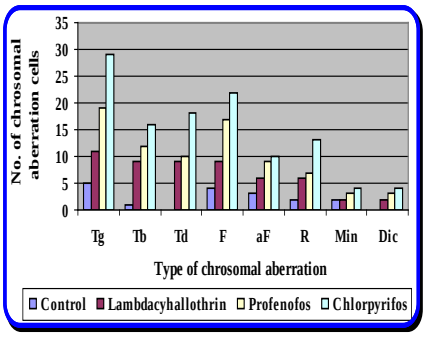

Fig. (2): Comparison between the types of chromatid and chromosome aberrations induced by tested pesticides with contr

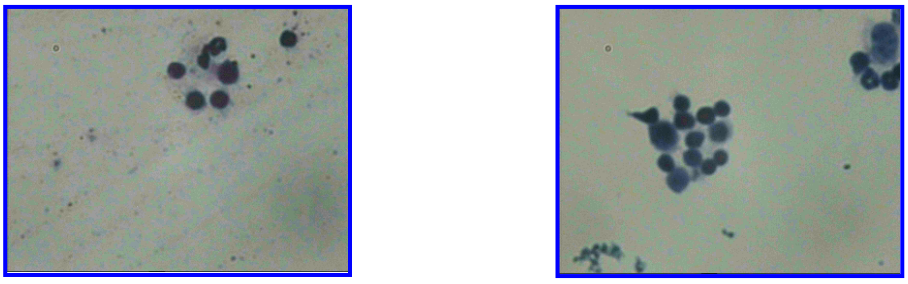

Fig. (3): Chromosomal aberrations in bon-marrow cells after 24 hours as a negative control.

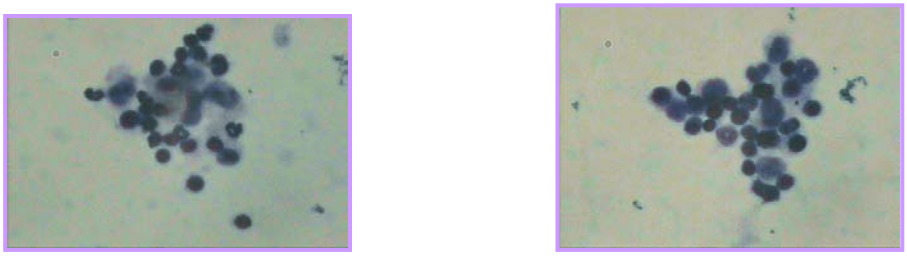

Fig. (4): Chromosomal aberrations in bon-marrow cells induced after treated with lambda- cyhalothrin, at (1/10 LD_{50}) for 24 hours.

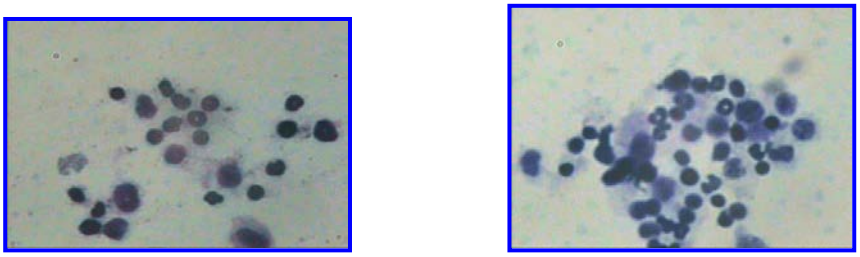

Fig. (5): Chromosomal aberrations in bon-marrow cells induced after treated with lambda- cyhalothrin, at (1/40 LD_{50}) for 24 hours.

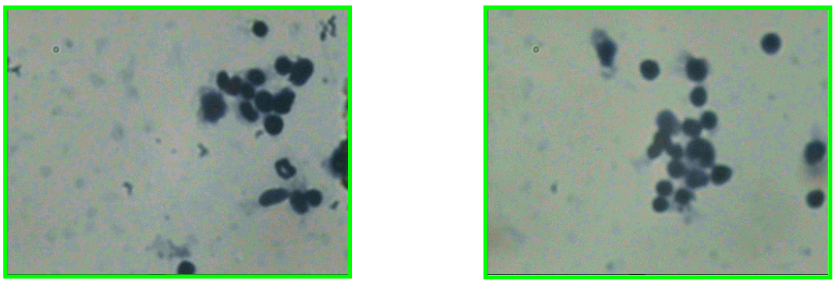

Fig. (6): Chromosomal aberrations in bon-marrow cells induced after treated with lambda-cyhalothrin at (ADI) for 24 hours.

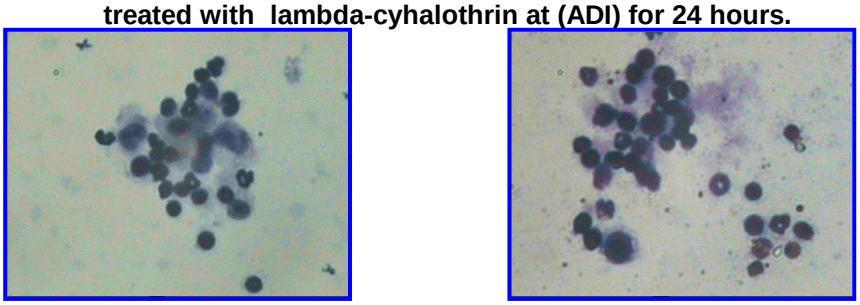

Fig. (7): Chromosomal aberrations in bon-marrow cells induced after treated with Profenofos at (1/10 LD_{50}) for 24 hours. (X 1000)

Fig. (8): Chromosomal aberrations in bon-marrow cells induced after treated with profenofos at (1/40 LD_{50}) for 24 hours. (X 1000)

Fig. (9): Chromosomal aberrations in bon-marrow cells induced after treated with profenofos at (ADI) for 24 hours. (X 1000)

Fig. (10): Chromosomal aberrations in bon-marrow cellsinduced after treated with chlorpyrifos at (1/10 LD_{50}) for 24 hours. (X 1000)

Fig. (11): Chromosomal aberration in bon-marrow cells induced after treated with chlorpyrifos at (1/40 LD_{50}) for 24 hours. (X 1000)

Fig. (12): Chromosomal aberrations in bon-marrow cell induced after treated with chlorpyrifos at (ADI) for 24 hours. (X 1000)

Micronucleus test of polychromatic erythrocytes on bon marrow cells:

Tardiff *et al.*, (1994) stated that, micronuclei serve as an important endpoint to detect the genetic damage by chemical or radiation in cultured cell and intact organism. Compared to traditional approaches involving the analysis of metaphase chromosomes, micronucleus methods are rapid and easy to learn, and have comparable sensitivity. For these reasons, micronucleus assays are being used with increasing regularity.

In our study the polychromatic erythrocytes micronucleus (PCEM) was scored as the individual erythrocytes containing, one, two, three, or more than three micronuclei in the cytoplasm of the cell, and also scored small micronucleus (size of micronucleus less than quarter of the cell) or big micronucleus (size of micronucleus more than quarter of the cell).

The data are presented in Table (2) and illustrated in Fig (13-24) reveal that the pesticide tested induced highly significant increase of (PCEM) within both dose level in comparison with control group and also the data showed dose response relationship that, at high dose 1/10 LD_{50} the total micronucleated were more than at low dose 1/10 LD_{50} and (ADI).

The experiments carried out using 1/10 LD_{50} for 90 days with lambda-cyhalothrin show a total of 47 (PCEM) among 1500 examined cells with a percentage of 3.1 %, while a total of 34 (PCEM) cells were obtained after the treatment with the 1/40 LD_{50} among 1500 cells with a percentage of 2.3 %, on the other hand (ADI) show a total of 13 PCEM) among 1500 examined cells with a percentage of 0. 9 %.

While, the experiments carried out using 1/10 LD_{50} for 90 days with profenofos show a total of 76 (PCEM) among 1500 examined cells with a percentage of 5.1 %, while a total of 66 (PCEM) cells were obtained after the treatment with the 1/40 LD_{50} among 1500 cells with a percentage of 4.4 %, on the other hand (ADI) show a total of 15 (PCEM) among 1500 examined cells with a percentage of 1.0 %.

However, the experiments carried out using 1/10 LD_{50} for 90 days with chloropyrifos show a total of 92 (PCEM) among 1500 examined cells with a percentage of 6.1 %, while a total of 79 (PCEM) cells were obtained after the treatment with the 1/40 LD_{50} among 1500 cells with a percentage of 5.3 %, on the other hand the lowest dose show a total of 22 (PCEM) among 1500 examined cells with a percentage of 1.5 %.

Statistical analysis of these results revealed that chloropyrifos highly significant increase the frequencies of (PCEM) at 1/10 and 1/40 LD_{50} doses compared with the control and other tested pesticides, but lambda-cyhalothrin is the lowest one. Generally it could be that all tested pesticides induce significant increase in micronuclei, given evidence that tested pesticides have clastogenic effect.

Table (2): Frequency of mice bone marrow polychromatic erythrocytes micronucleus (PCEM) induced by lambda-cyhalothrin, profenofos and chlorpyrifos at (1/10, 1/40 from LD_{50} and (ADI) for 30, 60, and 90 days as respectively.

Pesticides	Doses	Period	Exam-ined cells	No. of micronuclei								Total No. PCE cells	% PCEM cells	Means + S.E
				Big				Small						
				1	2	3	>3	1	2	3	>3			
Control	-	30	1500	2	0	0	0	7	0	0	0	9	0.6 %	1.1
		60	1500	2	1	0	0	6	1	0	0	10	0.7 %	1.3
		90	1500	3	0	0	0	8	1	0	0	12	0.8 %	1.5
Lamba-cyhalothrin	1/10	30	1500	11	1	0	0	18	4	1	1	36	2.4 %	4.0
		60	1500	13	1	0	0	21	5	2	1	43	2.9 %	5.4
		90	1500	14	2	1	0	22	5	2	1	47	3.1 %	5.9
	1/40	30	1500	8	0	1	0	14	3	1	0	27	1.8 %	3.4
		60	1500	9	1	0	0	14	3	1	1	29	1.9 %	3.6
		90	1500	11	1	1	0	16	3	1	1	34	2.3 %	4.3
	ADI	30	1500	3	0	0	0	5	1	0	0	9	0.6 %	1.1
		60	1500	3	0	0	0	7	1	0	0	11	0.7 %	1.4
		90	1500	4	0	0	0	6	2	1	0	13	0.9 %	1.6
Profenofos	1/10	30	1500	14	0	0	0	35	8	3	2	62	4.3 %	7.8
		60	1500	16	1	0	0	36	9	4	1	67	4.5 %	8.4
		90	1500	16	2	1	0	39	12	3	3	76	5.1 %	9.2
	1/40	30	1500	13	0	1	0	34	5	2	0	55	3.7 %	6.9
		60	1500	15	1	0	0	36	5	2	1	60	4.0 %	7.5
		90	1500	16	1	0	0	37	7	3	2	66	4.4 %	8.3
	ADI	30	1500	5	1	0	0	6	1	1	0	14	1.2 %	1.8
		60	1500	6	0	0	0	7	2	1	0	16	1.1 %	2.0
		90	1500	5	0	0	0	7	1	2	0	15	1.0 %	1.9
Chlorpyrifos	1/10	30	1500	20	3	0	0	44	9	4	1	81	5.4 %	10.1
		60	1500	24	3	1	0	47	11	4	0	90	6.0 %	11.3
		90	1500	25	2	1	0	48	11	5	1	92	6.1 %	11.5
	1/40	30	1500	19	2	0	0	41	4	1	0	67	4.6 %	8.3
		60	1500	20	2	0	0	43	6	2	0	73	4.9 %	9.1
		90	1500	23	1	1	0	44	7	2	1	79	5.3 %	9.9
	ADI	30	1500	5	0	0	0	7	3	1	1	17	1.1 %	2.1
		60	1500	5	0	0	0	6	3	2	0	16	1.1 %	2.0
		90	1500	7	1	0	0	8	4	2	0	22	1.5 %	2.8

The previous mentioned data was agree with data obtained by Stachetti Rodrigues G. *et al.,* (1997) who reported that, chlorpyrifos showed clastogenic potency at doses between 10 and 50 ppm, also showed significant increases in micronuclei frequency, also Titenko-Holland N., *et al.,* (1997) reported that, malathion caused significant increase in micronucleated cells, and Rosadele Cicchetti, *et al.,* (1999) organophosphate phosphamidon induce a dose dependent increase of micronucleated polychromatic erythrocytes.

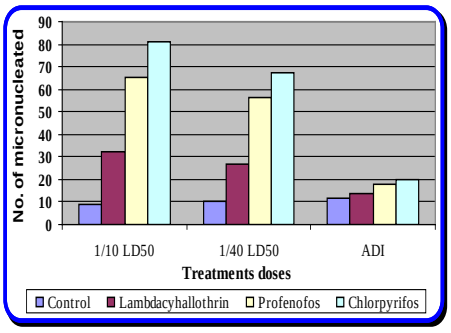

Fig. (13): Comparison between the scored polychromatic erythrocytes micronucleus (PCEM) induced by tested pesticides with control.

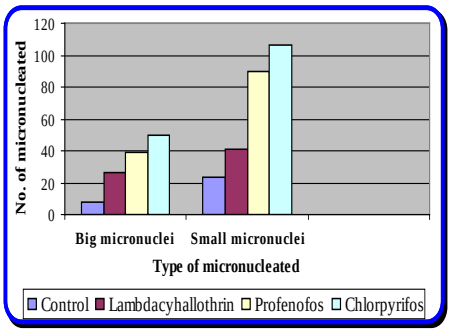

Fig. (14): Comparison between the scored small and big micronucleated (PCEM) induced by tested pesticides with control.

Fig. (15): Photomicrograph of mice bone marrow polychromatic erythrocyte micronucleus (PCEM) as a negative control.

Fig (16): Photomicrograph of mice bone marrow polychromatic rythrocyte micronucleus(PCEM) induced by lambda-cyhalothrin, at (1/10 LD_{50}) for 30 days

Fig (17): Photomicrograph of mice bone marrow polychromatic erythrocyte micronucleus (PCEM)induced by lambda cyhalothrin, at (1/40 LD_{50}) for 60 days

Fig. (18): Photomicrograph of mice bone marrow polychromatic erythrocyte micronucleus(PCEM) induced by lambda-cyhalothrin, at (ADI) for 90 days

Fig (19): Photomicrograph of mice bone marrow polychromatic erythrocyte micronucleus (PCEM) induced by profenofos at (1/10 LD_{50}) for 30 days.

Fig. (20): Photomicrograph of mice bone marrow polychromatic erythrocyte micronucleus (PCEM) induced by profenofos at (1/40 LD_{50}) for 60 days.

Fig. (21): Photomicrograph of mice bone marrow polychromatic erythrocyte micronucleus (PCEM) induced by profenofos at (ADI) for 90 day.

Fig. (22): Photomicrograph of mice bone marrow polychromatic erythrocytemicronucleus (PCEM) induced after treated with chlorpyrifos (1/10 LD_{50}) for 30 days.

Fig. (23): Photomicrograph of mice bone marrow polychromatic erythrocyte micronucleus (PCEM) induced by chlorpyrifos (1/40 LD_{50}) for 60 days.

Fig. (24): Photomicrograph of mice bone marrow polychromatic erythrocyte micronucleus (PCEM) induced after treated with Chlorpyrifos at (ADI) for 90 days.

REFERENCES

Alder, I. D. and A. El-Tarras (1989): Clastogenic effects of cis-diamminedichloroplatinum. I. Induction of chromosomal aberration in somatic and germinal cell of mice. J. Mut. Res., 198:1-26.

Aline Pértile Remor, Carla Caprini Totti, Dariele Alves Moreira, Gustavo Pimentel Dutra, Vanina Dahlström Heuser and Jane Marlei Boeira (2009): Occupational exposure of farm workers to pesticides: Biochemical parameters and evaluation of genotoxicity. Environment International, 35:273-278.

Ayla Çelik, Birgül Mazmanci, Yusuf Çamlica, Ülkü Çömelekoğlu, Ali Aşkin (2005): Evaluation of cytogenetic effects of lambda-cyhalothrin on Wistar rat bone marrow by gavage administration. Ecotoxicology and Environmental Safety, 61:128-133.

Bhaskar Gollapudi B., Mendrala Alan L., Ann Linscombe V., (1995): Evaluation of the genetic toxicity of the organophosphate insecticide chlorpyrifos. Toxicology, 342: 25-36.

Bhunya S. P. and Jena G. B. (2003): Mutagenicity assay of organophosphate pesticides, monocrotophos in mammalian. Cytologia, 53: 801-807.

Brusick, J. E. (1980): Reaction of certain phosphorothionate insecticides with alcohols and potentialation by breakdown products. J. Agric. Food. Chem, 11: 91- 96.

De ferrar, J., Yarrow, S and Cox, H. (1991): The effects of capsaicin upon electrogenic ion transport in rat descending colon. Naunyn Schmiedebergs Arch Pharmacl, 344: 557-563.

Donbak, Y. and Kenan Daglioglu, (2008): Evaluation of cytogenetic effects of lambda-cyhalothrin on Wistar rat bone marrow. Mutat Res, 239: 91-97.

Giri, S. Sharma, G. D. Giri, A. Prasad S. B. (2002): Fenvalerate-induced chromosome aberrations and sister chromatid exchanges in the bone marrow cells of mice in vivo. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 520: 125-132.

International Programme on Chemical Safety (IPCS), (1990) under the joint sponsorship of the United Nations Environment Programme, the International Labour Organisation and the World Health Organization. Environmental Health Criteria, 99:15-21.

Kourakis, A., Mouratidou, M., Barbouti, A. and Dimikiotou, M. (1996): Cytogenic effects of occupational exposure in the peripheral blood lymphocutes of pesticide sprayers. Carcinogenesis. Jan; 17: 99-101.

Lucy R. Webster, Geoff H. McKenzie and Helen T. Moriarty (2002): Organophosphate-based pesticides and genetic damage implicated in bladder cancer. Cancer Genetics and Cytogenetics, 133:112-117.

Nehéz, M. Selypes, A. Scheufler H. and Fischer G. W. (2006): Effect of dimethoate and O-demethyldimethoate on bone marrow cells of mice. Regulatory Toxicology and Pharmacology, 3:349-354.

Noël Degraeve, Marie-Christine Chollet and Jean Moutschen (2002): Cytogenetic effects induced by organophosphorus pesticides in mouse spermatocytes. NeuroToxicology. Elsevier, Amsterdam, Netherlands, 26:651-659.

Rahman, M. F. Mahboob, K. Danadevi, B. Saleha Banu and Paramjit Grover (2002): Assessment of genotoxic effects of chloropyriphos and acephate by the comet assay in mice leucocytes. Medical and Veterinary Entomology, 12(2): 187-191.

Rosadele Cicchetti, Monica Bari and Gabriella Argentin (1999): Induction of micronuclei in bone marrow by two pesticides and their differentiation with CREST staining: an in vivo study in mice. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 439: 239-248

Sailaja N., Chandrasekhar M. , Rekhadevi P.V. , Mahboob M., Rahman M.F., Saleha B. Vuyyuri, Danadevi K., Hussain and Paramjit Grover S.A. (2006): Genotoxic evaluation of workers employed in pesticide production. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 74-80

Schmid. W. (1975): The micronucleus teste. Mutation. Res., 31: 9-15.
Soheir M et al., (2002): Induction of micronuclei in mouse bone marrow by the insecticide gardona. Mutation Research/Genetic Toxicology, 47: 329-336.
Soheir M. Amer and Maha A. Fahmy (2007): Induction of micronuclei in mouse bone marrow by the insecticide Dursban. Mutation Research/Genetic Toxicology.,101(3): 247-255.
Stachetti Rodrigues G., Pimentel D., Weinstein L. H. (1998): Assessment of pesticide genotoxicity in an integrated pest management program I-Tradescantia micronucleus assay. Mutation research. Genetic toxicology and environmental mutagenesis, 412(3): 235-244.
Tardiff, P. H. M. Lohman and G. N. Wogan (1994): Hand Book of "Methods to Assess DNA Damage and Repair; Interspecies Comparision" Published by John Wiley & Sons Ltd.
Tian Y., T. and Yamauchi, (2003): Micronucleus formation in 3-day mouse embryos associated with maternal exposure to chlorpyrifos during the early preimplantation period Reproductive Toxicology, 17: 401-405
Titenko-Holland, N. Windham, P. Kolachana, F. Reinisch, S. Parvatham, A. M. Osorio and M. T. Smith (1997): Genotoxicity of malathion in human lymphocytes assessed using the micronucleus assay in vitro and in vivo: A study of malathion-exposed workers. Mutation Research/ Genetic Toxicology and Environmental Mutagenesis, 388: 85-95.

السمية الوراثية والتأثير الطفرى المحتمل لبعض المبيدات فى خلايا نخاع عظام الفئران البيضاء

حلمى محمد البندارى * ، سلوى السعيد نجم ** ، عادل عبد المنعم صالح ** ، محمد أبراهيم قاضى و فؤاد عبد اللة حسام الدين****
*** قسم وقاية النبات - كلية الزراعة - جامعة الفيوم**
**** قسم المبيدات - كلية الزراعة - جامعة المنصورة**

غالبا ما ترتبط زيادة أستخدام المبيدات فى كثير من بلدان العالم بزيادة التلوث البيئى مما يؤدى الى تغيرات بيئية وصحية خطيرة للانسان والحيوان . وتكمن الخطورة فى أن بعض المبيدات الحشرية لها القدرة على أحداث تلف واضرار بالمادة الوراثيه للخلايا الحية بصورة مباشرة أو غير مباشرة.

وقد أجرى هذا البحث لدراسة التغيرات الوراثية الخلوية والضرر الخلوى، ودراسة الآثر الضار على المادة الوراثية الناتج عن أستخدام مبيد حشرى من مجموعة البيروثرويد وهو مبيد اللامباداسيهالوثرين ومبيدين من مجموعة الفوسفات العضوية وهم البروفينوفوس والكلوربيروفوس. وذلك لتقييم وأختبار قدرة هذة المبيدات الحشرية على أحداث التغيرات الخلوية والتأثير الطفرى المحتمل والتغيرات الكيماوية المصاحبة لها فى خلايا نخاع العظام وذلك بأجراء أختبارين هما: **1-** أختبار التغيرات الكروموسومية التركيبية والعددية. **2-** أختبار القدرة على أحداث النويات الصغيرة فى خلايا الدم الحمراء غير الناضجة.

وقد أستخدم فى دراسة هذا الاختبار 180 فأر من ذكور الفئران البيضاء حيث قسمت عشوائيا الى 4 مجموعات رئيسية متساوية (1 مجموعة كنترول + 3 مجموعات للمبيدات تحت الاختبار) 45 فأر فى كل مجموعة. ثم قسمت كل مجموعة رئيسية الى 3 مجموعات ليكون هناك 10 معاملات (1 معاملة كنترول + 3 معاملات لكل مبيد x 3 مبيد تحت ألاختبار) 5 فأر فى كل

قفص. وأجريت المعاملة بتجريع الفئران عن طريق الفم 10/1، 40/1 ، (ADI) من الجرعة المميتة النصفية LD_{50} للمبيدات تحت الاختبار وذلك لمدة 24 ساعة، وقبل أنتهاء فترة التعريض بساعتين تم حقن مادة الكوليشيسن فى الغشاء البريتونى بتركيز 4 مجم/كجم وذلك فى نصف عدد الحيوانات تحت ألاختبار، ثم أجراء التشريح والحصول على نخاع العظام لدراسة التغيرات الكروموسومية. أما النصف ألاخر من الحيوانات تحت ألاختبار تركت للمعاملة عن طريق الفم مرتين أسبوعيا بجرعات 10/1، 40/1 ، (ADI) من الجرعة المميتة النصفية LD_{50} للمبيدات تحت ألاختبار وذلك لمدة 30، 60، 90 يوم وفى نهاية فترة المعاملة تم الحصول على نخاع العظام لدراسة القدرة على أحداث النويات الصغيرة فى خلايا الدم الحمراء غير الناضجة .

وقد أوضحت التحاليل الاحصائية النتائج التالية: أظهر تحليل الشذوذ الكروموسومى على قدرة المبيدات تحت الاختبار فى كل الجرعات المستخدمة على أحداث ضرر كبير للمادة الوراثية وظهور عديد من الطرز الكروموسومية الشاذة بصورة معنوية مقارنة بالمجموعة الضابطة، خاصة الفجوة والشظية ، كما أظهر التحليل ألاحصائى وجود علاقة خطية موجبة بين التركيز وبين زيادة تكرار التغيرات الكروموسومية.

كما أن المبيدات الثلاثة أظهرت تفاوت نسبى فى أحداث التأثر الطفرى معنويا حيث كان مبيد الكلوربيروفوس اكثر المبيدات أحداثا للتأثير الطفرى يلية مبيد البروفينوفوس بينما اللامباداثيهالوثرين لم يظهر ألا تأثير طفرى طفيف. كما أظهر تحليل النويات الصغيرة فى خلايا الدم الحمراء غير الناضجة قدرة المبيدات على زيادة فى تكرار طفرات النويات الصغيرة بمستويات عالية المعنوية ومرتبطة بالجرعة مقارنة بالمجموعة الضابطة.

قام بتحكيم البحث

أ. د/ على على عبد الهادى — كلية الزراعة – جامعة المنصورة

أ. د/ محمد ابراهيم عبد المجيد — كلية الزراعة – جامعة عين شمس

J. Plant Protection and Pathology, Mansoura University,Vol.1(9): 681 - 696, 2010