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Original article

MUTAGENIC AND RECOMBINOGENIC ASSESSMENT OF WIDELY USED PESTICIDES ON *DROSOPHILA MELANOGASTER*

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Summary

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The mutagenic potential of selected widely used pesticides: p,p'-dichlorodiphenyltrichloroethane (DDT); fenitrothion; propoxur; deltamethrin, bifenthrin; imidacloprid and thiametoxam was assessed using the wing spot test. Third-instar larvae of standard *Drosophila melanogaster* cross (ST), trans-heterozygous for the third chromosome recessive markers, multiple wing hairs (*mwh*) and flare (flr^3) were chronically exposed to test compounds. Feeding ended with pupation of the surviving larvae. Genetic changes induced in somatic cells of the wing's imaginal discs, mutant spots observed in marker-heterozygous (MH) and balancer-heterozygous (BH) flies were compared using the wing spot test, to estimate the genotoxic effects of these pesticides. In conclusion, exposure to 30 mg/mL deltamethrin, 40 mg/mL imidacloprid, 100 µg/mL DDT showed mutagenic and recombinagenic effects in the *Drosophila* wing spot test. In addition the results of chronic treatments performed at high doses showed mutagenic and recombinagenic effects in both genotypes.

Key words: *Drosophila melanogaster,* genotoxicity, pesticides, SMART, wing spot test

INTRODUCTION

The widespread use of pesticides may cause toxic, genotoxic or carcinogenic hazards. One of the best known was DDT, organochlorine group (OC); it is presently banned based on a cancellation order issued by the U.S. EPA for adverse environmental effects to wildlife, as well as human health risks. However it has been widely used to control agricultural insect pests and to control vectors of diseases (US HHS, 2002). Today, DDT is classified as a probable human carcinogen by international organisations. Although use discontinued, residues still pose concern from previous heavy uses due to persistence (Anonymous, 2017a). Fenitrothion,

an organophosphate (OP), is a contact insecticide and selective acaricide of low ovicidal properties. Fenitrothion is effective against a wide range of pests, i.e. penetrating, chewing and sucking insect pests, including public health programmes, listed by the World Health Organization (WHO) as vector control agent for malaria (Anonymous, 2017b). Propoxur is a non-systemic, general use insecticide used on a variety of insect pests such as chewing and sucking insects, ants, cockroaches, crickets, flies, and mosquitoes, available in several types of formulations and products, including emulsifiable concentrates, wettable powders, baits, aerosols, fumigants, granules, and oilsprays (Anonymous, 2017c). Deltamethrin is a synthetic pyrethroid, with a quick knockdown effect. Powerful for use to control the vectors of "non-persistent" viruses (viruses that can be passed on by the vector within a few minutes of starting to feed on the plant) (Anonymous, 2017d). Bifenthrin is another pyrethroid insecticide and acaricide which affects the nervous system and causes paralysis in insects; highly toxic to fish and aquatic organisms. The U.S. EPA has classified bifenthrin as Toxicity Class II – moderately toxic (Jonhson *et al.,* 2010). Neonicotinoid pesticides have been put on the market to replace the persistent and resistance developing insecticides by causing blockage in a type of neuronal pathway (nicotinergic) that is more abundant in insects than in warm-blooded animals (making the chemical selectively more toxic to insects than to warm-blooded animals). They are widely used for agricultural and household pest control (Tomizawa & Casida, 2003). This blockage leads to the accumulation of acetylcholine, an important neurotransmitter, resulting in the insect's paralysis, and eventually death. It is effective on contact and via stomach action (Anonymous, 2017e). Thiamethoxam's chemical structure is slightly different than that of the other neonicotinoid insecticides, and because of its greater water solubility, it moves readily in plant tissue. Products are labelled for soil, seed, and foliar treatments to a wide range of vegetable and field crops (Fishel, 2013). However, the genotoxic potentials of neonicotinoids have not yet been investigated in the *in vitro* SMAR test. In addition, recently non-target organisms have been increasingly reported to be affected together with target organisms. As a result of these effects, resistance development in pest populations has become a very important problem, yet to be solved. Emerging concern and controversy exists in ecotoxicology and eventually human risks of the tested pesticides; which led us to study in detail the genotoxic potential of pesticide class representatives in the present study. Previous works on the genotoxicity of pesticides used in this study report quite conflicting results depending on the genetic test system or assay used. To obtain more detailed knowledge of the genotoxic potential of these pesticides, we employed the standard version of the wing spot test in *Drosophila melanogaster.* The mutational and recombinational potential as well as the total genotoxicity as a function of exposure concentration was determined for these compounds.

The wing somatic mutation and recombination test in *Drosophila melanogaster* has been used to estimate the genotoxicity of pharmaceuticals, chemicals and environmental pollutants. The frequency of somatic recombination and mutations increases under the effect of mutagens and, as a result, the quantity of cells with mutant hairs raises in the *D. melanogaster* wing cells. The wing spot

test has gained wide recognition because 75% of known human disease genes have a recognizable match in the genome of *Drosophila*, with 50% of fly protein sequences having mammalian homologs. Results of the test agree well with *in vivo* mammalian genetic tests. It was found that the frequency of somatic mutant spots is dependent on the genetic background of *D. melanogaster* strains (Fortini *et al.* 2000). The objective of the present study was to examine genotoxicity potentials of some widely used pesticides belonging to different chemical classes, by using the wing spot test.

MATERIALS AND METHODS

Chemicals

DDT (CAS Registry number 50-29-3, purity 98%); fenitrothion (CAS Registry number is 94650-98-3, purity 95%); propoxur (CAS Registry number 127779-20-8, purity 96%); deltamethrin (CAS Registry number 64121-95-5, purity 98%), bifenthrin (CAS Registry number 82657-04-3, purity 92.2%), imidacloprid (CAS Registry number 138261-41-3, purity 97%); and thiametoxam (CAS Registry number 153719-23-4, purity 99.7%); used in this study were obtained from Hacettepe University Insecticide Test and Analysis Laboratory, Beytepe, Turkey. Except for DDT and fenitrothion, all chemicals were dissolved in acetone (2%). Ethyl-methane sulphonate (EMS, 0.5 mM) was used as positive control. EMS (100% purity; CAS No. 62-50-0) was obtained from Sigma Chemical (St. Louis, MO). Three negative control groups were used: pure distilled water, acetone (2%) and ethyl alcohol (0.5%) solvents.

Strains

Two mutant *Drosophila melanogaster* strains were used: The *multiple wing hairs* strain with genetic constitution: (*mwh/ mwh*) and the *flare-3* strain: (*flr*^{3}*/ In (3LR) TM3, ri p*^{p} *sep bx34e es Bd*^{S}*)*. Both strains were provided by Prof. F. E. Würgler (University of Zurich, Switzerland). For genetic symbols and description, see Lindsley & Zimm (1992). The standard cross (ST) was used in the experiments.

Treatment procedure

Final experimental exposure concentrations were based on pilot dose-range finding preliminary data. The wing spot test in *D. melanogaster* monitors loss of heterozygosity induced by point mutation, deletion, unbalanced half-translocation and mitotic recombination, as described by Graf *et al.* (1984). Two different genotypes of larvae were used in the tests: *mwh* and *flr*^{3}, which are wing markers located on the left arm of chromosome 3. Larvae were obtained by parental crosses between virgin females of *flr*^{3}*/TM3, Bd*^{S} and *mwh* males. From the crosses, eggs were collected during 8 h periods in culture bottles containing standard *Drosophila* medium (Carolina Biological Supply Company Burlington, NC). After 72 h the larvae were washed and selected for the treatments. For chronic feeding, small plastic vials were prepared with 1.5 g dry standard *Drosophila* medium and 5 mL of the respective test solutions (DDT and bifenthrin doses in µg/L; all others in mg/L; Table 1) were added; then 100 larvae were embedded in the medium. The larvae fed on the medium until pupation of the surviving larvae. After metamorphosis, all surviving flies were scored. Each cross produces two types of progeny, i.e. marker-heterozygous (*mwh* +/+ *flr*^{3}) and balancer-heterozygous (*mwh* +/+ *TM3*

Bd^S) flies. The dominant *Bd*^{S} marker allows the wings of these two genotypes to be distinguished. The hatched flies were stored in 70% ethyl alcohol. All experiments were performed at 25±1 °C, and 65% relative humidity.

EMS was used as positive control at a concentration of 0.1 mM. Acetone (2%) and ethyl alcohol (0.5%) were used as solvent controls.

Preparation and microscopic analysis of the wings

All wings were mounted on slides with Faure's solutions (gum arabic 30 g, glycerol 20 mL, chloral hydrate 50 g, water 50 mL) and examined for spots at 400× magnification. Frequency and size of single and twin spots were recorded. Single spots (mostly mwh but also rarely flr3) can result from various types of mutational events (deletions, point mutations, specific chromosome aberrations, etc.) or from recombination if mitotic crossing-over takes place between the two marker genes. Twin spots (composed of a *mwh* and a *flr*^{3} area) are produced by mitotic recombination between the proximal marker *flr*^{3} and the centromere of chromosome 3. Only mwh single spots can be recovered on the wings of balancer-heterozygous flies. They are all due to mutational events because recombinational events are suppressed in inversion-heterozygous cells with the multiply inverted TM3 balancer chromosome (Kaya *et al.,* 2000).

Data evaluation and statistical analyses

The wing spot data were evaluated with SMART PC-Version 2.1. For the frequencies of spots per wing, a multiple-decision procedure is used to decide whether a result is positive, weakly positive, inconclusive or negative. More details on the statistical procedure are given in Frei & Würgler (1995). The wing spot data of treated and distilled water control series were compared by conditional binomial test (Kastenbaum & Bowman, 1970). Each statistical test was performed at 5% significance level (Frei, 1991; Frei *et al.* 1992). Statistical comparisons of survival rates were made by chi-square test for ratios for independent samples. The clone formation frequency per cell cycle and 10^5 cells were calculated (Frei *et al.,* 1992).

RESULTS

In this study, some widely used pesticides representing different classes (DDT; fenitrothion; propoxur; deltamethrin, bifenthrin; imidacloprid and thiametoxam) were investigated for toxic effects and also possible genotoxic potential by using the wing spot test. Survival percentage of experimental groups and survival percentage of relative negative control groups' data were statistically compared by the use of chi-square test. The results are shown in Table 1. Acording to our results 100 µg/L DDT; 15 mg/L deltametrin; 5, 10, 20 and 40 mg/L imidacloprid; 2.5, 5, 10 mg/L thiamethoxam; 15 µg/L bifenthrin were found to be toxic compared with control group. The results showed dose-effect relationships. Range of toxicity from the highest to the lowest was: imidacloprid, thiamethoxam, deltametrin, DDT, bifenthrin. In this study, none of the exposure concentrations of fenitrothion and propoxur had toxic effects.

The data collected in the wing spot test are presented in Tables 2 and 3. The wings of the two types of offspring were scored: those with the inversion-free transheterozygous genotype, marker-heterozygous wings of flies (results are shown in

Table 1. Survival percentages of the flies exposed to different concentration of tests compounds.

Compounds	Concentration	Survival (%)	X^2 value
Distilled water		98	–
EMS (mM)	0.5	32	–
Acetone control (%)	2.0	77	–
Ethyl alcohol control (%)	0.5	78	–
DDT (µg/L)	20	62	1.82
	50	59	2.64
	100	31	20.27***
Deltamethrin (mg/L)	5	77	0
	10	78	0.006
	15	26	25.25***
Imidacloprid (mg/L)	5	47	7.25**
	10	39	12.45***
	20	36	14.88***
	40	21	32***
Propoxur (mg/L)	0.63	73	0.11
	1.25	81	0.11
	2.6	76	0.007
Fenitrothion (mg/L)	3.92	75	0.06
	7.88	61	2.08
	15.68	66	1
Thiamethoxam (mg/L)	1.25	86	0.5
	2.5	47	7.26**
	5	41	10.98***
	10	21	32***
Bifenthrin (µg/L)	3.75	87	0.61
	7.5	83	0.23
	15	47	7.26**

** P<0.01, *** P<0.001 survival statistics (Chi-square test).

Table 2), and those with balancer heterozygotes (results are shown in Table 3).

EMS induced all kinds of spots (except for twin spot with balance-heterozygous wings). The data demonstrated the strong mutagenic and recombinogenic activity of EMS as originally reported by Graf *et al.* (1984). All results show that the types of mutations encountered most frequently were small single spots, and mutation frequency increased by increasing concentration. Genetic changes induced in somatic cells of the wing's imaginal discs lead to the formation of mutant clones on the wing blade. Single spots are produced by somatic point mutation, deletion, non-disjunction and somatic recombination occurring between the two markers. Twin spots are produced exclusively by somatic recombination occurring between the proximal marker *flr* and the centromere of chromosome 3.

According the data observed from ST cross both MH flies and the BH flies at all exposure concentrations of thiamethoxam and fenitrothion gave negative results. Except for the 10 mg/L imidacloprid concentration in BH genotypes; all imidacloprid concentrations showed positive results. In addition, only the highest exposure concentrations of DDT, propoxur,

Table 2. Somatic Mutation and Recombination Test data obtained with the pesticides tested. Results with marker-heterozygous wings (n=80)

Test compound	Concentration	Small single spots (1–2 cells) (m=2)			Large single spots (>2 cells) (m=5)			Twin spots (m=5)			Total *mwh* spots (m=2)			Total spots (m=2)			c
		No	Fr.	D	No	Fr.	D	No	Fr.	D	No	Fr.	D	No	Fr.	D	
EMS 0.5 mM	Positive control	40	0.50		5	0.06		2	0.03		46	0.58		47	0.59		2.36
Distilled water	Negative control	20	0.25		1	0.01		1	0.01		19	0.24		22	0.28		0.97
Acetone (2%)	Negative control	18	0.23		0	0		0	0		18	0.23		18	0.23		0.92
Ethanol (0.5%)	Negative control	16	0.20		4	0.05		1	0.01		20	0.25		21	0.26		1.03
Deltamethrin (mg/L)	5	23	0.29	i	5	0.06	+	0	0		28	0.35	i	28	0.35	i	1.43
	10	25	0.31	i	2	0.03	i	1	0.01	i	28	0.35	i	28	0.35	i	1.43
	15	28	0.35	i	1	0.01	i	1	0.01	i	30	0.38	i	30	0.38	i	1.53
	30	38	0.48	+	5	0.06	+	2	0.03	i	45	0.56	+	45	0.56	+	2.31
Imidacloprid (mg/L)	5	21	0.26	i	10	0.13	+	1	0.01	i	31	0.39	+	32	0.40	+	1.59
	10	29	0.36	i	10	0.13	+	0	0		39	0.49	+	39	0.49	+	2.00
	20	30	0.38	i	5	0.06	+	1	0.01	i	36	0.45	+	36	0.45	+	1.84
	40	40	0.50	+	6	0.08	+	0	0		46	0.58	+	46	0.58	+	2.36
Propoxur (mg/L)	0.63	16	0.20	–	4	0.05	i	1	0.01	i	21	0.26	i	21	0.26	i	1.07
	1.25	18	0.23	–	3	0.04	i	3	0.04	i	24	0.30	i	24	0.30	i	1.23
	2.6	22	0.28	i	2	0.03	i	1	0.01	i	25	0.31	i	25	0.31	i	1.28
	5.2	30	0.38	i	2	0.03	i	2	0.03	i	33	0.41	+	34	0.43	+	1.69
DDT (µg/L)	20	20	0.25	i	5	0.06	i	0	0	i	25	0.31	i	25	0.31	i	1.28
	50	28	0.35	+	2	0.03	–	0	0		30	0.38	i	30	0.38	i	1.54
	100	40	0.50	+	3	0.04	–	2	0.03	i	45	0.56	+	45	0.56	+	2.31
Thiamethoxam (mg/L)	1.25	12	0.15	–	3	0.04	i	3	0.04	i	15	0.19	–	18	0.23	–	0.76
	2.5	17	0.21	–	1	0.01	i	0	0	–	18	0.23	–	18	0.23	–	0.92
	5	22	0.28	i	0	0	–	0	0	–	22	0.28	i	22	0.28	i	1.13
	10	16	0.20	–	0	0	–	1	0.01	i	17	0.21	–	17	0.21	–	0.87
Fenitrothion (mg/L)	3.5	15	0.19	i	4	0.05	i	0	0	i	19	0.24	–	19	0.24	–	0.97
	7	16	0.20	i	4	0.05	–	1	0.01	i	20	0.25	–	21	0.26	–	1.03
	15	17	0.21	i	2	0.03	–	3	0.04	i	21	0.26	–	22	0.28	–	1.08
Bifenthrin (µg/L)	3.75	8	0.10	–	4	0.05	i	2	0.03	i	14	0.18	–	14	0.18	–	0.72
	6	14	0.18	–	2	0.03	i	1	0.01	i	17	0.21	–	17	0.21	–	0.87
	7.5	24	0.30	i	2	0.03	i	0	0	–	26	0.33	i	26	0.33	i	1.33
	15	31	0.39	+	6	0.08	+	0	0	–	37	0.46	+	37	0.46	+	1.89

Fr: frequency, D: statistical diagnosis according to Frei & Wurgler (1988); += positive, – = negative, i= inconclusive, m= multiplication factor, c= frequency of clone formation per 10^5cells.

Table 3. Somatic Mutation and Recombination Test data obtained with the pesticides tested. Results with balancer-heterozygous wings (n=80)

Test compound	Concentration	Small single spots (1–2 cells) (m=2)			Large single spots (>2 cells) (m=5)			Twin spots (m=5)			Total *mwh* spots (m=2)			Total spots (m=2)			c
		No	Fr.	D	No	Fr.	D	No	Fr.	D	No	Fr.	D	No	Fr.	D	
EMS 0.5 mM	Positive control	45	0.56		7	0.09		0	0		51	0.64		52	0.65		2.36
Distilled water	Negative control	16	0.20		2	0.03		2	0.03		20	0.25		22	0.28		1.03
Acetone (2%)	Negative control	18	0.23		0	0		0	0		18	0.23		18	0.23		0.92
Ethanol (0.5%)	Negative control	16	0.20		4	0.05		1	0.01		20	0.25		21	0.26		1.03
Deltamethrin (mg/L)	5	19	0.24	–	2	0.03	i	0	0		21	0.26	i	21	0.26	i	1.08
	10	22	0.28	i	3	0.04	i	0	0		25	0.31	i	25	0.31	i	1.28
	15	26	0.33	i	0	0		0	0		26	0.33	i	26	0.33	i	1.33
	30	40	0.50	+	6	0.08	+	0	0		46	0.58	+	46	0.58	+	2.36
Imidacloprid (mg/L)	5	24	0.30	i	7	0.09	+	0	0		31	0.39	+	31	0.39	+	1.59
	10	21	0.26	i	9	0.11	+	0	0		29	0.36	i	29	0.36	i	1.49
	20	28	0.35	i	4	0.05	i	0	0		32	0.40	+	32	0.40	+	1.64
	40	37	0.46	+	2	0.03	i	0	0		39	0.49	+	39	0.49	+	2.00
Propoxur (mg/L)	0.63	18	0.23	–	2	0.03	i	0	0		20	0.25	i	20	0.25	i	1.03
	1.25	15	0.19	–	3	0.04	i	0	0		18	0.23	–	18	0.23	–	0.92
	2.6	22	0.28	i	4	0.05	i	0	0		26	0.33	i	26	0.33	i	1.33
	5.2	32	0.40	+	1	0.01	i	0	0		32	0.40	+	33	0.41	+	1.64
DDT (µg/L)	20	18	0.23	i	6	0.08	i	0	0		24	0.30	i	24	0.30	–	1.23
	50	32	0.40	+	4	0.05	–	0	0		35	0.44	+	36	0.45	+	1.79
	100	42	0.53	+	2	0.03	–	0	0		44	0.55	+	44	0.55	+	2.25
Thiamethoxam (mg/L)	1.25	17	0.21	–	0	0		0	0	–	17	0.21	–	17	0.21	–	0.87
	2.5	23	0.28	i	2	0.03	i	0	0	–	23	0.29	i	25	0.31	i	1.18
	5	16	0.20	–	1	0.01	i	0	0	–	16	0.20	-	17	0.21	–	0.82
	10	24	0.30	i	0	0		0	0	–	24	0.30	i	24	0.30	i	1.23
Fenitrothion (mg/L)	3.5	15	0.19	–	2	0.03	–	0	0	i	14	0.18	–	17	0.21	–	0.72
	7	18	0.23	i	1	0.01	–	0	0	i	17	0.21	–	19	0.24	–	0.87
	15	20	0.25	i	1	0.01	–	0	0	i	20	0.25	–	21	0.26	–	1.03
Bifenthrin (µg/L)	3.75	18	0.23	–	1	0.01	i	0	0	–	19	0.24	i	19	0.24	i	0.97
	6	15	0.19	–	0	0	–	0	0	–	15	0.19	–	15	0.19	–	0.77
	7.5	25	0.31	i	2	0.03	i	0	0	–	26	0.33	i	27	0.34	i	1.33
	15	32	0.40	+	1	0.01	i	0	0	–	33	0.41	+	33	0.41	+	1.69

Fr: frequency, D: statistical diagnosis according to Frei & Wurgler (1988); += positive, – = negative, i= inconclusive, m= multiplication factor, c= frequency of clone formation per 10^5 cells.

deltamethrin and bifenthrin gave positive results both for the marker-heterozygous and balancer-heterozygous genotypes. We conclude that the results showed dose-effect relationships.

The clone-formation frequency was also estimated in this research. It is well established that clone formation frequency of per 105 cells higher than 2.0 are indicative of genotoxic effect of the particular treatment (Graf *et al.,* 1994). In the MH genotypes, exposure to 30 mg/mL deltamethrin, 40 mg/mL imidacloprid, 100 µg/mL DDT the clone formation frequencies were higher than 2.0. Also this value was equal to 2, at an exposure concentration of 10 mg/mL imidacloprid (Table 2). In the BH genotypes, the clone formation frequencies were higher than 2.0 clones per 10^5 cells at concentrations of 30 mg/mL deltamethrin and 100 µg/mL DDT. This value was 2 at an exposure concentration of 40 mg/mL imidacloprid (Table 3).

The genotoxicity observed in MH flies is mainly due to mitotic (homologous) recombination and only to a lesser extent to point mutations. Homologous recombination is possibly an errant DNA repair mechanism that can result in a loss of heterozygozity or genetic rearrangements. Some of these genetic alterations may play a primary role in carcinogenesis.

DISCUSSION

Our results showed that 100 µg/L DDT; 15 mg/L deltametrin; 5, 10, 20 and 40 mg/L imidacloprid; 2.5, 5, 10 mg/L thiamethoxam; 15 µg/L bifenthrin were toxic to *Drosophila melanogaster* when compared with the respective control group. In addition, our study strongly suggests that exposure to 30 mg/mL deltamethrin, 40 mg/mL imidacloprid, 100 µg/mL DDT showed mutagenic and recombinagenic effects in the *Drosophila* wing spot test. Although these genetic alterations may have impacts in primary carcinogenesis; they are more likely to be involved in secondary and subsequent steps of carcinogenesis by which recessive oncogenic mutations are revealed (Bishop & Schiestl, 2003; Reliene *et al.*, 2007).

Previous works on the genotoxicity of pesticides used in this study report quite conflicting results depending on the genetic test system or assay used. Our results are in good agreement with those of other researchers. Ismail & Mohamed (2012) found deltamethrin-based commercial formulation to induce genotoxicity. Deltamethrin-treated rats showed decreased serum testosterone, luteinizing and follicle-stimulating hormone levels. Testicular total oxidant capacity, poly (ADP-ribose) polymerase, lactate dehydrogenase and DNA damage were significantly increased. *In vivo* cytogenetic effects of deltamethrin were evaluated by induction of micronuclei in adult mice (Ozkan & Ustuner, 2010). All doses (i.p. 50, 100, 200 mg/kg) deltamethrin significantly increased the frequency of micronuclei in erythrocytes and splenocytes; with a linear relationship between doses used and micronucleus frequencies. Additionally cytogenic effects of deltamethrin were reported in human lymphocytes using chromosomal aberration and cytokinesis-block micronucleus assays by Chauhan *et al.* (2007). Peripheral lymphocytes were exposed to deltamethrin at concentrations of 2.5, 5, 10, and 20 µM; and significant increase in chromosomal aberration frequency at 10 µM was reported showing the potential of commercial deltamethrin formulations for genotoxicity to mammals. In humans, blood cell cultures of men occupationally exposed to DDT showed increases in chromosomal damage.

In a separate study, significant increases in chromosomal damage were reported in workers who had direct and indirect occupational exposure to DDT showing potential to cause genotoxic effects; but does not support strong mutagenic effects (ATSDR). Similarly we found <100 µg/mL DDT to be negatively mutagenic and recombinagenic in the *Drosophila* wing spot test. In addition, Canales-Aguirre *et al.* (2011) investigated genotoxicity (micronuclei and comet assay) of DDT on systemic and mammary gland cells obtained from adult female Wistar rats. In addition, oxidative stress was studied in mammary tissue showing increased lipid peroxidation leading to oxidative stress. Their results confirmed DDT genotoxicity, not only for lymphocytes but also to mammary epithelial cells. Synergistic genotoxicity caused by low concentration of titanium dioxide nanoparticles and p,p'-DDT on human embryo L-02 hepatocytes were investigated by Shi *et al.* (2010). Nano-TiO_2 (0.1 g/L) was mixed with 0.01–1 mmol/L p, p'-DDT. Their results showed that low concentrations of nano-TiO_2 and p,p'-DDT increased oxidative stress. Oxidative stress is a major pathway for DNA and chromosome damage (Yildiz, 2004).

Frantzios *et al.* (2008) reported LC_{50} of imidacloprid to *Drosophila melanogaster* as 7.59×10^{-5} M for larvae after acute exposure; 1.43×10^{-4} M for adults, and after chronic exposure, it was 2.67×10^{-5} M and 6.09×10^{-5} M for larvae and adults. Their results show strong insecticidal effects and are similar to our results. Lopez-Antia *et al.* (2013) tested the lethal and sub-lethal effects of treated seed ingestion by the red-legged partridge (*Alectoris rufa*) in one year-old partridges (n=42 pairs) in a 10-day (prior to breeding) feeding study with imidacloprid-treated wheat. The high exposure doses produced decreased cellular immune response; and also reduced fertilization rate and chick survival. Vlastos *et al.* (2004) and Karabay & Oguz (2005) showed imidacloprid to increase the incidence of sister chromatid exchange, micronuclei formation and genetic damage in human lymphocytes. Stivaktakis *et al.* (2012) evaluated the genotoxic effect of two subacute doses of imidacloprid in rabbits over a period of four months by using the Cytokinesis Block Micronuclei method; and reported genotoxic effects on rabbit lymphocytes. Demsia *et al.* (2007) studied *in vivo* micronucleus assay with rat bone marrow polychromatic erythrocytes and found a statistically significant effect after treatment with imidacloprid at doses of 300 mg/kg body weight. Costa *et al.* (2009) found significant increase in DNA damage and genotoxicity endpoints in human peripheral blood lymphocytes using the comet assay and micronucleus test at 20 µM imidacloprid exposure. In addition Feng *et al.* (2005), using human peripheral blood lymphocytes with comet assay and cytogenetic tests demonstrated imidacloprid to induce DNA strand breakages and chromosome and/or genome mutations. Furthermore, Feng *et al.* (2004) reported 0.05 to 0.5 mg/L for imidacloprid to cause DNA damage in erythrocytes of the frog Rana nigromaculata (Hallowell). To conclude, imidacloprid has mutagenic and recombinagenic effects as tested by various cellular and animal models, bioassays and biochemical markers.

Bu *et al.* (2011) reported fenitrothion to induce micronuclei in root tip cells of *Vicia faba* in a dose dependent manner. In a separate study, induction of mitotic crossing-over, mitotic gene conversion and reverse mutation were demonstrated in *Saccharomyces cerevisiae* (Yadav *et*

al., 1982). Their results showed that fenitrothion treatment did not induce any of these genetic events. Their results agreed well with ours since fenitrothion was negative in terms of mutation induction at all application concentarions tested.

Evidence of mutagenic effects from exposure to bifenthrin are inconclusive. Studies of mouse leukocytes were positive for gene mutation effects. However, other tests of bifenthrin's mutagenic effects, including the Ames test and studies in live rat bone marrow cells, were negative (Johnson *et al.* 2010). In our study 15 µg/L bifenthrin demonstrated positive results for small single spots, total *mwh* spots and total spots. It is well established that clone formation frequency of per 10^5 cells higher than 2.0 are indicative of genotoxic effect of the particular treatment (Graf *et al.* 1994). However the clone-formation frequency is lower than 2.0; leading us to conclude that bifenthrin does not have genotoxic potential.

Propoxur did not cause mutations in six different strains of bacteria and no mutagenicity was reported (Hallenbeck & Cunningham-Burns, 1985). Wang *et al.* (1998) showed N-nitroso propoxur (derivative) to be more cytotoxic than propoxur (with LC_{50}s 20 and six times smaller, respectively in V79 and RTE mammalian cells). N-nitroso propoxur significantly induced sister chromatid exchange (≥0.01 mg/mL), chromosome aberration (≥2.5 mg/mL) and hprt gene mutation (≥0.5 mg/mL) in V79 cells, and cell transformation (≥0.2 mg/mL) in RTE cells; but was not mutagenic to either type of cells. Gul *et al.* (2012) studied genotoxic effects of sublethal exposure concentration of 5 mg/L propoxur on juvenile common carp (*Cyprinus carpio* L., 1758). They found no statistically significant increases in micronuclei frequencies. Moreover, Ündeğer & Başaran (2005) studied effects of propoxur on human peripheral lymphocytes and although did not find significant cytotoxic effects, reported 50, 100 and 200 µg/mL propoxur exposure to induce DNA damage. Besides, a 28-day oral exposure study using 8.51, 3.40, and 0.851 mg/kg propoxur was performed in male Wistar rats, and the occurrence of numerical and structural chromosome aberrations were investigated by Siroki *et al.* (2001). They found only the high propoxur dose increased the number of numerical, but not the structural, chromosome aberrations. These results are in good agreement with ours, since the highest propoxur concentration (5.2 mg/L) revealed positive results for total mwh spots and total spots. These results suggest that at concentrations <5.2 mg/L propoxur is not genotoxic to *Drosophila* larvae.

Šojic *et al.* (2012) investigated genotoxic effects of thiamethoxam metabolites by using Ames test and comet assay; and recorded genotoxic intermediates less frequently. Similarly thiamethoxam showed low mutation frequency in our study.

In conclusion, exposure to 30 mg/mL deltamethrin, 40 mg/mL imidacloprid, 100 µg/mL DDT showed mutagenic and recombinagenic effects in the *Drosophila* wing spot test. The results presented here contribute to databases on the genotoxicity of insecticides and eventually used in risk assessment.

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