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ANALYSIS OF GENOTOXIC ACTIVITY OF 16 COMPOUNDS AND MIXTURES BY THE *DROSOPHILA* MOSAIC TEST

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Summary - *The mutagenicity of 16 compounds and mixtures were tested by the* Drosophila melanogaster *wing mosaic test. Fourteen of them gave negative results, two proved to be mutagenic. The positive compounds were chlor-diamino-toluene and 2-(2,4-dichlorophenoxy) propionic acid. Chlor-diamino-toluene increased the frequency of mitotic recombinations and gene mutations although it was found negative by the sex linked recessive lethal test. 2-(2,4-dichlorophenoxy) propionic acid caused only mitotic recombinations.*

Riassunto (Analisi dell'attività genotossica di 16 composti e miscele nel test del mosaico somatico in *Drosophila*). - *E' stata provata la mutagenicità di 16 composti e miscele con il test* Drosophila melanogaster *wing mosaic. Quattordici sostanze erano negative e 2, cloro-diammino-toluene e acido 2-(2,4-dicloro-fenossi propionico), erano mutagene. Il cloro-diammino toluene aumentava la frequenza di ricombinazione mitotica e mutazione genica, sebbene fosse negativo nel test dei letali recessivi. L'altra sostanza positiva causava soltanto ricombinazione mitotica.*

Introduction

We report results that concern the mutagenic activity of 16 compounds and mixtures tested by the *Drosophila* wing mosaic test.

Mutagens are known to be able to cause mosaic spots in the fruit fly [1, 2]. Mollet and Würgler suggested in 1974 to use somatic cells (eye *primordium*) of *Drosophila melanogaster* for mutagenicity screening testing. The wing spot test we used in our experiments has been described later [3-5]. By now the *Drosophila* somatic mutation and recombination test has become a useful method for indentifying mutagens and carcinogens [6].

We cross in the wing mosaic test two mutant stocks to obtain larvae which are trans-heterozygous for two recessive visible wing cell mutations (*mwh* and *flare*^{3}) or heterozygous for a chromosome with the *mwh* marker and a homologue chromosome (TM2) carrying multiple inversions. Multiple wing hair (*mwh*) homozygous or hemizygous wing blade cells develop 3-7 processes instead of the usual one, characteristic to wild-type and *mwh* heterozygous cells. Flare^3 (*flr*^{3}) homozygous cells have irregular shaped hairs. *mwh* and flare genes are located on the left arm of chromosome 3, 3-0.0 and 3-39.0, respectively. The phenotype of the hairs of the test animals is wild. In the case of mutagenesis, recessive phenotypes are re-expressed in the form of mosaic spots on the body of the adult flies. Any increase in the clone frequency, as compared to control reflects mutagenic activity. Sources of clone induction are chromosome breaks and/or point mutations. Chromatid breaks proximal to the *flr locus* may lead to mitotic recombination that is visualized as twin spots. Breaks can also eliminate the wild-type alleles in heterozygous cells and thus single spots develop. These events are detected in both *mwh* +/+ *flr*^{3} and *mwh*/TM2 wing blade cells. Chromosome breaks are unlikely to be the source of *mwh* mosaic spots in *mwh*/TM2 wings, because mitotic recombination that involves multiple-inversion-bearing chromosomes (like TM2) results in aneuploidy and this stage is incompatible with cell viability. This *mwh* spots on *mwh*/TM2 wings reflect most probably induction of point mutations in the *mwh*$^+$ allele [3]. The difference in the frequency of mosaic spots on *mwh*/*flr*^{3} and on *mwh*/TM2 wings gives the number of mitotic recombinations.

Materials and methods

The substances to be tested (Table 1) were mixed with standard *Drosophila* food just prior to pouring it into glass vials at a temperature of about 45-50 °C. Larvae (50 per vial) were transferred into the vials shortly after it cooled down. Larvae deriving from a 8 h egg collection were 68-76 h old after egg laying. During the treatment larvae developed on the test substance-containing food up to pupariation. This period covers approximately 96-98% of the mitotic cell cycles in a wing primordium. Any delay in development and the rate of survival to adulthood (as indicators of toxicity) were recorded. Experiments were

and *mwh*/TM2 females were sorted out and analysed separately. Wings were mounted and screened to detect *mwh* mosaic spots under a compound microscope at a magnification of 400x. Data were analysed by the χ^2 test (2x2 contingency, with Yates correction).

The test was applied at the 5% level. A replicate experiment was carried out with each compound.

Flies to be tested came from a cross in which w^{co}/w^{co}; *flr se*/TM2 *Ubx*^{130} *se e* virgin females were mated with *fs(l)K10w/Y: mwh se e/mwh se e* males. In one experiment we crossed *fs(l)K10w/ClB; mwh se e/mwh se e* females with *mwh*$^+$ yw^{co} *f/Y; mwh se/mwh se* males and *mwh*$^+$ yw^{co} *f/fs(1)K10w; mwh se e/mwh se* female larvae were used for the test [6]. Mitotic recombination, deletion or gene mutation on the first chromosomes result in mosaic spots in these animals. The tester strains were provided by J. Szabad, Institute of Genetics, Biological Research Center, Szeged, Hungary.

Results and discussion

The results are summarized in Table 2. All chemicals used in agriculture proved to be nonmutagenic in the mosaic test except 2-(2,4-dichlorophenoxy) propionic acid sodium salt (Diclorprop, see Table 3).

Table 1. - *Composition of products and chemical names of active ingredients*

Copper oxychloride 450 FW	fungicide product
Bacillus thuringiensis	insecticide
Mavilex Diuron 315 g/l 3-(3,4-dichlorophenyl)-1,1-dimethylurea Atrazin 235 g/l 2-chloro-4-ethylamino-6-isopropylamino-1,3,5- triazine Dicamba 78 g/l 3,6-dichloro-2-methoxy benzoic acid	herbicide product
Fenitropan (1*RS*, 2*RS*)-2-nitro-1-phenyltrimethylene-di(acetate)	fungicide
Potassium ethyl-xanthogenate	
Prodiglyk (Mixture of Proflavine-diglucoside Proflavine-monoglucoside)	plant growth regulator
Heptopargil (E)-[(1*RS*, 4*RS*)-bornan-2-one *O*-prop-2-ynyloxime]	plant growth regulator
Dithane Kuprokelat (40% Mancozeb and mixture of chelating agents containing trace amount of Fe, Mn, Zn, Cu, B, Mo, Mg)	fungicide product
Biosperse (didecyl-dimethyl ammoniumchloride)	tenside
EGYT 2509 (2-chloro-1,2-[dimethylamino-isobutyl]-dibenzo[d,g][1,3,6]dioxazocine hydrochloride)	drug
Chlor-diamino toluene 92% CAT III	

Table 2. - *Characteristics of wing mosaicism*

Compound	Concentration w/w (%)	Genotype	no. of wings analysed	no. of *mwh* clones	Clone/wing
Agricultural chemicals					
1. *Copper oxychloride 450 FW*	0.063	*mwh/flr*^{3}	24	24	1.0
	0.031		40	33	0.83
	control		40	36	0.9
	0.031	*mwh*/TM2	40	10	0.25
	control		40	8	0.20
2nd experiment	0.016	*mwh/flr*^{3}	40	34	0.85
	control		40	39	0.98
2. *Bacillus Thuringiensis*	14.1	*mwh/flr*^{3}	40	38	0.96
	control		40	45	1.1
	14.1	*mwh*/TM2	40	12	0.3
	control		40	11	0.28
2nd experiment	14.1		39	41	1.1
	control		40	46	1.2
3. *Mavilex H-125-E FW*	0.125	*mwh/flr*^{3}	40	40	1.0
	control		40	43	1.1
	0.125	*mwh/TM2*	40	5	0.13
	control		40	7	0.18

Table 2. - *(continued)*

Compound	Concentration w/w (%)	Genotype	no. of wings analysed	no. of *mwh* clones	Clone/wing
2nd experiment	0.125	*mwh/flr³*	40	36	0.90
	control		40	38	0.95
	0.056	*mwh*/TM2	40	7	0.18
	control		40	9	0.22
4. *Fenitropan*	0.056		40	35	0.87
	control		40	9	0.22
2nd experiment	0.056	*mwh⁺, flk10w*	40	50	1.25
	control		40	4	1.20
5. *Potassium ethyl-xanthogenate*	0.1	*mwh/flr³*	40	44	1.1
	control		40	47	1.2
	0.1	*mwh*/TM2	40	10	0.25
	control		40	15	0.37
2nd experiment	0.2	*mwh/flr³*	16	15	0.94
	0.1		45	47	1.3
	0.05		12	13	1.0
	control		50	49	0.98
6. *Prodiglyk (contains 0.89% proflavine)*	5	*mwh/flr³*	40	30	0.75
	2.5		14	12	0.86
	control		40	46	1.2
2nd experiment	2.5		40	33	0.83
	control		40	34	0.85
	2.5	*mwh*/TM2	40	7	0.18
	control		40	7	0.18
7. *Heptopargil*	0.011	*mwh/flr³*	40	37	0.93
	control		40	36	0.9
2nd experiment	0.019		40	41	1.0
	control		40	50	1.3
	0.019	*mwh*/TM2	40	11	0.28
	control		40	8	0.20
8. *Dithane Kuprokelat*	0.125	*mwh/flr³*	40	36	0.9
	control		40	40	1.0
2nd experiment	0.063		40	42	1.1
	control		40	3	0.93
	0.063	*mwh*/TM2	40	10	0.25
	control		40	9	0.23
Miscellaneous					
9. *Biosperse (tenside)*	0.5	*mwh/flr³*	20	16	0.8
	0.25		40	50	1.3
	control		40	52	1.3
	0.25	*mwh*/TM2	40	8	0.2
	control		40	10	0.25
2nd experiment	0.25	*mwh/flr³*	40	38	0.95
	control		40	4	1.1
10. *EGYT-2509 (drug)*	0.33	*mwh/flr³*	40	40	1.0
	control		40	50	1.3
	0.33	*mwh*/TM2	40	5	0.13
	control		40	9	0.23
2nd experiment	0.59	*mwh/flr³*	16	11	0.69
	0.33		40	44	1.1
	control		40	52	1.3
11. *Chlor-diamino toluene 92% CAT III*	1		28	65	2.3 *(*)*
	control		28	31	1.1
2nd experiment	1		37	84	2. 3 *(*)*
	0.5		40	55	1.4
	0.25		40	50	1.3
	control		39	40	1.0
	1	*mwh*/TM2	40	18	0.45
	control		41	7	0.17
12. *Four samples of powdered bovine plasma*	10-20	*mwh/flr³* *mwh*/TM2	-	-	not mutagenic

(*) Significantly different from the control, $p < 0.05$

Table 3. - *Wing spot data obtained after exposure 2-(2.4-dichlorophenoxy) propionic acid*

Concentration w/w (%)	Genotype	Total spots wing	Large spots wing	Twin spots wing	Wings with spot no. of wings
1st experiment					
0.063	*mwh/flr*^{3}	60/59 = 1.0	7/59 = 0.12	3/59 = 0.05	37/59 = 0.63
0.031		94/62 = 1.5	21/62 = 0.34 (*)	6/62 = 0.096	53/62 = 0.85 (*)
control		84/86 = 0.98	10/86 = 0.12	3/86 = 0.035	48/86 = 0.56
0.031	*mwh*/TM2	7/40 = 0.18			
control		7/40 = 0.18			
2nd experiment					
0.063	*mwh/flr*^{3}	31/16 = 1.9 (*)	8/16 = 0.5 (*)	2/16 = 0.13	12/16 = 0.75
0.031		114/24 = 4.8 (*)	36/24 = 1.5 (*)	17/24 = 0.71	19/24 = 0.79
0.016		25/18 = 1.4	10/18 = 0.56	0/18 = 0.0	12/18 = 0.67
control		25/32 = 0.78	2/32 = 0.062	0/32 = 0.0	25/32 = 0.78

(*) Significantly different from the control, $p < 0.05$

In the assays with Diclorprop the following types of spots were evaluated separately: small single spots with only one or two mutant cells, large single spots with more than two affected cells, twin spots, total spots and wings with mosaic spot/number of wings. Since much higher frequency of clones developed on *mwh/flr*^{3} than on *mwh*/TM2 wings, we concluded that Diklorprop induced more breaks than point mutations at one *locus*.

Four samples of powdered bovine plasma, made by heating, gave unequivocally negative results at 10-20%, although the extract of one of them was found to be mutagenic by the *Salmonella*/microsome test.

We analysed the mutagenic activity of EGYT-2509, of Prodiglyk and of chlor-diamino-toluene by the sex-linked recessive lethal test too.

EGYT-2509 and Prodiglyk gave the same result in both tests. Chlor-diamino-toluene was efficient in producing somatic recombinations and mutations but proved to be negative in the sex-linked recessive lethal test.

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