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Studies on the genotoxicity of cypermethrin in *Drosophila melanogaster*.

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Cypermethrin (CAS No. 52315-07-8) is a synthetic pyrethroid insecticide used against a wide range of crop pests. It is reported to decrease the activities of acid and alkaline phosphatases in the liver and muscle of fishes (Bhatnagar *et al.*, 1995). It also depletes blood cholesterol, free fatty acids and mitochondrial enzymes in different tissues of fishes (Ghosh, 1990).

According to Pluijmen *et al.* (1984) and Klopman *et al.* (1985) this compound failed to induce gene mutations in *Salmonella typhimurium*. In *E. coli*, cypermethrin did not induce gene mutations (Brooks, 1976). Atale *et al.* (1993) observed a reduction in mitotic index and induction of chromosomal aberrations in *Capsicum annuum*. It also induced chromosomal aberrations in the spermatogonial cells of grasshopper *Poecilocerus pictus* (Shyin and Usha Rani, 1994). According to Amer and Aboul-ela (1985) this compound induced micronuclei in high frequencies in the bone marrow cells of mouse. The present communication describes the results obtained in the *Drosophila* wing spot as well as sex-linked recessive lethal tests following larval exposures.

Wing mosaic test is a fast one-generation test which assays several genetic end points induced by a mutagen in the wing primordial cells of *Drosophila*. The *mwh* +/+ *flr*^{3} trans-heterozygous larvae were obtained from the cross of high bioactive strains of *mwh* females and *flr*^{3}/TM3, *Ser* males. The allele *mwh* (*multiple wing hairs*, 3-0.3) and *flr*^{3} (*flare*, 3-38.8) are recessive genetic markers expressed autonomously as multiple trichomes or thick, misshapen trichomes on an otherwise normal adult wing. For details of the markers please refer to Lindsley and Zimm (1992). Third instar larvae were exposed to the LD_{50} (1×10^{-4} %) and lower doses of the test compound in instant food for the rest of the larval life, *i.e.*, for 48 h (Graf *et al.*, 1984). The wings of the eclosing flies were mounted and observed under a compound microscope to record the size and frequency of the mosaic spots (clones). Each experiment was repeated and the data were pooled and are represented in Table 1. For each experiment a concurrent control experiment was run where the larvae were exposed to the solvent (distilled water). The data were statistically evaluated following the conditional binomial test (Frei and Wurgler, 1988).

The sex-linked recessive lethal (SLRL) test, although time consuming as it involves more than one generation, is regarded as the best validated genotoxicity test in *Drosophila*. In this test, Oregon R larvae of same age were exposed to similar doses of cypermethrin as in the wing mosaic assay. The adult males, on eclosion, were crossed with 3 *Basc* homozygous females for 3 days. The resulting *Basc*/Y males were mated to their *Basc*/Ore-R sibs at a ratio of 1:1 in individual vials. The F_2 progeny were checked for the presence/absence of males with wild type eyes. The data on the frequency of lethal induction (Table 2) were evaluated statistically following Kastenbaum and Bowman (1970).

The frequency of small singles (*mwh* or *flr*^{3}) with 1-2 cells, large singles with 3 or more affected cells and twin (*mwh*/*flr*^{3}) spots were evaluated separately and the statistical outcomes were inconclusive for all types of spots. In this assay single spots originate due to the induction of gene mutations or gene conversions in the corresponding wild-type genes, deletion of chromosome parts carrying the wild-type alleles (Graf *et al.*, 1984) or induction of mitotic recombination in the chromosome region between the *mwh* and *flr*^{3} loci (Garcia-Bellido and Dapena, 1974). Twin spots with *mwh* and *flr*^{3} subclones, on the other hand, stem from the induction of mitotic recombination in the chromosome region between the *flr*^{3} locus and the centromere (Becker, 1976). In the present experiments, since the frequencies of different wing spots were not significantly higher than the control frequencies, it is concluded that cypermethrin is nongenotoxic in the wing primordial cells of *Drosophila*.

In the SLRL test, the frequency of lethals although higher than the control frequency was not significantly different from the control. Normally the sex-linked recessive lethals arise due to induction of

gene mutations, deletions of small chromosome parts and certain types of chromosomal aberrations (Lee *et al.*, 1983).

Cypermethrin, although it has been reported to induce sex chromosome loss in *Drosophila* (Marcos *et al.*, 1986), has failed to induce sex-linked recessive lethals in the present experiments. Thus it is concluded that this synthetic pyrethroid is nongenotoxic both in the somatic and germ line cells of *Drosophila melanogaster* following larval treatments.

Table 1. Summary of data obtained in the wing mosaic assay.

Treatment (hr)	Conc. (%)	No. of wings observed	Spots per wing (No. of spots) Small singles (s = 1-2) [m =2.0]	Large singles (s > 2) [m = 5.0]	Statistical Dignoses* Twins (t) [m = 5.0]	Total (T) [m = 2.0]
	Control	80	0.25(20)	0.04 (3)	0.00 (0)	0.29 (23)
	1×10^{-4}	80	0.36 (29) i	0.03 (2) -	0.01 (1) i	0.40 (32) i
48	5×10^{-5}	80	0.31 (25) i	0.04 (3) i	0.00 (0) i	0.35 (28) i
	2.5×10^{-5}	80	0.31 (25) i	0.01 (1) -	0.00 (0) i	0.33 (26) i

*Statistical diagnoses according to Frei and Wurgler (1988). + = positive, i = inconclusive, - = negative, m = multiplication factor. Probability levels: Alpha = Beta = 0.05. One-sided statistical tests.

Table 2. Summary of data obtained in the sex-linked recessive lethal test.

Treatment (hr)	Conc. (%)	Males tested	Tested chromosomes per male (Mean ± SD)	X chromosomes Total	Lethal	%	Conclusion*	Lethals per male 0	1	2
	Control	134	18.51 ± 3.08	2480	7	0.28		147	7	0
	1×10^{-4}	52	20.37 ± 1.63	1059	5	0.47	NS	47	5	0
48	5×10^{-5}	68	18.69 ± 2.41	1271	4	0.31	NS	64	4	0
	2.5×10^{-5}	64	20.16 ± 1.75	1290	4	0.31	NS	60	4	0

* Conclusion on the basis of Kastenbaum and Bowman (1970). NS = not significant, level of significance $P < 0.05$.

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