Research Notes

Genotoxicity of Sevin in wing primordial cells of *Drosophila melanogaster* in vivo.

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Abstract

The genotoxicity of Sevin, a systematic carbamate pesticide, has been evaluated in the wing primordial cells of *Drosophila melanogaster* by the larval feeding method. Larvae (48 ± 4h/72 ± 4h) heterozygous for mutations were cultured on the Sevin-containing media for their remaining life. Emerged flies were screened for mosaics on their wings to know the genotoxicity of Sevin.

Introduction

With the increased production and application of pesticides all over the world, it is essential that maximum effort should be geared to generate the knowledge about the hazardous impact on non target organisms. The impact in non-targets may influence the genetic systems and cause mutations that ultimately influence the progeny by heredity. Carbaryl, a carbamate pesticide, causes behavioral and neurological (Moser, 1988; Sideroff and Santolucito, 1972; Dsi, 1974; Anger and Setzer, 1979; Branch and Jacqz, 1986), reproductive (Cantor, 1992; Zahm, 1988; Davis, 1993), and carcinogenic problems (Smalley *et al*., 1968; Whorton, 1979) in exposed systems. However, there are inconclusive reports on the genotoxicity of Carbaryl in *Drosophila* (Dey *et al*., 1987). Hence, the present investigation is an endeavor to explore the genotoxicity of Carbaryl in *Drosophila melanogster*, by wing mosaic assay.

Materials and Method

Most widely used Carbary, that is Sevin (CAS No.63-25-2), was tested. Two *Drosophila* stocks, *mwh*/*mwh* and flr^3/flr^3 (Lindsley and Grell, 1968) were used in the present studies. All the culture stocks are maintained on standard *Drosophila* medium at 25 ± 1°C and RH 60%. The larvae (48 ± 4h/72 ± 4h) obtained after crossing males of the *mwh*/*mwh* stock with the virgin females of the flr^3/flr^3 stock were collected by floating them in 20% NaCl (Graf *et al*., 1984). The same larvae were exposed to non-toxic doses (5, 10, 15 and 20ppm) of Sevin (LC_{50} = 150ppm) for their rest of their larval life. Wings of the emerged flies were screened for the induction of mosaics by the Sevin. Mosaics were analyzed statistically using the SMART program (Frei and Wurgler, 1988).

Results and Discussion

Sevin was assayed separately in somatic cells of *Drosophila* by wing mosaic test at two instars using four different concentrations (5, 10, 15, and 20ppm). The results of the experiment

(Tables 1 and 2) reveal the frequency according to number of mosaics counted. There is an increase in mosaics with the concentration of the chemical fed to the larvae. Among the mosaics, single spots were predominating and twin spots are completely absent. However, at all the doses tested, Sevin induced insignificant mosaics to control indicating nongenotoxicity in wing primordial cells of *Drosophila*.

The wing mosaic assay is based on the formation of homozygous mutant clones on the adult's wing cuticle due to exposure to a mutagen during larval stage (Auerbach, 1946; Becker, 1975) and is a tool to generate the structure activity relation of chemicals (Graf *et al*., 1992). Single spots arise due to gene mutation, gene conversion, aneuploidy, mitotic recombination, or nondisjunction, while twin spots are the result of mitotic recombination (Graf *et al*., 1984; Garcia-Bellido and Dapena, 1974).

Table 1. Data on wing mosaic assay after 48 ± 4h aged larval exposure to Sevin.

Larval age	Sevin (ppm)	No.of wings screened	Small single spots	Large single spots	Twin spots	Total spots
48 ± 4h	0.0(control)	40	3(0.07)	0(0)	0(0)	3(0.07)
	5	40	4(0.01)-	1(0.02)-	0(0)-	5(0.15)-
	10	40	4(0.01)-	2(0.05)-	0(0)-	6(0.15)-
	15	40	5(0.012)-	2(0.05)-	0(0)-	7(0.17)-
	20	40	6(0.15)-	3(0.07)-	0(0)-	9(0.22)-

Statistical diagnosis according to Frei and Wurgler (1988):
+ positive, - negative, i inconclusive.
Probability levels: α=β=0.05. One sided statistical test.

Table 2. Data on wing mosaic assay after 72 ± 4h aged larval exposure to Sevin.

Larval age	Sevin (ppm)	No.of wings screened	Small single spots	Large single spots	Twin spots	Total spots
72 ± 4h	0.0(control)	40	4(0.1)	0(0)	0(0)	4(0.1)
	5	40	5(0.12)-	2(0.05)-	0(0)-	7(0.17)-
	10	40	6(0.15)-	2(0.05)-	0(0)-	8(0.2)-
	15	40	6(0.15)-	3(0.07)-	0(0)-	9(0.22)-
	20	40	7(0.17)-	4(0.1)-	0(0)-	11(0.25)-

Statistical diagnosis according to Frei and Wurgler (1988):
+ positive, - negative, i inconclusive.
Probability levels: α=β=0.05. One sided statistical test.

The present study infers the nongenotoxicity of Sevin in wing disc cells of *Drosophila*. But Sevin had induced Sex Linked Recessive Lethal (Brzheskii, 1972) in *Drosophila*. However, the inconclusive results of Sex Linked Recessive Lethal (Dey *et al*., 1987) made it important to reevaluate the effect of Sevin. The microsomes of insects function similarly to those in mammalian liver (Vogel and Sobels, 1976). Reactions generating O_2 also generate H_2O_2 by dismutase reaction that results in -OH, a potent oxidant (Haber and Wiss, 1934). Dismutases, catalases, and peroxides

are maintained the homeostasis (Fridovich, 1975) that results in the integrity and fidelity of the DNA. Carbamates are known to inhibit proteins (Cornman, 1954; Rannug and Rannug, 1984). But from the present results of the wing mosaic assay, it is possible that the same mechanism may not be operating with Sevin in wing disc cells of *Drosophila*.

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Drosophila lowei collections from Mount Lemmon, Arizona, in 2009.

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The *Drosophila pseudoobscura* subgroup inhabits western North America and contains seven species (Lakovaara and Saura, 1982; Heed and O'Grady, 2000), but four of these species have reportedly not been collected in over a decade nor are there living cultures of them. The relationships of four of the species within the pseudoobscura subgroup are well supported by allozymes (Figure 1; Lakovaara *et al*., 1976), and a phylogeny reconstructed using the mitochondrial gene *cytochrome oxidase II* (Beckenbach *et al*., 1993) revealed that *D. lowei* diverged from *D. pseudoobscura* approximately 8.4 Mya (Aquadro *et al*., 1991; Beckenbach *et al.*, 1993). The next closest ancestors to *D. pseudoobscura*, species of the *D. affinis* subgroup, diverged approximately 17Mya (Aquadro *et al*., 1991; Beckenbach *et al*., 1993). Genomic sequence data and establishment of laboratory stocks of *D. lowei* may enhance comparative studies within the pseudoobscura subgroup. Scientific collections of *D. lowei*, however, have not been undertaken since those made for Beckenbach *et al*. (1993).

First collected in 1960 in Santa Catalina Mountains near Tucson, Arizona (Heed *et al*., 1962), *D. lowei* has also been documented in the Chiricahua Mountains and Mongollon Rim in Arizona and near Pikes Peak, Colorado (Heed *et al*., 1969). Like other southern species of the pseudoobscura subgroup (Heed and O'Grady, 2000), *D. lowei* is restricted to highlands and is found in highest